UNIVERSITAT DE BARCELONA

# Physical activity and sleep in extreme weight conditions

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# PHYSICAL ACTIVITY AND SLEEP IN EXTREME WEIGHT CONDITIONS


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A thesis submitted to the

University of Barcelona, Faculty of Medicine

PhD Program: Medicine 2016

*Alla mia famiglia.*
*A la meva familia.*

“The important thing is not to stop questioning.
Curiosity has its own reason for existing.”

ALBERT EINSTEIN *(Ulm, 14th of March 1879–Princeton, 18th of April 1955).*

# ABSTRACT

There is an increasing worldwide prevalence of obesity and anorexia nervosa. These conditions are having important health consequences. Among the numerous factors linked to these extreme weight conditions, deviations in physical activity and sleep patterns have been identified. However, the underlying mechanisms are not yet clear. Compared to healthy-weight individuals, those with obesity and anorexia nervosa present a distinctive temperament and cognitive profile as well as specific alterations in plasma endocannabinoid and endocrine hormone levels. Separately, these psychobiological processes are being associated with sleep-wake behaviour.

This thesis gathers six studies to examine sleep and physical activity patterns in extreme weight conditions and the modulating role of several neuroendocrine factors, temperament, psychopathological indexes and cognitive functioning. A further aim, exclusive to anorexia nervosa, was to evaluate the effects of these mechanisms on the outcome of a partial, day hospital treatment.

An inverse association was found between moderate-to-vigorous physical activity (MVPA) and body mass index, in part mediated via the actions of exercise on endocannabinoid system functioning. A temperament profile of low novelty seeking and high harm avoidance, as was in the participants with obesity, may be implicated in the adversity of these individuals towards exercise. Additionally, individuals with obesity were found to have difficulties in decision-making (selecting choices that provided immediate reward despite long-term loss), which was linked to both elevated plasma concentrations of irisin and less MVPA.

Contrarily, a large variation was identified in the physical activity patterns of patients with anorexia nervosa; the majority presented daytime physical activity and MVPA habits that are similar to those of individuals without an eating disorder, except for a group who were particularly active. Furthermore, regulated and supervised MVPA may be a useful strategy for reducing the symptoms of depression identified in these patients and to promote treatment success.

Regarding sleep, both individuals with obesity and anorexia nervosa reported several sleep difficulties, suggesting a u-shaped relationship between sleep and body mass index. Poor sleep quality was associated with increased plasma orexin-A in both

conditions. In obesity the interaction between poor sleep and raised plasma orexin-A was associated with elevated body mass index. The interplay between sleep and orexin-A may have an adverse effect on treatment outcome.

In conclusion, the present thesis demonstrates that both physical activity and sleep are highly relevant components in the psychopathology of obesity and anorexia nervosa, contributing to the development and maintenance of these conditions via their interaction with other altered psychobiological processes.

# ACKNOWLEDGEMENTS

I would like to thank my supervisors, Prof. Fernando Fernández-Aranda and Prof. Jon Arcelus for their guidance and aid throughout the process of writing this thesis and the development of the studies included. I would like to thank Dr. Roser Granero for her assistance in statistical analysis, and Dr. Susana Jiménez-Murcia, who gave me the opportunity to additionally study the gambling disorder.

To Dr. Cristina Giner-Bartolomé, Dr. Ines Wolz and Dr. Inés Hilker, thank you for accompanying me both as colleagues and friends throughout the past four years and these last few months as you were also completing your own PhD. Thank you Dr. Zaida Agüera, for your assistance across all aspects of the research process, and to Gemma Mestre, Iris Tolosa, Dr. Núria Mallorquí and Trevor Steward; I have only been able to work with you for the last stages of this process, but it feels like you were present from the start. I would like to acknowledge everyone who has been part of the eating disorders unit of the University Hospital of Bellvitge during my work: Salomé, Moha, Melania, Edu and Maria Rita. Dr. Bea Fagundo, I will always appreciate your help as I was first learning about neurotransmission and brain function. To Dr. Nadine Riesco and Dr. Isabel Sánchez, thank you for sharing with me your immense clinical knowledge and experience; I will never stop being fascinated by your work.

I deeply appreciate all the support I have received from my friends, old and new, with origins from across the world: Spain, Italy, United Kingdom, France, Netherlands, Belgium, Germany, Turkey, Iran, India, China, Malasya, South Africa, Equador, Mexico, United States.

Most importantly, I need to give my thanks to my family. To my father and mother, who were constantly with me, during the good and difficult moments of this process, and for providing their support and patience as I attempted to express complex concepts and ideas. Papà, grazie per aiutarmi ogni volta che ne ho avuto bisogno. Mamma, grazie per condividere i tuoi conoscimenti come disegnatrice grafica. To my brothers, Marc and Oscar, thank you for all of your encouragement; spero che continuate a seguire le vostre passioni.

I deeply thank my Italian grandparents, without whom I would not be where I am now; my Spanish grandfather, who was a friend and not only family; and my Spanish

grandmother, who showed me the strength of determination. To my aunt and uncle; although far away, you were always close. I would also like to thank Montse, Eni and Esteve, who have always been family.

"No matter what accomplishments you make, somebody helped you"

ALTHEA GIBSON *(Clarendon County, 25th of August, 1927 – East Orange, 28th of September 2003).*

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# TABLES AND FIGURES

# LIST OF ABBREVIATIONS

**AEA:** Anandamide

**2-AG:** 2-Arachidonoylglycerol

**5-HT1A:** 5-Hydroxytryptamine (serotonin) receptor 1A

**5-HT2A:** 5-Hydroxytryptamine (serotonin) receptor 2A

**ACC:** Anterior cigulate cortex

**AEE:** Activity-based energy expenditure

**AGB:** Adjustable gastric banding

**AgRP:** Agouti gene-related peptide

**AN:** Anorexia nervosa

**BDNF:** Brain-derived neurotrophic factor

**BED:** Binge eating disorder

**BM:** Bulimia nervosa

**BMI:** Body mass index

**CAT:** Cognitive-analytic therapy

**CBT:** Cognitive-behavioural therapy

**CBT-E:** Enhanced cognitive-behavioural therapy

**DRD4:** Dopamine receptor D4

**ED:** Eating disorders

**EDNOS:** Eating disorders not-otherwise-specified

**FAAH:** Fatty acid amide hydrolase

**FBT:** Family-based treatment

**FNDC5:** Fibronectin type III domain containing 5

**FPT:** Focal psychodynamic therapy

**FTO:** Fat mass and ovesity-associated gene / lpha-ketoglutarate-dependent dioxygenase

**GABA:** Gamma-aminobutyric acid

**GLP-1:** Glucagon-like peptide-1.

**GWAS:** Genome-wide association studies

**IGT:** Iowa gambling task

**LSG:** Laparoscopic sleeve gastrectomy

**MANTRA:** Maudsley model of anorexia nervosa treatment for adults

**MOB:** Morbid obesity

**MVPA:** Moderate-vigorous physical activity

**non-REM:** Non-rapid eye movement

**NAEs:** N-acylethanolamides

**NAPE:** N-arachidonoyl phosphatidylethanolamine

**NEAT:** Nonexercise activity thermogenesis

**NPY:** Neuropeptide Y

**OB:** Obesity

**OEA:** *N*-oleylethanolamide

**PA:** Physical activity

**PC1/3:** Prohormone convertase 1/3

**PCSK1:** Proprotein convertase 1

**PEA:** Palmitoylethanolamide

**PFC:** Prefrontal cortex

**PGCI α:** PPARγ coactivator-1 α

**POMC:** Pro-opiomelanocortin

**PPAR:** Peroxisome proliferator-activated receptors

**PPY:** Pancreatic polypeptide

**RCT:** Randomised controlled trial

**REM:** Rapid eye movement

**RYGB:** Roux-en Y gastric bypass

**SCN:** Superchiasmatic nucleus

**SEM:** Structural equation modelling

**SPA:** Spontaneous physical activity

**SSCM:** Specialist Supportive Clinical Management

**SSRI:** Selective serotonin reuptake inhibitors

**sgACC:** Subgenual anterior cigulate cortex

**SWA:** Slow wave activity

**SWS:** Slow wave sleep

**UCP1:** Uncoupling protein 1

**WCST:** Wisconsin card sorting test

**WHO:** World health organization

**α-MSH**: α-melanocyte-stimulating-hormone

# PREFACE

This thesis was presented to the University of Barcelona in fulfilment of the requirements for a Doctor's degree. Six articles are included, conducted at the Eating Disorders Unit at the University Hospital of Bellvitge (Barcelona, Spain) with the collaboration of other research centres part of the CIBERobn research network (see *Section 3.1*). The articles have been published in international scientific journals, and achieve a global impact factor (IF) score of **22.27**. The IF of overall published collaborations accumulates to **39.7**. [Science Citation Index, 2015]

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## COLLABORATING INSTITUTIONS





## FINANCIAL SUPPORT

**IDIBELL - Bellvitge Biomedical Research Institute.** Predoctoral grant. Centre: Department of Psychiatry, University Hospital of Bellvitge, Barcelona. Support period 01.2013-01.2017. Beneficiary: Sarah Sauchelli Toran

**CIBER Fisiopatología de la Obesidad y Nutrición – CB06/03 (CIBERobn).** Research travelling grant. Centre: University of Tübingen, Tübingen, Germany. Support period: 01.2016-04.2016. Beneficiary: Sarah Sauchelli Toran

**CIBER Fisiopatología de la Obesidad y Nutrición- CB06/03 (CIBERobn)**. Instituto Carlos III - Ministry of Health. Centre: Department of Psychiatry, University Hospital of Bellvitge, Barcelona. Support period: 01.2007- . IP: F. Fernández Aranda. (http://www.ciberobn.es/)

**Research Project (FIS), Instituto de Salud Carlos III.** Neurocognition and emotion regulation in extreme weight conditions: Study of cerebral activity and changes associated to an intervention based on a therapeutic videogame (PI14/00290). Centre: Department of Psychiatry, University Hospital of Bellvitge, Barcelona. Support period 20134-2017. IP: Fernando Fernández-Aranda.

# CHAPTER 1. INTRODUCTION

Over the past few decades there has been a worldwide exponential rise in the prevalence of Obesity (OB) and anorexia nervosa (AN). Projections to the future suggest that by 2030, 1.12 billion individuals will be obese [1]. Individuals with OB have an elevated risk of cardiovascular and metabolic diseases, and this poses an important public health cost [2]. On the opposite extreme of the Body Mass Index (BMI) continuum, the incidence of AN is also rapidly increasing [3]. AN is a chronic psychiatric disorder characterized by the need to maintain a low body weight despite the broad range of severe medical risks that emerge. Although the aetiology of OB and AN is not yet clear, the literature highlights the involvement of multiple psychological, biological and sociocultural factors [4,5].

Among the factors associated with fluctuations in BMI, physical activity (PA) appears to be one of the main contributors. Individuals with OB present prolonged sedentary behaviour and low levels of moderate-vigorous PA (MVPA), such as walking rapidly or exercising [6]. Professionals worldwide have therefore emphasized the need to encourage PA and lifestyle intervention programs for OB that incorporate PA [7,8]. However, overtraining may result in fatigue, bradycardia, immune deficiency, insomnia, agitation, hypertension, and, if it is not compensated by an adequate diet, it can lead to dangerous weight loss [9]. Excessive exercising is considered a highly problematic and frequent facet of AN [10]. Hence, AN is more common among athletes and ballet dancers [11,12].

Disturbances in sleep are also a frequent problem in these extreme eating/weight conditions (EWC). Poor sleep is a common complaint among patients with AN, and greater symptom psychopathology has been associated with sleep maintenance difficulties, early morning awakening, difficulties in sleep initiation, and short sleep [13,14]. Similarly, insufficient or very long sleep has been reported in OB [15], and a reduction in fat mass has been found to take place concurrent to enhanced sleep [16]. Given the increasing prevalence of OB and AN, and the clinical significance of inadequate sleep and PA patterns for the development, maintenance, and prognosis in these conditions, there is an urgent need to understand the underlying etiopathological mechanism.

## 1.1. OBESITY

The World Health Organization (WHO)[17] defines OB as an abnormal or excessive accumulation of fat that may be harmful for health, and establishes a state of OB using the threshold marker body mass index (BMI). This is an index defining weight for height squared (klm/$m^2$), which is used to identify weight categories from underweight to OB. According to the WHO [17], a BMI greater than or equal to 30kg/$m^2$ is indicative of OB. OB impoverishes quality of life and augments the risk for various chronic diseases. It is one of the main causes of worldwide mortality, and has been labelled as the epidemic of the XXI century [18].

It is generally accepted that OB emerges as a consequence of an energy imbalance. Energy balance remains stable when energy intake (energy gathered from the ingestion of food and liquids that can be metabolised in the body) equates energy expenditure (energy used to maintain stable conditions in the body and to be physically active). In OB, energy intake surpasses energy expenditure. This can result in an increase in the energy stored in the body and weight gain takes place. Human and animal research has shown that multiple psychological, biological and social factors are implicated.

### 1.1.1. CLASSIFICATION

The most commonly used tool to define OB in adults is BMI [2] despite being unable to distinguish between lean mass and body fat [19–21]. The WHO makes the following subdivisions of OB [2].

| BMI (kg/$m^2$) | | Classification |
|---|---|---|
| From | To | |
| 30kg/$m^2$ | 34.9 | Class I OB |
| 35 kg/$m^2$ | 39.9 | Class II OB |
| 49 kg/$m^2$ | | Class III OB (morbid OB) |

***Table 1.*** *Classification of obesity according to body mass index.*

Differently, in children and teenagers OB is defined using age and gender as normograms for BMI. A child is considered as obese when their age-gender-specific BMI is equal to or above the 95$^{th}$ percentile [22].

### 1.1.2. PREVALENCE

Since 1980, a rise in the prevalence of OB by 27.5% for adults and 47.1% for children has been reported [23]. According to the WHO [17], in 2014 (crude estimates) 39% of the adult population was identified as being overweight (38% of men and 40% of women), reaching 1.9 billion people, and 13% were classified as obese (11% of men and 15% of women), which corresponds to around 600 million worldwide. An increasing trend has also been seen in developing countries, and theprevalence of OB among men from Tonga is greater than 50% and that among South African women is of 42% [23].

In the European Region, 50% of adults of 46 countries considered (87% of the region) were classified as overweight or obese in 2008 [24]. In Spain, the annual statistics for the year 2011/2012 showed the prevalence of OB in adults to be 17% (male = 18, female = 16) and 36.7% (male = 45.1%, female = 28.1) for overweight. Among children, 9.6 % were classified as obese (females = 9.6%, males = 9.6%) and 18.3% overweight (females = 16.9, males= 19.5) (Ministerio de Sanidad, Servicios Sociales e Igualdad, 2015).

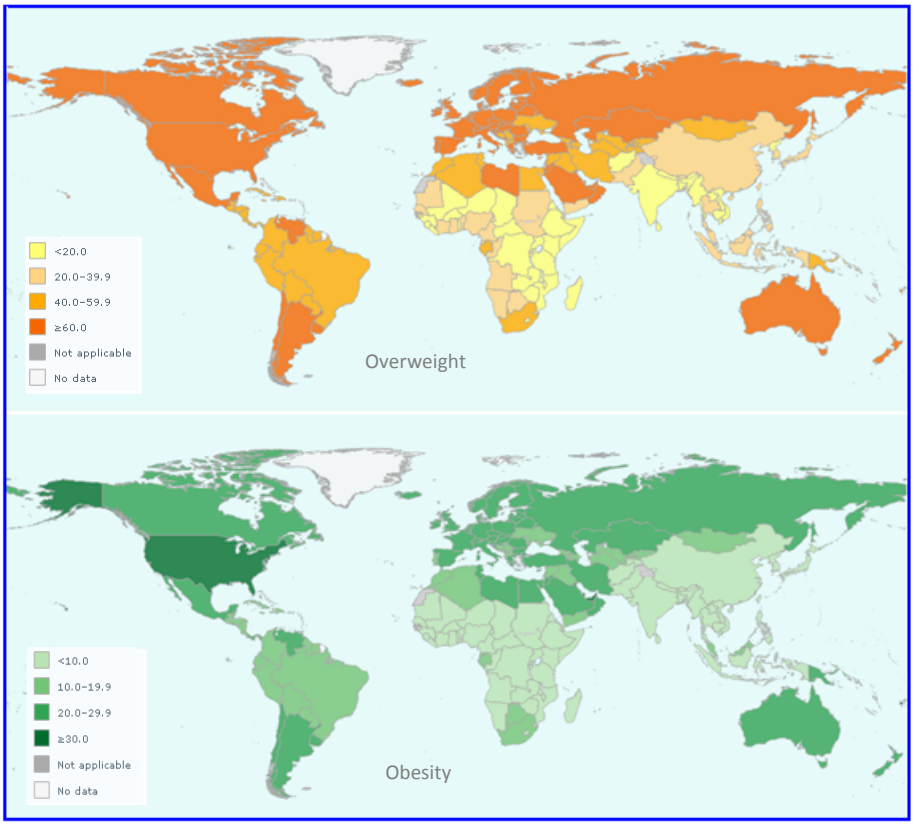


**Figure 1.** *Global overweight and obesity in 2014 by country. (Global Health Observatory data; WHO)*

## 1.1.3. HEALTH CONSEQUENCES

The health consequences of overweight and OB are extensive and range from moderate complaints that have a negative effect on quality of life, to early-age death. Greater body weight, abdominal fat, weight gain during adulthood, and a sedentary lifestyle increase the risk of health problems. Given the aggravating health consequences, the control of OB has been set as a global target for 2025 [26].

A continuous, almost linear trend from healthy-weight exists between BMI and mortality risk [27]. In Western Europe alone, an estimated 320 000 men and women die per year as a result of OB [26]. Among the most life-threatening medical complications are cardiovascular diseases, which include coronary heart disease, stroke, hypertension and coronary artery disease [2,28–30]. Frequent in OB is also type II Diabetes [31,32].

Additionally, a higher BMI increases the incidence of several cancers, both hormone dependent such as endometrial, breast and prostate cancer, and gastrointestinal/hepatic/renal cancers [2,33,34]. The excess weight also contributes to musculoskeletal disorders [35], impaired reproductive ability[36], and several lung diseases [37].

*Psychiatric comorbidity*

Comorbidity exists between OB and psychopathology, particularly depression and anxiety [38–40]. Around 40% to 70% of bariatric surgery patients have shown some form of mental illness [41,42]. Systematic reviews and meta-analyses of both cross-sectional and prospective studies have reported bidirectional associations between OB and both mood and anxiety disorders [43,44]. On the one hand, it has been postulated that stigmatization and pressure to be thin yields poor self-esteem and a negative self-image, thus perpetuating depression [45,46]. On the other hand, individuals with mood disorders are at greater risk of developing OB later in life [47]. OB also shares several symptoms with eating disorders (EDs) [48]. Some individuals with OB are diagnosed with the binge eating disorder (BED), an ED characterized by the ingestion of unusually large quantities of food while experiencing a loss of control [49]. Longitudinal studies have also shown that an elevated weight status can be a precursor of the development of an ED later in life [50, 51]. This may be a consequence of an internalized pressure to lose weight [50,51].

### 1.1.4. PSYCHOSOCIAL CONSEQUENCES

A prevailing problem in OB is the stigmatization often attached to it. Individuals with OB are often viewed as lazy, incompetent and lacking willpower, and face discrimination in education, employment and romantic relationships [52]. Stigmatization leads to several negative psychosocial outcomes (e.g. depressed mood, low self-esteem, poor body image, withdrawal from social interactions and psychological stress), immediate and/or delayed increase in food consumption and binge episodes [53,54].

### 1.1.5 ECONOMIC BURDEN

The health consequences of OB are an important public health concern, given that they require a great deal of health care utilization and expenses. In the United States, around 10% ($86 billion per year) of medical costs per year were attributed to excess weight [55]. An estimate across several European countries indicated that costs in 2002 associated with OB ranged from 0.09% to 0.61% of national gross domestic income, in Spain being 0.28% [56,57]. An increase in the incidence of OB will continue to raise these expenses substantially.

### 1.1.6. AETIOLOGY AND RISK FACTORS

#### 1.1.6.1. Diet

A systematic assessment of global trends from 1990 to 2010 in the dietary patters of adults showed that while there has been a small improvement in the consumption of healthy items, the consumption of unhealthy food has worsened notably [58]. There has been a shift towards the consumption of energy dense food, especially that containing a large amount of saturated fat and added sugar, and away from the intake of complex carbohydrates, dietary fibre, fruits and vegetables [59].

An energy-dense diet has long been considered as one of the primary causes of OB [60,61]. Particularly problematic is the continuous consumption of foods high in fat, being the macronutrient with the highest energy density. Additionally, a high fat diet ablates gastric afferent vagal satiety signalling that is generated by the mechanical distension of the stomach when food enters this organ [62]. Unlike in the case of other macronutrients, such as proteins, the expression of satiety signals is weaker when ingesting fat, which means that the feeling of satiety takes longer to emerge [63]. An increased consumption of energy-dense food augments daily caloric intake, consequently producing an increased vulnerability to weight gain [63].

Furthermore, research is increasingly demonstrating that foods high in fat and sugar content have strong addictive-like components [64,65]. Overlaps exist in the neural mechanisms associated with drug use and impulsive food intake [66]. This is reflected in the overconsumption of food in OB and compulsive drug-use in addicts [66]. Specifically,

alterations in the reward circuits such as the dopamine pathway (contributes to reward sensitivity, incentive motivation, conditioning, inhibition and stress reactivity) have been identified in both OB and addiction [66]. Excessive food intake, as seen in binge eating, occurs especially with foods high in fat. Rats administered with a high-fat diet present compulsive eating and neuroadaptive responses in reward circuits such as the dopamine system [67]. Along the same line, similar to other forms of addiction, sugar elicits gradual sensitization to its rewarding properties, opiate-like withdrawal, sensitization to other addictive substances, excessive intake and binge-related release of dopamine in the nucleus accumbens [68,69]. Given their rewarding effects, foods high in fat and sugars are highly palatable and frequently induce overconsumption, thus increasing the likelihood of OB [69].

1.1.6.2. Sedentary lifestyle

Further than changes in diet, a worldwide shift towards physical inactivity is another of the key contributors to the development of OB. PA refers to "any bodily movement produced by skeletal muscle that requires energy expenditure" [70]. This includes leisure-time PA (exercise and sport), activity that is part of the occupation, and activity carried out for household or other chores. The amount of energy expenditure achieved from PA depends on the mode, intensity, duration and frequency of the activity, as well as the individual (body weight, age, level of habituation, fitness etc.). Sedentary behaviour (or inactivity) is considered as "a state when body movement is minimal and energy expenditure approximates resting metabolic rate" [71]. This definition refers to passive activity such as TV viewing, reading, eating, driving, and working at the computer.

An inverse correlation exists between BMI and PA, and this relationship seems to follow a secular trend over time; there has been a decrease in PA concurrent to the increase in BMI [72,73]. An analysis of PA and OB trends from 1988 to 2010 in the U.S. has shown a gradual yearly increase in BMI and waist circumference of around 0.37% (greater in women than men), and a rise in the prevalence of physical inactivity from 19.1% to 51.7% in women and from 11.4% to 43.5% in men [74]. The escalation in BMI and waist circumference after adjusting for baseline PA, caloric intake and age, correlated significantly with the changes in leisure-time PA [74]. Similarly, PA has been found to have a protective role against weight gain 20 years later [75], and the genetic

contribution to BMI (see in *Section 1.1.6.4.)* seems to be reinforced by an increase in time spent watching television and weakened from an increment in PA [76]. The decline in PA has been observed in both wealthy (e.g. the U.S. and U.K.)[77] and middle- to low-income countries (e.g. China)[79].

1.1.6.3. Sociocultural factors

Modernization is considered as one of the main factors underlying the existing PA trends. In the last 50 to 60 years modernization has resulted in the development of numerous daily, energy saving activities. Among these are the use of cars for transport and the development of robotics, computerization and control systems that facilitate household and other tasks [80]. A shift has also taken place in the workplace, from physical to office-based labour [81]. The development of new entertainment technology such as computers and television can also be linked to the progressive decline in leisure-time PA, as well as augmented food intake [82].

Furthermore, studies have consistently shown that in developed countries, a higher income facilitates the purchase of nutritious foods of higher quality and the consumption of what is considered as a healthy diet, while a lower income has been associated with a diet of highly processed foods and less PA [83]. Adults with a superior socioeconomic status are more likely to meet the recommended guidelines on the consumption of vegetables, fruits and whole grains [84,85]. Accordingly, in developed countries the prevalence of OB is significantly greater in low-income families [86]. This is the opposite in developing countries, where there is food shortage and more manual work and a higher socioeconomic status is associated with a higher rate of OB [87].

Ethnicity and culture also play a role in BMI by transmitting a series of "learnt behaviours" [2]. There are important discrepancies in the body image development across ethnicities. For example, the perceived ideal body size in african-american women is larger and preferences are more heterogeneous than that of white non-hispanic women [88,89] who additionally experience body dissatisfaction when surpassing a lower BMI threshold [90]. Similarly, attempts to lose weight have been found to be associated with a larger body size and negative self-perception only in caucausian and Latino women, not in african-american women [91]. Cultural differences also exist in the nutritional

composition of traditional foods and the cultural understanding of healthy foods; Hmong immigrants to the U.S. consider healthy food only if fresh, while frozen or canned food is avoided [92]. Ethno-cultural preferences are likely to influence eating behaviour and food choice, which might be reflected in the disparities in the frequency of OB [93].

Finally, changes in the food industry are also highly involved in the increasing prevalence of OB, especially in developed countries. The availability of "fast foods" (normally high in fat, energy dense and low in complex carbohydrates) is increasing and they are often included as part of the weekly diet rather than consumed on special occasions [94]. Beverages that are high in sugar and alcohol often accompany these meals. Moreover, fast foods are commercialized by large manufacturing companies that spend a substantial amount of money in advertisement campaigns and sell these unhealthy products in large portions in order to create the illusion of getting the best value for money [94].

1.1.6.4. Genetic factors

An estimated 31% to 91% of variability in BMI has been attributed to genetic composition, depending on sociodemographic factors and time point of assessment [95,96]. The heritability of OB is particularly visible in the early stages of life [97,98]. The resemblance between monozygotic twins is present even if they are separated by adoption [99]. Genome-wide association studies (GWAS) have also identified several genetic variants linked to fluctuations in BMI, such as the fat mass and OB-associated gene (lpha-ketoglutarate-dependent dioxygenase; FTO) [100]. The extensive evidence for strong genetic and environmental influences has pointed towards a gene-environment interplay in the development of OB; the genetic basis of an individual may intensify the individual's vulnerability to an "obesogenic" environment (environmental factors supporting OB) [101]. For example, individuals who are more are more responsive to food cues (smell, taste, and sight) but are less able to recognize signals of satiety are more vulnerable to the food environment [102].

1.1.6.5. Neuroendocrinology

Abnormalities in numerous neurotrasmitters and hormones have been found to be implicated in OB. Leptin is a peptide synthesized in adipose tissue according to energy status and is involved in the short- and long-term regulation of food intake and energy balance via its actions on satiety-signalling (anorexigenic) and hunger-signalling (orexigenic) peptides across a hormonal gut-brain axis [103,104]. When released during a meal, leptin signals satiety such that food intake is halted [103,104]. Differently, in conditions of energy deficit, circulating leptin concentrations decrease, which consequently up-regulates neuropeptide Y (NPY) and agouti gene-related peptide (AgRP) in order to stimulate food intake and down-regulates the expression of pro-opiomelanocortin (POMC) and α-melanocyte-stimulating-hormone (α-MSH) to inhibit energy expenditure [105,106]. Studies in OB, however, have point towards a resistance to the effects of leptin [104]. Administration of leptin in obese rodents and humans with congenital leptin deficiency results in diminished food intake and weight loss [101, 107]. Obese obese rodents without a leptin-deficiency are little to non-responsive to peripheral and central leptin infusion [107].

High rates of insulin resistance have also been detected in OB [108]. Insulin is produced by βcells in the pancreas when there is an increase in blood glucose levels in order to facilitate glucose uptake into adipose tissue and skeletal muscle, and to inhibit glucose release from the liver [109]. However, when there is an excess in adipose tissue (as in the case of OB), insulin resistance develops: insulin-stimulated glucose transport and metabolism decrease and/or an inefficient suppression of output from the liver takes place [108,109]. Insulin resistance has been closely related to leptin function, possibly in the form of a feedback loop; impairment in the actions of leptin may result in hepatic insulin resistance, while insulin resistance in the hypothalamus stimulates overfeeding and consequently leptin resistance [110].

Differently, the serum levels of the hunger-stimulating hormone ghrelin have been found to be lower in individuals with OB compared to healthy controls [111]. Via peripheral and central actions on the hypothalamus, pituitary gland and stomach, ghrelin induces the sensation of hunger and promotes food intake by increasing before the start of the meal and decreasing once ingestion is terminated [111]. Although generally

downgraded in OB compared to healthy weight [112], the expected decrease in plasma concentrations of ghrelin after a meal is blunted in individuals with OB [113]. Therefore, the experience of hunger remains for longer even though further energy intake is no longer needed [113].

Ghrelin and leptin may also act on the dopaminergic pathway and modulate hedonic eating [114]. Impaired function of these peptides has been associated with the heightened sensitivity towards the rewarding effects of palatable foods observed in OB that sometimes presents itself in the form of "food addiction" or BED and results in overconsumption [114,115]. Additionally, alterations have also been observed in the plasma concentrations of NPY; a peptide that stimulates hunger, reduces energy expenditure and stimulates fat growth [116,117].

Animal and human studies have found the involvement of orexin neuropeptides in the development of OB [118]. Orexins, comprising of orexin-A and orexin-B, are neuropeptides primarily expressed in the lateral hypothalamic area and are believed to play an important role in energy homeostasis [118]. They monitor humoral and neural indicators of energy balance and stimulate the experience of hunger in conditions of a negative energy balance [118,119]. Intracerebroventricular (i.c.v.) injection of orexins in the light period induces food consumption in rodents [120], while orexin-knockout mice display hypophagia [121]. Narcolepsy is a sleep disorder consisting in the sudden intrusion of sleep during the day (sometimes accompanied by cataplexy) and inefficient sleep [122]. Affected individuals have altered orexin neuron functioning and have an increased BMI [122] despite decreased caloric intake [123]. Deficiencies in the orexin neuron pathway may therefore contribute to weight gain and an incrementation in the risk of OB. Interestingly, orexins also seem to have a paradoxical effect on homeostasis, simultaneously contributing to energy expenditure via the modulation of theremogenesis-based energy expenditure [118] and the stimulation of skeletal glucose metabolism [124].

A dysregulation of the endocannabinoid system is also implicated in the development of OB. This central regulatory system plays a modulator role across a wide range of biological and cognitive processes [125], and consists of lipid endocannabinoids and the

corresponding cannabinoid receptors (CB1 and CB2). The endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) replicate the actions of the exogenous plant cannabinoid tetrahydrocannabinol (THC) and, by intensifying the rewarding properties of food, stimulate appetite and food intake [126]. Studies have reported increased plasma concentrations of 2-AG and AEA in OB [127,128]. Alterations in the plasma concentrations of 2-AG and OEA have been associated with changes in lipid metabolism, visceral adipose tissue, triglyceride levels and HDL-cholesterol concentrations [129,130].

Differently, the more recently identified peptide irisin was proposed to be implicated in the beneficial effects of PA on energy expenditure and OB resistence [131,132]. Irisin is secreted from the cleaving of the membrane protein fibronectin type III domain containing 5 (FNDC5) induced by exercise. Once released, irisin seems to stimulate white adipose cells (uncoupling protein 1; UCP1) expression to turn white adipose tissue (primary site for lipid and energy storage) into brown adipose tissue that can then be dispersed via the process of thermogenesis [132]. In mice, this process has been associated with improved glucose tolerance, greater insulin sensitivity, reduced body weight and a fall in fat mass [132,133]. In humans however findings are inconsistent; some studies have identified a positive correlation between plasma irisin concentrations or FNDC5 expression and BMI [134–136], whereas others have obtained an inverse interaction [137,138].

1.1.6.6. Personality traits

Temperament can also be another risk factor for the development of OB, given its role in both cognition and behaviour [139]. Cloninger[139] proposed a psychobiological and multi-dimensional model of personality comprising four temperament traits, which are more stable throughout the lifespan, and three character traits, which are more vulnerable to change [139,140]. Individuals with OB have shown a temperament profile that differs from that of healthy controls [141]. Specifically, they tend to be more withdrawn and experience worry towards uncertainity and possible harm (high harm avoidance) [142,143]. They are often less persistent [143,144], more cooperative [144] and studies have found self-directedness to be a protective factor from the development of OB [141]. In addition, those individuals who show traits of BED are also more impulsive (high novelty-

seeking) compared to healthy controls and other individuals with OB who do not experience binge eating [145,146].

1.1.6.7. Cognition and neuropsychology

Individuals with OB have been found to have impaired executive function [147,148], which refers to all self-regulatory processes that monitor goal-oriented thought and behaviour. In particular, these individuals tend to be more impulsive, with poorer decision-making skills and abated inhibitatory behaviour [147]. Studies have demonstrated the hyperactivation of reward/motivation brain regions such as the causate/putamen, anterior insula, amygdale, hippocampus, medial prefrontal cortex (PFC) and anterior cingulate cortex (ACC) in response to high-calorie food cues [149,150]. This heightened sensitivity promotes the experience of hedonic hunger (food intake despite homeostatic satiety). Unlike lean individuals, those with OB also have alterations in the orbitofrontal cortex, which has been associated with impaired inhibitory behaviour [148]. Consequently, there is an increased susceptibility to uncontrolled eating and weight gain [151].

## 1.1.7. TREATMENT

The majority of interventions aimed at managing OB have focused on regulating dietary intake and promoting PA in order to achieve lifestyle behaviour change. In addition, bariatric surgery and drugs are sometimes applied for rapid weight/fat loss and the inhibition of appetite.

### 1.1.7.1. Dietary and lifestyle interventions

The aim of these interventions is to reduce energy intake and augment energy expenditure [152]. Diets are specifically designed to generate a negative energy balance by decreasing daily calorie intake [153]. Although diets are able to induce short-term weight loss [154,155], the extent of weight loss achieved by diet alone is only limited and success rates on the long-term are also poor since the low-calorie diet needs to be permanently kept in order to ensure that the weight lost is maintained [156]. Interventions therefore often incorporate PA programmes. Several interventions have been developed to increment PA and reduce sedentary behaviour [7,8]. The PA programmes vary in terms of the modality, location and setting of the intervention, as well as the practitioner providing the support. The combination of low-calorie diets and increased PA seem to

produce improved results [157,158]. Yet, adherence to these lifestyle changes range between 5%-20% [159].

1.1.7.2. Pharmacological interventions

Drugs administered for inducing weight loss act in different manners. Xenical, for example, is a lipase inhibitor that reduces the absorption of fat from food consumption by 30% [160]. Lorcaserin influences serotonin activity in order to stimulate an anorexic effect that suppresses appetite [161]. Finally, other drugs such as Contrave (bupropion and naltrexone) and Qnexa (phentermine and topiramate) have also been found to have moderate effects in reducing waist circumference and body weight and inhibiting appetite [162,163].

1.1.7.3. Bariatric surgery

By influencing gut hormone function and neural activity, bariatric surgery (adjustable gastric banding (AGB), Roux-en Y gastric bypass (RYGB), or laparoscopic sleeve gastrectomy (LSG)) [164] is the intervention for OB that has thus far demonstrated the best long-term effectiveness [165]. RYGB produces alterations in gut peptides [166], brain activation [167], appetite [168], and food preferences [164,167]. A decrease in fasting ghrelin and enhanced post-prandial increase in pancreatic polypeptide (PPY) and glucagon-like peptide-1 (GLP-1) after surgery have been associated with a faster and stronger sensation of satiety after food intake [169]. PPY and GLP-1 inhibit gastric emptying and delay postprandial plasma glucose and insulin. It has been proposed that gastric bypass may also change food choice and energy intake via the mediation of neural activity [170]. Lower activation of the cerebral areas participating in the mesolimbic reward pathway co-occuring with a decrease in appetite has been found one month post-intervention [167] Moreover, altered brain dopamine system and activation of brain regions related to the perception of reward have been reported, which could result in a reduced preference for high-calorie foods and weight loss [164,170].

## 1.2. ANOREXIA NERVOSA

AN is an ED characterized by low body weight, a fear of gaining weight, a desire for thinness and extreme food restriction. In some occasions, individuals with AN present binge behaviour and engage in compensatory actions such as vomiting, excessive exercise and misuse of laxatives and dieuretics [49]. Denial of the disorder is frequent and is accompanied by a biased overestimation of one's own body weight and size [49,171]. Several medical complications also occur with the chronicity of the disorder; amenorrhea being the most common [172]. AN is more common in females compared to males [49]. Severity of the disease, as indicated by the diagnostic manual DSM-5 is currently based on BMI: the disorder severity is considered as mild when BMI is greater than 17, moderate when between 17 and 16 and severe when BMI is inferior to 16 as severe [49]. Despite the extensive research, the aetiology of AN is unclear and treatment success remains limited.

### 1.2.1 CLASSIFICATION

The DSM-5 defines AN as a Feeding and Eating Disorder: disorders characterized by altered eating and/or eating-related behaviour that have important consequences for physical wellbeing and/or psycholosocial functioning. The criteria for the diagnosis of AN are the following:

A. Restriction of energy intake relative to requirements, leading to significantly low body weight in the context of age, sex, developmental trajectory, and physical health. Significantly low weight is defined as a weight that is less than minimally normal or, for children and adolescents, less than that minimally expected.

B. Intense fear of gaining weight or of becoming fat, or persistent behavior that interferes with weight gain, even though at significantly low weight.

C. Disturbance in the way in which one's body weight or shape is experienced , undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight.

Within the diagnosis of AN, two subtypes are identified:

| Subtypes: |
| --- |
| ***Restricting type:*** During the last 3 months, the individual has not engaged in recurrent episodes of binge eating or purging behavior (i.e., self-induced vomiting or the mis-use of laxatives, diuretics, or enemas). This subtype describes presentations in which weight loss is accomplished primarily through dieting, fasting, adn/or excessive exercise. |
| ***Binge-eating/purging type:*** During the last 3 months, the individual has engaged in recurrent episodes of binge eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas). |

***Table 2****. DSM-5 Diagnostic Criteria for Anorexia Nervosa* [49].

## 1.2.2. PREVALENCE

The estimated lifetime prevalence of AN is aproximally 0.4% to 0.6%, although an increasing trend across time has been found [3,173]. The onset is usually during adolescence, mean age being 17 [174], and it is more common among females than males with a 10 to 1 ratio [49,175].

## 1.2.3. HEALTH CONSEQUENCES

*Mortality risk*

AN is the psychiatric disorder with the highest risk of mortality [176,177]. Additionally, multiple medical sequels have been identified [172,176,178]. Chronic intake restriction and vomiting weakens the pharyngeal muscle, slows gastric emptying and generates early satiety, nauseas and bloating [179]. It can also lead to gastric perforation due to gastric dilatation [180], or the superior mesenteric artery syndrome [181], and/or both liver enzyme abnormalities and phosphatemia [182]. Furthermore, sudden cardiac arrest has been reported in the more severe cases of AN, probably a consequence of bradycardia [183], as well as a high prevalence of anemia, leukopenia and thrombocytopenia [184]. Particularly usual in AN is the development of low bone mineral density (osteoporosis)[185], which can be detected from the early stages of the disease, and consequently an increased vulnerability to fragility fractures [186,187]. Starvation also leads to endocrine dysfunction, characterized by elevated circulating cortisol and growth hormone concentrations [188],

thyroid function abnormalities [189] and hypoglycaemia [190]. Accordingly, many patients suffer of hypogonadism and in women amenorrhea develops [191]. Other medical complications include brain atrophy resulting in impaired neurocognitive functioning [192], xerosis cutis, lanugo hair growth and thinning of the hair [193].

*Psychiatric comorbidity*

Around 50% of adolescent patients with AN present some form of comorbid psychiatric illness [194]. The most common Axis I disorders in both adolescents and adults are affective disorders (major depression and dysthemia) and anxiety disorders (social phobia and obsessive-compulsive disorder) [194–196]. Comorbidity is greater in more severe cases and is more frequent in those patients with the AN binge-purge subtype [194]. The Axis II personality disorders are habitual, the most common being: avoidant (53%), dependent (37%), obsessive-compulsive (33%) and borderline personality disorder (29%) [197,198].

Given the strong comorbidity, pharmacotherapy has been proposed as a potential candidate in the treatment for EDs [199], however only a minimal to moderate effect has been found. An example is the use of antidepressants such as fluoxetine for which some studies have promoted the beneficial effects [200] but the results have not been replicated [201].

### 1.2.4. ECONOMIC BURDEN

The lifetime healthcare required by patients with AN is 78% greater than that used by individuals without an ED, although it is generally directed to the treatment of co-morbid psychiatric disorders and the management of weight loss rather than psychopathology of the disorder itself [202]. From a review of nine studies, Ágh and colleagues [202] reported that the annual individual costs of medical attention ranged between €2993 and €55,270, which are notably higher than those for individuals with other EDs and healthy individuals without a psychiatric disorder.

## 1.2.5. AETIOLOGY AND RISK FACTORS

Despite the extensive research, the exact cause of anorexia remains unknown. Yet, most professionals, researchers and clinicians alike, agree that multiple factors are involved.

### 1.2.5.1. Sociocultural factors

A psychosocial approach to the development and maintenence of AN places emphasis on the internalization of the "thin-ideal" that is perpetuated by the mass media of Western cultures [203,204]. This refers to the acceptance and integration of the cultural standard that thinness equates happiness, success and beauty. Exposure to thin-ideal images has been associated with ED symptoms [205]. However, not all women are equally vulnerable to this sociocultural pressure and do not develop eating-related pathology [206]. It has been suggested that the effects of the thinness standard occur via a gene-environment interaction [207,208]: women who are at "high genetic risk" are more propense to engage in behaviours such as excessive dieting and exercising (triggerers of ED) with media exposure.

### 1.2.5.2. Genetic factors

An emerging movement has focused on the *familial* nature of EDs [4]. Relatives of patients with ED have an almost 10-fold likelyhood of developing an ED compared to unaffected families [209,210]. Family and twin-studies have provided a plausible explanation of such familial aggregation in AN by identifying additive genetic effects [4,211]. The estimated heritability for AN ranges between 33% and 84% [212–214]. Candidate genes may also be associated with polymorphisms at the serotonin (5-HT2A) and dopamine (DRD4) receptor genes [215,216]. The serotoninergic and dopaminergic systems linked to many of the symptoms of AN [215,217].

### 1.2.5.3. Neuroendocrinology

Dysregulation of neuropeptides that modulate appetite and energy homeostasis have been found in AN [218]. For example, plasma concentrations of the brain-derived neurotrophic factor (BDNF) are lower in severely emaciated patients with AN compared to healthy individuals [219]. BDNF is as an inhibitor of food intake [219], and has also been linked to ED symptomatology, thus emphasizing its importance in the pathophysiology of EDs [220]. A disrupted circadian rhythm of leptin during the night has

also been reported; the expected physiological augmentation is reduced or is abolished [221]. Hyperleptinanemia often remains after weight recovery and is considered as one of the causes of the lengthly time required for weight gain and renewed weight loss that is sometimes reported to occur in these patients during the re-feeding stage of treatment [222].

Differently, concentrations of NPY, AgRP and obestatin are elevated in patients in the acute stage of AN [223,224]. Concentrations of NPY remain elevated despite the ingestion of high-carbohydrate and high-protein breakfasts, thus indicating a marked dysregulation [225]. Similarly, fasting plasma concentrations of ghrelin are high in underweight AN patients and normalize with weight gain [226], [227].

1.2.5.4. Personality

The ability to chronically maintain highly restricted eating, as seen in AN, is increasingly being linked to the personality/temperament and cognitive style of these patients [228,229]. Many individuals with AN display traits of anxiety that are often present prior to the development of the illness [230], and are both linked to lower BMI and more severe psychopathology [231]. Accordingly, elevated harm avoidance has been identified in AN [229,232]. These individuals are also particularly anhedonic and ascetic, being able to achieve self-denial across several aspects of their lives and not only in terms of food intake [233]. In support, studies have found an increased ability to delay reward in AN patients compared to healthy controls [234,235].

1.2.5.5. Cognition and neuropsychology

Cognitive inflexibility, perseverence and perfectionism are other aspects commonly seen in this ED. Patients with AN have difficulties in set-shifting, struggling to adapt to changes in rules [147,236,237]. Excessive perfectionism has been found to be particulalry problematic, as it contributes to the rentless dieting despite the deterioting health in order to achieve the “thinness ideal” promoted by society [238].

Although the literature is inconsistent [239], several anomalies have been detected in AN in the functioning of related brain networks. Overall grey and white matter volumes are reduced [239]. Decreased volume in the ACC has been associated with the poorer

perceptual organization and reasoning skills observed in these patients, as well as a worse prognosis of the disorder [240]. In addition, the activation of the ACC during a stop-signal (go/no-go) task was found to be lower in individuals with AN compared to healthy controls, suggesting that these patients require less cognitive resources to carry out tasks that require inhibitatory control [241]. A larger orbitofrontal gyrus rectus, which is believed to regulate sensory-specific satiety [242], might facilitate the termination of food intake before the physiological nutritional needs are met [243].

The insula may also play an important role [244,245]. This structure is functionally connected to multiple other brain regions and is implicated in appetite and satiety, pain processing, interoceptive awareness, compulsive-obsessive traits and the processing of reward and emotions [244,246]. Studies have demonstrated atypical activity in the insula of both ill and recovered patients with AN [247–250]. Furthermore, altered serotonin 5-HT1A and 5-HT2A receptor expression and increased dopamine D2 receptor binding have been recorded [228,251,252]. Functional Magnetic Resonance Imaging (fMRI) during food-related tasks has shown further abnormalities in limbic and paralimpic activation, which is also indicative of potential problems in reward and salience processing [253,254]. After an analysis of abnormalities in the frontostriatal circuits of patients with AN, Marsh and colleagues [255] detected an impairment in self-regulatory control. This might explain the constant preoccupation with shape and weight, the self-starvation, and the difficulties to halt the maladaptive behaviour.

### 1.2.6. TREATMENT

In light of the complexity of AN, several treatment interventions have been developed [243]. Psychological therapies aim to treat AN in itself or address certain aspects/symptoms of the disorder. Additionally, many patients with AN are treated with pharmaceutical treatments, although thus far a specific effective medication has not been identified.

#### 1.2.6.1. Psychological interventions

- ***Cognitive-Behavioural Therapy*** **(CBT)** [256,257] seeks to aid the individual overcome those difficulties that result from dysfunctional thinking, behaviour,

and emotional responses/behaviours. CBT is one of the most commonly used psychotherapy modalities for the treatment of AN.

- ***Enhanced CBT (CBT-E)*** is a newer version of CBT that was developed as an intensive psychotherapy based on CBT [257]. It is primarily applied in day patient and inpatient settings and consists in 4 stages during which the patient learns to modify and stabilize their eating patterns (Stage 1), to sustain the process of change (Stage 2), and to address the factors maintaining the eating problem (Stage 3). In a fourth stage, possible setbacks are discussed.

- ***Specialist Supportive Clinical Management*** **(SSCM)** [258], entails the standard use of outpatient individual treatment that includes management of the clinical status (information, advice and encouragement), psychotherapy in order to promote a positive therapeutic relationship and the encouragement of change [258].

- ***Maudsley model of AN treatment for adults (MANTRA)*** [259,260] is a more recent cognitive-interpersonal treatment that gathers diverse approaches such as motivational interviewing, cognitive remediation and a flexible involvement of carers. The focus is the obsessional and anxious/avoidant traits that are considered to be an essential component of the illness [260].

- ***Focal psychodynamic therapy (FPT)*** [261] is based on the use of the psychodynamic diagnostic interview via which the therapist identifies psychodynamic-related foci of the disorder. This psychotherapeutic approach comprises three phases: 1) To create a therapeutic alliance, to recognize pro-anorectic behaviour and ego-syntonic beliefs and to closely examine self esteem; 2) Relevant relationships and the association between those and abnormal eating behaviour are addressed; 3) Progress is transferred to real life.

- ***Interpersonal psychotherapy*** [256] approaches AN from the notion that ED symptomatology is interwined with interpersonal relationships. Among the topics addressed are grief, role transitions, and interpersonal disputes and

difficulties. Therefore, the objective is to improve the relationships of the patients with surrounding others.

- ***Cognitive-analytic Therapy (CAT)*** integrates aspects of cognitive therapy and brief, focal psychodynamic therapy [262,263]. With the aid of the therapist, the patient places the disorder in the context of their existent and early relationships. The therapist helps patients gain a multi-faceted understanding of themselves from which to achieve healthy relationships such that the disorder loses importance.

- ***Family-based treatment (FBT)*** [264] places emphasis on the potential of the family as a therapeutic tool given that the family is considered as playing an important role in the disorder. During the process of this treatment, responsibility is taken away from the parents, parents are complimented on their positive parenting skills, families work out the best way to manage the child's weight and eating behaviour in an age-appropiate manner, and a healthy relationship is established between the patient and the other family members.

Extensive research has been conducted into the efficacy of these treatment interventions. Though clinical improvements have been reported, evidence shows that further advancement is needed. The use of CBT as a treatment modality is considered to be a suitable option in AN as it seems to ameliorate adherence to treatment, reduce relapse rates, facilitate weight gain and attenuate ED symptoms [265]. However findings are inconsistent and this treatment approach has been found by some to have similar effects to others [265]. Differently, in a randomised controlled study (RCT) the comparison of SSCM, CBT and interpersonal therapy has shown a superior outcome after SSCM [266]. These results were not maintained after long-term follow-up [267]. When compared to SSCM, MANTRA was found to be equally effective, but patients allocated to this psychotherapy modality provided more positive feedback with MANTRA, and in the severe cases the weight gain was greater [259]. Finally, in a comparison between family psychotherapy, CAT, CBT and routine treatment, the specialized treatments proved to be more effective [268]. Yet, despite symptomatic improvement at 1-year

follow-up, there was an overall poor outcome in a large number of patients across groups (two thirds of the entire sample) [268].

1.2.6.2. Pharmacological interventions

Extensive drug treatments for AN have been examined, however incongruent results towards their efficacy has led professionals to consider the use of pharmacotherapy as a complementary strategy and not as the primary form of intervention [243,269]. The aim of pharmacotherapy is to facilitate weight gain, to restrain the core preoccupations or behaviours associated with the disorder (e.g. binge/purge), and to ameliorate mood [243]. The first attempt of pharmacotherapy was via the administration of Cyproheptadine, a first-generation antihistamine with anticholinergic and antiserotonergic properties. Initial studies showed a positive effect on weight gain [270,271], however later studies reported only a minimal effect and specific to the restricting AN subtype [272]. Since then multiple drugs have been tested, including antidepressants such as selective serotonin reuptake inhibitors (SSRI) and tricyclic antidepressants (e.g. clomipramine and amitriptyline), other antipsychotics (Olanzepine, Quetapine and Rispedone), nutritional supplements (e.g. zin), opiate antagonists (Naloxone), and hormones/drugs used to treat osteoporosis (e.g. insulin-like growth factor and gonadal steroid replacement). Although some drugs have shown positive results, the effects are only weak and have been questioned [243,273].

In summary, numerous interventions exist for the treatment of AN, and some have proven to be more effective than others. However, a single treatment approach that can successfully address the disorder to achieve complete and long-term remission has not yet been identified. Only a limited amount of trials have been conducted on new psychotherapies and pharmacological interventions, and despite evidence of moderate improvements in symptomatology and weight gain, many patients complete treatment with a poor outcome and a high risk of relapse. In all likelihood, the complexity of the disorder signifies that there are external factors, impaired or altered in AN, that are contributing to the treatment process.

# 1.3. PHYSICAL ACTIVITY

"If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health."

HIPPOCRATES *(Kos,Ancient Greece c.460BC – Larissa,Ancient Greece c.370BC)*

Regular PA has several health benefits [274–276]. It is associated with a lower risk of coronary heart disease and adverse cardiovascular events [277,278]. Dose-response studies have demonstrated that PA decreases the risk of several cancers [279,280] and diabetes [281]. Interventions promoting PA have resulted in a fall in the severity of arthritis [282], sexual dysfunction [283], and chronic fatigue syndrome [284]. Exercise is also beneficial for emotional well-being [274], and reducing the likelihood of developing depression and anxiety [285,286].

## 1.3.1. PHYSICAL ACTIVITY AND OBESITY

Prospective studies have shown that habitual MVPA (including brisk walking) facilitates the maintenance of a stable weight and waist circumference over time [287], whereas TV-watching has been independently associated with OB and weight gain [82]. Furthermore, regardless of diet and sex, PA and exercise training have been found to reduce total, abdominal and subcutaneous fat [288–290].

The WHO [291] recommends that in order to maintain weight, the individual should engage in a minimum of 150 minutes of moderate intensity aerobic PA per week (e.g. brisk walk or dancing) or 75 minutes if partaking in activities of vigorous intensity (e.g. running, aerobics, fast cycling), as well as muscle strengthening activities twice a week. Doubling these guidelines are recommended for further health benefits and weight loss [291].Yet, in 2011 the estimated worldwide prevalence of insufficient PA was of 31.1% in adults and around 80.3% in children (estimate based on the data of 122 countries stored in the WHO repository) [292]. Inactivity is more frequent in women (33.9%) than men (27.9%), and with both sexes combined prevalence rates range from 4.7% in Bangladesh to 71.9% in Malta. Walking a minimum of 10 minutes per day five times a week was reported in around 64% of studied countries, with little variation between

countries. In terms of vigorous-intensity PA (three or more days per week), the prevalence went down to 31.4% and there were large differences between countries [292].

### 1.3.2. PHYSICAL ACTIVITY AND ANOREXIA NERVOSA

It has been historically acknowledged that AN is sometimes accompanied by a marked motor restlessness [293]. Given the emanciated condition of the body, strenuous PA in AN can result in numerous medical complications such as overuse injuries, bone fractures, osteoporosis and hormonal imbalances [294,295].

Despite the extensive research that has been conducted in the PA of patients with AN, consensus has not yet been reached as to the definition of problematic PA [296]. Among the terms used are: "hyperactivity" [222,297], "exercise addiction" [298,299], "exercise dependence" [300,301], "obligatory exercise" [302–305], "excessive/high level exercise" [306–308] and "compulsive exercise" [309,310]. Reflecting the variety of terms published, it seems that assessments are based on different conceptualizations of problematic PA. These can be categorized into those that examine PA from a quantitative standpoint, thus examining the duration, frequency and/or intensity of exercise, and those who focus on the compulsive nature of the exercise (qualitative approach). The use of a quantitative approach has been highly questioned [296]. There is no consensus on the amount of exercise that can be considered as "excessive". In addition, it is unclear whether one should focus on exercise at diagnosis [311312] or on the lifetime incidence of excessive exercise [313]. It has therefore been suggested that it is not the amount of PA *per se* that is important, but the presence of a compulsive need to exercise [296,314].

In AN, PA often begins as a leisure time pleasurable activity, which progresses into a compulsive behaviour where exercise becomes "obsessive" and "out-of-control" [313]. The concept of compulsivity has been claimed to be a more adequate definition of problematic PA in AN [296]. Overall, PA can be considered as compulsive when it interferes with everyday life activities (at work or during leisure time) and relationships with others, and is continued despite injury and/or other medical complications [315,316]. Exercise for the need to control weight and shape, the experience of guilt and/or anxiety when unable to exercise and the need to compensate for missed exercise sessions have also been incorporated in the definition [314,316,317].

The qualitative (compulsivity) approach to problematic PA has received support from studies with clinical and non-clinical samples, in which an association has been found with ED cognitions and behaviours and general psychopathology [317–321]. Furthermore, while using a quantitative definition would require the type of activity studied to be established, such as routine daily activities (e.g. walking to work), structured sports (e.g. uncontrolled training) or abnormal exercising (e.g. extreme numbers of push ups), a compulsivity-based definition would cover all of these. Yet, discrepancies persist, and more research is required to obtain a more specific overview of the PA patterns in this clinical population [296,309].

1.3.2.1. Prevalence

The exact prevalence of high PA in AN has not been clearly established and estimates range from 34% to 80% [10,306,308]. Some studies have not found a difference in PA between patients with AN and controls [322,323]. This can be attributed to several factors. First, an operationalized definition of problematic activity is lacking (see above). Second, in some studies assessment took place via the use of subjective information (e.g. self-reported questionnaires and clinical interview), while in others objective instruments were employed (e.g. accelerometers, pedometers, actigraphy). Finally, differences in sample are also relevant, such as the duration of the disorder, age, and time point of assessment (e.g. baseline, lifetime prevalence, during treatment).

Patients with AN with high PA present more severe ED symptoms, in particular greater body dissatisfaction and drive for thinness [308,309]. They report more depression, anxiety, anhedonia and obsessive-compulsive symptoms [308,324,325]. These patients also present a specific personality profile characterized by higher levels of perfectionism and obsessive-compulsive traits, and lower reward dependence and novelty seeking [309].

1.3.2.2. Physical activity and treatment outcome

Some studies have found an association between PA and a longer treatment duration [326], less improvement in ED symptoms at the completion of the treatment programme [309], a higher risk of dropout [310] and an increased likelihood of relapse after recovery [327,328]. Yet, these findings are not consistent throughout the literature. Other studies have not

found an effect on treatment response [297] or recovery rates [329]. Finally, other authors have proposed that only prolonged light PA hinders treatment [330].

Based on the potential health benefits of PA, some centres have incorporated a PA intervention to the treatment process and have obtained positive results [295]. For example, Calogero and Pedrotty [331] found an overall decrease in compulsive attitudes towards exercise and an additional gain of around 30% in weight in the more severe cases if patients attended an exercise programme. Additionally, Tokumura and colleagues [332], found further improvements in BMI, percentage body fat and exercise capacity at 1-year follow-up. However, scepticism among professional prevails. Given the fragile state of individuals with acute AN and the heterogeneity in the findings, further research is needed.

# 1.4. SLEEP

"I love sleep. My life has the tendency to fall apart when I'm awake, you know?"

ERNEST HEMINGWAY *(Oak Park, 21st of July 1899 – Ketchum, 2nd of July 1961)*

Sleep is a physiological state during which the body can recover from fatigue. In order to allow this process to occur, it is recommended that adults sleep 7 to 9 hours daily [333]. Yet, many adults struggle with sleep and do not meet these guidelines [334]. An analysis of sleeping habits across countries in 2011 demonstrated that in some countries up to an estimated 11.5% of the population slept less than seven hours and that in certain countries such as Italy and Norway the prevalence had been increasing exponentially over the previous ten years [335]. From a survey administered to 10132 individuals, the prevalence of self-reported sleep problems in 2008 was 56% in U.S.A, 31% in Western Europe and 23% in Japan [336].

Short sleep duration, poor sleep quality and a disruption in circadian timing have extensive detrimental effects on health [337]. Habitual sleep curtailment is a predictor of all-cause mortality [338], glucose intolerance and diabetes [339], OB [340,341], cardiovascular diseases [342–344], an impoverished immune system [345,346] and a non-alcoholic fatty liver disease [347]. Poor sleep can cause anxiety and depression [348–350], and may result in impaired attention capacity and cognitive performance [351].

## 1.4.1. SLEEP IN OBESITY

Epidemiological studies have shown a strong link between sleep and OB [340,352]. In a meta-analysis of 45 studies covering 604,509 adults, the odds ratio for OB with short sleep duration was found to rise to 1.55 [340]. The odds augment with a progressive drop in hours of sleep [353]. In a population-based study of 5,549 women, those who reported less than six hours of sleep per night in the recent past were 1.89 times more likely of having OB and 3.12 of having extreme OB [15]. In addition, in line with others [354,355], the authors obtained a U-shaped relationship between sleep and OB; sleeping more than nine hours was also linked to greater odds of OB [15]. In a longitudinal study, the odds

ratio for the incidence of a BMI ≥ 25 (6% of total sample) after 1 year was 1.50 in those participants who slept between five and six hours per night at baseline, and 1.91 for those who slept less than five hours [356]. An inverse association between sleep duration and BMI was also observed in a 13-year prospective study of young adults [357], and reduced sleep duration in children has been found to augment the risk of OB later in life [358]. Support also comes from objective measures. Actigraphy has shown adiposity and BMI to be associated with reduced sleep duration and inconsistent sleep patterns [359,360], and lower sleep efficiency has been associated with greater fat mass [361].

Further than sleep duration, diminished sleep quality and the use of sleep medication are frequent in OB [362]. OB is common among those individuals with obstructive sleep apnea [123]; a condition of shallow or disrupted breathing that occurs when sleeping as a result of a collapse or blockage of the respiratory pathway. Individuals diagnosed with night eating syndrome, a disorder characterized by the consumption of the majority of daily food intake at night, and the sleep-related ED, defined as continuous eating throughout the night, are more likely to have sleep disturbances and develop OB [363,364].

### 1.4.2. SLEEP IN ANOREXIA NERVOSA

Poor sleep is one of the regular complaints among individuals with AN when seeking treatment [14]. In a recent study by Kim and colleagues [13], 58% of patients with AN reported sleep disturbances, including difficulties falling asleep, midsleep awakenings, early morning awakening, parasomnia and hypersomnia. Several electroencephalogram (EEG) studies have obtained similar results; the key differences between patients with AN and healthy controls being sleep efficiency and sleep architecture [365]. In their acute state, patients with AN have been found to present greater sleep fragmentation and more frequent arousals, as well as weak slow wave sleep (SWS) and slow wave activity (SWA) [366,367]. Finally, a study examining both self-reported (Pittsburgh Sleep Quality Index) and objectively measured (polysomnography) sleep quality, observed poorer sleep compared to healthy controls and improvements after treatment only in the subjective experience of sleep [368].

## 1.5. SUMMARY

OB and AN are conditions located in the extreme ends of the eating/weight continuum. They are both receiving increasing attention from the scientific and health sectors given the rapidly augmenting prevalence and medical complications associated. Numerous interacting psychological, social and biological factors are being identified to contribute to the aetiology, development and maintenance of these conditions. Among these, physical activity and sleep habits may play an important role. It may therefore be particularly useful to understand the psychobehavioural mechanisms related to the physical activity and sleep patterns observed in individuals with OB and AN in order to develop effective prevention and intervention strategies.

# CHAPTER 2. AIMS AND HYPOTHESES

## 2.1. GENERAL AIMS

1) **To present the PA (daytime and MVPA) and sleep patterns of individuals with OB and AN.**

**Hypothesis:** It was hypothised that both individuals with OB and patients with AN would show patterns of PA and sleep different from those of individuals with a healthy-weight.

2) **To explore the neuroendocrinological and psychopathological mechanisms underlying PA and sleep in these extreme weight conditions.**

**Hypothesis:** Anomalies in endocrine function were expected to contribute to and be moderated by the PA and sleep patterns of individuals with OB and AN. Psychopathological indexes were also predicted be implicated in the difficulties faced by these individuals to achieve healthy PA and sleep habits.

3) **To examine how abnormal sleep and PA patterns may act as mediators in the treatment outcome of a CBT for AN.**

**Hypothesis**: It was postulated that the interaction between PA and psychopathology and between sleep and impaired biological processes would contribute to the efficacy of the psychotherapy for AN.

## 2.2. SPECIFIC AIMS

1) **To examine the link between MVPA and BMI, and the moderating role of temperament and endocannabinoids.**

**Hypothesis:** Individuals with OB were expected to show less MVPA compared to a nonclinical group. The amount of time spent in MVPA was predicted to be influenced by the individual's temperament. Furthermore, plasma concentrations of endocannabinoids were anticipated to be partial mediators in the mechanism by which PA contributes to BMI.

2) **To evaluate the role of plasma concentrations of irisin and MVPA in the modulation of executive functions in OB and MOB.**

**Hypothesis:** The OB and MOB groups were hypothized to perform poorly in the cognitive tasks, as well as show less MVPA and augmented circulating levels of irisin. Performance was also predicted to be related to both MVPA and serum irisin.

3) **To assess the PA profile of patients with AN (daytime and MVPA), and how MVPA may influence the outcome of a CBT treatment for AN.**

**Hypothesis:** A group of patients with AN were expected to display notably higher levels of MVPA. Additionally, MVPA was predicted to inversely correlate with depression symptoms. Given the incongruency in the existing literature a hypothesis on the effects on outcome could not be established.

4) **To explore the presence of a compulsive component to exercise in AN and other EDs and to validate a Spanish translation of the Compulsive Exercise Test (CET).**

**Hypothesis:** The Spanish version of the CET was predicted to be a valid instrument to assess compulsive exercise in EDs. The participants with an ED were hypothized to show elevated scores in the test compared to a healthy control group. Accordingly, greater ED severity and symptoms of general psychopathology were anticipated to be associated with higher the scores in the test.

5) **To evaluate the role of sleep in the relationship between serum orexin-A levels and BMI.**

**Hypothesis**: Poorer self-reported sleep quality was expected in individuals with OB and MOB in comparison to lean individuals. In addition, circulating levels of orexin-A were predicted to influence BMI, mediated by poor sleep quality.

6) **To examine the function of orexin-A in AN, its effects on treatment outcome, and whether sleep may be implicated as a mediating factor.**

**Hypothesis:** Plasma concentrations of orexin-A were predicted to be altered in patients with AN compared to healthy weight/eating controls. High plasma concentrations were anticipated to have a negative influence on treatment outcome due to a link to impovireshed sleep quality.

## 2.3. ARTICLES INCLUDED IN THE THESIS

In order to assess the hypotheses, the following studies were conducted:

**Study 1:** Fernández-Aranda F.*, **Sauchelli S.*,** Pastor A., Gonzalez ML, de la Torre R., Granero R., Jiménez-Murcia S., Baños R., Botella C., Fernández-Real JM., Fernández-García JC., Frübeck G., Gómez-Ambrosi J., Rodríguez R., Tinahones FJ., Arcelus J., Fagundo AN., Agüera Z., Miró J., Casanueva FF. (2014) Moderate-vigorous PA across BMI in female: moderating effect of endocannabinoids and temperament. PLoS One, 9(8): e104534.

**Study 2:** Fagundo AB., Jiménez-Murcia S., Giner-Bartolomé C., Agüera Z., **Sauchelli S.,** Pardo M., Crujeras AB., Granero R., Baños R., Botella C., de la Torre R., Fernández-Real JM., Fernández-García JC., Frühbeck G., Rodríguez A., Mallorquí-Bagué N., Tárrega S., Tinahones FJ:, Rodríguez R-., Ortega F., Menchón JM., Casanueva FF., Fernández-Aranda F. (2016) Modulation of itisin and PA on executive functions in OB and morbid OB. Scientific Reports, 6: 30820.

**Study 3:** **Sauchelli S**., Arcelus J., Sánchez I., Riesco N., Jiménez-Murcia S., Granero R., Gunnard K., Baños R., Botella C., de la Torre R., Fernández-García JC., Fernández-Real JM., Frübeck G., Gómez-Ambrosi J., Tinahones FJ., Casanueva FF., Menchón JM., Fernández-Aranda. (2015). PA in AN: how relevant is it to therapy response? European Psychiatry, 30(8): 924-931.

**Study 4:** **Sauchelli S.**, Arcelus, J., Granero R., Jiménez-Murcia S., Aguera Z., Fernandez-Aranda F. (2016). Dimensions of compulsive exercise across eating disorder diagnostic subtypes and the validation of the spanish version of the Compulsive Exercise Test. Frontiers in Psychology, 7:1852.

**Study 5:** **Sauchelli S.,** Jiménez-Murcia S., Fernández-García JC; Garrido-Sánchez, L., Tinahones, FJ., Casanueva FF., Baños R., Botella C., Crujeiras AB., de la Torre R., Fernández-Real JM; Frühbeck G., Granero R., Ortega FJ., Rodríguez A., Zipfel S., Giel KE., Menchón JM., Fernández-Aranda F**.** (2016) Interaction between orexin-A and sleep quality in females in extreme weight conditions. European Eating Disorders Review, 24(6):510-517.

**Study 6:** **Sauchelli S**., Jiménez-Murcia S., Sánchez I., Riesco N., Custal N., Fernández-García JC., Garrido-Sánchez L., Tinahones FJ., Steiger H., Israel M., Baños RM., Botella C., de la Torre R., Fernández-Real JM., Ortega FJ., Frühbeck G., Granero R., Tárrega S., Crujeiras AB., Rodríguez A., Estivill X., Beckmann JS., Casanueva FF., Menchón JM., Fernández-Aranda F. (2016). Orexin and sleep quality in AN: Clinical relevance and influence on treatment outcome. Psychoneuroendocrinology, 65:102-108.

# CHAPTER 3. METHODS

## 3.1. PARTICIPANTS

The participants of the studies included in this thesis were recruited from the University Hospital of Bellvitge-IDIBELL, as well as other collaborating centres part of the CIBERobn Spanish Research Network (Instituto de la Salud Juan Carlos III). These were: the Department of Endocrinology at the University Hospital of Santiago (Santiago de Compostela); the Department of Diabetes, Endocrinology and Nutrition of the Clinic University Hospital Virgen de Victoria (Malaga); the Department of Endocrinology and Nutrition of the University of Navarra (Pamplona); the Diabetes, Endocrinology and Nutrition Department of the Biomedical Research Institute of Girona (IdIBGi-Doctor Josep Trueta Hospital, Girona); the Hospital del Mar Medical Research Institute (IMIM, Barcelona) and the Department of Basic Psychology, Clinic and Psychobiology of the University Jaume I (Castellón). The clinical samples were individuals referred to the corresponding clinics, and the healthy eating/weight samples were gathered from the same catchment areas. In general, participants were excluded if they were younger than 18 years of age, had suffered a lifetime history of other Axis I mental disorders, had a history of chronic medical illness or a neurological condition (e.g. Parkinson's disease) and/or used psychoactive medication or drugs. None of the control groups had a BMI below 18kg/m$^2$ or above 29.9kg/m$^2$, and did not have a lifetime history of an ED. Written and informed consent was given by all participants. The ethics committees of each institution approved the study and the studies were conducted in accordance with the declaration of Helsinki.


***Figure 2.*** *CIBERobn collaborating centres.*

## 3.2. ASSESSMENT OF PHYSICAL ACTIVITY

### 3.2.1. ACCELEROMETER-BASED PHYSICAL ACTIVITY

PA was measured by using the tri-axial accelerometer Actiwatch AW7 (Figure 4; CamNtech Ltd, Cambridge Neurotechnology, Cambridge, UK). This is a small (39x32x9mm) and light-weight (10.5g) instrument that measures movement across a 24 hour period. In the studies part of this thesis, the Actiwatch AW7 was worn for six consecutive days (five week days and one weekend) on the non-dominant wrist from 00:00h on day 1 to 00:00h on day 7. Movement is calculated in the form of activity counts in a 1 minute epoch-length and data is extracted with the Actiwatch 7 software (CamNtech Ltd). Only the data between 07:00h and 23:00h was analysed. Cases of less than 4 days of recording were excluded and no detected movement for 10 or more consecutive epochs (10 minute period) were considered as missing (seen as implausible counts or as periods in which the participant was sleeping). These procedures have been carried out in previous studies [369,370]. Three types of PA were examined:

- ***Daytime PA*** refers to the intensity of activity in a given minute. This was calculated in the form of average mean counts per minute (counts·min$^{-1}$) over the six days of assessment.

- ***Time in moderate-to-vigorous PA (MVPA)*** refers to the average amount of minutes per day (over the days assessed) during which the individual was in MVPA. PA was considered as MVPA based on an algorithm proposed by Heil [371]. By using another activity monitor (the Actical @), Heil [371] proposed that activity-based energy expenditure (AEE) in adults could be estimated with wrist-worn accelerometers by applying the formula: *AEE = 0.02013 + (1.282E-5) x HAC*. By introducing the value 0.0310 for AEE, which corresponds to 3 Metabolic Equivalent of Task (3 METS) and represents an activity intensity of a brisk walk, a cut point of 848 (counts·min$^{-1}$) was derived. MVPA corresponds to all activities of an intensity ranging from a brisk walk to sports that generate greater AEE. The described algorithm has been previously employed to evaluate

AEE in children using the Actiwatch AW4 (produced by the same manufacturer) [372].

- ***Low versus high PA* levels** refers to the differentiation between individuals who present a profile of high PA levels (high exercisers) and those who show low PA levels (low exercisers). Classification was achieved based on the time spent in MVPA. If greater than 300 minutes over the 6 days of analysis, the individual was considered as a being highly active.


***Figure 3.*** *Accelerometer Actiwatch AW7.*

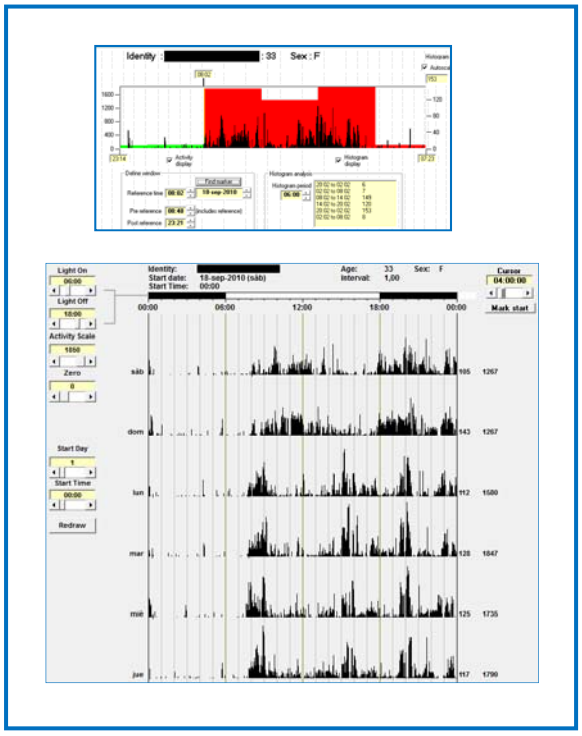

***Figure 4.*** *Graphs displaying different types of PA*

### 3.2.2. COMPULSIVE EXERCISE TEST

The CET (see *Appendix I*) is a self-reported questionnaire first developed by Lorin Taranis, Stephen Touyz, and Caroline Meyer and validated against a healthy adult population [317]. It aims to explore the concept of compulsive exercise by examining the underlying emotional, cognitive and behavioural characteristics. The questionnaire consists in 24 items answered on a 6-point Likert scale, from 0 (never true) to 5 (always true). The components evaluated are five: 1) avoidance and rule-driven exercise behavior, which refers to exercising in a repetitive manner in order to avoid negative feelings and/or the experience of guilt that might arise when not exercising; 2) exercise for weight control; 3) exercise for mood improvement; 4) lack of exercise enjoyment, which signifies continued exercise even though the individual no longer enjoys it; and 5) exercise rigidity, where exercise is driven by the need to maintain a strict exercise schedule. The scores in each subscale are summed in order to obtain the more global CET total score. The psychometric properties of the CET have been validated in non-clinical and clinical samples, showing high concurrent and convergent validity and a Cronbach's (alpha) ranging from 0.72 to 0.88 [317,318].

## 3.3. ASSESSMENT OF SLEEP

### 3.3.1. ACCELEROMETER-ASSESSED SLEEP TIME

The previously described accelerometer was also used as an actigraph to calculate sleep time. Bed time and get up time were indicated by pressing a marker button at the centre of the device. Via the use of several algorithms the Actiwatch Sleep Analysis 7 software (CamNtech Ltd) derives daily actual sleep time. Actigraphy has shown to correlate with polysomnography recording, the gold standard of sleep, with an intra-class correlation co-efficient of 0.76 for sleep time [373].

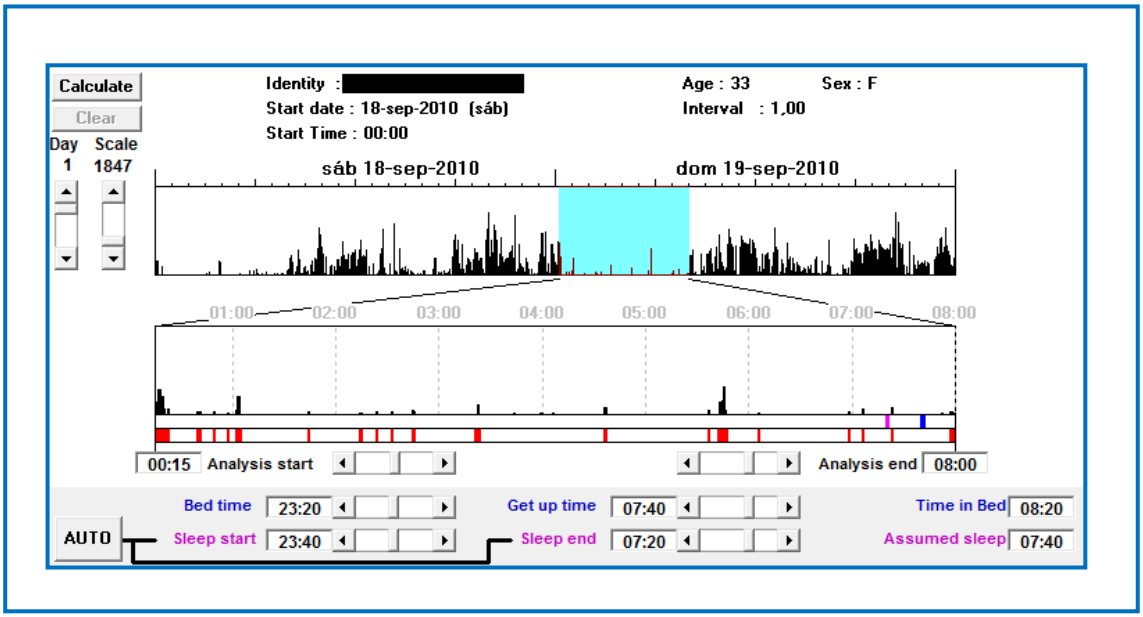


***Figure 5.*** *Graphs displaying sleep time extracted from the accelerometer*

## 3.3.2. SELF-REPORTED SLEEP QUALITY

The Pittsburgh Sleep Quality Index (PSQI) [374] was employed to evaluate possibly problematic sleep. This is a 19-item questionnaire that provides information regarding seven "components" of sleep: 1) subjective sleep quality; 2) sleep latency; 3) sleep duration; 4) sleep alterations; 5) use of sleeping medication; 6) daytime dysfunction; 7) sleep efficiency. Each item is scored on a Likert scale ranging from 0 (no difficulty) to 3 (severe difficulty). The sum of the scores of each scale provides a global sleep quality index of 0 to 21. Scores above 5 are indicative of problematic sleep. This cut-off score adequately differentiates between healthy-weight controls and patients with sleep disturbances with a sensitivity of 89.6% and a specificity of 86.5% [374]. A Spanish version of this questionnaire has been validated, with an internal consistency of (Cronbach alpha) 0.81 [375].

# CHAPTER 4. RESULTS.

## 4.1. STUDY 1: MODERATE-VIGOROUS PHYSICAL ACTIVITY ACROSS BMI IN FEMALES: MODERATING EFFECT OF ENDOCANNABINOIDS AND TEMPERAMENT

**Background:** The crucial role of PA in the prevention and management of OB is widely accepted [376]. Understanding the mechanisms that promote PA and their effect on BMI is essential for the development of future effective interventions. Studies have found that the endocannabinoids AEA and 2-AG and endocannabinoid-related compounds such as *N*-oleylethanolamide (OEA) are involved in both PA [377,378] and OB [126,128,379]. Temperament, as defined by Cloninger [140], also seems to be implicated [141,143,380,381]. In addition, there is some evidence of an association between endocannabinoid system functioning and temperament; reduced CB1 receptor availability has been found to be associated with higher levels of novelty-seeking [382]. Therefore an interaction between MVPA, temperament and endocannabinoids may exist that contributes to fluctuations in BMI. This has not been previously examined.

**Objectives:** To examine differences in the PA, temperament and serum endocannabinoid profile of individuals with distinct weight status (from healthy-weight to morbid OB (MOB), and to explore how these factors may interact to influence fluctuations in BMI.

**Methods:** A total of 189 female individuals participated in this study; 30 with OB (BMI = 30–39.9, kg/m2), 43 with MOB (BMI ≥ 40, kg/m2) , and 116 healthy-weight controls (BMI = 18.5–29.9 kg/m2). The Tanita Multi-Frequency Body Composition Analyzer MC-180MA (Tanita Corporation, Tokyo, Japan) was used to obtain BMI after measuring height with a stadiometer. Data on daytime PA and time in MVPA were extracted from the Actiwatch AW7. The Temperament and Character Inventory-Revised (TCI-R; 140) was used to assess four temperament traits of interest: novelty-seeking, harm avoidance, reward dependence and persistence. Finally, blood extractions were conducted between 08:00h and 09:00h after an overnight fast to measure plasma

concentrations of AEA (ng/mL), 2-AG (ng/mL), OEA (ng/mL) and palmitoylethanolamide (PEA; ng/mL).

**Results:** A negative linear trend was found across weight status in time spent in MVPA ($p = .008$); the MOB group showed the least MVPA. However, differences in daytime PA were not significant. Alterations were also found in serum 2-AG levels, which were elevated in both obese groups compared to the control group ($p < .001$). Structural equation modelling (SEM) showed that MVPA was related to high novelty-seeking ($b = 0.18$, $p = .026$) and low harm avoidance ($b = -0.16$, $p = .048$). MVPA was directly associated with a decrease in BMI ($b = -0.13$, $p = .039$), and an increase in AEA ($b = 0.22$, $p = .004$) and OEA ($b = 0.18$, $p < .001$) concentrations. AEA and OEA partly mediated the link between MVPA and BMI, being both directly related to BMI (AEA: $b = 0.26$, $p < .001$; OEA: $b = -0.15$, $p = .041$).

# MODERATE-VIGOROUS PHYSICAL ACTIVITY ACROSS BODY MASS INDEX IN FEMALES: MODERATING EFFECT OF ENDOCANNABINOIDS AND TEMPERAMENT

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**Journal:** PloS One

**Impact Factor:** 3.06

**Abstract**

**Background:** Endocannabinoids and temperament traits have been linked to both physical activity and body mass index (BMI) however no study has explored how these factors interact in females. The aims of this cross-sectional study were to: 1) examine differences among distinct BMI groups on daytime physical activity and time spent in moderate-vigorous physical activity (MVPA), temperament traits and plasma endocannabinoid concentrations; and 2) explore the association and interaction between MVPA, temperament, endocannabinoids and BMI. **Methods:** Physical activity was measured with the wrist-worn accelerometer Actiwatch AW7, in a sample of 189 female participants (43 morbid obese, 30 obese, and 116 healthy-weight controls). The Temperament and Character Inventory-Revised questionnaire was used to assess personality traits. BMI was calculated by bioelectrical impedance analysis via the TANITA digital scale. Blood analyses were conducted to measure levels of endocannabinoids and endocannabinoid-related compounds. Path-analysis was performed to examine the association between predictive variables and MVPA. **Results:** Obese groups showed lower MVPA and dysfunctional temperament traits compared to healthy-weight controls. Plasma concentrations of 2-arachidonoylglyceryl (2-AG) were greater in obese groups. Path-analysis identified a direct effect between greater MVPA and low BMI ($b$=-0.13, $p$=. 039) and high MVPA levels were associated with elevated anandamide (AEA) levels ($b$=0.16, $p$=. 049) and *N*-oleylethanolamide (OEA) levels ($b$=0.22, $p$=. 004), as well as high Novelty seeking ($b$=0.18, $p$<. 001) and low Harm avoidance ($b$=-0.16, $p$<. 001). **Conclusions:** Obese individuals showed a distinct temperament profile and circulating endocannabinoids compared to controls. Temperament and endocannabinoids may act as moderators of the low MVPA in obesity.

**Introduction**

There is a growing prevalence of obesity and overweight worldwide. A fall in energy expenditure is believed to be one of the leading lifestyle changes boosting the notable spread of obesity [1]. Prolonged sedentary behavior has been strongly associated with this extreme weight condition and a consequent increase in the likelihood of cardiovascular diseases, hypertension, type 2 diabetes, and osteoporosis [2]. Differently, regular exercise or structured moderate-vigorous physical activity (MVPA) facilitates weight control [3].

These changes in physical activity (PA) patterns are of particular concern for females. The prevalence of obesity has been estimated to be greater among females than males (11.9% versus 7.7%) across all studied world regions [2]. In addition, females spend less time engaging in MVPA and more in sedentary activities, a gender difference especially seen among younger adults [4]. Given this evidence, and that the decline in PA over time is especially present among females [5], there is an urgent need to understand the mechanisms underlying the inter-individual fluctuations in structured PA and their relationship to body mass index (BMI) in females.

Moderate-Vigorous Physical Activity /BMI and Endocannabinoids

Biological models have been proposed to explain individual differences in MVPA. Studies have identified the role of the endocannabinoid (eCB) system in the engagement and maintenance of structured PA, interacting with the reward neurosystem of exercise [6,7]. It has been suggested that the beneficial effects of PA for cognitive function may be partly related to the eCB system [8]. The eCB system is also known for its modulation of cognitive and emotional behavior [9] and for its extensive central and peripheral control of energy balance [10]. The eCBs 2-arachidonoylglycerol (2-AG) and anandamide (AEA) are the endogenous lipid mediators of this system and have similar actions to those of the exogenous plant cannabinoid Δ9-tetrahydrocannabionol (THC). ECBs stimulate appetite and food intake by intensifying the orosensory reward of food, which takes place via the activation of the CB1 receptors of the central nervous system. It is also believed that the motivation to ingest is modulated by interactions between the eCB and opioid systems [11]. ECBs may additionally be involved in the peripheral regulation of feeding since intestinal levels of AEA have been found to increase under food deprivation and decrease during re-feeding [12]. The opposite pattern occurs with N-oleoylethanolamide (OEA), an eCB-related compound but non-CB1 receptor ligand with anorectic effects [13]. Upon the ingestion of fat, OEA is formed in the intestine and activates the intestinal peroxisome proliferator-activated receptor alpha (PPARα), which sends a satiety signal through the vagus nerve [14].

An over-activation of the eCB system has been associated with obesity and abnormal eating behavior [15]. The eCB system is also involved in lipid and glucose metabolism and its peripheral dysregulation in obesity affects several organs that participate in energy homeostasis including the liver, pancreas, adipose tissue and skeletal muscle [16]. Studies of the eCB system in human subjects have reported that in the obese condition plasma levels of 2-AG are increased [17,18], while other studies have also reported elevated AEA plasma levels in obese subjects compared to lean subjects [17].

In animal and human studies [11,19,20], both N-acylethanolamides AEA and OEA, as well as other analogs such as N-palmitoylethanolamide (PEA), have been found to increase shortly after intense exercise, while 2-AG seems to remain stable. You et al [21] found that the gene expression of fatty acid amide hydrolase (FAAH), enzyme that degrades the N-acyl-ethanolamides, is lower in the abdominal adipose of obese women on a program combining exercise training and a caloric restriction diet. Dubreucq et al [22] reported CB1 knockout mice to display 30-40% less running behavior, and proposed a functional loop between the eCB system and MVPA. In support, studies have found that acute administrations of CB1 receptor antagonists or knocking down CB1 receptors in brain GABA neurons have a negative effect on wheel running activity in rats and mice [23]. Further, Avraham et al. [24] administered the eCBs-related compound 2-arachidonylglyceryl-ether

(2-AGE, Noladin) in Sabra mice, detecting that high doses of 2-AG did not alter food intake, but resulted in weight loss and increased PA. These findings may suggest an interaction between eCBs, BMI, MVPA and reward circuits.

Moderate-Vigorous Physical Activity/BMI and Temperament

Cloninger [25] proposed a psychobiological model of personality comprising four heritable temperament traits: Novelty seeking, Harm avoidance, Reward dependence, and Persistence. Some of Cloninger's temperament traits have been associated with MVPA. Authors found a negative correlation between MVPA and Harm avoidance (characterized by inhibition, anxiety and a pessimistic attitude) and a positive link to low Novelty seeking (characterized by introversion, lack of enthusiasm, tolerance to monotony, low response to novelty and low dynamism and curiosity)[26]. In a meta-analysis, the personality trait extraversion, conceptually related to Novelty seeking, and conscientiousness appeared to have a positive effect on structured PA, while high scores of neuroticism, opposite to extraversion, to be inversely associated with MVPA [27]. A relationship between specific temperament traits and BMI has also been described in the literature. Several studies have connected Novelty seeking with obesity, although some observe a positive association [28], while others did not find significant differences [29]. Further, greater Harm avoidance scores were found in obese compared to lean participants [30].

Endocannabinoids and Temperament

The eCB system plays a modulator role in many cognitive and emotional processes [9]. Studies have shown a link between the eCB system and Cloninger's temperament traits. An inverse relationship was observed between CB1 receptor availability and Novelty seeking [31]. Further, AEA has been identified as a substrate of the cytochrome P450 2D6 [32], and genotypic variations of this enzyme have been linked to individual differences in Harm avoidance, socialization ability, and anxiety [33]. Navarrete et al [34] compared the genetic expression of dopamine (DRD2) and cannabinoid (CB1, CB2) receptors in two mouse strains, observing that the mouse strain displaying greater motor behavior also presented more exploratory behavior, impulsivity, and lower attention capacity, and that these were related to CB2 receptor regulation.

Aims of the study

The literature demonstrates significant relationships between the eCB system, Cloninger's temperament traits and MVPA, all associated with BMI. However, no study has analyzed these factors together to assess the modulating effect of eCB functioning and temperament traits on MVPA and lifestyle PA, and the links to BMI in females. Therefore, the aims of the present study were to: 1) examine differences among distinct BMI groups on lifestyle PA levels and time spent in MVPA, temperament traits and plasma eCB concentrations; and 2) explore the association and interaction between MVPA, temperament, eCBs and BMI. Based on the literature, we hypothesized

that: 1) Higher BMI would be associated with greater sedentary behavior, altered plasma eCBs levels and a specific temperament profile; 2) MVPA levels would be linked to specific temperament traits, in particular Novelty seeking, and altered eCB concentration, in particular augmented plasma AEA levels; 3) Both temperament and eCBs would be implicated in the relationship between MVPA and BMI.

## Method and Materials

Ethics statement

All participants gave written informed consent and the Ethics Committees of all the research institutions involved in the data collection approved the study: Comité Ético de Investigación Clínica del Hospital Universitari de Bellvitge; Comitè Ètic d'Investigació Clínica del Hospital Universitari de Girona Doctor Josep Trueta; Comité Ético de Investigación Clínica del Consorci Mar Parc de Salut de Barcelona-Parc de Salut Mar; Subcomisión de Investigación Clínica del Hospital Universitario "Virgen de la Victoria"; Comité Ético de Investigación Clínica de la Universidad de Navarra; Comité Ético de Investigación Clínica de Galicia & Universidad de Santiago de Campostela; Comissió Deontológica de la Universitat Jaume 1. The study was conducted in accordance with the Declaration of Helsinki.

Participants

The sample comprised 189 female individuals, distributed along the BMI continuum, and included: 30 obese participants (BMI=30-39.9, kg/m$^2$), 43 morbid obese (BMI $\geq$ 40, kg/m$^2$), and 116 healthy-weight controls (BMI=18.5-29.9 kg/m$^2$). Participants were Spanish speakers, with a mean age of 34 years (SD=12.3) (distribution of mean age by group was: control 27.6 –SD=7.9–, obese 44.9 –SD=12.9– and morbid obese 43.5 –SD=10.2–). Seven centers, all involved in the CIBERobn Spanish Research Network, participated: the Eating Disorders Unit (Department of Psychiatry, University Hospital of Bellvitge-IDIBELL, Barcelona), the Department of Endocrinology at the University Hospital of Santiago (Santiago de Compostela); the Department of Diabetes, Endocrinology and Nutrition (Clinic University Hospital Virgen de Victoria, Malaga); the Department of Endocrinology and Nutrition (University of Navarra, Pamplona); the Diabetes, Endocrinology and Nutrition Department, Biomedical Research Institute of Girona (IdIBGi-Doctor Josep Trueta Hospital, Girona); the Hospital del Mar Medical Research Institute (IMIM, Barcelona) and the Department of Basic Psychology, Clinic and Psychobiology (University Jaume I, Castellón). The obese participants were patients who had been consecutively referred to the clinics mentioned above. Recruitment of the controls took place by means of word-of-mouth and advertisements at the local universities. All controls were from the same catchment area as the obese patients.

Exclusion criteria were: a) having suffered a lifetime history of Axis I mental disorders since many are linked to altered PA and eCB levels and temperament styles, especially depression

and eating disorders, which are highly co-morbid with obesity [35, 37]; b) having a history of chronic medical illness or a neurological condition (e.g. Parkinson's disease) that may affect motor capacity; c) use psychoactive medication or drugs that influence PA (e.g. cocaine, beta blockers or thyroid medication) or plasma endocannabinoid concentrations (e.g. cannabis); d) being under 18, as adolescence is characterized by psychobiological changes, or over 60 given that age-related medical conditions (e.g. arthritis) affect physical functioning in daily life. Substance abuse/dependence (including cannabis) and eating disorder diagnoses were conducted face-to-face using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) [38]. The evaluation of general health or mental illnesses was based on the General Health Questionnaire-28 (GHQ-28) [39]. Enrolment into the study was between January 2010 and March 2013.

Measures

**Temperament and Character Inventory-Revised** (TCI-R) [40]. This questionnaire is composed of 240-items scored on a 5-point Likert scale and measures personality derived from three character and four temperament dimensions. The dimensions reflecting temperament (Harm Avoidance, Novelty seeking, Reward Dependence and Persistence) were assessed, which entailed the analysis of 133 items of the total items in the questionnaire. Evaluation of the Spanish revised version [41] generated an internal consistency (coefficient alpha) of 0.87.

**Physical Activity** was evaluated with Actiwatch AW7 (Actiwatch AW7; CamNtech Ltd, Cambridge Neurotechnology, Cambridge, UK), a small (39x32x9 mm), light-weight (10.5 g) accelerometer that measures activity. The Actiwatch is worn on the non-dominant wrist for 6 days (4 week days and 1 weekend), from 00:00 hr on day 1 to 00:00 hr on day 7. PA data was calculated in the form of activity counts in a 1-minute epoch length over 24 hours. The counts represent the peak intensity of the movement detected by the Actiwatch AW7. Only the data between 7:00hr and 23:00hr was analyzed; a data reduction procedure that has been recommended and conducted in previous studies [42,43]. No detected movement for 10 or more consecutive epochs (10 minutes) was considered as missing (seen as implausible counts or as periods in which the participant was sleeping). In addition, a minimum of 4 days of wear was used as criterion to accept the case. This is the lower recommended minimum to accurately estimate daily PA in adults [42]. Upon analysis of the data, there were no cases of 4 or less days of wear. The Actiwatch 7 software (CamNtech Ltd) was used to extract the data. Two PA variables were assessed:

*Daytime PA* Daily PA was calculated in the form of mean counts per minute (counts·min$^{-1}$) over the 6 days.

*Time in SLPA and MVPA* The average amount of time during the day spent in sedentary-light PA (SLPA) and MVPA was calculated using an algorithm proposed by Heil [44]. Employing the activity monitor Actical (Mini Mitter Co., Inc., Bend, OR), another Actiwatch produced by the same manufacturer,

which was placed on the ankle, hip and wrist, Heil [44] developed algorithms to predict activity energy expenditure (AEE) in children and adults. To obtain the cut point for MVPA, the formula: *AEE = 0.02013 + (1.282E-5) x HAC* (elaborated for wrist worn accelerometers) was used. This yielded a cut point of 848 counts·min$^{-1}$. This value predicts a PA intensity of 3 MET, which corresponds to a brisk walk. The algorithm to predict AEE in children has been used in a previous study to identify MVPA from the wrist-worn Actiwatch AW4 (CamNtech Ltd, Cambridge Neurotechnology, Cambridge, UK), an earlier version of the Actiwatch AW7 [45].

The Actiwatch AW4 has reliability as a measure of PA similar to other accelerometers [46]. Wrist worn accelerometers have been used to measure PA in various studies [45,47]. They have also been found to predict a similar amount of variance in energy expenditure to the hip-placed accelerometers [48,49].

**Body Composition** was assessed using the Tanita Multi-Frequency Body Composition Analyzer MC-180MA (Tanita Corporation, Tokyo, Japan). The Tanita is a weighting instrument utilizing bioelectrical impedance analysis for the screening of body fat and composition. This instrument is repeatedly revised in relation to the reference standards dual-energy X-ray absorptiometry (DEXA) (http://www.bl-biologica.es/tanita_tbf.htm) and has been validated against other weighing methods [50]. Height was calculated using a stadiometer.

**Endocannabinoids quantification method**. Blood samples were collected from participants between 8 and 9 am after at least 12 hours of fasting. The blood was centrifuged at 3500 rpm at 4ºC for 15-20 min. Plasma aliquots were stored at -80ºC until analysis. Plasma concentrations of the eCBs AEA (ng/mL) and 2-AG (ng/mL) were assessed. In addition, the following acylethanolamides OEA (ng/mL) and PEA (ng/mL) were assessed.

The eCB quantification was done with modifications of a previously described methodology of eCB analysis in brain tissue [51]. After adding the following amounts of deuterated analogues (Cayman Chemical, USA) 0.25 ng AEA-d4, 1 ng PEA-d4 and OEA-d4, 5 ng 2-AG to a 0.5 mL aliquot of plasma, eCBs were extracted with a liquid-liquid extraction in tert-butyl-methyl-ether (Merck, Germany) and the extracts analyzed in a LC/MS-MS system (Agilent 6410, USA). ECBs were separated in a C8 column (2.1 x 100 mm x 1.8 µm particle size, Zorbax, Agilent) by gradient chromatography of a mobile phase of water and acetonitrile containing 0.1% formic acid (Merck, Germany). The source operated on the positive electrospray ionization mode and the detection was done by the multiple reactions monitoring mechanism (MRM). The following precursor to product ion transitions were used: m/z 379→287 for 2-AG, m/z 348→62 for AEA, m/z 326→62 for OEA, m/z 300→62 for PEA, m/z 384→287 for 2-AG-d5, 352→66 for AEA-d4, m/z 330→66 for OEA-d4 and m/z 304→66 for PEA-d4. ECB quantification was done by isotopic dilution of the deuterated analogues

response. Variations in precision and accuracy were < 15% for the individual sample replicates.

Procedure

Experienced psychologists and psychiatrists (all extensively trained in the use of the instruments) completed the clinical and physical assessment in two structured face-to-face interviews. In addition to the first clinical interview, temperament and general health status information was obtained through self-report questionnaires. Prior to assessment, basic anthropometrical features were determined by the TANITA and blood samples were obtained after overnight fasting. The accelerometers provided in the first interview were collected after 7 days in a second face-to-face assessment session.

Statistical Analysis

Statistical analysis was carried out with STATA13 for Windows. Analysis of variance (ANOVA) was used to compare BMI, PA level and eCBs between diagnostic subtypes (controls, obese and morbid obese). Polynomial contrasts into ANOVA were explored by means of linear and quadratic trends, and post-hoc comparisons and Cohens'-d coefficients for the effect size of differences between groups (moderate effect size was considered for $|d|\geq 0.50$ and good effect size for $|d|\geq 0.80$).

Structural equation models (SEM) tested the mediational pathway between temperament scores, MVPA levels, eCBs and BMI, adjusted by the covariate participants' age. The mediational path was considered as adequate when it met previously described criteria [52]. Overall goodness-of-fit statistics were assessed with the $\chi^2$ test, the root mean squared error of approximation (RMSEA), baseline comparison indexes (Comparative Fit Index CFI and Tucker-Lewis Index TLI) and residuals size (Standardized Mean Squared Residual SMSR). A fit was considered to be good if [53]: a non-significant result ($p > .05$) was achieved for the $\chi 2$ test, the RMSEA was < .08, the CFI-TI coefficients were > .90 and SRMR was limited to 0.08. The equation level goodness-of-fit and the effect sizes were estimated through multiple correlation (mc) and Bentler-Raykov multiple correlation (mc2) [54].

## Results

Comparison of Physical Activity measures, BMI, Temperament and Endocannabinoids between groups

Results obtained in ANOVA procedures (Table 1) showed differences between groups of weight for the MVPA means: a negative linear trend emerged (the higher the weights the lower the MVPA mean levels, $p$=. 008) and statistical differences for the pairwise comparison between morbid obese versus controls (f=-19.2, $p$=. 005) were found. No statistical association emerged between groups of weight and daytime PA levels. Linear trends appeared for TCI-R temperament scales: Novelty seeking (decreasing trend), Harm avoidance (increasing trend) and Persistence scales (increasing trend), and an additional quadratic trend for Reward dependence. Statistical differences were found between obese and controls for Novelty seeking ($p$=. 015), Harm avoidance ($p$<.001) and

Reward dependence ($p$=.002), and between morbid obese and controls for Novelty seeking ($p$=.038) and Harm avoidance ($p$<.001). As shown in Table 2, the eCB 2-AG was associated with the distinct BMI groups: positive linear and quadratic trends were obtained for this biological measure and statistical differences emerged in the post-hoc comparison between both obese and morbid obese versus controls.

Mediation model of Moderate-Vigorous Physical Activity level when including temperament and biological parameters

Figure 1 shows the path-diagram and standardized structural coefficients for the mediational model between temperament traits, eCBs, MVPA level and BMI. Results were adjusted by the covariate participants' age. Variables selected for the model accomplished Baron-Kenny's requirements for mediational paths. No reciprocal association between eCBs and BMI were retained since no statistical effect of eCBs on BMI emerged, and retaining these parameters affected the fitting. Both low Novelty seeking and high Harm avoidance scores were predictive of lower MVPA levels. The eCBs measures AEA and OEA mediated the association between MVPA levels and BMI: a) high MVPA levels were associated with high AEA measures, and elevated AEA values were related to high BMI, b) high MVPA levels were also related to high OEA levels, and low levels for this cannabimimetic were associated with higher BMI. MVPA levels and Harm avoidance scores also showed direct effects with BMI (high BMI was predicted by high Harm avoidance and low MVPA levels). Pathway of Figure 1 achieved goodness-of-fit: $\chi^2$=8.36 ($p$=. 30), RMSEA=. 036, CFI=. 99, TLI=. 98 and SRMR=. 033. Considering each equation level, MVPA level achieved low effect size values (mc=. 26 and $mc^2$=. 07), while BMI obtained higher ones (mc=. 69 and $mc^2$=. 48). The overall $R^2$ (coefficient of determination) was very good ($R^2$=0.47).

## Discussion

The aims of the present study were to analyze the differences between extreme BMI groups on daytime PA, MVPA, temperament and eCBs, and explore whether temperament traits and eCB concentrations are mediators of MVPA in females.

Physical Activity and BMI

As expected, comparison among weight categories showed that the morbid obese participants displayed the least MVPA compared to the healthy-weight participants. Differently, daytime PA did not vary between groups. These findings are in line with the existing literature. A recent study assessing the effects of adherence to MVPA guidelines on BMI and waist circumference detected an inverse association between meeting the PA guidelines and baseline BMI and waist circumference. However, later linear regressions demonstrated that only vigorous PA was significantly correlated with lower BMI [55]. In addition, the authors observed that only high adherence to the MVPA guidelines resulted in decreases in BMI and waist circumference, while stable PA had no effect. Similarly, in another study, a

significant trend across weight categories in PA among females was only observed for the time spent in vigorous PA [1]. A hypothesis may be that it is a gradual decrease in time spent in MVPA, rather than daytime activity per se that increases the risk and maintains the global prevalence of obesity. As the present study was a cross-sectional, causality cannot be determined, but our findings suggest that the relationship is more complex.

Temperament and BMI

Regarding temperament, both obese groups (obese and morbid obese), presented a distinct temperament profile from the controls, characterized by greater scores in Harm avoidance and Reward dependence, but lower Novelty seeking. Whereas those studies that included obese participants with comorbid eating disorders (namely Binge Eating Disorder) found higher impulsivity scores [36], those who excluded this group of obese patients reported lower impulsivity levels [30]. This may explain the reason why the high Novelty seeking in obese individuals described in the literature [28] was not found in the present study, as individuals with a history of eating disorders were excluded from it. Similar to previous studies [30], the obese individuals in our study presented a temperament profile characterized by passivity, sensitivity, nervousness, insecurity, and social dependence.

Endocannabinoid concentrations and BMI

One remarkable finding in our study was the elevated level of plasma 2-AG concentrations found in the obese groups, which is keeping with the literature [15]. Di Marzo et al [56] observed that a lifestyle modification program for obese patients resulted in a fall of both 2-AG and AEA concentrations, however only 2-AG was associated with a decrease in visceral adipose tissue, triacylglycerol concentrations, and HDL3-cholesterol concentrations, which suggests that these eCBs may have distinct metabolic roles. The present study therefore provides further support to the increasing evidence for the involvement of eCBs in fat metabolism development. Additional research must be conducted to determine the role of the eCB system in obesity.

Temperament traits and endocannabinoid factors as mediators of Moderate-Vigorous Physical Activity and BMI

As expected, objectively measured MVPA was found to be inversely associated with BMI. Noteworthy, the majority of studies assessing the relationship between BMI and PA have employed subjective measures to assess the latter, namely self-reported questionnaires and surveys. Self-reported PA has been found to differ from more direct assessments, which questions the reliability of self-report measures [57]. The use of an accelerometer in a large sample in the present study therefore overcomes this methodological limitation. In relation to the association between MVPA and temperament traits, an interesting link was found between MVPA and Novelty seeking and Harm avoidance scores. This is in line with other studies, whereby aspects of Novelty seeking, namely energetic attitude and exploratory behavior, have been found to

be related to both motoric activity in animals [34] and weekly hours spent exercising in humans [58]. Whereas individuals with high scores in extraversion (corresponding to high Novelty seeking) have a more sociable and interactive lifestyle, and are thus more likely to be active [27], those with low levels of Novelty seeking tend to be passive, inhibited, and less dynamic, and are therefore expected to adopt a more sedentary lifestyle [26]. In contrast, the Harm avoidance temperament trait is associated with the inhibition of behaviors due to greater pessimistic worry or avoidance [59] and sensitivity to pain expectancy [60]. Individuals scoring higher in this trait are likely to be averse to engaging in MVPA, especially high contact and risky sports, as they avoid any activity in which they may be vulnerable to injury.

Consistent with prior animal [6] and human [20] studies, our results showed that AEA and OEA plasma concentrations but not 2-AG were positively associated with MVPA. Authors have proposed that this may be due to differences in the biosynthesis and degradation mechanisms [61]. Whereas AEA and OEA are N-acylethanolamides (NAEs) synthesized from the hydrolysis of *N*-arachidonoyl phosphatidylethanolamine (NAPE) and degraded by the FAAH, 2-AG is synthesized from other precursors (diacylglycerols) and enzymes (diacylglycerol lipases-a and b) and is primarily degraded by monoacylglycerol lipase (MAGL) [16]. Therefore, given that these eCBs have distinct metabolic pathways, a different interaction with MVPA has been proposed [6].

One discrepancy between our results and those of Heyman et al [57], is that we did not find an association between MVPA and PEA despite that PEA shares the same biosynthetic and degradation pathways of AEA and OEA. This could be because the eCB levels in this study were measured at the basal state, while Heyman et al [61] measured eCB levels after intense exercise. In support to our data, regarding the specific relationships between some NAEs and PA, Gasperi et al [62] found increased activity of FAAH in the lymphocytes of physically active subjects at resting condition. It must be noted that blood eCB levels represent the spillover from many sources and it is not possible to differentiate the tissue of origin. For instance, Caraceni et al [63] found that blood AEA, PEA and OEA levels were correlated with liver function, but eCB levels may vary differently in different tissues such as in the intestine or brain [12, 56], or in different depots of the same tissue such as in subcutaneous or visceral fat [14]. The skeletal muscle itself is altered in obesity, with increased expression of CB1 receptors and elevated levels of 2-AG without there being changes in AEA. It has been suggested that, in contrast to 2-AG, AEA and possibly OEA may have beneficial effects on glucose uptake and mitochondrial biogenesis in the muscle through the activation of other receptors such as peroxisome proliferators (PPARs) or the transient receptor potential vanilloid receptor 1 (TPRV1). In this regard, it has been proposed that PA could be a complementary approach for the treatment of obesity without the side effects of CB1 antagonists [19]. In addition to the PA peripheral effects, the

potentiation of the eCB system after MVPA also has positive effects on cognitive functions, again linked to AEA [8], which could facilitate the implementation of both preventive and treatment programs for obesity.

Furthermore, in the current study the eCBs AEA and eCB-related compound OEA seem to act as contrasting mediators in the relationship between MVPA and BMI. The underlying mechanism between MVPA and the eCB system activation is not yet clear. It could be due to the increases in stress and glucocorticoid hormones (particularly cortisol) that occur with structured PA and seem to be implicated in the activation of eCB signaling [64]. Separately, eCBs have been found to be implicated in the regulation of appetite (as is the case of marijuana) via the activation of the reward system [65]. When energy homeostasis is challenged, such as in situations of food deprivation, an increase in endocannabinoid levels takes place [66]. The process is associated with a reinforced pleasure obtained from ingestion and from the rewarding properties of food [11,67], which may lead to hyperphagia, overconsumption and consequentially weight gain [68]. Differently, the eCB-related compound OEA is a putative, peripheral satiety factor and anorexigen mediator, which promotes satiety and reduces weight gain by stimulating the vagal sensory nerves that in turn stimulate the brainstem and hypothalamus [13]. These findings are reflected in the mediation effect of AEA and OEA on the relationship between MVPA and BMI obtained in the current study. MVPA has an inverse direct effect on BMI, which can be attributed to energy expenditure, and a similar indirect relationship may also exist mediated by OEA. Yet, MVPA may also be associated with augmented BMI through the orexigen effect of AEA.

Finally, Novelty seeking and plasma AEA concentrations were both found to be positively linked to MVPA. Van Laere et al [31] evaluated CB1 receptor availability in temperament, finding greater global cerebral CB1 receptor availability to be inversely related to Novelty seeking. Novelty seeking may interact with the eCB system via the engagement in MVPA. Furthermore, the activation of this system with exercise appears to result in exercise-induced analgesia and may be responsible for the reported runner's high, a transient and intense feeling of happiness, elation, and energy [20]. Our results support the concept that individuals who are high in impulsive traits may engage in PA to achieve a gratifying state, following a positive reinforcement conditioning, whereas more passive and less energetic individuals, such as those who present elevated Harm avoidance, present more sedentary behavior. Reduction in the BMI might be a consequent effect, but also may act as a maintaining factor of this vicious circle. Further research is needed to understand the mechanisms of these associations. For instance OEA is not a CB1 agonist and it is best known for acting as a fat sensor in the intestine, but in a recent report it has been suggested that OEA is also involved in the reward system by stimulating central dopamine activity [69] and may participate in the control of reward-related behaviors

through a PPARα receptor-independent mechanism [70].

Limitations

The study has a number of limitations that should be considered. First, the study focused of females. Future studies should also assess male participants who are likely to present distinct PA patterns. Second, the accelerometer was placed in the non-dominant wrist. Though this instrument permits a more accurate assessment of PA compared to subjective measures, PA entailing only lower-body movement may not be adequately captured. Future studies should place accelerometers to both the wrist and waist, as well as use self-reported questionnaires in order to obtain a more complete description of the activity patterns of participants. It must also be noted that in the current study plasma eCBs levels were assessed in the morning after an overnight fast, while MVPA was evaluated throughout the day. Although a link between time spent in MVPA and circulating eCBs was observed, a more controlled design should be developed in order to demonstrate the exact cause-effect mechanism. Finally, the cross-sectional nature of the study does not permit causality to be determined. Longitudinal studies should be conducted to evaluate how temperament in adolescence or young adulthood may predict the interaction between eCBs, temperament, and MVPA later in life, and to assess how lifestyle changes with increases in the time spent engaging in MVPA may be related to alterations in the eCB system and BMI.

Despite its limitations, this study has several important strengths, including the substantial sample size. Furthermore, path-analysis is used in this study as a case of structural equation modeling (SEM) with exploratory aims, with the advantage (compared to classical regression models) of allowing the inclusion of multiple relationships among a set of variables, including mediational associations. The results obtained constitute empirical evidence for the development of further theories about the role of MVPA, endocannabinoids and temperament on BMI.

In conclusion, the present study provides further understanding of the pathophysiological mechanism involved in PA and obesity by integrating previously described links between the eCB system, temperament, MVPA, and BMI, to generate a psychobiological model of the relationship between engagement in MVPA and BMI. It was shown that decreases in time spent in MVPA, rather than overall daytime PA, may be underlying the augmentation in obesity, and this may occur through the interaction with both psychological and biological factors. Important clinical conclusions may be drawn to confront the excess obesity among females. Future therapeutic approaches aiming at preventing obesity by reducing sedentary behavior and encouraging exercise, should consider both physiological and behavioral maintaining factors (e.g. attitude and motivation, behavioral tasks, environmental factors, locus of control), and temperament traits. It may be hypothesized that additional psychological interventions focusing on improving enthusiastic and inquiring attitudes, positive own reactions in front of novelty and new goals, might have a

positive secondary influence on patients' attitude towards MVPA.

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**Table 1**. BMI, PA, temperament, endocannabinoids and endocannabinoids related compounds among study groups.

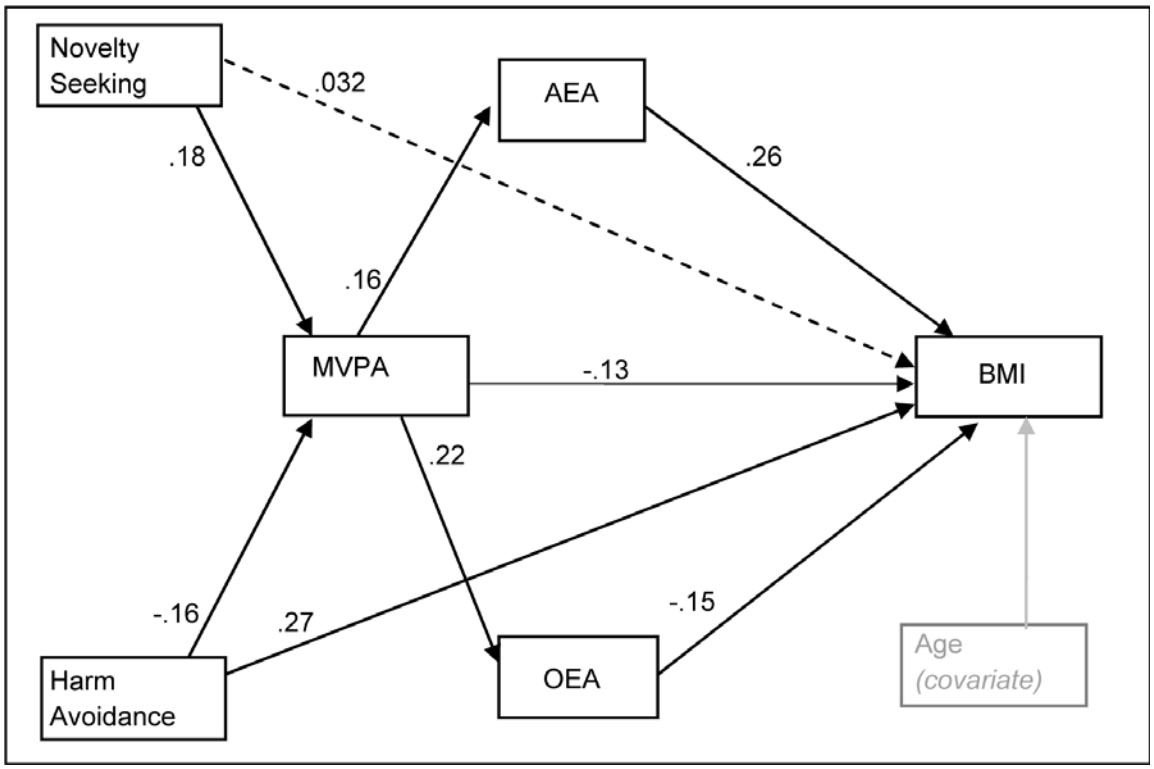
| | Controls (*n*=116) | | Obese (*n*=30) | | Morbid-obese (*n*=43) | | ANOVA: p-value, trends and contrasts | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Group | LT | QT | OB vs CO | | MO vs CO | | MO vs OB | |
| | Mean | SD | Mean | SD | Mean | SD | *p* | *p* | *p* | ϕ | \| d \| | ϕ | \| d \| | ϕ | \| d \| |
| Body mass index | 21.6 | 2.8 | 35.5 | 2.3 | 46.2 | 4.8 | **<.001** | **<.001** | **.019** | **13.9*** | *5.42†* | 24.6* | *6.24†* | **10.7*** | *2.81†* |
| MVPA | 67.20 | 35.18 | 65.84 | 51.03 | 48.02 | 33.03 | **.016** | **.008** | .283 | -1.35 | 0.03 | **-19.18*** | *0.56†* | **-17.82*** | 0.41 |
| Daytime PA | 297.97 | 68.77 | 305.31 | 93.16 | 273.71 | 72.02 | .120 | .109 | .196 | 7.34 | 0.09 | -24.26 | 0.34 | -31.60 | 0.38 |
| TCI-R: Novelty-seeking | 100.3 | 12.9 | 92.9 | 18.1 | 94.8 | 14.1 | **.017** | **.038** | .120 | **-7.35*** | 0.47 | **-5.51*** | 0.41 | 1.84 | 0.11 |
| TCI-R: Harm avoidance | 92.2 | 16.1 | 106.7 | 18.7 | 113.0 | 19.3 | **<.001** | **<.001** | .261 | **14.5*** | *0.83†* | **20.8*** | *1.17†* | 6.29 | 0.33 |
| TCI-R: Reward depend. | 99.6 | 14.4 | 109.0 | 14.6 | 103.0 | 13.9 | **.008** | .194 | **.010** | **9.38*** | *0.65†* | 3.40 | 0.24 | -5.98 | 0.42 |
| TCI-R: Persistence | 111.4 | 16.8 | 112.8 | 20.0 | 104.9 | 19.6 | .100 | **.050** | .210 | 1.44 | 0.08 | -6.51 | 0.36 | -7.95 | 0.40 |
| 2-AG (ng/mL) | 1.60 | 1.02 | 3.34 | 1.65 | 3.82 | 2.55 | **<.001** | **<.001** | .070 | **1.74*** | *1.27†* | **2.22*** | *1.14†* | 0.48 | 0.23 |
| AEA (ng/mL) | 0.55 | 0.17 | 0.59 | 0.26 | 0.62 | 0.20 | .160 | .069 | .874 | 0.04 | 0.19 | 0.07 | 0.39 | 0.03 | 0.13 |
| PEA (ng/mL) | 2.48 | 0.61 | 2.62 | 0.94 | 2.41 | 0.50 | .503 | .603 | .254 | 0.13 | 0.17 | -0.07 | 0.12 | -0.20 | 0.27 |
| OEA (ng/mL) | 4.07 | 1.39 | 4.13 | 1.58 | 3.86 | 0.89 | .672 | .425 | .567 | 0.06 | 0.04 | -0.22 | 0.19 | -0.28 | 0.22 |

ϕ: Adjusted mean difference in ANOVA. LT: linear trend. QT: quadratic trend. | d |:Cohen's-d.
*Bold: significant contrast (.05 level). †Italics: moderate to good effect-size (| d |≥0.5)
BMI: Body mass index; MVPA: moderate-vigorous physical activity; PA: physical activity; 2-AG: 2- arachidonoylglycerol; AEA: anandamide; OEA: *N*-oleylethanolamide; PEA: *N*-palmitoylethanolamine.

**Table 2**. Results of the SEM evaluating the pathways between personality, MVPA, eCBs and BMI.

| | | Std.Coef. | SE | z | *p* | 95% CI | |
|---|---|---|---|---|---|---|---|
| MVPA | Novelty seeking | 0.1761 | 0.0792 | 2.22 | 0.026 | 0.0208; | 0.3314 |
| | Harm avoidance | -0.1575 | 0.0795 | -1.98 | 0.048 | -0.3132; | -0.0017 |
| | *Constant* | *1.2444* | *0.7831* | *1.59* | *0.112* | *-0.2904;* | *2.7792* |
| BMI | MVPA | -0.1285 | 0.0622 | -2.06 | 0.039 | -0.2505; | -0.0065 |
| | AEA | 0.2649 | 0.0739 | 3.58 | <0.001 | 0.1200; | 0.4099 |
| | OEA | -0.1514 | 0.0740 | -2.05 | 0.041 | -0.2964; | -0.0065 |
| | Novelty seeking | 0.0317 | 0.0623 | 0.51 | 0.611 | -0.0904; | 0.1537 |
| | Harm avoidance | 0.2686 | 0.0608 | 4.42 | <0.001 | 0.1494; | 0.3879 |
| | AGE (covariate) | 0.5229 | 0.0551 | 9.49 | <0.001 | 0.4149; | 0.6309 |
| | *Constant* | *-0.3051* | *0.6229* | *-0.49* | *0.624* | *-1.5260;* | *0.9157* |
| AEA | MVPA | 0.1558 | 0.0791 | 1.97 | 0.049 | 0.0007; | 0.3109 |
| | *Constant* | *2.7223* | *0.2386* | *11.41* | *<0.001* | *2.2546;* | *3.1900* |
| OEA | MVPA | 0.2233 | 0.0771 | 2.90 | 0.004 | 0.0722; | 0.3743 |
| | *Constant* | *2.6724* | *0.2423* | *11.03* | *<0.001* | *2.1974;* | *3.1473* |

**Figure 1.** The moderating role of temperament and endocannabinoids on physical activity levels and body mass index.




Continuous line: significant parameter. Structural Equation Model analysis shows that the temperaments low Novelty seeking and low Harm avoidance were predictive of low physical activity (MVPA) levels. High MVPA levels were associated with high anandamide (AEA) levels and high AEA levels were associated with high body mass index (BMI). In addition, high MVPA levels were associated with high *N*-oleylethanolamine (OEA) levels and low OEA levels were associated with high BMI. A direct effect was found between high MVPA and low BMI and between high Harm avoidance and high BMI.

## 4.2. STUDY 2: MODULATION OF IRISIN AND PA ON EXECUTIVE FUNCTIONS IN OBESITY AND MORBID OBESITY.

**Background:** Cognitive defecits have been found in OB, particularly in cognitive flexibility and decision making skills [147]. This seems to be partly due to abnormaities in the brain circuitry responsible for reward processing [383,384]. Evidence exists for beneficial effects of PA on executive functions such as response inhibition, working memory and decision-making abilities [385]. Being secreted in response to exercise to promote energy expenditure, irisin is believed to play an intermediatory role between PA and BMI [131,132]. However, the incongruency in findings advocates the need to conduct further research. Furthermore, irisin has been detected in human cerebrospinal fluid and hypothalamic sections [386], and animal research has shown FNDC5 expression in the brain [387]. Although it remains to be shown if irisin can cross the blood-brain barrier, the potential involvement of irisin in the nervous systems suggests this myokine may contribute to the influence of PA on cognition.

**Objectives:** To compare executive functioning (decision-making response inhibition and cognitive flexibility) between individuals with OB, MOB and healthy-weight controls, and to examine the relationships between MVPA, circulating irisin levels, and pre-frontal-mediated executive function.

**Methods:** The sample comprised 114 participants, of which 21 had OB, 44 MOB, and 49 were lean controls. The participants were female, aged between 18 and 60 years and Spanish was their native language. Exclusion criteria were: a history of a chronic medical illness or neurological condition that might affect cognitive function, having experienced a head trauma with a loss of consciousness that lasted longer than 2 minutes, currently using psycho-active medication or drugs, being male, was aged under 18 or over 60, having received a diagnosis of diabetes (type I or type II) or an ED. Neurological assessment took place via the use of the Wisconsin Card Sorting Test (WCST; [388]), the Stroop Color and Word Test [389], and the Iowa Gambling Task (IGT; [390]). Time in MVPA was used as a measure of PA. Irisin was extracted from blood samples taken in the morning between 08:00h and 09:00h after an overnight fast.

Results: In comparison to the healthy controls, the participants with OB and MOB had a lower academic level, were older, and had a greater BMI and percentage body fat (all $p < .001$). After adjusting for age, a linear trend was found in circulating irisin levels across the three weight groups, being highest in the MOB group, followed by the OB group, and finally the control group ($p < .001$), whereas the opposite occurred in terms of MVPA ($p < .001$). Pairwise comparisons indicated that a significant difference in MVPA was only present between the MOB and control groups ($p < .001$). Regarding the neuropsychological assessment, group differences were only detected in the IGT-total score, where the controls obtained a higher score than the participants with OB and MOB (both $p < .01$). The score did not differ significantly between these two groups ($p = .815$). Lastly, in the control group, MVPA correlated positively with the WISC-total correct items score ($r = .223$) and inversely with the IGT-total score ($r = -.283$), while irisin correlated positively with WCST-total errors ($r = .223$) and negatively with the Stroop interference and WCST categories completed scores ($r = -.228$ and $r = -.204$ respectively). In the OB group, MVPA correlated positively with the IGT-total score and negatively with the WISC-total correct hits ($r = -.332$), WISC conceptual answers ($r = -.315$) and WISC categories completed ($r = -.335$) scores, whereas a positive correlation was present between MVPA and IGT-total ($r = .491$). In the MOB group, MVPA correlated positively with the Stroop interference score ($r = .311$) and negatively with the IGT-total score ($r = -.293$), while circulating irisn correlated negatively with the IGT-total score ($r = -.335$).

# MODULATION OF IRISIN AND PHYSICAL ACTIVITY ON EXECUTIVE FUNCTIONS IN OBESITY AND MORBID OBESITY

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**Journal:** Scientific Reports

**Impact Factor: 5.23**

**Keywords:** Irisin; Executive functions; Decision making; Response inhibition; Cognitive flexibility; Obesity; Morbid obesity.

**Abstract**

Whether the executive profile is different between obesity (OB) and morbid obesity (MO) remains unclear. Recent evidence suggests that physical activity (PA) can act as a cognitive enhancer. Irisin is a recently discovered hormone associated with some of the positive effects of PA. The objective of the study was to investigate the executive profile in OB and MO, and to explore the role of PA and irisin. 114 participants were included (21 OB, 44 MO and 49 healthy controls-HC) in the study and assessed with the Wisconsin Card Sorting Test, Stroop Color and Word Test, and Iowa Gambling Task. All participants were female, aged between 18 and 60 years. Results showed a similar dysfunctional profile on decision making in OB and MO compared with HC. Thus, no specific neuropsychological profiles between OB and MO can be clearly observed in our sample. However, a negative correlation was found between irisin and executive functioning. These results demonstrate a specific executive profile in OB and a relevant and negative modulation of irisin on executive functioning. Although irisin might be a promising target for the treatment of obesity, its effects on cognition might be considered when thinking about its therapeutic use.

## Introduction

Obesity has been associated with important biological and environmental risk factors [1]. It has been demonstrated that these patients have a dysfunctional neural pattern, characterized by an alteration in the brain circuitry associated with the reward system [2–4]. From a neuropsychological perspective, deficits in attention, memory and mainly executive functions are major domains defining this pathology [5]. In a previous study, we have demonstrated a dysfunctional executive profile in obesity, mainly in the cognitive flexibility and decision making domains [6]. However, whether this cognitive profile differs between obese (BMI= 30-39.9, kg/m2), and morbid obese subjects (BMI ≥ 40, kg/m2) remains unclear.

The mechanisms underlying the cognitive deficits in obesity are also poorly understood. Some hypotheses, concerning vascular and metabolic states associated with obesity, have been considered as influencing cognitive performance in obesity [7–9]. However, it is well-accepted that cognitive functions are complex and are influenced by a number of social and environmental factors [10]. In this line, recent evidence in both human and animal studies suggests that exercise can act as a cognitive enhancer [11–13]. Of interest, the most important effects of physical activity appear to be on tests measuring executive functions such as response inhibition, working memory and decision making [14].

Specifically, meta-analyses in humans indicate that physical exercise correlates positively with cognitive performance in healthy subjects and in patients with dementia or mild cognitive impairment [14–16]. Recent neuroimaging studies also suggest that a combination of omega-3 fatty acids, aerobic exercise and cognitive stimulation prevents a decline in the gray matter volume of the frontal, parietal and cingulate cortex in patients with mild cognitive impairment [17]. Particularly

interesting are those studies suggesting that physical activity, a balanced diet, cognitive stimulation or the management of conditions such as diabetes and obesity are preventive factors in Alzheimer's disease [18]. Alongside pharmacological research, these approaches may help slow down the progression of the disease. However, in order to develop successful treatments that complement these strategies, the understanding of the underlying mechanisms is essential.

It has been postulated that irisin, a recently discovered hormone, might be associated with some of the effects of physical exercise such as energy expenditure and thermogenesis [19], even though its role is far from being clear. Irisin is secreted after the cleaving of the membrane protein fibronectin type III domain containing 5 (FNDC5) and is related to the conversion of white adipose tissue into beige adipose tissue by means of its action in the expression of the uncoupling protein 1 (UCP1). Animal studies suggest that increased plasma levels of irisin are associated with an increase in energy expenditure [19]. In humans, irisin has been linked to body mass index (BMI) despite results being inconsistent. Some authors have reported a positive correlation between circulating irisin levels and BMI [20–23], whereas others a negative correlation [24,25]. Some variables that can explain these differences are the methods used for measuring circulating levels of irisin and the presence of diabetes. Our results could be crucial since all published findings regarding irisin, and particularly circulating irisin levels, are actually under a cloud of suspicion [26,27]. At this point, as we have previously stated in a recent review [28], there is no doubt as to the need to discern which fraction of FNDC5 is cleaved to produce the soluble portion of irisin. For these reasons, in this study we used the Phoenix pharmaceuticals ELISA kit Ref. EK-067-52, being the only available kit that has been validated [20]. Regardless, based on these findings, it might be postulated that irisin could be a target hormone in conditions characterized by pathological food intake and extreme BMI, such as obesity.

Additionally, previous reports have evidenced that irisin may have a role in the nervous system. This conclusion is based on the expression of FNDC5 in the brain [29], as well as the presence of irisin in human cerebrospinal fluid and hypothalamic sections [30]. Thus, although it is currently unknown whether irisin can cross the blood-brain barrier and whether irisin may function as a messenger between the skeletal muscle or adipose tissue and brain, previous studies demonstrated that, for example, peripheral administration of irisin is able to reduce blood pressure induced by sympathetic out-flow [31]. Thus, considering the association between irisin levels and physical activity, it is reasonable to consider the role of this hormone on the cognitive effects of exercise in humans, especially taking into account their expression in the brain, as found in several animal studies [29,32].

Thus, the objective of the study was to investigate differences in the cognitive profile of obese subjects versus that of morbid obese subjects, mainly in executive functioning (decision making,

response inhibition and cognitive flexibility). We also aimed at exploring the relationship between levels of physical activity and circulating levels of irisin and prefrontal-mediated executive functions in these patients. We hypothesized that both obese and morbid obese subjects would show alterations in executive performance compared to healthy controls. We also hypothesized a role of irisin levels on this dysexecutive profile. For this purpose, we used three neuropsychological tasks (Wisconsin Card Sorting Test; Stroop Color and Word Test; and Iowa Gambling Task) known to be mediated by the prefrontal and orbitofrontal cortex functioning [33].

**Results**

*Sample characteristics*

Table 1 includes the descriptive data of the participants of the study. Statistically significant differences were observed across all variables. HC were the group with the highest proportion of university-level education while obese group presented the highest proportion of low academic levels. Mean age was statistically equal for OB and MO ($p$=.214) but patients of these two groups were statistically older than HC ($p$<.001 for both pairwise comparisons). Means for BMI and body-fat were higher for MO, followed by OB and HC (for these two variables linear and quadratic trends achieved significant results indicating that differences between MO vs OB were lower than those obtained for HC vs OB).

--- Table 1 ---

*Group comparison for plasma irisin and activity levels*

Table 2 contains the comparison between weight groups for circulating levels of irisin and activity levels (MVPA), adjusted by the covariates participants' age and years of education. Regarding plasma irisin levels, a significant linear trend was obtained (means for irisin tended to increase comparing HC, OB and MO), but a quadratic trend was not statistically significant (mean difference for HC vs OB is similar to mean difference between OB and MO). All pairwise comparisons for plasma irisin levels were statistically significant and obtained moderate to high effect size ($|d|$>0.50). With respect to activity levels, a linear trend was also achieved (means for MVPA tended to decrease comparing HC, OB and MO) and a quadratic trend was not significant. For MVPA, the only significant pairwise comparison with moderate effect size was that of MO versus HC. Figure 1 shows the radar chart for the means of the z-scores of irisin and activity levels, illustrating that the morbidly obese group was characterized by the highest irisin levels and low MVPA-IGT levels, obese patients by medium MVPA-irisin levels and low IGT measures, while the control group by high IGT scores, medium MVPA and very low irisin levels.

--- Table 2 ---

--- Figure 1 ---

*Group comparison for neuropsychological performance*

Table 3 depicts the ANOVA results comparing neuropsychological test performance between weight groups (HC, OB and MO), adjusted by patients' age and years of education. Only the total IGT scores were different across the three diagnostic conditions: means tended to decrease when comparing HC versus OB and MO (significant linear trend) and the statistical quadratic trend indicates that the difference comparing HC versus OB (means 31.7 vs -0.81) was higher than the difference of OB versus MO (means -0.81 vs 3.40). Pairwise comparisons achieved significant results and a high effect size for HC compared to OB and MO, but no statistical differences were found upon comparing OB and MO. Figure 1 also included the distribution of means for z-IGT scores. No significant differences between groups were observed for the WCST. Lastly, the OB subjects displayed the worse performance in Stroop test (interference score), although no significant differences between the three groups were observed.

---Table 3 ---

*Association between irisin and activity levels with neuropsychological measures*

Table 4 contains the partial correlations, adjusted by participants' age and years of education, between irisin and MVPA with the cognitive outcomes. The r-coefficients were estimated separately (stratified) according to weight group. For HC, activity levels correlated positivity with the WCST-total correct items and negatively with the IGT total score, while irisin correlated negatively with interference and WCST-categories completed and positively with WCST-total errors. For OB, activity levels correlated positively with the IGT-total score and negatively with WCST total correct hits, conceptual answers and categories completed. No association was found between irisin levels and cognitive measures. For MO, the activity levels correlated positively with the interference measures (Stroop interference score) and negatively with the IGT-total score. Irisin levels also correlated negatively with the IGT total score.

--- Table 4 ---

**Discussion**

This study set out to examine the executive profile of obese and morbidly obese subjects and the role of irisin and activity on prefrontal-dependent cognitive functions in healthy controls and obese subjects. The primary finding of this study was the similar executive profile observed between obese and morbidly obese subjects. According to our results, neither obese nor morbidly obese patients showed significant impairment in the cognitive flexibility capacity or the inhibition response compared to the healthy controls. However, they showed a significant impairment in the decision making capacity. Finally, although not statistically significant a moderate to high effect size of correlations between levels of irisin and physical activity with neuropsychological measures were found. Although physical activity has been previously related to executive functions [12], this is to the best of our knowledge, the first time that not only physical

activity, but also irisin is to some extent associated with executive functioning in humans.

As for decision making performance, both OB and MO subjects went for choices that result in elevated immediate gains despite important future losses, thus showing a similar level of impairment. This profile has been previously described in obese patients [6], suggesting that reduced decision making abilities are core characteristics of obesity and result in inadequate self-control [6]. Of interest, a recent systematic review examining the relationship between obesity and cognition concluded that decision making is one of the functions most affected in these patients [34]. From a clinical perspective, it might be postulated that there are rational similarities between OB and MO subjects in terms of decision making performance and day to day eating behavior.

These neuropsychological results are also consistent with the hypothesis of food addiction in obesity [4,35]. According to this theory, the neural substrates of the decision making's deficits in obesity are similar to those found in drug addiction. Specifically, previous results have suggested that executive functions, such as decision making, are modulated by dopaminergic functioning (D1 receptors and D2 receptors) in prefrontal cortex and fronto-subcortical circuits [33]. In human and animal models, it has been demonstrated that D2R downregulation mediates signaling in the striatal indirect circuits associated with prefrontal areas [36]. In addition, impairments in those circuits have been linked to less executive control [37], thus suggesting that DA's impaired modulation of these areas might be contributing to the impulsive drug intake seen in addiction [38,39]. In support, neuroimaging studies in both drug addicted and obese subjects have evidenced low availability of D2R in the striatum to be associated with reduced activity in the ACC and deficient control over food and drug consumption [40,41], which points to the similar brain deregulation in obesity and addiction.

However, we failed to find an association between obesity and inhibition response or cognitive flexibility. According to our results, the executive profile in obesity is more associated with impairments in 'hot' executive functions (related to emotional or motivational processes) than 'cold' executive functions (more rational or logical processes)[42]. Our results are not in line with those of other studies, showing alterations in cognitive flexibility and inhibition response in obesity [5]. These differences might be explained by the characteristics of the sample. A relevant strength of our study is the exclusion of patients with eating disorders, such as Binge eating disorder (BED) or Bulima nervosa, and diabetes. It has been broadly demonstrated that obese patients with eating disorders or impaired glucose regulation show deficits in inhibition and flexibility [43]. Furthermore, it has been demonstrated that obese individuals with obesity-related somatic comorbidity (i.e., hypertension, diabetes) perform worse in neurocognitive tasks compared to obese individuals without any somatic disorder [44]. Additionally, neuroimaging studies have demonstrated that individuals with BED have a

diminished ability to recruit impulse-control-related brain regions compared with obese subjects without BED. The authors concluded that the observed differences in neural correlates of inhibitory processing in BED relative to OB suggest distinct neurobiological contributions to binge eating as a subgroup of obese individuals [45]. All in all, these discrepant results highlight the importance of controlling for these confounder variables in cognitive studies, and conducting a more comprehensive assessment of inhibition response and cognitive flexibility in obesity.

The effects of activity in executive functions are in line with the positive effects of physical activity in other cognitive functions. It has been demonstrated that exercise positively activates cognitive performance [11–13]. In studies with healthy controls, physical activity has been positively correlated to improvements in executive functions such as cognitive flexibility and working memory [14,15]. It has also been demonstrated that older adults that engage in physical exercise show a better performance in reasoning capacity, working memory and speed of processing than those maintaining a sedentary lifestyle [46]. Similarly, physically active retired subjects perform better on a series of cognitive tests than retired individuals who are inactive [47].

However, our results might indicate that the positive effects of physical activity on cognition would not be mediated by the action of irisin. Elevated levels of irisin were associated with disruption in cognitive flexibility performance and inhibition response in healthy controls and with disruption of decision making capacity in OB. Recent studies have been focusing on irisin as a potential treatment for obesity [48,49]. However, according to these results a potential cognitive effect of irisin might be considered when thinking about its therapeutic use. Nevertheless, we should keep in mind that this is a correlation study. Thus, future studies should confirm this hypothesis.

Although it is currently unknown whether irisin can cross the blood-brain barrier and whether irisin may function as a messenger between the skeletal muscle or adipose tissue and brain, previous studies have demonstrated, for example, that peripheral administration of irisin is able to reduce blood pressure induced by sympathetic out-flow [31]. From a neuropsychological perspective, the effects of irisin on executive functions might be explained by its action on some neurotransmitters, such as GABA and BDNF [29]. Irisin has recently been detected in the cerebellar Purkinje cells [29], specifically in GABAergic cells. Although until now it has not been detected in other brain regions, this study points to a novel cerebral pathway that might also be implicating more cortical regions. Dysfunction of the GABAergic system may contribute to cognitive impairment in humans. Specifically, individuals with Alzheimer's disease have decreased cerebral GABA in the brain and CSF [50]. Furthermore, GABA levels in human CSF decrease with aging [51], which has been associated with cognitive impairment. According to these studies, the expression of irisin in the

GABAergic brain cells might, to some extent, explain its effects on the central nervous system-mediated functions.

From a molecular perspective, the effects of irisin in the prefrontal-related cognitive functions might be mediated by its association with the PPARγ coactivator-1 α (PGC1α). As explained above the effects of irisin are mediated by its action on the expression of UCP1, regulated by PGC1α [19]. Specifically, higher expression of PGC1α is associated with higher levels of irisin. PGC1α is the main transcriptional regulator of mitochondrial function, associated with neurological deficits and cerebral anomalies [52,53]. Of interest, it has been demonstrated that PGC1α deficient mice show a significant brain deficiency [54]. In humans, genetic mutations on genes that affect mitochondrial functions have been found to contribute to the pathogenesis of neurodegenerative diseases such as dementia [55,56]. In particular, genes that are expressed in response to PGC-1α are under-expressed in Parkinson's disease and Lewy body disease patients [56]. It has also been suggested that enhancement of PGC-1α by dietary treatment might benefit cognitive function and synaptic plasticity in Alzheimer's disease by preventing Aβ production in the brain [53,55,57]. This positive correlation between PGC-1α and cognition was not observed in our study. One possible explanation could be that increased irisin levels in obese subjects result from physiological compensatory mechanisms that may involve decreased sensitivity to irisin or an attempt to increase glucose sensitivity [28].

This study has several strengths, including the large sample size. Biological approaches, as applied in the present study, are an additional level of analysis on top of neuropsychological assessment, which provides a practical tool for the study of executive processes. Additionally, our study was specifically designed to comprehensively test the executive dysfunction in obese and morbidly obese subjects by using three well validated executive tasks. However, the results of this study should be interpreted in the context of some limitations. First, measures of intelligence quotient (IQ) were not considered, which might have influenced executive performance. Nonetheless, years of education, as a measure of cognitive capacity have been considered in the statistical analysis. Second, only females were included in the study, making the results not applicable to males. Replication of these results with a group including males should be considered. Future studies should also consider including further decision-making, inhibition response and cognitive flexibility tasks in order to shed more light on the mechanisms underlying the executive functions profile in obesity. Moreover, the cross-reactivity of the ELISA immunoassay on detecting irisin FNDC5 precursor at circulating level should be taken into account [28]. Also, although we have used multivariate methods to control the potential biases due to potential confounder variables[58], the control group is not matched for age and education. Future studies should replicate this study with a control group matched for age and education. Additionally, results reflect moderate to

high effect size of correlations between levels of irisin, physical activity and cognition. Future studies should further explore these associations with bigger sample populations to check for p-value statistical significances. Finally, altered irisin levels have been associated with fatty liver, renal, and autoimmune diseases [59]. Future studies including these variables should also be considered.

In summary, our results provide novel information regarding the executive pattern of obesity and the influence of the physical activity and irisin on the cognitive profile. This study is particularly timely given the important public-health impact of obesity and the potential significance of accurately-defined biological variables that are associated. Efforts should focus on determining new biomarkers in order to accelerate the detection of potential drug targets and the implementation of new therapeutic approaches. According to our results, the undesirable effects of irisin on cognition should be considered before using it as a target for the treatment of these extreme eating/weight conditions.

**Material and methods**

*Sample*

Seven centers, all involved in the CIBERobn Spanish Research Network, participated: the Eating Disorders Unit (Department of Psychiatry, University Hospital of Bellvitge-IDIBELL, Barcelona), the Department of Endocrinology at the University Hospital of Santiago (Santiago de Compostela); the Department of Diabetes, Endocrinology and Nutrition (Clinic University Hospital Virgen de Victoria, Málaga); the Department of Endocrinology and Nutrition (University of Navarra, Pamplona); the Diabetes, Endocrinology and Nutrition Department, Biomedical Research Institute of Girona (IdIBGi-Doctor Josep Trueta Hospital, Girona); the IMIM (Hospital del Mar Medical Research Institute, Barcelona) and the Department of Basic Psychology, Clinic and Psychobiology (University Jaume I, Castelló). Enrolment into the study took place between January 2010 and September 2013.

One hundred and fourteen participants were included, distributed along the BMI continuum: 21 obese subjects (BMI= 30-39.9, kg/m$^2$), 44 morbid obese subjects (BMI $\geq$ 40, kg/m$^2$) and 49 healthy controls (BMI= 18.97-24.61 kg/m$^2$). BMI was calculated as the body mass divided by the squared height of the subject. Height was calculated using a stadiometer. All participants were female, aged between 18 and 60 years and spoke Spanish as their first language. Participants were informed about the research procedures and gave informed consent in writing. Procedures were approved by the Ethical Committee of each of the aforementioned institutions (Ref. 048/10; 307/06; 2010/3914/I; 110/2010). The methods were carried out in accordance with the approved guidelines.

The exclusion criteria were: (1) History of chronic medical illness or neurological condition that might affect cognitive function; (2) Head trauma with loss of consciousness for more than 2 min,

learning disability or mental retardation; (3) Use of psycho-active medications or drugs (4) Being male; (5) Age under 18 or over 60 (to discard neuropsychological deficits associated with age); (6) Having diabetes type I or II. (7) Obese patients who have comorbid binge eating disorder (DSM-IV criteria [60]). Healthy controls were recruited through several sources including word-of-mouth and advertisements in the local university. Prior to assessment, HC were asked about lifetime or current presence of an eating disorders or obesity. The lifetime history of health or mental illnesses profile was based on the general health questionnaire GHQ-28 [61].

*Neuropsychological assessment*

As described in a previous study [6], all participants underwent a comprehensive neuropsychological and clinical assessment. The neuropsychological tests were selected to cover various aspects of executive functions including decision making, response inhibition, strategic planning and cognitive flexibility and were administered by a trained psychologist in a single session and in a randomized order. All participants were assessed with the following neuropsychological tests: (a) Wisconsin Card Sorting Test (WCST) [62], (b) Stroop Color and Word Test (SCWT) [63] and (c) Iowa Gambling Task (IGT) [64].

- Wisconsin Card Sorting Test

The WCST is a classical measure of planning capacity, cognitive flexibility, capacity of shifting among stimulus, and control of impulsive responses not aimed at achieving an objective. Subjects have to match a target card with one of four category cards: a single red triangle, two green stars, three yellow crosses, and 4 blue circles. Cards might be matched by color, number, or shape. After each trial a feedback is given to the participant, indicating if they have correctly matched the card. However, along the task the classification rule is unpredictably changing. The test ends when the participant has completed 6 categories or 128 trials. For the purpose of this study the following measures of the test were considered: Total corrects responses, Total errors, Conceptual answers, and Total Categories completed.

- Stroop Color and Word Test

This paper and pencil test has shown adequate reliability and construct validity for the assessment of inhibition and switching skills. The SCWT measures interference control, flexibility and attention. The task included three pages: (1) a page with color words printed in black ink; (2) a page with “Xs” printed in color; (3) a page with names of colors printed in an incongruent color (i.e. word “blue” printed in red ink). Participants have 45 seconds to read as many words as possible in the first page and name the ink in pages 2 and 3. Three scores are obtained after task completion: number of words (W) (page 1), number of colors named “X” (C) (page 2) and number of color-named words (CW) (page 3). With these scores, the CW estimated (CW’) is calculated by using the formula: CW’= (W*C)/ (W+C). An additional “interference score” is obtained with the formula: Interference=CW-CW’. This is the main variable and higher scores in

this variable indicate better capacity of inhibition response.

- Iowa Gambling Task

This computer task evaluates decision-making, risk and reward and punishment value. The subject has to select 100 cards from four decks (A, B, C and D). After each card selection an output is given: gain or a gain and loss of money. Two decks (A and B) are not advantageous as the final loss is higher than the final gain. Decks C and D, however, are advantageous since the punishments are smaller. The final objective of the task is to make the most of profit and gain as much money as possible. This test is scored by subtracting the amount of cards selected from decks A and B from the amount of cards selected from decks C and D. Higher results point to better performance while negative results point to preference for the not advantageous decks.

*Physical Activity assessment*

Physical Activity was evaluated with Actiwatch AW7 (Actiwatch AW7; CamNtech Ltd, Cambridge Neurotechnology, Cambridge, UK), a small (3963269 mm), light-weight (10.5 g) accelerometer that measures activity. The Actiwatch is worn on the non-dominant wrist for 6 days (4 weekdays and 1 weekend), from 00:00 hr on day 1 to 00:00 hr on day 7. Physicacl activity data was calculated in the form of activity counts in a 1-minute epoch length over 24 hours. The counts represent the peak intensity of the movement detected by the Actiwatch AW7. Only the data between 7:00hr and 23:00hr is analyzed; a data reduction procedure that has been recommended and conducted in previous studies [65,66]. No detected movement for 10 or more consecutive epochs (10 minutes) is considered as missing (seen as implausible counts or as periods during which the participant is sleeping). In addition, a minimum of 4 days of wear is used as criterion to accept the case. This is the lower recommended minimum to accurately estimate daily PA in adults [67]. Upon analysis of the data, there were no cases of 4 or less days of wear. The Actiwatch 7 software (CamNtech Ltd) is used to extract the data. For the purpose of this study the time in Moderate-Vigorous Physical Activity (MVPA) was considered. The average amount of time during the day spent in MVPA was calculated using an algorithm proposed by Heil [67]. Employing the activity monitor Actical (Mini Mitter Co., Inc., Bend, OR), another Actiwatch produced by the same manufacturer, which was placed on the ankle, hip and wrist, Heil [67] developed algorithms to predict activity energy expenditure (AEE) in children and adults. To obtain the cut point for MVPA, the formula, AEE=0.02013+(1.282E-5) x HAC (elaborated for wrist worn accelerometers) was used. This yielded a cut point of 848 counts x $min^{-1}$. This value predicts a PA intensity of 3 MET, which corresponds to a brisk walk. The algorithm to predict AEE in children has been used in a previous study to identify MVPA from the wrist-worn Actiwatch AW4 (CamNtech Ltd, Cambridge Neurotechnology, Cambridge, UK), an earlier version of the Actiwatch AW7 [67]. This method has been previously used in obese population [69].

*Irisin quantification method*

Blood samples from overall participants were obtained under basal conditions after a 12-h overnight fast. EDTA-plasma and serum of specimen were separated from whole blood and immediately frozen at -80º C until assay.

The quantitative measurement of irisin in human plasma samples was performed using a commercial enzyme-linked immunosorbent assay (ELISA) kit directed against amino acids 31-143 of the FNDC5 protein (Irisin ELISA Kit EK-067-52; Phoenix Pharmaceuticals, INC, CA) according to the manufacturer's instructions. Absorbance from each sample was measured in duplicate using a spectrophotometric microplate reader at wavelength of 450 nm (Versamax Microplate Reader; Associates of Cape Cod Incorporated, East Falmouth, MA).

*Statistical analysis*

Analyses were carried out with SPSS21 for Windows. First, analysis of variance (ANOVA) valued the association between the diagnosis subtype and the neurocognitive measures. Due the ordinal scale for the weight measure (HC, OB and MOB), ANOVA included polynomial contrasts to explore the presence of trends (linear and quadratic). In addition, considering the importance of age and education on the cognitive performance, these two variables were included as covariates in the ANOVA procedures. The effect size for each pairwise comparison was valued through Cohen's-*d* coefficient (moderate effect size was considered for $|d|>0.50$ and high for $|d|>0.80$). Second, the association between physical activity and irisin with cognitive outcomes was assessed with partial correlations, also adjusted by covariates patients' age and years of academic studies. Due the strong association between *R*-coefficients and significant test (only large *R*-coefficients achieve significant results in small size samples while small *R*-coefficients achieve significant results in large samples), correlations were interpreted as relevant based on their sample size (moderate effect-size was considered for $|R|\geq.20$ and good effect-size for $|R|\geq.25$ [70-71].

Due the multiple statistical tests, potential increases in Type I error was controlled through Bonferroni's method.

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**Acknowledgement**

This work was supported by Fondo de Investigación Sanitaria-FIS (PI14/290; PI11/210) and Fondos FEDER; AGAUR (2009SGR1554, 2009SGR718); ABF was supported by Ministerio de Ciencia e Innovación Subprograma Juan de la Cierva (JCI-2011-09248); CGB was supported by Ayudas Predoctorales de Formación en Investigación en Salud-PFIS (FI12/00470). ABC is funded by the ISCIII through a research contract "Sara Borrell" (C09/00365). MP is a Miguel Servet Fellow (ISCIII/SERGAS). JCFG is recipient of a "Rio Hortega" contract from"Instituto de Salud Carlos III,"

Madrid, Spain (CM12/00059). CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn) and CIBER Salud Mental (CIBERsam), are supported by ISCIII.

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**Competing Financial Interests statement:** We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

**Table 1.** Sociodemographics, irisin and activity levels.

| | | HC normal-weight ($n = 49$) | Obesity ($n = 21$) | Morbid-Obesity ($n = 44$) | Statistic | $p$ |
|---|---|---|---|---|---|---|
| Academic level; *%* | *Primary* | 8.2% | 70.6% | 61.9% | $\chi^2_{(df:4)} = 43.65$ | **<.001** |
| | *Secondary* | 38.8% | 23.5% | 31.0% | | |
| | *University* | 53.1% | 5.9% | 7.1% | | |
| Education (years); | *Range* | 9 to 20 | 7 to 20 | 0 to 20 | $F_{(df:3;110)} = 35.33$ | **<.001** |
| | *Mean (SD)* | 18.47 (2.52) | 11.76 (3.82) | 13.00 (4.70) | | |
| Age (years); | *Range* | 19 to 46 | 26 to 68 | 22 to 63 | $F_{(df:3;110)} = 42.31$ | **<.001** |
| | *Mean (SD)* | 29.04 (6.22) | 49.19 (11.69) | 42.25 (10.84) | | |
| BMI (kg/m$^2$); | *Range* | 18.97 to 24.61 | 30.79 to 39.11 | 40.35 to 59.52 | $F_{(df:3;110)} = 624.4$ | **<.001** |
| | *Mean (SD)* | 21.61 (1.54) | 35.52 (2.40) | 46.32 (4.91) | | |
| Body-fat (%); | *Range* | 18 to 35 | 34 to 50 | 38 to 53 | $F_{(df:3;110)} = 287.3$ | **<.001** |
| | *Mean (SD)* | 26.57 (4.50) | 41.41 (3.84) | 46.55 (3.66) | | |
| Irisin (ng/ml); | *Range* | 60.5 to 171.7 | 92.3 to 176.0 | 68.7 to 219.8 | $F_{(df:3;110)} = 20.86$ | **<.001** |
| | *Mean (SD)* | 104.00 (24.32) | 122.38 (23.63) | 141.40 (32.96) | | |
| Activity: MVPA; | *Range* | 14.0 to 154.2 | 9.0 to 151.5 | 3.7 to 126.8 | $F_{(df:3;110)} = 5.04$ | **.008** |
| | *Mean (SD)* | 71.37 (34.09) | 54.47 (43.82) | 47.40 (34.15) | | |

BMI: Body mass index. SD: standard deviation.

**Table 2.** Polynomial trends and pairwise comparisons for Irisin and Physical activity (ANOVA adjusted by covariates participants' age and years of education).

| | Adjusted means | | | Factor | | Trends | | | | Pairwise-comparisons: mean differences | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HC | OB | MO | Group | | Linear | | Quadratic | | OB vs HC | | | MO vs HC | | | MO vs OB | | |
| | $n$=49 | $n$=21 | $n$=44 | $F_{(2;111)}$ | $p$ | $F_{(1;111)}$ | $p$ | $F_{(1;111)}$ | $p$ | MD | $p$ | $\lvert d\rvert$ | MD | $p$ | $\lvert d\rvert$ | MD | $p$ | $\lvert d\rvert$ |
| Irisin (ng/ml) | 104.0 | 122.4 | 141.4 | 20.86 | .002 | 41.72 | .002 | 0.002 | .999 | 18.38 | .026 | 0.77* | 37.40 | .002 | 1.29* | 19.02 | .022 | 0.66* |
| Activity: MVPA | 71.37 | 54.47 | 47.40 | 5.04 | .016 | 9.74 | .004 | 0.192 | .999 | -15.90 | .198 | 0.41 | -23.97 | .004 | 0.70* | -8.07 | .830 | 0.20 |

Abbreviations: HC, Healthy controls normal-weight; OB, Obesity; MO, Morbid-Obesity; $|d|$: Cohen's-d.
*Moderate to high effect size ($|d|$>0.50).
$|d|$: Cohen's-d. *Moderate to high effect size ($|d|$>0.50).
Results include Bonferronis-correction for multiple comparisons.

**Table 3.** Polynomial trends and pairwise comparisons for neuropsychological variables: ANOVA adjusted by the covariates participants' age and years of education.

| | | Adjusted means | | | Factor | | Trends | | | | Pairwise-comparisons: mean differences | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | OB vs HC | | | MO vs HC | | | MO vs OB | | | |
| | | HC | OB | MO | Group | | Linear | | Quadratic | | | | | | | | | | | |
| | | n=49 | n=21 | n=44 | $F_{(2,109)}$ | $p$ | $CE$ | $p$ | $CE$ | $p$ | MD | $p$ | $\lvert d\rvert$ | MD | $p$ | $\lvert d\rvert$ | MD | $p$ | $\lvert d\rvert$ |
| STROOP | Interference | 2.52 | -1.61 | 1.27 | 2.05 | .348 | -0.89 | .442 | 2.86 | .132 | -4.13 | .173 | 0.45 | -1.25 | .583 | 0.19 | 2.88 | .436 | 0.46 |
| WCST | Total corrects | 64.02 | 67.49 | 69.22 | 1.25 | .497 | 3.68 | .119 | -0.71 | .850 | 3.48 | .571 | 0.30 | 5.20 | .316 | 0.43 | 1.73 | .815 | 0.11 |
| | Total errors | 30.13 | 36.38 | 34.13 | 0.47 | .692 | 2.83 | .454 | -3.47 | .676 | 6.25 | .571 | 0.29 | 4.01 | .583 | 0.18 | -2.25 | .815 | 0.08 |
| | Conceptual answers | 54.76 | 56.65 | 60.17 | 0.88 | .555 | 3.82 | .224 | 0.66 | .857 | 1.90 | .759 | 0.11 | 5.41 | .398 | 0.31 | 3.51 | .815 | 0.16 |
| | Categories completed | 4.64 | 4.43 | 4.64 | 0.13 | .881 | 0.00 | .997 | 0.18 | .774 | -0.22 | .759 | 0.12 | 0.00 | .997 | 0.01 | 0.22 | .815 | 0.09 |
| IGT | Total | 31.73 | -0.81 | 3.40 | 11.24 | **.001** | -20.04 | **<.001** | 15.00 | **.041** | **-32.5** | **.001** | **1.28*** | **-28.3** | **.001** | **1.20*** | 4.21 | .815 | 0.19 |

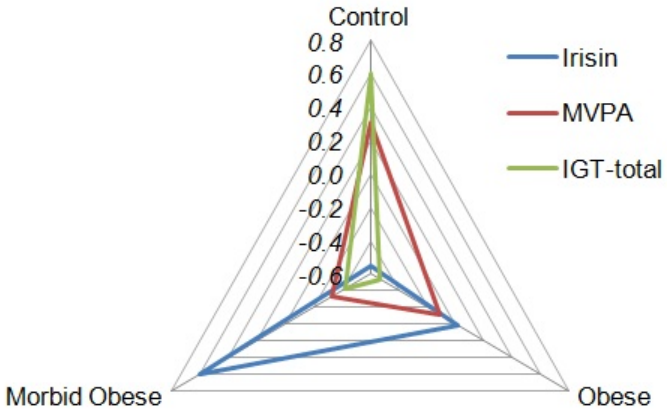
Abbreviations: HC, healthy controls normal-weight; OB, Obesity; MO, Morbid-Obesity.
SCWT, Stroop Color and Word Test; WCST, Wisconsin Card Sorting Test; IGT, Iowa Gambling Task; Conc.answers, conceptual answers; Cat.complet., categories completed.
$|d|$: Cohen's d. *Moderate to high effect size ($|d|>0.50$).
Results include Bonferroni's-correction for multiple comparisons.

**Table 4.** Partial correlations (adjusted by the covariates age and years of education) between cognitive variables, physical activity and irisin.

| | | HC ($n$ = 49) | | | | OB ($n$ = 21) | | | | MO ($n$ = 44) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Activity | | Irisin | | Activity | | Irisin | | Activity | | Irisin | |
| | | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ |
| STROOP | Interference | -.081 | .594 | **-.228*** | .128 | -.070 | .781 | .169 | .501 | **.311*** | .078 | -.038 | .835 |
| WCST | Total corrects | **.223*** | .136 | .033 | .828 | **-.332*** | .179 | .170 | .500 | .154 | .392 | .088 | .624 |
| | Total errors | -.056 | .711 | **.233*** | .119 | .136 | .591 | .159 | .530 | -.057 | .754 | -.148 | .410 |
| | Conceptual answers | .193 | .200 | -.072 | .632 | **-.315*** | .203 | -.091 | .719 | .150 | .405 | .143 | .426 |
| | Categories completed | .157 | .299 | **-.204*** | .173 | **-.335*** | .175 | -.083 | .743 | .157 | .384 | .012 | .948 |
| IGT | Total | **-.283*** | .056 | .151 | .317 | **.491*** | .038 | -.194 | .440 | **-.293*** | .098 | **-.335*** | .057 |

HC, healthy control normal-weight; CO-over., control over-weight; OB, Obesity; MO, Morbid-Obesity.
Activity: MVPA score. SCWT, Stroop Color and Word Test; WCST, Wisconsin Card Sorting Test; IGT, Iowa Gambling Task.
*Bold: moderate to high effect size for correlation (|$R$|>0.20).
Correlation matrix includes Bonferroni's correction for multiple comparisons.

**Figure 1.** Radar-chart for the distribution of the mean levels of irisin, activity and IGT-total measure in the three groups of weight (Z-scores).




*Radar chart displays multivariate results in a two-dimensional chart, representing different axes for each category/group starting from the same point (center of the graphic) and extending outward from the center. Each color-line represents a different variable, and the line size drawn connecting the data to each axis represents the relative magnitude of the variable for the group. So, this graph displays what groups-observations are most similar/different and the connected pattern allows seeing the relative position of each group compared to others. To plot z-scores instead of raw-scores each metric gets equal weight and to simplify the interpretation.

## 4.3. STUDY 3: PHYSICAL ACTIVITY AND ANOREXIA NERVOSA: HOW RELEVANT IS IT TO THERAPY RESPONSE?

**Background:** Excessive PA has been historically considered as a key characteristic of AN [10]. However, discrepancies exist regarding the effects it has on treatment outcome [309,310,329,391]. This can be attributed to several factors, including differences in the treatment received by the studied sample (full-hospitalization, day hospitalization or outpatient), in the measuring instrument (self-reported questionnaires, interviews or objective devices such as accelerometers), in the definition of excessive PA (an excess in the time and intensity of exercise or a compulsive drive to exercise), and, importantly, in the type of PA evaluated (time spent in intense exercising, overall activity, activity at inappropriate times).

**Objectives:** To compare patients with AN versus healthy controls on different forms of PA, and to evaluate the possible effect of MVPA on treatment outcome taking into consideration additional clinical variables.

**Methods:** 88 patients with AN (BMI < 18.5 kg/m2) who attended the Eating Disorder Unit of the University Hospital of Bellvitge were compared to 116 healthy eating/weight controls (BMI = 18.5–29.9 kg/m2) recruited from the same catchment area. Three types of PA were measured with the Actiwatch AW7: daytime PA, MVPA and the prevalence of high levels of PA (high exercisers). The Eating Disorder Inventory-2 (EDI-2) [392] was used to measure ED psychopathology, and symptoms of general psychopathology were examined with the Symptom-Checklist-revised (SCL-90-R) [393]. Treatment outcome was defined in terms of “full remission”, “partial remisson” and “poor outcome” (no remission/dropout)

**Results:** A significant difference in the time spent in MVPA and daytime PA was not found between the patients with AN and healthy controls ($p = .21$ and $p = .34$ respectively). The prevalence of high PA among the patients was of 37%, while that among the healthy controls was of 61% ($p = .014$). MVPA was associated with a decreased likelihood of poor treatment outcome ($b = -.23$) and less depressive symptoms ($b = -.33$). Age positively correlated with time in MVPA ($b = .43$) and

depressive symptoms (b = -.37), whereas an inverse link was found between the duration of the disorder and these variable (b = .25 and b = -.22 respectively). ED psychopathology was associated with both greater depressive symptoms (b = .71) and a poor treatment outcome (b = 0.31).

# PHYSICAL ACTIVITY IN ANOREXIA NERVOSA: HOW RELEVANT IS IT TO THERAPY RESPONSE?

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**Journal:** European Psychiatry
**Impact Factor:** 3.44

**Key words:** Physical Activity; Anorexia Nervosa; Treatment Outcome; Depression; Partial hospitalization

**Abstract**

*Objective:* Elevated physical activity has been observed in some patients with anorexia nervosa (AN) despite their emaciated condition. However, its effects on treatment outcome remain unclear. This study aimed to examine objectively measured physical activity in this clinical population and how it might be related to a partial hospitalization therapy response, after considering potential confounders.

*Method:* The sample comprised 88 AN patients consecutively enrolled in a day hospital treatment program, and 116 healthy-weight controls. All participants were female and a baseline assessment took place using an accelerometer (Actiwatch AW7) to measure physical activity, the Eating Disorders Inventory-2 and the Depression subscale of the Symptom Checklist-Revised. Outcome was evaluated upon the termination of the treatment program by expert clinicians.

*Results:* Although AN patients and controls did not differ in the average time spent in moderate-to-vigorous physical activity (MVPA) (p=.21), nor daytime physical activity (p=.34), fewer AN patients presented a high physical activity profile compared to the controls (37% vs. 61%, respectively; p=.014). Both lower levels of MVPA and greater eating disorder severity had a direct effect on a poor treatment outcome. Depression symptoms in the patients were associated with lower MVPA, as well as with an older age, a shorter duration of the disorder and greater eating disorder psychopathology.

*Conclusions:* There is a notable variation in the physical activity profile of AN patients, characterized by either low or very high patterns. Physical activity is a highly relevant issue in AN that must be taken into account during the treatment process.

## 1. Introduction

Anorexia Nervosa (AN) is a severe and chronic Eating Disorder (ED) characterized by self-induced dieting, low body weight and an intense fear of gaining weight [1]. Baseline physical activity (PA) levels have been associated with longer treatment duration [2], poorer outcome when considered as less change in ED symptoms [3] and with an increased likelihood of dropout [4], in others no influence has been observed in therapy response [5] or recovery rates [6]. However, others have proposed as a relevant predictor the type of PA rather than the time spent in PA [7]. These authors found that a poor outcome was predicted by the time spent engaging in light PA (e.g. walking) rather than the time spent in intensive PA (e.g. swimming or running) [7].

The discrepancies in the literature assessing PA in EDs may be attributed to several factors. First, there is a lack of an operationalized definition of what elevated PA constitutes [8], the most common terms being: “hyperactivity” [5,9], “excessive/high-level exercise” [10–12] and “compulsive exercise” [3,13]. Second, there are several methodological gaps in the assessment of PA as some studies have used subjective instruments rather than objective measures. This is particularly important since subjective self-reports of PA have

been found to be unreliable in patients with AN given that they seem to underreport/underestimate the amount of PA they engage in [14]. Third, some researchers have considered PA as a continuous variable, while others as a categorical construct [15].

The discrepancies in the literature concerning the possible effect of PA on treatment outcome might also be due to the heterogeneity of the therapy approaches and settings used in the treatment. Whereas in some studies PA was measured while the patients were in full-time inpatient hospitalization [5], in others the patients were receiving either inpatient or outpatient treatment [6] or a combination of inpatient and day hospital [3,4,7]. Additionally, in most of these studies the relevant variable depression was inconsistently considered. Although depressive symptoms is frequent in AN [16] and its inverse link to PA has been documented [17–19], previous studies have not taken it into account.

The present study attempts to overcome some of the aforementioned methodological gaps by using an objective instrument to examine various types of PA with a continuous measure approach, in a partial Day Hospital treatment, which is less restrictive in terms of PA and is a more realistic setting, and after considering those factors that may be influencing both PA and treatment outcome (such as depression symptoms).

The purpose of this study were twofold: a) To examine the differences between patients with AN and healthy-weight controls in terms of daytime PA levels and time spent in moderate-to-vigorous PA (MVPA); b) To assess the relationship between MVPA and treatment outcome and the potential modulating role of additional clinical variables. The inconsistencies in the literature do not permit a hypothesis to be made on the effects of MVPA on treatment outcome. Nonetheless, it is expected that a subgroup of the AN patients in our sample will present particularly high levels of MVPA compared to the controls, and that MVPA levels in AN patients will be inversely associated with depression symptoms.

## 2. Methods

### *2.1 Participants*

A total of 88 AN patients (BMI<18.5, $kg/m^2$) and 116 healthy eating/weight controls (HC) participated in the study. The AN participants were patients consecutively admitted to the Day Hospital Treatment Program of the ED Unit at the University Hospital of Bellvitge, and were diagnosed according to the DSM-IV-TR criteria [20] by means of a semi-structured clinical interview (SCID-I) [21]. Of the AN patients, 52 were diagnosed with restrictive AN, 16 with purging AN and 20 with binge-purging AN. The mean age of onset of the AN was 21.2 years (SD=8.4) and the mean duration of the disorder was 7.2 years (SD=6.4); difference by group was not significant: control 27.5 – SD=7.9, AN 27.9 –SD=9.0, p=.74). All participants were female and Spanish speakers. The mean age of the HC was 27.5 years (SD=7.9), while that of the AN patients was 27.94 (SD=9) years. The

body mass index (BMI) of the HC was 21.7 (SD=2.8), while that of the AN patients was 16.6 (SD=6.3).

Exclusion criteria for all the participants were: (a) a history of a medical illness or neurological condition that could influence PA; (b) being male; (c) using psychoactive medications or drugs; (d) being under 18 or over 60. Controls were also excluded if they had suffered a lifetime ED. Face-to-face interviews employing a Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I) [21] were carried out to detect possible ED diagnoses. The evaluation of general health or mental illnesses was based on the General Health Questionnaire-28 (GHQ-28) (22]. There were no statistical differences between AN-subtypes in mean age (p=.42), age of onset (p=.68), duration of the disorder (p=.10) or SCL-90-R depression score (p=.09).

The HC were recruited via word-of-mouth and advertisements at the local universities of the following centers (CIBERobn Spanish Research Network): Department of Diabetes, Endocrinology and Nutrition (Clinic University Hospital Virgen de Victoria, Malaga); the Department of Endocrinology and Nutrition (University of Navarra, Pamplona); the Hospital del Mar Medical Research Institute (IMIM, Barcelona) and the Department of Basic Psychology, Clinic and Psychobiology (University Jaume I, Castellón). Enrolment took place between January 2010 and June 2013.

*2.2. Treatment protocol*

Following assessment and diagnosis, AN patients received treatment as usual, which consists in the manualized Day Hospital Program; previously described [23]. The program takes place from 9:00h to 15:00h during week days. It comprises supervised breakfast and lunch as well as group therapy covering nutritional and dietary patterns and psychological-psychiatric factors in several group sessions (cognitive restructuring, social skills training, body image therapy, stress management strategies, problem solving, art therapy). The day hospital treatment lasts around 12 weeks, based on a Cognitive-Behavioral Therapy approach, after which the patients continue regular, individual, therapy. The outcome of the day hospital intervention is classified by expert clinicians in terms of three categories based on the DSM-IV-TR criteria and described previously [24]: a) "full remission", which signifies that the patient has reached a BMI greater or equal to 18.5 (threshold for a healthy weight according to the World Health Organization), with an absence of both anorexic cognitions and bingeing/purging behaviors for a continuous period of time; b) "partial remission", when the patients present a notable improvement in ED symptoms but there are still residual symptoms; c) "no-remission", which refers to those patients that have dropped out of the program or terminated the day hospital treatment with a poor result. In the present article, no-remission was considered as a poor treatment outcome.

*2.3. Measures*

*Physical Activity.* The Actiwatch AW7 (Actiwatch AW7®; CamNtech Ltd, Cambridge Neurotechnology, Cambridge,

UK) was used to measure PA. This is a small (39x32x9 mm), light-weight (10.5 g) accelerometer that evaluates PA. It is worn on the non-dominant wrist for 6 days (4 week days and 1 weekend), from 00:00h on day 1 to 00:00h on day 7. The PA data was calculated in terms of activity counts (peak intensity of detected movement) in a 1-minute epoch length across 24 hours. The data between 7:00h and 23:00h was extracted and examined. This data reduction technique has been recommended and carried out in previous studies [25–27]. Ten or more consecutive epochs (10 minutes) of no detected movement were considered as missing (seen as implausible counts or periods in which the participant was sleeping). Furthermore, a minimum of 4 days of wear was used as criterion to accept the case. This corresponds to the recommended minimum to adequately estimate daily PA in adults [25]. No cases of 4 or less days of wear were detected. The Actiwatch 7 software (CamNtech Ltd) was used to extract the data. Three PA variables were assessed:

*Daytime PA* Daytime PA refers to the intensity of the physical activity presented in a given minute. This was calculated in the form of the average mean counts per minute (counts·min$^{-1}$) over the 6 days.

*Time in MVPA* The average amount of time per day (over the 6 days) the participant spent in MVPA was calculated based on an algorithm suggested by Heil [28] to predict activity energy expenditure (AEE) in both children and adults [28]. Using the activity monitor Actical@ (Mini Mitter Co., Inc., Bend, OR), another Actiwatch produced by the same manufacturer, which was placed on the ankle, hip and wrist, Heil (28) proposed the use of the following formula for wrist-worn accelerometers in adults: $AEE = 0.02013 + (1.282^{E-5}) \times Activity\ Monitor\ Output$. A cut point of 848 counts·min$^{-1}$ was then derived to establish the time in MVPA (minutes), deemed to predict a PA intensity of 3 Metabolic Equivalent of Task (METs) (the standard measure of PA intensity) that corresponds to a brisk walk. This cut point was based on the use of the value 0.0310 for AEE, assumed to equate 3 METs (Heil, 28). Therefore, MVPA refers to all activities ranging from a brisk walk to those sports producing greater AEE. The algorithm proposed by Heil (28) has been used previously [26].

*High versus low PA levels* PA level refers to the classification of participants as presenting high versus low PA levels based on the amount of time spent in MVPA. Participants who spent more than 300 minutes in MVPA over the 6 days of assessment were considered as showing a high PA level profile (high exercisers). Low PA levels were assumed when the participant did not reach this threshold (low exercisers). Of interest was the prevalence (percentage) of participants within each group (AN and HC) showing high (versus low) MVPA levels. The proposed threshold of 300 minutes corresponds to the definition provided in other studies [6].

The Actiwatch AW4 provides a reliable measure of PA corresponding to other accelerometers [29]. Wrist-worn acceler-

ometers have been found to predict a parallel amount of variance in energy expenditure to hip-placed accelerometers [30].

*Body mass index and body composition.* The Tanita Multi-Frequency Body Composition Analyzer MC-180MA (Tanita Corporation, Tokyo, Japan) is a weighting instrument that calculates body composition using bioelectrical impedance analysis. This device is repeatedly revised to meet the reference standards dual-energy X-ray absorptiometry (DEXA) (http://www.bl-biologica.es/tanita_tbf.htm). and has been validated against other weighing methods [31].

*Depression symptoms.* The depression scale of the Symptom Checklist-revised (SCL-90-R) [32] was employed to measure the presence of depression symptoms. This test, which consist of 90 items, evaluates psychological problems and symptoms of psychopathology subdivided in 9 primary symptom dimensions: 1) Somatization; 2) Obsession-Compulsion; 3) Interpersonal Sensitivity; 4) Depression; 5) Anxiety; 6) Hostility; 7) Phobic Anxiety; 8) Paranoid Ideation; and 9) Psychoticism. Three global indices are also included: the global severity index (GAI), which indicates overall distress, the positive symptom distress index (PSDI), designed to assess the intensity of the symptoms, and the positive symptom total (PST), which assesses self-reported symptoms. The scale of interest was the fourth scale: "depression". This test has been validated in a Spanish population [33] obtaining a mean internal consistency of 0.75 (Coefficient alpha).

*Eating Disorder Psychopathology*. The Eating Disorder Inventory-2 [34] was used to examine ED characteristics. This 91-item questionnaire explores cognitive and behavioral characteristics typically associated with EDs. The items, scored on a six-point Likert scale, are grouped into 11 scales: 1) Drive for thinness; 2) Bulimia; 3) Body dissatisfaction; 4) Ineffectiveness; 5) Perfectionism; 6) Interpersonal distrust; 7) Interoceptive awareness; 8) Maturity fears; 9) Ascetism; 10) Impulse regulation; and 11) Social insecurity. This instrument was validated in a Spanish population [35] with a moderate mean internal consistency of 0.63 (Coefficient alpha).

### *2.4. Procedure*

Experienced psychologists and psychiatrists (all extensively trained in the use of the instruments) completed the clinical and physical evaluations during two initial clinical structured face-to-face interviews. Self-report questionnaires were used to obtain baseline information on the general health status (including depression). The duration of the disorder was established by subtracting the age of onset of the disorder from the patients' age at the time of the first interview. The Actiwatch 7 was given upon admission to the day hospital and was returned after 7 days. Treatment outcome was assessed at the termination of the day hospital program by the expert clinicians.

Written informed consent was obtained from all participants and the Ethics Committees of each institution approved the study. The study was conducted in

accordance with the Declaration of Helsinki.

*2.5. Statistical Analysis*

The statistical analysis was carried out with Stata13 for windows. Correlation coefficients were used to estimate the associations of both the age of the participants and body composition (height, weight, BMI, muscle mass, percentage of body fat) with MVPA, daytime MVPA, ED severity, depressive symptoms and treatment outcome (AN). A small correlation effect size was assumed at $|r|<0.30$. Comparisons of PA measures between AN patients and HC were conducted with analysis of variance (ANOVA) and logistic regression, both adjusted by the SCL-90-R depression score. Cohen's *d* coefficient evaluated the effect size for mean differences and proportion differences (moderate effect size was considered for $|d|>0.50$ and high for $|d|>0.80$).

The mediational hypotheses were tested through the use of Structural Equation Models (SEM). The overall goodness-of-fit was evaluated via the $\chi^2$ test, the Root Mean Squared Error of Approximation (RMSEA), baseline comparison indexes (Comparative Fit Index CFI and Tucker-Lewis Index TLI) and the residuals' size (Standardized Mean Squared Residual SMSR). A good fit was considered for [29]: a non-significant result ($p>.05$) in the $\chi^2$ procedure, RMSEA<0.10, CFI>0.90, TI>0.90 and SRMR<0.08. The effect sizes and the equation-level-goodness-of-fit were also estimated via the $R^2$ coefficients for each equation and for the global model, multiple correlation (mc) and Bentler-Raykov multiple correlation ($mc^2$) [37]. These last two coefficients report the relatedness of each dependent variable with the model's linear prediction (in non-recursive models $mc^2$ is computed to avoid the problem of achieving inconsistent negative multiple correlations).

## 3. Results

*3.1. Primary characteristics of the sample*

Participant characteristics with regard to their age and body composition are presented in Table 1. Although the age was similar (p=.74), there were statistically significant differences between the AN and HC samples across all body composition measures.

[TABLE 1]

*3.2. Comparison between AN and HC in PA measures and ED/depression symptoms.*

Table 2 presents the comparison between groups in the levels of depression symptoms, ED severity (measured through the EDI-2 total score), daytime PA, time spent in MVPA, and the prevalence of participants with high MVPA levels. The results indicate that the patients with AN presented a significantly higher depression score and higher ED psychopathology levels compared to the HC. The probability that the AN patients presented a profile of high PA levels (more than 300 minutes of MVPA over the 6 days) was 2.7 times less compared to that of the HC. There were no differences in terms of the amount of MVPA or daytime PA the

groups engaged in. Although no significant differences appeared, the distribution of the MVPA values showed a larger dispersion for the AN patients compared to those of the HC and a positive skewness was also present in the AN group (the outside values were in the right tail of the distribution, $p<.001$ in the Skewness/Kurtosis test). [SFigure 1].

[TABLE 2]

*3.3. Associations between age and body composition with PA measures, ED/depression symptoms and treatment outcome.*

Table 3 shows the correlation coefficient matrix between the participants' age and body composition measures with time in MVPA, daytime PA, the EDI-2 total and the SCL-90-R depression scale, stratified by diagnostic subtype (AN and HC groups). This table also includes the point-biserial correlation between age and body composition with the presence of a poor therapy outcome (drop-out or no-remission) in the AN group. All the correlation coefficients achieved small effect size ($|r|<.30$), which indicates the lack of association between the set of variables. The only exception was for BMI, which positively correlated with EDI-2 total (the higher the BMI, the higher the EDI score, for both the HC and AN groups).

[TABLE 3]

*3.4. Underlying process comprising time in MVPA, depression symptoms and treatment outcome*

In the AN sample, the risk of a poor outcome (drop-out or no-remission) was 37.2% (95%CI: 27.7% to 47.8), of partial remission was 30.2% (95%CI: 21.5% to 40.6) and of full remission 32.6% (95%CI: 23.6% to 43.0).

Figure 1 presents the pathway-diagram of the SEM evaluating the role of MVPA and depression symptoms on treatment outcome, taking into consideration the variables age, duration of the disorder and ED psychopathology. Goodness-of-fit was good for this model ($\chi^2=4.601$ ($p=.203$), RMSEA=.090; CFI=.977; TLI=.907, SRMR=.051) and the global predictive capacity was high ($R^2=.638$). Results show that the EDI-total score and MVPA had a direct effect on treatment outcome: high EDI-total scores and less time in MVPA predicted a high probability of a poor outcome. Furthermore, MVPA had a meditational role in the relationship between age and treatment outcome, as well as between the duration of the disorder and the result of the treatment: the older patients and those with a shorter duration of the disorder showed greater MVPA, and thus a decreased probability of a poor outcome. The SCL-90-R depression score was higher in older patients, those patients with a shorter duration of the disorder, those who reported a higher EDI-2 total score and those who spent less time in MVPA. However, depression symptoms were not related to poor treatment outcome.

[FIGURE 1]

**4. Discussion**

The purpose of this study was to examine PA in patients with AN in comparison to healthy-weight controls, the role that PA may play in the outcome of a Day Hospital treatment for AN and the potential modulating role of additional clinical variables.

Although the AN patients presented a slightly lower average amount of daytime PA and spent less time in MVPA compared to the controls, the differences were not statistically significant. This is in line with previous studies that have not found differences between these groups [38]. However, in this study, when MVPA was subdivided into high and low MVPA levels, a greater number of the controls presented overall high MVPA levels compared to the patient sample. The prevalence of 37% of high MVPA levels in the AN patients, as measured with the accelerometer, is consistent with that reported in the literature [10,39]. Yet, 61.1% of the controls were classified as showing high MVPA levels. This might be attributed to differences in the distribution of PA [38]. The results of this study show that, although on average both groups were equally active, while the controls were evenly spread out with respect to the time they spent in MVPA, the patient sample presented either low or very high levels: the majority of patients spent less time in MVPA compared to the controls, but a small percentage displayed particularly high levels of MVPA.

It has been hypothesized that PA may play an important role in the development and maintenance of AN [40,41], which is congruent with the "compulsive" exercise observed in a subgroup of patients [3]. However, the energy deficiency yields several medical complications [42], which may reduce the patients' physical fitness and thus causing a decrease in the ability to engage in PA. In support, an inverse association was observed in this study and others [7] between the duration of the disorder and MVPA. This suggests that patients with a longer duration of the disorder, and therefore in poorer physical and health conditions, are those who presented less MVPA.

Interestingly, time spent in MVPA was inversely associated with a poorer outcome. Some studies have shown that moderate to high PA does not result in poorer treatment outcome, while prolonged engagement in light PA may hinder improvement [5–7]. Others, however, have reported a negative effect on treatment duration and outcome [2,3,7]. This discrepancy may be due to the lack of an operationalized definition of exercise or differences in the measurement protocol of PA. The treatment type examined also differed between the studies and may be influencing the findings, which reduces the comparability of results. It is also important to note that, on average, the patients in this study presented highly similar MVPA to that of the controls, suggesting that, on average, these patients presented relatively normal levels of MVPA.

Exercise protocols have been implemented in various treatment programs and some have obtained positive results, whereby exercise interventions have resulted in greater weight gain, as well as

a decrease in obligatory attitudes towards exercise, an increased exercise capacity, reduced emotional stress and improved quality of life and have thus been encouraged [43,44]. The process by which moderate PA might be beneficial to treatment outcome is not yet clear. Our results suggests that some of the physiological changes that are known to take place with exercise, such as increased muscle mass or decreased percentage of body fat [45;46], might not be directly implicated; the body composition of the AN patients in our study was not found to be associated to neither MVPA nor treatment outcome. Other authors have proposed that, when exercise is separated from the "compulsive" component experienced by AN patients, PA can have positive effects on psychological well-being [47].

In the present study, MVPA was also linked to depression: those patients who showed elevated levels of depression symptoms engaged less in MVPA. As expected, greater ED psychopathology was related to higher depression symptoms. These findings suggest that both less time in MVPA and lower ED psychopathology may be linked to the strong comorbidity between depression symptoms and AN [48,49]. The inverse association between MVPA and depression symptoms is in line with the theory that PA in AN is not only the means to further lose weight, but may also have a mood regulatory function [9,11,14,40,50–52]. However, the assessment of these factors in this study was cross-sectional in nature, therefore direction cannot be determined; it is likely that these relationships are bi-directional. For example, patients with AN have reported the use of a disordered eating behavior to avoid and suppress feelings of sadness [53]. Future studies examining the link between PA and treatment outcome should take into consideration depression symptoms.

This study has several strengths. First, PA was objectively assessed with an accelerometer, which has been proven to be more accurate than self-reported measures, especially in AN [14]. In addition, accelerometry does not inhibit the patients from engaging in the activity they would have engaged in if they were not wearing the accelerometers. Past studies have reported that some patients forgot that they were wearing the accelerometer [39]. The large sample size also facilitates the generalizability of the results. By including a healthy-weight control group, this study is able to examine the extent to which PA patterns in AN differ from those of healthy individuals. Finally, this is the first study to demonstrate the importance of taking into consideration age, duration of the illness and depression symptoms as potential confounders that must be controlled in future studies.

However, some limitations need to be considered. The accelerometer was placed on the non-dominant wrist, which signifies that lower-body movement may have not been accurately captured. The accelerometer should be placed in both wrist and waist, and the analysis of PA should be complemented by self-reported measures. Furthermore, the identification of MVPA was based on a formula developed by Heil [28] and considering AEE as 0.0310 as proposed by the same

author. This is not the standard approach to predicting MET values, the established measure of PA intensity. The purpose of using the equation was due to the output generated by our specific acceleromenter. There is a wide diversity in the existing monitors for the detection of movement and acceleration that provide distinct output units as well as measurement mechanisms that depend on several factors such as placement and number of movement detection planes. Such differences imply that individual formulas for these devises should be produced for an accurate detection of MVPA. Consequently, Heil [28] suggested a specific equation to estimate AEE for the specific characteristics of the Actical@. Given that this Actiwatch was developed by the same manufacturer with highly similar characteristics to our accelerometer, this formula was believed to be the most appropriate for our monitor. Another limitation is that, as previously mentioned, parts of the study were cross-sectional. Future studies should examine whether PA and ED psychopathology may precede the depression symptoms. Finally, the patient sample in this study comprised treatment-seeking individuals with AN, therefore the results obtained may not represent PA patterns of individuals with AN not seeking treatment.

## 5. Conclusion

In the present study it was observed that, in spite of being in an emaciated condition, about a third of patients with AN showed high MVPA levels, a prevalence smaller than that of healthy-weight controls. However the average daytime PA and time in MVPA were similar. Moreover, MVPA was a predictor of treatment outcome and was associated with decreased depression symptoms. From a clinical perspective, there is an urgent need for studies to further explore the role of PA in this clinical population in order to understand how it should be addressed in the treatment process.

## Acknowledgments

This manuscript was supported by grants from Instituto Salud Carlos III (FIS PI11/210, FIS14/290 and CIBERobn) and AGAUR de la Generalitat de Catalunya (2014 SGR 1672). CIBERObn is an initiative of ISCIII. Sarah Sauchelli is recipient of a pre-doctoral Grant (2013–17) by IDIBELL. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Conflict of Interests

None declared.

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**Table 1.** Profile of the sample in terms of age and body composition

| | Means and SD | | | | | | |
|---|---|---|---|---|---|---|---|
| | HC ($n$=116) | | AN ($n$=88) | | $F_{(1,202)}$ | $p$ | $\|d\|$ |
| Age (years-old) | 27.54 | 7.90 | 27.94 | 8.98 | 0.11 | .736 | 0.05 |
| Height (cm) | 165.21 | 6.59 | 161.88 | 6.30 | 10.82 | **.001** | **0.52** |
| Weight (kg) | 60.15 | 4.82 | 54.98 | 13.86 | 12.10 | **.001** | **0.50** |
| Body mass index (BMI, kg/m$^2$) | 21.70 | 2.84 | 16.60 | 1.34 | 234.1 | **<.001** | **2.29** |
| Muscle mass | 41.23 | 3.96 | 37.62 | 3.71 | 35.5 | **<.001** | **0.94** |
| Percent of body fat | 26.04 | 5.91 | 15.08 | 6.29 | 133.6 | **<.001** | **1.80** |

SD: standard deviation. $|d|$: Cohen's-*d* coefficient measuring mean difference effect size.
*Bold: significant pairwise comparison. *Bold: moderate ($|d|$>0.5) to good ($|d|$>0.8) effect size.

**Table 2.** Comparison between AN patients and HC in ED/depressive symptoms and PA measures.

| | Adjusted means and SD | | | | Factor group | | | |
|---|---|---|---|---|---|---|---|---|
| | HC ($n$=116) | | AN ($n$=88) | | $F_{(1,202)}$ | $p$ | MD | $\|d\|$ |
| *SCL-90-R scale:* | | | | | | | | |
| Depression symptoms | 0.69 | 0.50 | 2.04 | 0.99 | 152.53 | <.001 | **1.35*** | **1.72*** |
| *EDI-2 scale:* | | | | | | | | |
| EDI-2: total score | 24.94 | 20.3 | 76.84 | 41.3 | 131.6 | <.001 | **51.91*** | **1.59*** |
| *Activity measures* | | | | | | | | |
| Time in MVPA (minutes) | 63.13 | 35.2 | 55.15 | 42.1 | 1.14 | .288 | 7.98 | 0.21 |
| Daytime PA (counts·minute) | 290.5 | 68.8 | 264.1 | 85.7 | 3.22 | .074 | 26.40 | 0.34 |

| | HC | AN | $Wald_{(1)}$ | $p$ | OR | $\|d\|$ |
|---|---|---|---|---|---|---|
| High PA levels (% within each group) | 61.1% | 37.1% | 6.02 | .014 | **0.37*** | **0.50*** |

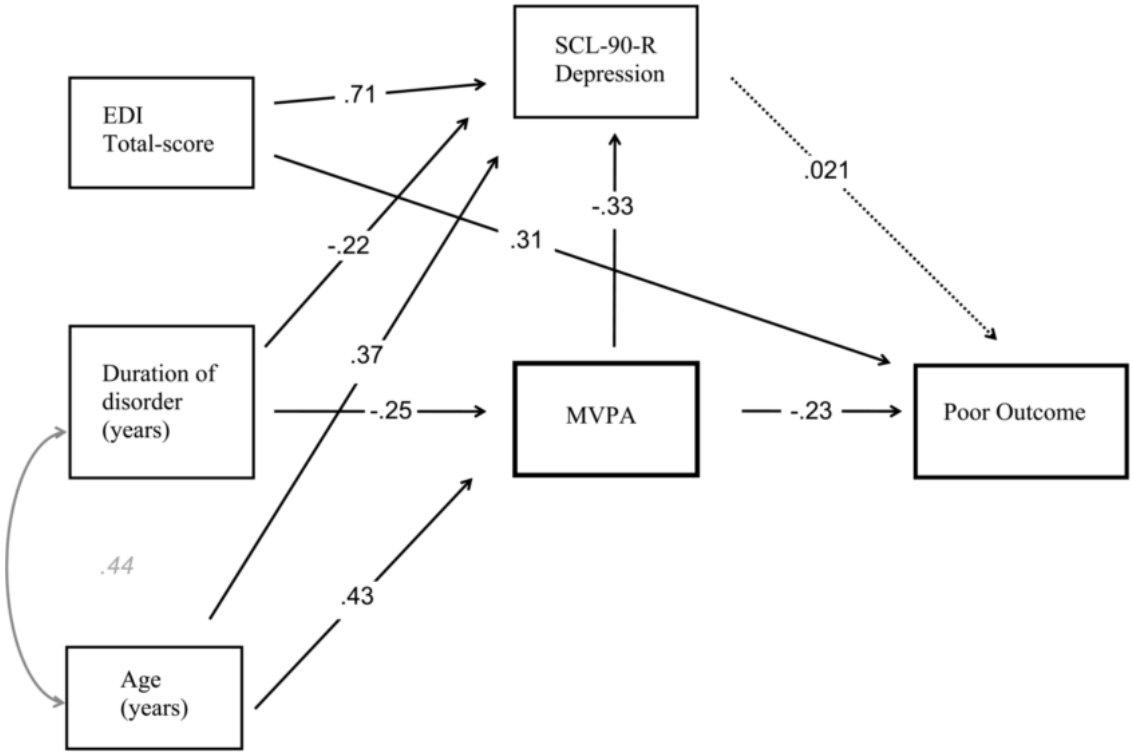
SD: standard deviation. MD: mean difference. OR: odds ratio.
$|d|$: Cohen's-*d* coefficient measuring mean difference effect size.
*Bold: significant pairwise comparison. *Bold: moderate ($|d|$>0.5) to good ($|d|$>0.8) effect size.
Comparison of activity measures adjusted by the SCL-90-R depression score.
MVPA: Moderate-to-vigorous Physical Activity; EDI: Eating Disorder Inventory; SCL-90-R: Symptom Checklist-90-Revised

**Table 3.** Correlations of both age and body composition with PA measures, ED/depression symptoms and treatment outcome

| | Healthy Controls (*n*=116) | | | | Anorexia (*n*=88) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | MVPA | DPA | EDI-2 | SCL-dep | MVPA | DPA | EDI-2 | SCL-dep | Poor out. |
| Age (years-old) | .006 | .205 | -.034 | -.051 | .114 | .127 | .052 | .203 | -.029 |
| Height (cm) | -.239 | -.180 | -.022 | -.056 | -.148 | -.021 | .063 | -.051 | -.026 |
| Weight (kg) | -.238 | -.176 | -.023 | -.054 | .047 | .102 | .259 | .199 | .021 |
| BMI (kg/m$^2$) | -.053 | -.003 | **.321** | -.103 | -.054 | -.065 | **.317** | .171 | -.178 |
| Muscle mass | -.171 | -.095 | .176 | -.132 | -.069 | -.027 | .265 | .047 | -.180 |
| % body fat | -.120 | -.063 | .244 | -.099 | .017 | .069 | .102 | .003 | -.165 |

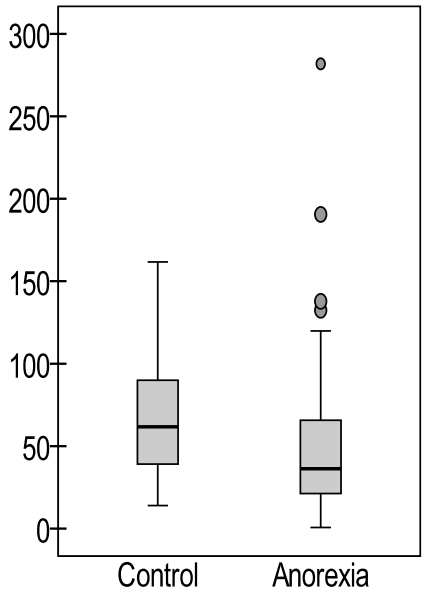
MVPA: Moderate-to-vigorous physical activity (minutes). DPA: daytime physical activity (counts·minute).
EDI-2: total score. SCL-dep: SCL-90-R depression score.
Poor out: Poor outcome (dropout or no-remission).

**Figure 1.** The role of MVPA and depression symptoms on treatment outcome.




Continuous line: significant parameter. Structural Equation Model analysis shows that high MVPA levels were inversely associated with SCl-90-R depression scores and a poor outcome. Greater Eating Disorder psychopathology (EDI Total-score) was associated with a poor outcome and high depression scores. Finally, a longer duration of the disorder was associated with lower depression scores and MVPA levels, while being older was associated with elevated depression scores and MVPA levels.

**Supplementary figure S1.** Distribution of the moderate-to-vigorous physical activity scores for the control and anorexia nervosa groups.




A wider dispersion is visible in the distribution of the moderate-to-vigorous physical activity levels of the anorexia nervosa patients compared to that of the healthy controls, and a positive skewness is also present in the anorexia nervosa group.

**Supplementary file 2**. Structural standardized coefficients and goodness-of-fit of the final structural equation model.

| Structural | Coefficient | SE | z | *p* | 95%CI (coefficient) | |
|---|---|---|---|---|---|---|
| MVPA | | | | | | |
| Duration of Disorder | -.2534633 | .1290799 | -1.96 | 0.050 | -.5064552 | -.0004714 |
| Age | .4296475 | .1223294 | 3.51 | <0.001 | .1898863 | .6694087 |
| *constant* | *.2004552* | *.4114381* | *0.49* | *0.626* | *-.6059487* | *1.006.859* |
| Depression symptoms | | | | | | |
| MVPA | -.3281986 | .0943437 | -3.48 | 0.001 | -.5131088 | -.1432884 |
| Duration of disorder | -.220353 | .0992689 | -2.22 | 0.026 | -.4149165 | -.0257895 |
| Age | .3697475 | .1043504 | 3.54 | <0.001 | .1652245 | .5742705 |
| EDI-2_total | .7102573 | .0705665 | 10.07 | <0.001 | .5719495 | .848565 |
| *constant* | *.2821625* | *.3605492* | *0.78* | *0.434* | *-.4245009* | *.9888259* |
| Poor Outcome | | | | | | |
| MVPA | -.2251082 | .1222733 | -1.84 | 0.066 | -.4647594 | .0145431 |
| Depression symptoms | .0210216 | .1629458 | 0.13 | 0.897 | -.2983464 | .3403895 |
| EDI-2_total | .3131678 | .1569371 | 2.00 | 0.046 | .0055766 | .6207589 |
| *constant* | *.3480422* | *.3753754* | *0.93* | *0.354* | *-.3876802* | *1.083.764* |
| cov(Duration,Age) | .4358053 | .1045801 | 4.17 | <0.001 | .2308322 | .6407785 |

| Equation-level goodness-of-fit | Variance | | | | | |
|---|---|---|---|---|---|---|
| | Fitted | Predicted | Residual | R-squared | mc | $mc^2$ |
| MVPA | 1.143.538 | 176.016 | 9.675.216 | .1539223 | .3923294 | .1539223 |
| Depression symptoms | .967485 | .5559579 | .4115271 | .5746424 | .7580517 | .5746424 |
| Poor Outcome | .213169 | .0367363 | .1764327 | .1723342 | .4151315 | .1723342 |

mc = correlation between depvar and its prediction.
$mc^2$ = Bentler-Raykov squared multiple correlation coefficient.
MVPA: Moderate-to-vigorous Physical Activity; EDI: Eating Disorder Inventory.

## 4.4. STUDY 4: DIMENSIONS OF COMPULSIVE EXERCISE ACROSS EATING DISORDER DIAGNOSTIC SUBTYPES AND THE VALIDATION OF THE SPANISH VERSION OF THE COMPULSIVE EXERCISE TEST.

**Background:** Exessive exercise is a frequent characteristic of several EDs, believed to play an important role in the etiopathology and maintenance of the disorders [308–310,328]. Increasingly however, it is being argued that it is not the amount of exercise *per se* that is problematic, rather the patient's experience of a compulsive urge to exercise and/or difficulties/unwillingness to stop exercising [296,314]. Compulsive exercise has been detected in up to 55% of ED patients [306,309], and has been attributed to the need for control over body weight and shape [309,394]. Recent studies, however, have argued that there may be more than one factor that drive compulsive exercising in patients with ED [296,314]. Meyer and colleagues [314] therefore proposed a cognitive-behavioural model of compulsive exercise and the self-administered questionnaire CET [317] was developed to tap into the cognitive, emotional and behavioural characteristics of compulsive exercise.

**Objectives:** The present study aimed to validate a Spanish version of the CET, to examine differences across ED diagnostic subtypes in terms of the cognitions driving compulsive exercise as defined by the CET, and to assess the possible links between the CET dimensions, ED severity and symptoms of general psychopathology.

**Methods:** The sample consisted of 157 patients (40 AN, 56 BN, 61 EDNOS) who attended the University Hospital of Bellvitge for a clinical evaluation, and 128 healthy controls recruited from the local university. All participants were 18 years of age or older and Spanish speakers at a native level. None of the healthy controls had a history of an ED. Participants were asked to complete the CET, along with the EDI-2 to measure ED severity and the SCL-90-R to evaluate general psychopathology.

**Results:** A Confirmatory Factor Analysis demonstrated the Spanish version of the CET to be a valid instrument (Cronbach's alpha ranging from .79 to .94 across the CET scales) that was also able to differentiate between patients with ED and healthy controls in four of five subscales. Overall, CET scores were highest in the BN group, followed

by the EDNOS group, and finally both the AN and healthy control groups, who presented statistically similar scores across all scales except for the mood improvement scale (the AN group obtained lower scores in this scale). There were no differences between groups in scores in the exercise rigidity scale. Scores in the total CET scale correlated with overall ED severity (total EDI-2; r = .316), drive for thinness (r = .487), body dissatisfaction (r = .301), interoceptive awareness (r = .248), and interpersonal sensitivity as measured by the SCL-90-R.

# DIMENSIONS OF COMPULSIVE EXERCISE ACROSS EATING DISORDER DIAGNOSTIC SUBTYPES AND THE VALIDATION OF THE SPANISH VERSION OF THE COMPULSIVE EXERCISE TEST

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**Journal:** International Journal of Eating Disorders

**Impact Factor:**2.46

**Key Words:** Compulsive exercise, Eating disorders, Psychopathology, Compulsive Exercise Test, Spanish validation

**Abstract**

**Objectives:** Compulsive exercise in eating disorders has been traditionally considered as a behavior that serves the purpose of weight/shape control. More recently, it has been postulated that there may be other factors that drive the compulsive need to exercise. This has led to the development of the Compulsive Exercise Test (CET); a self-reported questionnaire that aims to explore the cognitive-behavioral underpinnings of compulsive exercise from a multi-faceted perspective. The objectives of this study were threefold: 1) To validate the Spanish version of the CET; 2) To compare eating disorder diagnostic subtypes and a healthy control group in terms of the factors that drive compulsive exercise as defined by the CET; 3) To explore how the dimensions evaluated in the CET are associated with eating disorder symptoms and general psychopathology. **Methods:** The CET was administered to a total of 157 patients with an eating disorder (40 anorexia nervosa, 56 bulimia nervosa, 61 eating disorder not-otherwise-specified (EDNOS)) and 128 healthy weight/eating controls. Patients were assessed via a semi-structured interview to reach a DSM-IV-TR diagnosis. Additionally, all participants completed the Symptom Checklist-90-Revised (SCL-90R) and the Eating Disorders Inventory-2 (EDI-2). **Results:** Confirmatory factor analysis demonstrated adequate goodness-of-fit to the original five-factor model of the CET. Bulimia nervosa and EDNOS patients scored higher in the avoidance and rule-driven behavior, weight control and total CET scales in comparison to the healthy controls, and higher across all scales apart from the exercise rigidity scale compared to the anorexia nervosa patients. Mean scores of the anorexia nervosa patients did not differ to those of the control participants, except for the mood improvement scale where the anorexia nervosa patients obtained a lower mean score. Mean scores between the bulimia nervosa and EDNOS patients were equivalent. The CET scales avoidance and rule-driven behavior, weight of control and total CET scores were positively correlated with the clinical assessment measures of the SCL-90R and EDI-2. **Conclusion:** Compulsive exercise is a multidimensional construct and the factors driving compulsive exercise differ according to the eating disorder diagnostic subtype. This should be taken into account when addressing compulsive exercise during the treatment of eating disorders.

## 1 Introduction

Excessive exercise is a recurrent behavior in eating disorders (ED), believed to be implicated in the aetiology, development and maintenance of these disorders (Davis et al., 1997; Taranis et al., 2011). Prevalence rates range between 34% and 55% (Bewell-Weiss and Carter, 2010; Dalle Grave et al., 2008; Sauchelli et al., 2015), and it has been associated with poorer treatment outcome (Dalle Grave et al., 2008; Stiles-Shields, Bamford,2015; Shroff et al., 2008), an increased likelihood of dropout during treatment (El Ghoch et al., 2013) and a shorter time to relapse (Strober et al., 1997; Carter, Blackmore, 2004). However, some studies have not found an association between exercise and treatment response (Kostrzewa et al., 2013a; van Elburg et al., 2007), and there is evidence

suggesting that supervised physical activity may be somewhat beneficial for outcome and attitudes toward exercise (Calogero and Pedrotty, 2004; Sauchelli et al., 2015).

The inconsistencies in the literature regarding the pathology of exercise in ED are likely to be due to discrepancies in the methodology of the studies. First, studies differ in the samples assessed; the duration of the disorder, age, and time point of assessment (baseline, lifetime prevalence, during treatment). Second, an operationalized definition of excessive exercise is lacking. A quantitative approach to excessive exercise has been questioned; it is unclear whether focus should be placed on current or lifetime exercise or the amount/type of exercise required for it to be considered as excessive (Meyer and Taranis, 2011). It has therefore been argued that it is not the amount/intensity of exercise *per se* that is problematic in EDs, but the presence of a compulsive drive to engage in exercise (Meyer et al., 2011; Meyer and Taranis, 2011). Exercise can be considered as compulsive when the individual feels compelled to exercise, the behavior interferes with everyday life, relationships with others, and is continued despite potential harm to the self (APA, 1994; Yates, 1991). Compulsive exercise may be a more accurate representation of the pathological exercise profile of these patients given that it emphasizes the cognitions that maintain the behavior despite the damaging effects and that may hinder treatment progress. Yet, the majority of existing literature in adults with EDs has focused on the assessment of the total amount of exercise and the associated medical complications; only limited attention has been placed on its nature within these disorders.

It was originally believed that compulsive exercise in EDs was related to a need for body weight/shape control (Achamrah et al., 2016; Dalle Grave et al., 2008). More recent studies have suggested a multifactorial etiology (Meyer, Taranis, Goodwin, & Haycraft, 2011; Meyer & Taranis, 2011). Based on a cognitive behavioral model, Meyer and colleagues (Meyer et al., 2011) proposed four key constructs underlying compulsive exercising: 1) eating psychopathology; b) obsessive compulsiveness; c) mood regulation; and d) perfectionism.

This multidimensional definition of compulsive exercise led to the development of a new measure, the Compulsive Exercise Test (CET; Taranis et al, 2011), which examines the emotional, cognitive and behavioral characteristics of compulsive exercise from a multi-facet perspective. Further than exercising as a way to regulate mood and control body weight/shape, the instrument also evaluates the extent to which the maintenance of such unhealthy exercise behavior may be driven by the need to sustain a rigid schedule, to improve one's mood despite a lack of enjoyment when exercising, or as a way to avoid negative emotions and feelings of guilt that may emerge when not exercising (Meyer et al., 2011; Taranis et al., 2011). In addition, it can be employed to distinguish a clinical from a non-clinical group (Meyer et al., 2016). The CET, with a five-factor structure, was first psychometrically tested in a non-clinical population (Taranis et al., 2011)

and recently in a clinical setting as well (Meyer et al., 2016).

Meyer and colleagues' (2011) model has been supported by several studies in both community (Goodwin et al., 2016, 2014a, 2014b, 2012, 2011) and clinical adolescent groups (Formby et al., 2014; Noetel et al., 2016; Swenne, 2016). Fewer studies have examined a multidimensional function of exercise in adults with EDs. These studies, however, have found several associations between some of the dimensions described by Meyer and colleagues (2011) and greater exercise frequency, ED psychopathology and clinical characteristics such as obsessions and compulsivity (Taranis & Meyer, 2010, 2011; Taranis et al., 2011; Naylor, Mountford, & Brown, 2011).

Despite the consensus on the relevance of exercise in the etiology of EDs, contradictory findings persist. Furthermore, thus far no study has examined the various factors (cognitive/emotional/behavioral) driving compulsive exercise, rather than actual exercise patterns, across ED diagnostic subtypes, and how each might be linked to ED symptoms and general psychopathology. The aims of this study were threefold: 1) To validate the Spanish version of the CET; 2) To compare eating disorder diagnostic subtypes and a healthy control group in terms of the factors that drive compulsive exercise as defined by the CET; 3) To explore how the dimensions evaluated in the CET are associated with ED symptoms and general psychopathology. Based on the available literature we anticipated that the Spanish version of the CET would also allow us to differentiate between clinical and non-clinical groups, where participants with EDs would show elevated compulsive cognitions and behaviors towards exercise compared to healthy controls. We also hypothesized that greater compulsive cognitions would be associated with increased ED and general psychopathology.

## 2 Methods

### 2.1 Participants

The sample comprised 157 patients with EDs; 40 (25.5%) patients with anorexia nervosa (AN), 56 (35.7%) patients with bulimia nervosa (BN), 61 (38.8%) patients with an ED not-otherwise-specified (EDNOS). The mean body mass indices of each group were 15.9 (SD = 1.6), 25.48 (SD = 6.6) and 22.97 (SD = 5.89) respectively. These patients were compared to 128 healthy weight/eating controls (mean body mass index = 22.06; SD = 2.54). A total of 228 (79.7%) participants were female (AN = 37; BN = 53; EDNOS = 53; controls = 85) and 57 (20.3%) were male (AN = 3; BN = 3; EDNOS = 8; controls = 43). The ED groups were patients consecutively assessed at the ED unit of the University Hospital of Bellvitge, Spain. Diagnosis was made on the basis of the DSM-IV-TR (during the recruitment phase the DSM-5 had not yet been published), based on a semi-structured clinical interview (First et al., 1997). Controls were students from the local university (University of Barcelona, Spain). All participants had to be 18 years and over and speak Spanish at the level of a native speaker. In addition, the patients had to fully meet the diagnostic criteria.

Potential control participants were excluded if they had a history of an ED, which was not the case. Mean ages were: 28.88 years (SD = 10.36) for the AN patients, 20.50 years (SD = 10.46) for the BN patients, 25.31 years (SD = 8.27) for the EDNOS patients, and 21.04 years (SD = 4.96) for the control participants.

### 2.2 Measures

*Compulsive Exercise Test* (CET; Taranis et al., 2011). This self-reported questionnaire is designed to explore the emotional, cognitive and behavioral characteristics of compulsive exercise. It comprises 24 items answered on a 6-point Likert scale, from 0 (never true) to 5 (always true). Five subscales are derived: 1) avoidance and rule-driven behavior; 2) weight control exercise; 3) mood improvement; 4) lack of exercise enjoyment and 5) exercise rigidity. Mean averages of each subscale are summed to obtain a CET total score. The psychometric properties of the CET have been validated in a non-clinical and clinical sample, showing high concurrent and convergent validity and Cronbach's alpha ranging from 0.72 to 0.88 (Meyer et al., 2016; Taranis et al., 2011).

The translation into Spanish was conducted via the use of back-translation (Brislin, 1970). Two translators, experts in the field of EDs, provided a first translation from English to Spanish. A third examined the document for possible errors and resolved any doubts or inconsistencies. The Spanish version was then translated back into English, which was compared to the original English version. Throughout the process, language, grammar and cultural discrepancies that might influence the interpretation of the questionnaire items were taken into account. Bilingual individuals overviewed the entire translation process.

*Eating Disorder Inventory-2* (Garner, 1991). This is a reliable self-reported questionnaire that explores typical cognitive and behavioral characteristics of ED. A total of 91 items, answered on a 6-point Likert scale, provide standardized scores on 11 subscales: drive for thinness, body dissatisfaction, bulimia, effectiveness, perfectionism, interpersonal distrust, interoceptive awareness, maturity fears, asceticism, impulse regulation and social insecurity. The EDI-2 total score (sum of scores in each scale) was used to examine overall ED severity. Validation in a Spanish population (Garner, 1998) yielded a mean internal consistency of 0.63 (Cronbach's alpha).

*The Symptom Checklist-revised* (SCL-90-R)(Derogatis, 1990). This self-reported measure was used to assess general psychopathology. The 90-item questionnaire explores psychological problems and symptoms of psychopathology. A total of 9 primary symptom dimensions are extracted: 1) Somatization; 2) Obsession-Compulsion; 3) Interpersonal Sensitivity; 4) Depression; 5) Anxiety; 6) Hostility; 7) Phobic Anxiety; 8) Paranoid Ideation; and 9) Psychoticism. Three global indices are also present: the global severity index (GSI), designed to evaluate overall distress, the positive symptom distress index (PSDI), which indicates the intensity of the symptoms, and the

positive symptom total (PST), which assesses self-reported symptoms. The test has been validated in a Spanish population (Derogatis, 2002), with a mean internal consistency of 0.75 (Cronbach's alpha).

*Body Mass Index* of the patients was attained using the Tanita Multi-Frequency Body Composition Analyzer MC-180MA (Tanita Corporation, Tokyo, Japan). This device is repeatedly revised to meet the reference standards dual-energy X-ray absorptiometry (http://www.bl-biologica.es/tanita_tbf.htm), and has been validated against other weighing methods (Strain, Wang et al., 2008). A stadiometer was used to measure height.

The supplementary table 1 includes the distribution of the measures in the study (direct means and standard deviations) stratified according to diagnostic subtype, and the internal consistency in the sample (Cronbach's alpha coefficients). The raw scores (original data) of the questionnaires (CET, EDI-2 and SCL-90-R) was analyzed.

## 2.3 Procedure

Psychologists and psychiatrists (all extensively trained in the use of the instruments) conducted clinical and physical evaluations during two clinical structured face-to-face interviews. Assessment instruments were also administered upon first evaluation. The controls (students from the University of Barcelona) completed the necessary printed questionnaires throughout the academic year 2014-2015. Self-reported height and weight were provided alongside the questionnaires. Written informed consent was obtained from all participants prior to their inclusion in the study, and the Ethics Committee of the University Hospital of Bellvitge approved the study. The study was conducted in accordance with the Declaration of Helsinki.

## 2.4 Statistical Analysis

Analyses were carried out with SPSS20 and Mplus7.4 for Windows. Firstly, confirmatory factor analysis was used to assess the validity of the original CET construct model (based on the five dimensions: avoidance and rule-driven behavior, weight control exercise, mood improvement, lack of enjoyment, and exercise rigidity) for the groups studied. Goodness-of-fit was examined for (Kline, 2010): Root Mean Square Error of Approximation RMSEA<.10, Comparative Fit Index CFI>.90, Tucker-Lewis Index TLI>.90, and Standardized Root Mean Square Residual SRMR<.10. Next, analysis of variance (ANOVA) adjusted by the participants' sex and age was conducted to compare clinical measures between the groups studied (ED diagnostic subtypes and controls). Finally, partial correlations adjusted by participants' sex and age were used to analyze the association between CET scores and ED and general psychopathology scores (EDI-2 scales and SCL-90R scales respectively).

In this study, results were considered statistically significant at a significance threshold of $p \leq 0.05$. In addition, *p-values* depend on both the magnitude of associations and the precision of the

estimate; effects of small magnitude that may be clinically unimportant can seem "significant" when the sample size is large and the opposite can occur whereby a large effect size could emerge from a small sample without it reaching significance. Therefore, both the significance test and estimation of effect sizes were taken into account. Cohen's-*d* coefficients were used to assess the strength of the associations in the ANOVA procedures (moderate effect size was considered for $|d|>0.50$ and large for $|d|>0.80$) and partial correlations were considered moderate if $|r|>0.24$ and large if $|r|>0.30$.

## 3 Results

### 3.1 Psychometrical properties of the CET Spanish version

Confirmatory factor analysis generated adequate goodness-of-fit for the five dimension structure (RMSEA=.087, CFI=.910, TLI=.900 and SRMR=.080), with Cronbach's-alpha values for the CET dimensions ranging between $\alpha$=.79 (exercise rigidity scale) and $\alpha$=.96 (avoidance and rule-driven scale). Correlations between factors were high, ranging between $r$=.46 (between the weight control and mood improvement scales) and $r$=.69 (between the weight control and avoidance and rule-driven scales).

The model including the participants' gender as a group to assess the invariance of the internal structure by sex also displayed an adequate fit (RMSEA=.097, CFI=.900, TLI=.900 and SRMR=.090), and joint tests for parameter classes confirmed invariance by measurement coefficients ($\chi^2$=16.95, $p$=.458), covariance for measurement errors ($\chi^2$=28.64, $p$=.192) and covariance for exogenous variables ($\chi^2$=7.98, $p$=.158).

The supplementary table 2 includes the complete results from the confirmatory factor analysis.

### 3.2 Comparison of CET and clinical measures across diagnostic subtypes

Table 1 includes the results of the ANOVA, adjusted according to the covariates participants' sex and age, comparing the CET and other clinical measures between the groups evaluated. The first part of the table presents the descriptive data for each group (the raw mean scores and standard deviations obtained from the questionnaires adjusted by the covariates sex and age), followed by the global F-test exploring the presence of overall differences between the groups, and finally the pairwise comparisons estimating mean differences between the ED diagnostic subtypes. Based on the test of significance from pairwise comparisons (*p-value)*, the BN and EDNOS groups obtained higher mean scores compared to the control participants in the avoidance and rule-driven behavior, weight control exercise and total CET scales, and higher mean scores than the AN group across all scales except for the exercise rigidity scale. There were no differences between the AN patients and the control group in any of the CET scales, except for the mood improvement scale where the control participants scored higher. CET scores were statistically equal between the BN

and EDNOS groups. Differences between groups were found in the mean EDI-2 (ED symptoms) total score in all pairwise comparisons, being highest in the BN patients, followed by the EDNOS patients, the AN patients and finally the control participants. A similar pattern was observed in terms of the SCL-90R (general psychopathology) GSI mean score, although in this case there were no differences between the AN patients and the EDNOS patients. Regarding the effect size for the pairwise comparisons, overall the contrasts that reached significance ($p \leq 0.05$) were also of moderate to good effect size ($|d|>0.50$). The exceptions were: AN *vs* controls for mood improvement scale, EDNOS *vs* controls for CET total, AN *vs* BN for all the CET scales except for total score, AN *vs* EDNOS for weight control exercise and CET total and BN *vs* EDNOS for GSI scale.

### 3.3 Association between CET and clinical measures in the ED patients

Table 2 displays the partial correlations (adjusted by the covariates sex and age) between the CET scales and clinical profile of the ED sample ($n$=158). The CET scales mood improvement and lack of enjoyment only obtained poor correlations with the EDI-2 and the SCL-90R scores, and the CET exercise rigidity scale only positively correlated with the EDI-2 drive for thinness scale. The strongest associations were found between the scales avoidance and rule-driven behavior, weight control exercise and total CET, and: a) the EDI-2 scales drive for thinness, body dissatisfaction, interceptive awareness, ascetism, social insecurity and total EDI-2; and b) the SCL-90R scales interpersonal sensitivity, depressive symptoms and anxiety and the global GSI and PST indexes.

## 4 Discussion

The present study assessed the validity of a Spanish version of the CET, and reviewed the potential differences between ED diagnostic subtypes and a healthy comparison group in terms of the dimensions of compulsive exercise as defined by the CET. Associations between these dimensions and ED symptoms and general psychopathology were also examined.

After being translated into Spanish, the initial five factor structure of the CET was preserved. In line with the proposed multidimensional conceptualization of compulsive exercise (Meyer et al., 2011; Meyer & Taranis, 2011), this study demonstrates that compulsive exercise may be incited by multiple factors. It also shows that the instrument is able to successfully distinguish individuals with a diagnosis of an ED from a non-clinical group on four of the five subscales and global score of the CET.

Adding to the results obtained by Meyer and colleagues (2016), the findings in this study also show that exercise can be particularly compulsive in individuals with BN and EDNOS; the BN and EDNOS groups scored higher in the total CET scale compared to the healthy controls. These patients were more likely to engage in exercise because of body weight/shape concerns than the healthy controls. The CET scores in our study

also indicate that the drive to exercise compulsively might emerge from the need to follow strict exercise rules and the fear that the terminating the exercise pattern could result in a negative emotional state and/or feelings of guilt (avoidance and rule-driven behavior scale). Moreover, continued exercise driven by the need to follow a strict exercise schedule seems to be of equal importance across all ED diagnostic subtypes and healthy controls.

Interestingly, AN patients did not differ from healthy controls in overall (global) compulsive exercise as defined by Meyer and colleagues (Meyer et al., 2011; Meyer & Taranis, 2011). The only discrepancy was in exercising for mood improvement, where scores were lower in the AN group. In addition, the patients with AN scored lower across all subscales compared to the patients with BN (apart from exercise rigidity), and exercise driven by weight/shape control and exercising despite a lack of enjoyment was less prevalent among these patients compared to those with EDNOS. Previous studies have shown excessive exercise to be particularly high in only a subgroup of individuals with AN (Bewell-Weiss and Carter, 2010; Davis et al., 1997; Sauchelli et al., 2015; Shroff et al., 2006). Bratland-Sanda and colleagues (2010) also found that patients with AN underreported the amount of exercise they engaged in. One of the explanations proposed by the authors is that individuals with AN have a different conceptualization of exercise, which could be reflected in the low CET scores observed in this study when compared to the other ED subtypes (Bratland-Sanda et al., 2010). Furthermore, as the disorder progresses, the physiological deterioration associated with it may be impeding the patients from engaging in exercise, which then becomes a secondary factor and receives less attention. Duration of the AN disorder has been found to be inversely linked to physical activity (Sauchelli et al., 2015). This was not evaluated in the present study, but it would be interesting to further assess the influence of this variable. Nonetheless, it has been claimed that the compulsive cognitions underlying the motivation to exercise should receive considerable attention from clinicians (Meyer & Trananis, 2011; Meyer et al., 2011).

The use of exercise for the control of body weight/shape in EDs is considered as a fundamental feature of EDs (Dalle Grave et al., 2008; Hechler et al., 2008). Accordingly, the ED participants in our study with more compulsive exercise-related cognitions showed greater body dissatisfaction and drive for thinness. An association was also found between poor interpersonal sensitivity and compulsive exercise, specifically exercise to control body weight/shape and avoidance and rule-driven exercise behavior. Individuals with EDs show a heightened sensitivity to the opinions and expectancies of others, often assume negative judgments and report negative social interactions (Atlas, 2004). In EDs this is particularly strong in relation to one's own appearance (Atlas, 2004). Therefore, individuals with EDs may exercise to control body weight and shape, which consequentially improves their self-esteem. When the individual

believes that having an "ideal body" is the only way to achieve and maintain social approval, exercise might become ritualistic and compulsive; the fear of perceived criticism from others on appearance may result in an impulsive urge to exercise. In support, a recent study of college women has shown that body and eating-related social comparisons were directly associated with greater exercise-related thoughts and prolonged exercise behavior (Fitzsimmons-Craft et al., 2016).

Studies have reported continuous exercise in AN to serve the purpose of affect regulation (Bratland-Sanda et al., 2010; Peñas-Lledó et al., 2002). This study, parallel to that of Meyer and colleagues (2016), however shows that exercise for mood improvement might be more important for healthy individuals than individuals with AN. A possible hypothesis is that, contrary to healthy individuals, emotion regulation in AN is primarily achieved by other factors inherent of the pathology. For example, it has been found that starvation-induced weight loss in acute AN patients is associated with an amelioration in the ability to regulate negative emotions (Brockmeyer et al., 2013, 2012). Therefore, in comparison to healthy individuals, AN patients might rely mostly on alternative strategies, such as starvation, to improve mood. Exercise may serve the function of partially inhibiting a negative state and not as much to induce positive feelings (Boyd et al., 2007; Vansteelandt et al., 2007).

Furthermore, in the present study compulsive exercise was predominant in the BN diagnostic category, but the bulimia subscale of the EDI-2 did not correlate with any of the CET subscales. This may be relevant when examining the origin of the pathological cognitions towards exercise. The EDI-2 subscale focuses primarily on the binge/purge symptoms of BN, such as eating when upset, eating/drinking in secret, or thinking about eating/bingeing/vomiting. It does not examine other facets of the disorder. One of these may be temperament. Cloninger (1987, 1999) described a psychobiological model that defines personality from a psychobiological and multi-dimensional perspective. It comprises four temperament traits, which have an inherited component and remain relatively stable across the lifespan, and three character traits, which are more modifiable in response to the environment. Individuals with BN show particularly high novelty-seeking in comparison to individuals with other ED and healthy controls (Atiye et al., 2015). This is a temperament trait characterized by both cognitive and behavioral impulsivity (Cloninger, 1999). Greater novelty-seeking has been associated with exercise behaviors in both non-clinical (Fernández-Aranda et al., 2014) and clinical (Dalle Grave et al., 2008) populations. It can therefore be hypothized that the cognitive urge to exercise, as described by Taranis and colleagues (2011), is not merely associated with psychopathology, but temperament may also be an important vulnerability factor. The role of temperament was not examined in the current study, but should be explored in more depth in future studies.

There are several strengths to the current study. Most importantly, this study is the first to validate a Spanish translation of the CET, thus providing the groundwork for further research into this multi-faceted perspective of compulsive exercise in both clinical and nonclinical Spanish-speaking samples. In addition, the use of a large sample and the inclusion of a healthy comparison group increases the generability of our findings, and the study compares ED diagnostic subtypes (AN, BN, EDNOS) that display varying psychopathology, personality and behavioral profiles. However, some limitations need to be considered. First, the assessment of compulsive exercise was made via the use of a participative measure, which is vulnerable to reporting bias. Future studies should consider the use of objective instruments to assess actual exercise (frequency, intensity, and duration) in order to evaluate whether the compulsion to exercise reported by patients is reflected in daily exercise patterns, how these might prospectively influence psychopathology, and whether reducing physical activity may influence ED symptoms (or viceversa). Second, the clinical sample in this study comprised patients who were actively seeking treatment, and findings cannot be generalized to those individuals with an ED who are not receiving clinical attention and who might display more/less compulsive exercising.

The current study highlights the need to acknowledge that compulsive exercise is not a unitary but a multidimensional construct. Furthermore, it seems that the greater the psychopathology, the more likely it is that the exercise behavior is compulsive; the BN group presented the worst psychopathology and higher mean scores in the CET. Compulsive exercise poses an obstacle for treatment progress (Dalle Grave et al. 2008; Solenberger, 2001; Carter et al. 2004). Many clinicians have therefore favoured the prohibition of exercise during treatment, however, some studies have shown that supervised, moderate physical activity programs may be beneficial (Alberti et al., 2013; Kostrzewa et al., 2013a; Sauchelli et al., 2015). The present study shows that compulsive exercise should not be addressed equally in all ED, and interventions incorporating physical activity programs should be adapted to the distinct ED diagnostic subtypes. For example, particular attention should be placed on the high levels of compulsivity that were observed in the bulimia nervosa and EDNOS patients. Furthermore, when examining the presence of compulsive exercise in eating disorders and throughout the intervention process, it is essential that professionals take psychopathology into account.

## 5 Abbreviations

ED: Eating disorder
CET: Compulsive Exercise Test
AN: Anorexia nervosa
BN: Bulimia nervosa
EDNOS: Eating disorders not-otherwise-specified
EDI-2: Eating Disorder Inventory-2
SCL-90-R: Symptom Checklist-90 Revised
RMSEA: Root mean square error of approximation
CFI: Comparative fit index

SRMR: Standardized root mean square residual

## 6 Funding

The study was supported by grants from Instituto Salud Carlos III (FIS PI11/210, FIS14/290 and CIBERobn) and AGAUR de la Generalitat de Catalunya (2014 SGR 1672). CIBERObn is an initiative of ISCIII. Sarah Sauchelli is recipient of a pre-doctoral Grant (2013–17) by Institut d'Investigació Biomèdica de Bellvitge (IDIBELL). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## 7 Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

## 8 Author contributions

All authors designed the work and revised it for important intellectual content. The data was gathered by **SS, ZA, SJM, FFA** and **APG**. **RG** conducted the statistical analysis. **SS, JA,** and **FFA** drafted the study. All authors revised, commented on and approved the final manuscript and are accountable for all aspects of the work.

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**Table 1:** Comparison of CET raw scores and clinical measures between groups: ANOVA adjusted by participants' sex and age.

| | controls ($n$=128) | | AN ($n$=40) | | BN ($n$=56) | | EDNOS ($n$=62) | | Factor group | | Pairwise comparisons: $p$-value and Cohen's $d$ | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | controls-AN | | controls-BN | | controls-EDNOS | | AN vs BN | | AN-EDNOS | | BN-EDNOS | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | $F_{(3,283)}$ | $p$ | $p$ | $\lvert d\rvert$ | $p$ | $\lvert d\rvert$ | $p$ | $\lvert d\rvert$ | $p$ | $\lvert d\rvert$ | $p$ | $\lvert d\rvert$ | $p$ | $\lvert d\rvert$ |
| **Avoidance and rule-driven behavior** | 1.28 | 1.07 | 1.69 | 1.55 | 2.27 | 1.50 | 2.12 | 1.65 | 7.48 | **<.001*** | .128 | 0.30 | **<.001*** | **0.76**† | **<.001** | **0.60**† | **.038*** | 0.39 | .124 | 0.27 | .538 | 0.10 |
| **Weight control exercise** | 2.15 | 1.28 | 2.54 | 1.46 | 3.36 | 1.37 | 3.08 | 1.49 | 10.97 | **<.001*** | .142 | 0.28 | **<.001*** | **0.91**† | **<.001** | **0.67**† | **.003*** | **0.58**† | **.046*** | 0.37 | .266 | 0.20 |
| **Mood improvement** | 3.47 | 1.04 | 2.97 | 1.65 | 3.53 | 1.29 | 3.46 | 1.45 | 1.87 | .135 | **.042*** | 0.37 | .810 | 0.05 | .944 | 0.01 | **.034*** | 0.38 | .060 | 0.32 | .771 | 0.05 |
| **Lack enjoyment** | 2.66 | 1.00 | 2.31 | 1.32 | 2.87 | 1.29 | 2.91 | 1.31 | 2.60 | .053 | .131 | 0.29 | .314 | 0.19 | .178 | 0.22 | **.022*** | 0.43 | **.013*** | **0.50**† | .862 | 0.03 |
| **Exercise rigidity** | 2.38 | 1.29 | 2.05 | 1.54 | 2.56 | 1.55 | 2.44 | 1.51 | 1.08 | .359 | .229 | 0.24 | .480 | 0.13 | .807 | 0.04 | .081 | 0.33 | .181 | 0.26 | .639 | 0.08 |
| **Total CET score** | 10.79 | 3.79 | 10.75 | 5.52 | 13.66 | 4.71 | 12.85 | 5.31 | 6.18 | **<.001*** | .956 | 0.01 | **.001*** | **0.67**† | **.005*** | 0.45 | **.002*** | **0.57**† | **.024*** | 0.39 | .350 | 0.16 |
| **EDI-2: Total score** | 37.4 | 26.0 | 80.5 | 43.4 | 122.4 | 38.0 | 100.0 | 45.5 | 68.90 | **<.001*** | **<.001*** | **1.21**† | **<.001*** | **2.61**† | **<.001*** | **1.69**† | **<.001*** | **1.03**† | **.009*** | 0.44 | **.001*** | **0.63**† |
| **SCL-90R: GSI score** | 0.8 | 0.5 | 1.6 | 0.8 | 1.9 | 0.7 | 1.7 | 0.7 | 48.42 | **<.001*** | **<.001*** | **1.25**† | **<.001*** | **2.02**† | **<.001*** | **1.53**† | **.003*** | **0.53**† | .257 | 0.19 | **.038*** | 0.35 |

*Note.* AN: anorexia. BN: bulimia. EDNOS: eating disorder non-otherwise-specified. SD: standard deviation. *Bold: significant result. †Bold: moderate ($|d|$>0.50) to good ($|d|$>0.80) effect size.

**Table 2:** Partial correlations (adjusted by participants' sex and age) between CET raw scores and clinical measures in the eating disorder sample ($n$=158)

| CET scales → | Avoidance rule-driven behavior | Weight control exercise | Mood improvement | Lack enjoyment | Exercise rigidity | Total CET score |
|---|---|---|---|---|---|---|
| EDI: Drive for thinness | **.408†** | **.578†** | .214 | .228 | **.300†** | **.487†** |
| EDI: Body dissatisfaction | **.277†** | **.399†** | .026 | .121 | .132 | **.301†** |
| EDI: Interoceptive awarness | **.312†** | **.342†** | .071 | .108 | .125 | **.284†** |
| EDI: Bulimia | .074 | .175 | -.012 | .074 | -.002 | .113 |
| EDI: Interpersonal distrust | .197 | .194 | .058 | -.005 | .031 | .136 |
| EDI: Ineffectiveness | .226 | .203 | -.010 | .015 | .070 | .174 |
| EDI: Maturity fears | .084 | .112 | -.044 | .023 | .009 | .038 |
| EDI: Perfectionism | .133 | .171 | .039 | .082 | .139 | .174 |
| EDI: Impulse regulation | .176 | .220 | .010 | .029 | .085 | .165 |
| EDI: Asceticism | .237 | **.355†** | -.017 | .095 | .082 | **.248†** |
| EDI: Social insecurity | **.248†** | .199 | .012 | -.002 | .081 | .179 |
| EDI: Total score | **.325†** | **.404†** | .048 | .107 | .145 | **.316†** |
| SCL-90: Somatization | .181 | .136 | -.002 | .042 | .065 | .131 |
| SCL-90: Obsessive/compul. | .212 | .125 | -.011 | .077 | .061 | .163 |
| SCL-90: Interponal sensit. | **.306†** | **.275†** | .065 | .127 | .137 | **.279†** |
| SCL-90: Depressive | **.267†** | .128 | .041 | .083 | .115 | .192 |
| SCL-90: Anxiety | **.243†** | .129 | .037 | .084 | .042 | .152 |
| SCL-90: Hostility | .151 | .094 | -.006 | .001 | .078 | .111 |
| SCL-90: Phobic anxiety | .227 | .151 | -.067 | -.036 | -.050 | .116 |
| SCL-90: Paranoid ideation | .184 | **.248†** | -.029 | .171 | .104 | .203 |
| SCL-90: Psychotic | .218 | .172 | .036 | .145 | .105 | .171 |
| SCL-90: GSI score | **.279†** | .192 | .029 | .101 | .097 | .211 |
| SCL-90: PST score | **.279†** | .204 | .042 | .169 | .076 | .218 |
| SCL-90: PSDI score | .239 | .156 | .066 | .045 | .144 | .200 |

†Bold: effect size into the moderate ($|r|$>0.24) to good range ($|r|$>0.30).

## 1 Supplementary Table 1

Distribution of the psychometrical measures in the study

| | | HC (*n*=128) | | AN (*n*=40) | | BN (*n*=56) | | EDNOS (*n*=62) | |
|---|---|---|---|---|---|---|---|---|---|
| | α | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Avoidance - rule/driven | .96 | 1.34 | 1.07 | 1.62 | 1.55 | 2.17 | 1.50 | 2.12 | 1.65 |
| Weight control | .85 | 2.23 | 1.28 | 2.44 | 1.46 | 3.22 | 1.37 | 3.10 | 1.49 |
| Mood improvement | .90 | 3.54 | 1.04 | 2.90 | 1.65 | 3.42 | 1.29 | 3.47 | 1.45 |
| Lack enjoyment | .82 | 2.80 | 1.00 | 2.18 | 1.32 | 2.67 | 1.29 | 2.89 | 1.31 |
| Exercise rigidity | .79 | 2.48 | 1.29 | 1.95 | 1.54 | 2.43 | 1.55 | 2.42 | 1.51 |
| Total score | .92 | 11.05 | 3.79 | 10.47 | 5.52 | 13.23 | 4.71 | 12.89 | 5.31 |
| EDI: Drive for thinness | .93 | 4.65 | 5.68 | 9.59 | 7.51 | 16.91 | 3.85 | 14.53 | 6.16 |
| EDI: Body dissatisfaction | .94 | 6.87 | 7.67 | 13.46 | 7.73 | 19.36 | 7.28 | 16.05 | 9.04 |
| EDI: Interpersonal awareness | .86 | 2.89 | 3.33 | 8.21 | 6.63 | 13.88 | 6.53 | 11.73 | 6.85 |
| EDI: Bulimia | .85 | 1.06 | 2.05 | 2.54 | 3.07 | 11.07 | 4.60 | 5.24 | 5.28 |
| EDI: Interpersonal distrust | .80 | 2.74 | 3.00 | 4.85 | 4.13 | 5.80 | 5.05 | 5.50 | 4.65 |
| EDI: Ineffectiveness | .91 | 2.80 | 4.17 | 10.72 | 8.51 | 12.88 | 7.04 | 11.03 | 7.23 |
| EDI: Maturity fears | .80 | 4.94 | 3.81 | 6.90 | 5.74 | 9.63 | 6.19 | 8.21 | 5.80 |
| EDI: Perfectionism | .71 | 4.84 | 3.66 | 4.69 | 4.01 | 6.14 | 4.18 | 6.87 | 4.36 |
| EDI: Impulse regulation | .82 | 1.47 | 2.55 | 5.87 | 6.72 | 8.04 | 6.03 | 6.50 | 5.51 |
| EDI: Ascetism | .70 | 2.76 | 2.55 | 5.59 | 4.31 | 8.80 | 3.46 | 7.52 | 4.56 |
| EDI: Social insecurity | .82 | 2.93 | 3.16 | 7.31 | 5.16 | 8.46 | 5.40 | 7.44 | 4.89 |
| EDI: Total score | .97 | 37.96 | 26.02 | 79.72 | 43.43 | 120.96 | 38.00 | 100.61 | 45.49 |
| SCL-90: Somatization | .92 | 0.70 | 0.58 | 1.67 | 0.93 | 2.06 | 0.81 | 1.86 | 0.97 |
| SCL-90: Obsessive/compulsive | .87 | 1.05 | 0.63 | 1.72 | 0.95 | 2.09 | 0.82 | 1.88 | 0.86 |
| SCL-90: Interpersonal sensitivity | .89 | 0.96 | 0.69 | 1.73 | 0.96 | 2.26 | 0.88 | 2.05 | 0.95 |
| SCL-90: Depressive | .94 | 0.90 | 0.65 | 2.14 | 0.97 | 2.54 | 0.84 | 2.16 | 0.91 |
| SCL-90: Anxiety | .91 | 0.71 | 0.56 | 1.53 | 0.96 | 1.96 | 0.89 | 1.73 | 0.85 |
| SCL-90: Hostility | .87 | 0.59 | 0.58 | 1.20 | 1.01 | 1.38 | 1.04 | 1.28 | 0.87 |
| SCL-90: Phobic anxiety | .85 | 0.21 | 0.35 | 0.90 | 0.96 | 1.22 | 0.91 | 0.98 | 0.85 |
| SCL-90: Paranoid ideation | .78 | 0.83 | 0.62 | 1.23 | 0.85 | 1.57 | 0.88 | 1.42 | 0.81 |
| SCL-90: Psychotic | .86 | 0.43 | 0.46 | 1.15 | 0.75 | 1.50 | 0.78 | 1.22 | 0.74 |
| SCL-90: GSI score | .98 | 0.74 | 0.47 | 1.58 | 0.77 | 1.97 | 0.69 | 1.72 | 0.73 |
| SCL-90: PST score | .98 | 40.05 | 18.48 | 59.20 | 17.50 | 68.50 | 14.82 | 64.02 | 17.76 |
| SCL-90: PSDI score | .98 | 1.55 | 0.37 | 2.27 | 0.61 | 2.52 | 0.57 | 2.32 | 0.57 |

*Note.* HC: healthy controls. AN: anorexia nervosa. BN: bulimia nervosa. EDNOS: eating disorder non-otherwise-specified. EDI: Eating Disorder Inventory-2; SCL-90: Symptom Checklist Revised 90
α: Cronbach's alpha in sample.

## 2. Supplementary Table 2

Confirmatory Factor Analysis (includes invariance of parameters for groups defined by sex)

| | Complete sample | | | | | Standardized coefficients including sex invariance | | | | | | Test of invariance (measurem.coeff) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Standardized coefficients | | | | | Women | | | Men | | | | |
| | B | SE | *p* | 95%CI (B) | | B | SE | *p* | B | SE | *p* | $\chi^2(df=1)$ | *p* |
| F1. Avoidance and rule/driven | | | | | | | | | | | | | |
| 9. Depressed | .856 | .024 | <.001 | 0.808 | 0.903 | .836 | .021 | <.001 | .829 | .039 | <.001 | 0.089 | 0.766 |
| 10. Guilty | .867 | .023 | <.001 | 0.823 | 0.911 | .879 | .016 | <.001 | .861 | .034 | <.001 | 0.063 | 0.801 |
| 11. Insistent | .714 | .043 | <.001 | 0.630 | 0.797 | .748 | .030 | <.001 | .627 | .057 | <.001 | 2.016 | 0.156 |
| 15. I don't miss | .693 | .045 | <.001 | 0.605 | 0.782 | .729 | .031 | <.001 | .622 | .058 | <.001 | 1.562 | 0.211 |
| 16. Feel agitated | .919 | .015 | <.001 | 0.890 | 0.949 | .900 | .014 | <.001 | .907 | .026 | <.001 | 1.094 | 0.296 |
| 20. Angry-frustrated | .924 | .014 | <.001 | 0.896 | 0.952 | .917 | .012 | <.001 | .853 | .035 | <.001 | 0.083 | 0.773 |
| 22. Let down | .838 | .027 | <.001 | 0.786 | 0.890 | .860 | .018 | <.001 | .824 | .040 | <.001 | 0.064 | 0.801 |
| 23. Anxious | .917 | .015 | <.001 | 0.886 | 0.947 | .871 | .017 | <.001 | .816 | .041 | <.001 | 0.146 | 0.703 |
| F2. Weight control | | | | | | | | | | | | | |
| 2. Appearance | .736 | .044 | <.001 | 0.650 | 0.821 | .817 | .025 | <.001 | .641 | .063 | <.001 | 2.566 | 0.109 |
| 6. More exercise | .866 | .026 | <.001 | 0.815 | 0.916 | .846 | .022 | <.001 | .789 | .056 | <.001 | 1.470 | 0.225 |
| 8. Not slim | .310 | .079 | <.001 | 0.154 | 0.465 | .377 | .057 | <.001 | .260 | .053 | <.001 | 0.263 | 0.608 |
| 13. Lose weight | .845 | .029 | <.001 | 0.789 | 0.902 | .820 | .025 | <.001 | .721 | .060 | <.001 | 0.533 | 0.466 |
| 18. Worry | .895 | .023 | <.001 | 0.850 | 0.940 | .851 | .022 | <.001 | .695 | .061 | <.001 | 0.399 | 0.528 |
| F3. Mood improvement | | | | | | | | | | | | | |
| 1. Feel happy | .834 | .031 | <.001 | 0.774 | 0.895 | .820 | .024 | <.001 | .722 | .057 | <.001 | 0.514 | 0.473 |
| 4. Less anxious | .769 | .038 | <.001 | 0.694 | 0.844 | .753 | .031 | <.001 | .657 | .061 | <.001 | 2.037 | 0.154 |
| 14. Less tense | .875 | .026 | <.001 | 0.825 | 0.925 | .904 | .016 | <.001 | .820 | .049 | <.001 | 2.147 | 0.143 |
| 17. Improves mood | .850 | .028 | <.001 | 0.795 | 0.906 | .855 | .021 | <.001 | .730 | .057 | <.001 | 0.744 | 0.388 |
| 24. Less depressed | .694 | .047 | <.001 | 0.601 | 0.787 | .782 | .028 | <.001 | .545 | .061 | <.001 | 2.785 | 0.095 |
| F4. Lack of enjoyment | | | | | | | | | | | | | |
| 5. Chore | .288 | .134 | .032 | 0.025 | 0.551 | .288 | .094 | .002 | .444 | .135 | .001 | 0.314 | 0.575 |
| 12. Enjoy | .321 | .144 | .025 | 0.040 | 0.603 | .365 | .110 | .001 | .486 | .142 | .001 | 0.229 | 0.632 |
| F5. Exercise rigidity | | | | | | | | | | | | | |
| 3. Organization | .660 | .055 | <.001 | 0.553 | 0.768 | .630 | .043 | <.001 | .560 | .073 | <.001 | 2.494 | 0.114 |
| 7. Repetitive | .852 | .036 | <.001 | 0.782 | 0.922 | .866 | .026 | <.001 | .753 | .069 | <.001 | 0.464 | 0.496 |
| 19. Routine | .829 | .037 | <.001 | 0.755 | 0.902 | .805 | .030 | <.001 | .709 | .070 | <.001 | 0.878 | 0.349 |
| Fit statistics: RMSEA: .087; CFI: .91; TLI: .90; SRMR: .08 | | | | | | Fit statistics: RMSEA: .097; CFI: .90; TLI: .90; SRMR: .09 | | | | | | | |

| Correlations between factors | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| F1. Avoidance and rule/driven | - | | | | |
| F2. Weight control | .693 | - | | | |
| F3. Mood improvement | .584 | .460 | - | | |
| F4. Lack of enjoyment | .624 | .549 | .660 | - | |
| F5. Exercise rigidity | .597 | .544 | .530 | .625 | - |

| Joint tests for parameter classes | $\chi^2$ | *df* | *p* |
|---|---|---|---|
| Measurement coefficients | 16.95 | 17 | .458 |
| Covariances measurement errors | 28.64 | 23 | .192 |
| Covariances exogenous variables | 7.977 | 5 | .158 |

## 4.5. STUDY 5: INTERACTION BETWEEN SLEEP QUALITY AND OREXIN IN EXTREME WEIGHT CONDITIONS.

**Background:** The evidence for an association between disturbances in sleep structure and OB is extensive [340,352]. Originally discovered as an orexigenic (hunger-stimulating) neuropeptide implicated in the regulation of energy expenditure, it was later found that orexin-A plays an important role in the sleep-wake cycle by promoting arousal and wakefulness [119]. Individuals with narcolepsy show minimal to no orexin neuron activity [395]. Furthermore, administration of orexin antagonists has been found to efficiently facilitate sleep [396,397]. Therefore, one of the functions of sleep may be to mediate some of the biological mechanisms underlying the development of OB.

**Objectives:** To evaluate serum concentrations of orexin-A and sleep patterns in individuals with OB and MOB without a sleep disorder, and to examine the possible contributing role of an interaction between poor sleep quality and elevated plasma orexin-A levels in the fluctuations in BMI.

**Methods:** Of the participants in this study, 26 had OB (BMI = 30-34.9 kg/m$^2$), 40 MOB (BMI ≥ 35 kg/m$^2$) and 32 were healthy-weight controls (BMI = 18.5-24.9 kg/m$^2$). BMI was calculated via the use of the Tanita Multi-Frequency Body Composition Analyzer MC-180MA (Tanita Corporation, Tokyo, Japan) after height was obtained using a stadiometer. Sleep quality was measured with the PSQI and the Actiwatch AW7 was used to assess the duration of sleep. Serum orexin-A levels were examined from blood samples taken between 08:00h and 09:00h after an overnight fast. Additionally, the SCL-90-R [398] was administered to examine symptoms of general psychopathology.

**Results:** A higher weight status was associated with elevated plasma orexin-A concentrations ($p = 0.50$), as well as greater anxiety ($p < .001$), depression ($p < .001$) and somatization symptoms ($p < .001$). Sleep quality was poorer in the individuals with OB and MOB compared to the healthy-weight controls (both: $p < .001$), and a quadratic trend was found in objective sleep time, being longer in the OB group ($p = .031$). SEM showed plasma orexin-A to be related to poor total sleep quality ($b = .18$), which in turn was associated with elevated BMI ($b = .23$). Elevated depression, anxiety and somatization symptoms were linked to poorer sleep quality ($b = .26$, $b = .20$, and $b = .26$ respectively). Somatization symptoms were also directly connected to increased BMI ($b = .41$).

# INTERACTION BETWEEN OREXIN-A AND SLEEP QUALITY IN FEMALES IN EXTREME WEIGHT CONDITIONS.

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Conflict of interest: The authors declare no conflict of interest.

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**Funding:**This manuscript was partially supported by grants from Instituto de Salud Carlos III (FIS PI14/290 and CIBEROBNn) and Fondo Europeo de Desarrollo Regional (FEDER) a way to build Europe, as well as AGAUR of la Generalitat de Catalunya (2014SGR1672) and PROMOSAM (PSI2014-56303-REDT), Spain. CIBEROBN and CIBERSAM are both initiatives of ISCIII. Sarah Sauchelli is recipientof a pre-doctoral Grant (2013–17) by IDIBELL. José C. Fernández-García is recipient of a research

contract from Servicio Andaluz de Salud (SAS) (B-0033-2014). Lourdes Garrido-Sánchez is supported by a fellowship from the Fondo de Investigación Sanitaria (FIS) "Miguel Servet I" CP13/00188. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Journal:** European Eating Disorders Review
**Impact Factor:** 2.9

**Keywords:** Obesity; Body Mass Index; Orexin; Sleep

**Abstract**

The current study examined the relationship between plasma orexin-A and sleep in obesity. Concentrations of orexin-A and sleep were evaluated in 26 obese, 40 morbid obese and 32 healthy-weight participants. The sleep monitor Actiwatch AW7 and the Pittsburgh Sleep Quality Index were used to evaluate sleep. The Symptom Checklist-90-Revised was administered to assess symptoms of psychopathology. A higher weight status was associated with elevated orexin-A levels ($p = .050$), greater depression, anxiety and somatization symptoms (all: $p < .001$), and impoverished self-reported sleep quality ($p < .001$). A quadratic trend was found in objective sleep time, being longest in the obese group ($p = .031$). Structural equation modeling showed plasma orexin-A to be related to poor total sleep quality, which in turn was associated with elevated body mass index. Our data confirm an interaction between elevated plasma orexin-A concentrations and poor sleep that contributes to fluctuations in body mass index.

## Introduction

With the steady rise in body mass index (BMI) over the past few decades, the prevalence of obesity (OB) and morbid obesity (MOB) is rapidly rising worldwide (Finucane et al., 2011). The increase in body fat and visceral obesity has been associated with multiple cardiovascular and metabolic illnesses, thus prevention of obesity is becoming a pressing public health issue (Bombak, 2014). Parallel to the increase in the prevalence of obesity, a rapid decline in sleep time has been observed in modern society (Bixler, 2009).

A meta-analysis of 18 studies examining a total of 604,509 adults, showed a progressive increase in the likelihood of obesity with a decrease in sleep time; odds ratio for obesity being 1.55 for less than 5 hours of sleep and a decrease of one hour/night of sleep yielding an increase of $0.35 kg/m^2$ in BMI (Cappuccio et al., 2008). Later, a meta-analysis of prospective studies also concluded that prolonged short sleep duration increased the risk of developing obesity later in life with an odds ratio of 1.45 (Wu, Zhai, & Zhang, 2014). Diminished sleep quality also seems to be present in obesity as well as a greater use of sleep medication (Bidulescu et al., 2010). Actigraphy (objective analysis of various aspects of sleep via the use of a sleep monitor), has also evidenced adiposity and BMI to be associated with reduced sleep duration, inconsistent sleep patterns and poor sleep efficiency (Appelhans et al., 2013; Bailey et al., 2014).

The relationship between poor sleep and weight has also been observed in several sleep and eating disorders. For example, narcolepsy is a chronic, neuro-degenerative sleep disorder characterized by recurrent intrusions of REM sleep during the day, sometimes accompanied by cataplexy (sudden muscle weakness). Affected individuals are frequently obese (Tsujino & Sakurai, 2013). Binge eating (the consumption of large quantities of food within a short period of time), as seen in the binge eating disorder, has been associated with sleep problems (Tzischinsky, Latzer, Epstein, & Tov,

2000) and obesity (Bulik and Reichborn-Kjennerud, 2003). Obesity is also common in the sleep-related eating disorder, consisting in recurrent episodes of eating at the transition from night-time sleep to arousal, and the night eating syndrome, in which a large proportion of daily food intake is consumed at night (Auger, 2006; Meule, Allison, & Platte, 2014).

Sleep regulation can be attributed to a wide range of environmental and biological mechanisms. One of these is the hypocretin (orexin) neuron system (Tsujino & Sakurai, 2009, 2013). Orexin A (hypocretin 1) and orexin B (hypocretin 2) are 33- and 28-amino-acide neuropeptides derived from the protein precursor labeled prepro-orexin primarily synthesized in the lateral-posterior-perifornical hypothalamus, and attach to the orexin receptor-1 and orexin receptor-2 spread across several brain sites. Orexins innervate an extensive number of areas throughout the central nervous system thus playing a role in multiple physiological processes, among which are arousal, behaviour, homeostatic mechanisms, reward seeking and autonomic function (Burdakov, Karnani, & Gonzalez, 2013; Mahler, Smith, Moorman, Sartor, & Aston-Jones, 2012; Sakurai, 2005; Tsujino & Sakurai, 2009, 2013).

It is widely agreed that orexins are involved in the sleep/wake cycle. Orexins innervate aminergic nuclei that promote arousal and inhibit rapid eye movement (REM) sleep (Tsujino & Sakurai, 2009; Sakurai, Mieda and Tsujino, 2010). Extracellular levels of orexin have been found to rise during wakefulness (Yoshida et al., 2001), and electro-physiology has identified wake-active neurons in animals, with firing rates that peak when the animals are active (Alam et al., 2002). Concordant, the administration of orexin receptor antagonists have proven to be effective sleep inducers (Winrow & Renger, 2014). The role of orexin-A in the sleep/wake system has also been consistently documented in narcolepsy (Tsujino & Sakurai, 2013). Orexin knockout mice display extreme sleepiness and frequent motoric arrests during the dark (active) phase (Chemelli et al., 1999). Long-term hypothalamic staining has shown a progressive decrease by 85%-95% in orexin-firing neurons in narcoleptic patients, not found in neurologically healthy controls (Thannickal et al., 2000).

Early studies in narcolepsy also suggested the involvement of orexin-A in energy balance (Tsujino & Sakurai, 2013). Neuronal orexin damage and irregularities in orexin function have been linked to the development of obesity despite hypophagia in both human narcoleptic patients and animal-models of narcolepsy (Tsujino & Sakurai, 2013). Initial reports in obesity have shown a similar pattern. In a study of 15 morbidly obese participants who underwent a surgical intervention, peripherally measured orexin-A was found to be inversely associated with BMI, and weight loss resulted in an increase of orexin-A levels (Adam et al., 2002). Similarly, BMI has been shown to inversely correlate to serum orexin-A in children (Tomasik, Spodaryk, & Sztefko, 2004), and an increase in plasma orexin-

A levels has also been observed in children after participating in a weight-loss programme (Bronský et al., 2007).
Later studies, however, have obtained contrasting results. Heinonen and colleagues (2005) reported elevated plasma orexin-A levels in obese individuals waiting for a gastric band operation compared to lean controls, which remained unchanged at a one-year post-surgery follow-up. Others found inter-individual differences in post-surgery changes in orexin-A; plasma concentrations increased in some obese participants after weight loss, while in others there was a decrease (Gupta et al., 2014). Furthermore, a study conducted in patients with chronic obstructive pulmonary disease, who are frequently malnourished, found that orexin-A did not correlate with BMI, but was only related to greater food intake (Akbulut et al., 2014).

In summary, an exponential increase in the prevalence of obesity has been reported, which seems to be partly related to poorer sleep quality. This is of particular concern for females, who are more likely to be obese (Kelly et al., 2008) and report difficulties in falling and maintinging sleep (Meyer, Wall, Larson, Laska & Neumark-Stzainer, 2012). Furthermore, evidence points to an interplay between the role of orexin-A in the regulation of sleep/arousal and energy balance to contribute (Nixon et al., 2015), however limited research has been conducted to examine the nature of this relationship in obese humans and studies thus far have obtained conflicting results. Our objective was to analyze plasma orexin-A levels in obese and morbid obese female participants without an eating nor sleeping disorder in comparison to a healthy-weight female comparison group, and to explore the interaction between plasma orexin-A levels, sleep quality and fluctuations in BMI. Based on the existing literature we were unable to predict possible deviations in the plasma concentrations of orexin-A in the obese groups, but we expected that an interaction between elevated orexin-A levels and poorer sleep would be related to raised BMI.

**Materials and methods**

Participants

The sample comprised a total of 98 female participants of which 26 were OB (BMI = 30-39.9 kg/m$^2$), 40 were MOB (BMI > 40 kg/m$^2$) and 32 were healthy-weight controls (BMI = 18.5-24.9 kg/m$^2$). Participants were excluded if they were male, younger than 18 years, had a lifetime history of a mental disorder, including any eating disorder (evaluated by means of the General Health Questionnaire-28; Goldberg, 1978), and/or had a sleep disorder. The possible presence of the obstructive sleep apnea syndrome, along with other sleep disorders, was based on a clinical evaluation from an expert professional and, if necessary, polysomnography. Several centers participated in this study that belong to our Spanish Research Network (CIBEROBN). The network comprises centers spread throughout Spain that provide treatment for eating disorders or obesity and collaborate together in numerous research projects. The centers that participated in order to conduct the present study were: The

Eating Disorders Unit (Department of Psychiatry, University Hospital of Bellvitge- IDIBELL, Barcelona), the Department of Endocrinology at the University Hospital of Santiago (Santiago de Compostela); the Department of Diabetes, Endocrinology and Nutrition (Clinic University Hospital Virgen de Victoria, Malaga); the Department of Endocrinology and Nutrition (University of Navarra, Pam- plona); the Diabetes, Endocrinology and Nutrition Department, Biomedical Research Institute of Girona (IdIBGi-Doctor Josep Trueta Hospital, Girona); the Hospital del Mar Medical Research Institute (IMIM, Barcelona) and the Department of Basic Psychology, Clinic and Psychobiology (University Jaume I, Castellón). The OB and MOB participants were patients referred to these centers, while the control group was a convenience sample (not matched by age) recruited via word-of-mouth and advertisements at the local universities from the same catchment areas.

Mean BMI of the healthy-weight controls was 22.6 kg/m² (*SD* = 2.7), of the OB group was 35.5 kg/m² (*SD* = 2.5), and of the MOB was 46.4 kg/m² (*SD* = 4.9) ($p <$ .001). Mean age of the controls was 37.3 years (*SD* = 5.6), which was significantly lower to that of the OB (*M* = 47.0 years, *SD* = 11.4) and MOB (*M* = 43.2 years, *SD* = 10.4) groups (controls vs OB: $p <$ .001; controls vs MOB: $p$ = .009). No difference was found between the OB and MOB participants ($p$ = .112). The presence of eating-disorder symptoms, such as drive for thinness, were evaluated using the Eating Disorder Inventory-2, (Garner, 1991). The total score was highest in the MOB group (*M* = 79.4, *SD* = 30.6), followed by the OB group (*M* = 66, *SD* = 28.7) and lowest in the controls (*M* = 23.6, *SD* = 24.6) (MOB vs controls: $p <$ .001; MOB vs OB: $p$ = .063; OB vs controls: $p <$ .001). However, none of the participants reached the clinical threshold, thus further indicating the absence of an eating disorder.

Measures

*Sleep quality.* The Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) was used to evaluate self-reported problematic sleep and sleep time. The questionnaire consists of 19 items scored on a Likert scale ranging from 0 (no difficulty) to 3 (severe difficulties). Seven "components" of sleep are obtained: 1) subjective sleep quality; 2) sleep latency; 3) sleep duration; 4) habitual sleep efficiency; 5) sleep duration; 6) use of sleeping medication; 7) daytime dysfunction. From the sum of the scores of each table, a global sleep quality score is obtained ranging from 0 to 21, with higher scores indicating poorer sleep. Scores above 5 are representative of sleep disturbance. Additionally, the PSQI includes five open questions habitual sleep-wake patterns. Test-retest reliability for the global score was acceptable (.85). Comparison between patients and lean controls using the 5 cut-off score to indicate pathology has shown a sensitivity of 89.6% and a specificity of 86.5% (Buysse et al., 1989). Internal consistency of the Spanish version of the PSQI is of (Chronbach alpha) 0.81 (Royuela & Macías, 1997).

*Objective sleep time.* The duration of nocturnal sleep was also evaluated using the movement monitor Actiwatch AW7

(Actiwatch AW7; CamNtech Ltd, Cambridge Neurotechnology, Cambridge, UK). This is a small (39×32×9 mm), light-weight (10.5 g), triaxial instrument that records movement intensity in the form of counts in a 1-minute epoch length. It can be utilized as an actigraph, whereby users press a marker button to indicate bed time and get up time and data of actual nocturnal sleep time is extracted. Participants were also asked to keep sleep logs. Sleep time is analyzed using the Actiwatch Sleep Analysis 7 software (CamNtech Ltd). Actigraphy has shown to correlate with polysomnography recording, the gold standard of sleep, with an intra-class correlation co-efficient of .76 for sleep time (Blackwell et al., 2008). The Actiwatch was purposely included in the procedure to avoid the possibility of incorrect estimates of sleep duration due to self-reported mis-perception.

*Orexin A plasma concentrations.* Blood samples were collected from all participants between 0800h and 0900h after an overnight fast. Method of analysis has been described elsewhere (Sauchelli et al., 2016).

*Body Mass Index.* The Tanita Multi-Frequency Body Composition Analyzer MC-180MA (Tanita Corporation, Tokyo, Japan) was used to measure BMI. This is a weighting instrument that provides information on body composition using bioelectrical impedance analysis. The device is repeatedly revised to meet the reference standards dual-energy X-ray absorptiometry (DEXA) (http://www.bl-biologica.es/tanita_tbf.htm) and has been validated against other weighing methods (Strain et al., 2008). Height was obtained by using a stadiometer.

*General psychopathology.* To examine general psychopathology, the Symptom-Checklist-revised (SCL-90-R) (Degoratis, 1990) was used. This self-administered questionnaire comprises 90 items answered on a 5-point Likert scale, from which scores on 9 dimensions are obtained: 1) somatization; 2) obsession-compulsion; 3) interpersonal sensitivity; 4) depression; 5) anxiety; 6) hostility; 7) phobic anxiety; 8) paranoid ideation; 9) psychoticism. Scores on three global indexes are also obtained: 1) the Global severity index (GSI) to appraise overall distress; 2) the positive-symptom total (PST), which indicates self-reported symptoms; 3) the positive symptom distress index (PSDI) to assess symptom intensity. The Spanish validation of this questionnaire (Derogatis, 2002) has revealed a mean internal consistency of .75 (coefficient alpha). The depression, anxiety, and somatization scales were considered in the current study due to their previously reported role in sleep and obesity (Algul et al., 2009).

Procedure

Clinical and physical assessments were conducted by experienced psychologists, psychiatrists and endocrinologists (all extensively trained in the use of the instruments). Subjective sleep quality and general health status were determined by using the questionnaires. Basic anthropometric information was obtained from the TANITA, and blood sampling took place prior to actigraphic recording

after an overnight fast. As described in previous studies (Sauchelli et al., 2016), the Actiwatch was provided on the first day of assessment and collected after 7 days. All participants gave written informed consent to participate. The study was approved by the Institutional Research Ethics Committee of each institution and was conducted in accordance with the Declaration of Helsinki.

Statistical analysis

Analyses were carried out with Stata13.1 for Windows. Analysis of variance (ANOVA) adjusted according to participants' age was conducted to compare plasma orexin A, sleep and symptomatology (SCL-90R depression, anxiety and somatization scales) between diagnostic conditions. Orthogonal polynomial contrasts were included in the ANOVA procedures to assess the presence of linear and/or quadratic trends between means and Cohen's-*d* indicated the effect size of pairwise comparisons (moderate effect size was considered for $|d| > 0.50$ and large effect size for $|d| > 0.80$). Bonferroni-Finner's correction controlled the inflation in type-I error because of the multiple statistical comparisons. Partial correlations adjusted according to age were also conducted to analyze the association between sleep time when measured with the PSQI (subjective assessment) and that indicated by the actigraph (objective assessment).
Structural Equation Modeling (SEM) in the OB and MOB subsamples was used to test the paths between BMI, orexin A concentrations, symptomatology levels and overall self-reported sleep quality. Path analysis procedures constitute in a straightforward extension of multiple regression modeling that can be used for both confirmatory and exploratory modeling, with the aim of estimating the magnitude and significance of "hypothesized" causal connections into a set of variables (von Oertzen, 2010). Goodness-of-fit was evaluated using the standard indices of fit, including chi-square ($\chi^2$), Comparative Fit Index (CFI), Root Mean Square Error of Approximation (RMSEA), Standardized Root Mean Squared Residual (SRMR), and the Tucker-Lewis Index (TLI). Goodness-of-fit was established when (Bentler, 1990): $\chi^2$ with a $p > .05$, a CFI and TLI > .9, a RMSEA < .08 and a SMSR < .10. The global predictive capacity was measured through the coefficient of determination (CD).

**Results**

Comparison between weight groups

Table 1 shows the results of the ANOVA, adjusted according to participants' age, comparing plasma orexin-A concentrations, self-reported sleep (PSQI scores), actigraphy-based sleep time and psychopathology (SCL-90R depression/anxiety/somatization) across the three groups (lean controls, OB and MOB). Elevated plasma orexin-A concentrations were found to be present in the OB and MOB groups compared to the lean participants. Self-reported sleep quality and symptoms of anxiety, depression and somatization followed significant linear trends, being highest in the MOB group and lowest in the healthy controls. A quadratic trend was also present in self-reported sleep quality and

sleep latency. When measured with the actigraph, only a quadratic trend was found in sleep time, whereby the OB participants slept longer than the MOB participants and controls, who did not differ in sleep time. Furthermore, sleep time measured via the actigraph did not correlate with that reported by the participants in the PSQI (HC: r = .306, $p$ = .089; OB: r = .194, $p$ = .342; MOB: r = .208, $p$ = .198).

Path analysis

Figure 1 presents the SEM (adjusted according to participants' age) portraying the relationships between plasma orexin-A levels, overall self-reported sleep quality (PSQI-total), anxiety, depression and somatization symptoms (SCL-90R scales), and BMI in the OB and MOB participants. Table S1 displays complete indexes of the SEM model. Adequate goodness-of-fit was achieved: $\chi^2$ = 0.38 ($p$ = .944), RMSEA = .01, CFI = .999, TLI = .999 and SRMR = .007. The global predictive capacity was high (CD = .577). The standardized coefficients of this model indicate that poorer sleep quality (a higher PSQI-total score) was associated with greater psychopathology (SCL-90R scales) and elevated plasma orexin-A levels. BMI was higher with impoverished sleep quality and increased symptoms of somatization. Orexin-A levels did not directly predict BMI. Sleep quality was a mediator role in the relationships between psychopathology and BMI, as well as in the link between plasma orexin-A concentrations and BMI.

**Discussion**

Given the reported relevance of poor sleep in obesity, and the role of orexin-A in the regulation of sleep and arousal, the present study aimed to evaluate whether an interaction between diminished sleep quality and plasma orexin-A concentrations could be influencing weight status. Both OB and MOB participants reported notably poorer sleep quality and less sleep compared to the lean participants. These findings concord with those of previous studies that have found that short sleep and sleep disturbances are related to an increased likelihood of obesity (Bailey et al., 2014; Bidulescu et al., 2010; Cappuccio et al., 2008). However, when sleep time was measured objectively with the actigraph, the OB group were found to sleep longer than the other two groups, whereas sleep time was similar between the MOB and control groups. These findings suggest that in our sample it is impoverished sleep quality (e.g. inefficient sleep and/or alterations during sleep) rather than the amount of sleep *per se* that is associated with obesity. In support, Meyer and colleagues (2012) found that short sleep correlated with BMI and greater odds of being obese only in the male participants but not in the female participants, while an association between difficulties in falling/maintaining sleep and BMI was only found in the female participants. The sample in the present consisted fully in adult women.

The link between poor sleep quality and obesity might be related to greater symptoms of depression and anxiety as seen in our OB and MOB groups. Evidence has shown an interaction

between obesity, psychological distress and problematic sleep (Algul et al., 2009). Furthermore, somatization symptoms are linked to several forms of pain that might also be having a detrimental effect on sleep. This could be particularly relevant in the reduced sleep time we observed in the MOB compared to OB group, whereby the pains might start to significantly interfere with restorative sleep. Studies in fibromyalgia, a chronic pain disorder of unknown etiology, show both morbid obesity and severe sleep disturbances that ameliorate after bariatric surgery (Masheb, White, & Grilo, 2016; Saber et al., 2008). However, in the present study weight-related pain was not evaluated, and needs be taken into consideration in future studies in order to confirm such hypothesis.

Our findings also suggest that sleep might act as a modulator in the role of plasma orexin A in BMI variation. Elevated plasma orexin A levels were identified in the OB and MOB participants compared to the lean controls. These findings are only partially consistent with the existing literature (Gupta et al., 2014; Heinonen et al., 2005). Studies have reported increases in prepro-orexin in the hypothalamus in situations of hypoglycemia or after fasting (Komaki et al., 2001; Sakurai et al., 1998), and intracerebroventricular injection of orexin in rats results in elevated food intake (Sakurai et al., 1998). Differently, the administration of SB-649868 or genetic ablation of orexin neurons inhibits such effects, resulting in increases in body weight despite hypophagia (Chemelli et al., 1999; Ishii et al., 2005). This contradictory influence has also been observed in patients with narcolepsy, who present a high prevalence of obesity despite hypophagia (Tsujino & Sakurai, 2013).

The exact mechanism that may be underlying the role of orexin-A in obesity is not fully understood. Orexin-A is generally considered as an appetite stimulator (Tsujino & Sakurai, 2009, 2013) that generates the experience of hunger to regulate eating behavior and food intake (Sakurai et al., 1998; Tsujino & Sakurai, 2013) by receiving metabolic cues from peripheral peptides (Sakurai, 2005; Toshinai et al., 2003). Orexin receptors have also been located in peripheral organs that are involved in feeding and metabolism such as adipose tissue, gastrointestinal tracts, pancreas and the adrenal gland (Kirchgessner & Liu, 1999; Ouedraogo, Näslund, & Kirchgessner, 2003). Yet, orexins also contribute to energy expenditure (Teske, Billington, & Kotz, 2010). They are implicated in the regulation of thermoregenesis-based energy expenditure (Tupone, Madden, Cano, & Morrison, 2011), and the stimulation of skeletal glucose metabolism (Shiuchi et al., 2009). Evidence has demonstrated increased locomotor activity in rats placed in conditions of limited food availability that is accompanied by an upregulation of rat orexin receptor mRNA (Lu, Bagnol, Burke, Akil, & Watson, 2000).

The findings from the present study imply that orexin may be related to weight gain by impeding adequate sleep. In line with others (Akbulut et al., 2014; Komaki et al., 2001), we did not find a direct link between serum orexin-A and BMI. Rather, our results showed increased

plasma orexin levels to be related to poorer sleep quality, which in turn was associated with greater BMI. Intracerebroventricular administration of orexin A results in a decrease of both rapid and non-rapid eye movement sleep and an increase in wakefulness (Hagan, Whitworth, & Moss, 1999). Orexin receptor antagonists have been found to facilitate restorative sleep (Winrow & Renger, 2014). In turn, sleep loss has been associated with a decrease in the appetite suppressing hormone leptin, which generates the experience of satiety and halts food intake, accompanied by an increase in the appetite stimulating hormone ghrelin, which promotes continued consumption (Spiegel, Tasali, Leproult, Scherberg, & Van Cauter, 2011; Taheri, Lin, Austin, Young, & Mignot, 2004). These alterations are believed to contribute to metabolic imbalance and an intense appetite for high-carbohydrate food that results in increased BMI (Schmid, Hallschmid, Jauch-Chara, Born, & Schultes, 2008; Taheri et al., 2004). One might suggest that fluctuations in orexin functioning may in part be contributing to weight status via the modulation of sleep and the associated changes in the activity of hormones implicated in the regulation of energy balance. This suggests the presence of a feedback loop between hormones, sleep quality and energy balance.

It must be noted that the involvement of ghrelin and leptin activity was not evaluated in the current study. Furthermore, this study is cross-sectional in nature, and directionality cannot be determined without further analysis. Another limitation is that only women were included in the study and therefore findings cannot be extrapolated to men. Findings might also be partially influenced by the menstrual status of participants, which was not taken into account during data gathering. Animal studies have demonstrated that hormonal changes occur during the estrous cycle that are tightly synchronized with orexin function (Porkka-Heiskanen, Kalinchuk, Alanko, Huhtaniemi, & Stenberg, 2004) and sleep architecture (Schwierin, Borbély, & Tobler, 1998). In fact, a rise in plasma orexin-A concentrations takes place when women enter menopause, which has been associated with the parallel decline in estrogen (El-Sedeek, Korish, & Deef, 2010), and women in the early transition to menopause also show increased sleep difficulties such as insomnia (Bruyneel, 2015; Joffe, Massler, & Sharkey, 2010). The OB and MOB women in the present study were significantly older than the lean controls. Last, the classification of weight groups was based on the participants' BMI, which is the most universally used measure (WHO Consultation, 2000) but is limited as it is unable to distinguish between lean mass and body fat. The assessment of waist circumference and/or percentage body fat in addition to BMI may improve the accuracy of group categorization.

Nonetheless, as far as known, this is the first study to examine the connections between sleep impairment and orexin-A in obese humans without a sleep disorder. Discrepancies from the lean population were found in OB and MOB in terms of plasma orexin-A concentrations and both self-reported and actigraphy-based sleep.

An interaction between these variables also seems to influence fluctuations in BMI. These findings have several clinical implications. First, behavioral interventions that are currently being applied for the prevention and management of obesity have focused on lifestyle changes directed towards adopting a healthy diet and an increase in physical activity. This study indicates that the incorporation of healthy sleeping habits may also be beneficial. From a pharmacological perspective, although orexin receptor antagonists have been primarily used for sleep disorders such as insomnia (Winrow & Renger, 2014), the results obtained in this study suggest that their effect as sleep inducers might be valuable for the treatment of obesity. Moreover, these pharmacological and behavioral interventions may also aid the treatment of eating disorders such as the binge eating disorder, night time eating disorder and the sleep-related eating disorder. Poor sleep has been associated with increased binge eating behavior (Tzischinsky et al., 2000). Therefore, inducing appropriate sleep (by establishing a lifestyle change and/or the use of orexin receptor antagonists to stimulate sleep), might reduce the binge eating episodes as well as the inappropriate eating behavior that occurs during night time. A future step in research could be to examine the interaction between orexin-A and sleep quality in these eating disorders and how it might contribute to their treatment.

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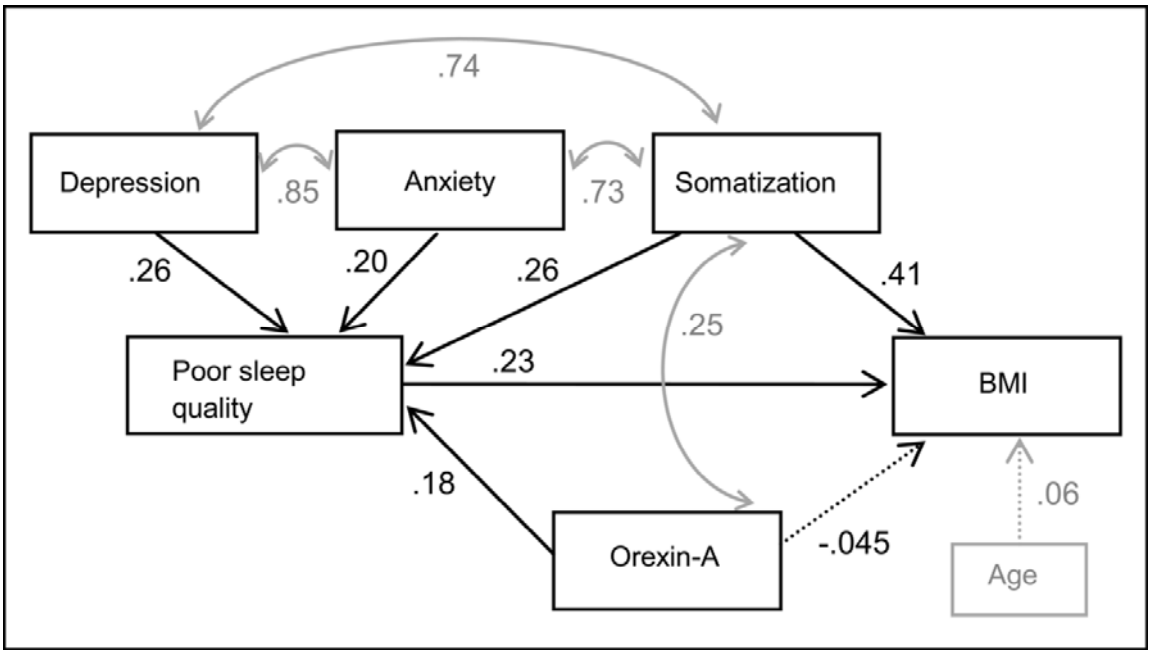
**Table 1.** Comparison of the main variables of the study between groups: ANOVA adjusted by age.

| | Means and SD | | | | Factor Group | | Polynomial Trends (*p*-val) | | Pairwise comparisons | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HC *n*=32 | | OB *n*=26 | | MOB *n*=40 | | | | HC versus OB | | | | HC versus MOB | | | | OB versus MOB | | | |
| | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | MD | *p* | *95%CI(MD)* | $\lvert d \rvert$ | MD | *p* | *95%CI(MD)* | $\lvert d \rvert$ | MD | *p* | *95%CI(MD)* | $\lvert d \rvert$ |
| Orexin-A | 2.87 | 0.69 | 3.42 | 0.92 | 3.30 | 0.94 | 3.11 | **.050*** | Linear **.042*** | Quad. .116 | -0.55 | **.028*** | -1.04 -0.06 | **0.68**† | -0.44 | **.042*** | -0.86 -0.02 | **0.53**† | 0.11 | .610 | -0.33 0.55 | 0.12 |
| PSQI: quality | 0.57 | 0.71 | 1.53 | 0.79 | 1.66 | 0.73 | 19.37 | **<.001*** | **<.001*** | **.023*** | -0.96 | **<.001*** | -1.38 -0.54 | **1.28**† | -1.09 | **<.001*** | -1.45 -0.73 | **1.52**† | -0.13 | .484 | -0.51 0.24 | 0.18 |
| PSQI: latency | 0.70 | 0.73 | 1.66 | 0.92 | 1.59 | 0.96 | 10.32 | **<.001*** | **<.001*** | **.017*** | -0.97 | **<.001*** | -1.47 -0.47 | **1.17**† | -0.89 | **<.001*** | -1.32 -0.46 | **1.04**† | 0.07 | .744 | -0.38 0.52 | 0.08 |
| PSQI: duration | 0.38 | 0.61 | 0.69 | 1.00 | 0.83 | 0.83 | 2.60 | .079 | **.025*** | .655 | -0.32 | .178 | -0.78 0.15 | 0.39 | -0.46 | **.025*** | -0.85 -0.06 | **0.63**† | -0.14 | .509 | -0.56 0.28 | 0.15 |
| PSQI: alterations | 1.04 | 0.25 | 1.77 | 0.61 | 2.05 | 0.60 | 32.62 | **<.001*** | **<.001*** | .073 | -0.73 | **<.001*** | -1.02 -0.44 | **1.56**† | -1.01 | **<.001*** | -1.25 -0.76 | **2.19**† | -0.28 | **.036*** | -0.54 -0.02 | 0.46 |
| PSQI: medication | 0.19 | 0.55 | 0.56 | 1.08 | 1.12 | 1.34 | 6.76 | **.003*** | **.001*** | .707 | -0.37 | .228 | -0.98 0.24 | 0.43 | -0.94 | **.001*** | -1.46 -0.42 | **0.91**† | -0.57 | **.042*** | -1.11 -0.02 | 0.46 |
| PSQI: dysfunction | 0.59 | 0.67 | 1.01 | 0.98 | 1.31 | 1.02 | 5.37 | **.007*** | **.001*** | .796 | -0.42 | .108 | -0.94 0.09 | **0.50**† | -0.73 | **.001*** | -1.17 -0.29 | **0.85**† | -0.31 | .191 | -0.77 0.16 | 0.31 |
| PSQI: efficiency | 0.10 | 0.30 | 0.72 | 0.99 | 0.79 | 0.91 | 6.84 | **.003*** | **.001*** | .163 | -0.62 | **.008*** | -1.07 -0.17 | **0.85**† | -0.70 | **.001*** | -1.08 -0.31 | **1.03**† | -0.08 | .704 | -0.48 0.33 | 0.08 |
| PSQI: total | 3.53 | 2.07 | 7.94 | 3.78 | 9.33 | 4.08 | 23.63 | **<.001*** | **<.001*** | .076 | -4.41 | **<.001*** | -6.38 -2.44 | **1.45**† | -5.80 | **<.001*** | -7.49 -4.11 | **1.79**† | -1.39 | .122 | -3.16 0.38 | 0.35 |
| Actigraph-based sleep time | 369.80 | 52.23 | 421.43 | 62.48 | 385.57 | 61.44 | 5.14 | **.031*** | .268 | **.002** | -51.63 | **.002*** | -84.37 -18.89 | **0.90**† | -15.77 | .268 | -43.86 12.33 | 0.28 | 35.86 | **.017*** | 6.43 65.29 | **0.58**† |
| SCL-90R: depres. | 0.67 | 0.52 | 1.36 | 0.90 | 1.70 | 0.78 | 15.95 | **<.001*** | **<.001*** | .337 | -0.69 | **.002*** | -1.11 -0.27 | **0.94**† | -1.03 | **<.001*** | -1.39 -0.67 | **1.55**† | -0.34 | .078 | -0.72 0.04 | 0.40 |
| SCL-90R: anxiety | 0.42 | 0.34 | 0.89 | 0.63 | 1.31 | 0.77 | 17.13 | **<.001*** | **<.001*** | .871 | -0.47 | **.010*** | -0.82 -0.12 | **0.92**† | -0.89 | **<.001*** | -1.19 -0.58 | **1.49**† | -0.42 | **.010*** | -0.74 -0.10 | **0.60**† |
| SCL-90R: somat. | 0.59 | 0.41 | 1.45 | 0.75 | 1.97 | 0.89 | 30.32 | **<.001*** | **<.001*** | .329 | -0.86 | **<.001*** | -1.27 -0.45 | **1.42**† | -1.38 | **<.001*** | -1.73 -1.03 | **1.97**† | -0.52 | **.006*** | -0.88 -0.15 | **0.63**† |

*Note.* SCL-90R: Symptom Check List questionnaire. HC: healthy controls. OB: obese. MOB: morbid obese. MD: mean difference. Depres: SCL-90R depression scale score. Anx: SCL-90R anxiety scale score. Somat.: SCL-90R somatization scale score.

*Bold: significant result (.05 level). †Bold: moderate to large effect size ($|d|$>0.50). Bonferroni's-Finner correction.

**Figure 1.** Structural equation model for the relationships between plasma orexin A levels, sleep quality, psychopathology symptoms and body mass index.




Continuous line: significant parameter Grey: covariates and co-variances between factors. (Higher scores in the sleep quality measure indicate poorer sleep)

***Table S1(supplementary):***

Structural equation model for the relationships between plasma orexin levels, sleep quality, psychopathology symptoms and body mass index (standardized coefficients).

| | B-standard | SE | z | p | 95%CI(B) | |
|---|---|---|---|---|---|---|
| PSQI: total sleep quality | | | | | | |
| Depression | 0.257 | 0.131 | 1.964 | .047 | 0.005 | 0.539 |
| Anxiety | 0.200 | 0.102 | 1.963 | .050 | 0.006 | 0.476 |
| Orexin-A | 0.184 | 0.074 | 2.480 | .013 | 0.039 | 0.329 |
| Somatization | 0.256 | 0.113 | 2.270 | .023 | 0.035 | 0.476 |
| *Constant* | *0.006* | *0.282* | *0.020* | *.982* | *-0.545* | *0.558* |
| BMI | | | | | | |
| PSQI: total sleep quality | 0.227 | 0.105 | 2.160 | .030 | 0.021 | 0.432 |
| Orexin-A | -0.045 | 0.085 | -0.340 | .735 | -0.194 | 0.137 |
| Somatization | 0.410 | 0.103 | 4.220 | <.001 | 0.232 | 0.635 |
| Age | 0.056 | 0.085 | 0.280 | .783 | -0.143 | 0.190 |
| *Constant* | *2.303* | *0.497* | *4.630* | *.000* | *1.329* | *3.278* |
| cov(Depres,Anx) | 0.851 | 0.028 | 30.480 | <.001 | 0.796 | 0.905 |
| cov(Depres,Somat) | 0.740 | 0.046 | 16.170 | <.001 | 0.650 | 0.829 |
| cov(Anx,Somat) | 0.732 | 0.048 | 14.960 | <.001 | 0.628 | 0.817 |
| cov(Orexin,Somat) | 0.254 | 0.096 | 2.450 | .014 | 0.046 | 0.421 |

## 4.6. STUDY 6: OREXIN AND SLEEP QUALITY IN ANOREXIA NERVOSA: CLINICAL RELEVANCE AND INFLUENCE ON TREATMENT OUTCOME

**Background:** Given the detected role of orexin neural pathways in the regulation of energy balance [118], the potential involvement of these neuropeptides in AN has been recently proposed [399,400]. However, the levels of serum orexin-A in patients with AN compared to those of healthy individuals is unclear given the lack of research [399,400]. Individuals with AN also report several sleep complaints, particularly in terms of sleep disturbances. As previous studies have shown a link between orexin-A, sleep and energy homeostasis [118], alterations in serum orexin-A may be implicated in the sleep difficulties reported by these patients.

**Objectives:** In this study we aimed to examine self-reported sleep quality and plasma orexin-A concentrations in patients with AN compared to healthy eating/weight controls, and to assess whether or not an interaction exists between serum orexin-A and sleep in this disorder. A third goal was to explore the possible influence of these factors on treatment outcome.

**Methods**: The participants in this study were 48 female patients with AN (BMI < 18.5 kg/m2) and 98 healthy weight/eating female controls (BMI = 18.5–24.9 kg/m2). During a preliminary evaluation, the PSQI was administered, along with the SCL-90-R [398] and the EDI-2 [392] to evaluate ED severity and general symptoms of psychopathology. Baseline orexin-A levels were obtained from blood samples collected between 08:00h and 09:00h after an overnight fast. After, all patients received a three-month psychotherapy treatment, and outcome was defined as “full remission”, “partial remission” and “poor outcome (no remission/dropout)”.

**Results:** Compared to the healthy controls, the patients reported greater sleep disturbances and poorer overall sleep quality ($p = .026$). There were no differences in baseline concentrations of plasma orexin-A levels, which were associated with sleep disturbances ($|r| = .30$), sleep inefficiency ($|r| = .22$) and overall poorer sleep ($|r| = .22$). With the use of SEM, an interaction between elevated orexin-A concentrations and poor sleep efficiency contributed to a poor treatment outcome.

# OREXIN AND SLEEP QUALITY IN ANOREXIA NERVOSA: CLINICAL RELEVANCE AND INFLUENCE ON TREATMENT OUTCOME

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**Journal:** Psychoneuroendocrinology
**Impact Factor:** 4.7

**Key words:** Anorexia Nervosa; Orexin-A; Sleep; Treatment Outcome; Partial hospitalization

**Abstract**

**Background and Aims:** Orexins/ hypocretins are orexigenic peptides implicated in the regulation of feeding behavior and the sleep/wake cycle. Little is known about the functioning of these peptides in anorexia nervosa (AN). The aims of the current study were to evaluate the extent to which orexin-A might be linked to sleep and treatment outcome in AN.

**Method:** Fasting plasma orexin-A concentrations were measured in 48 females with AN at the start of a day hospital treatment and in 98 normal-eater/healthy-weight controls. The Pittsburgh Sleep Quality Index was administered at the beginning of the treatment as a measure of sleep quality. Other psychopathological variables were evaluated with the Symptom Checklist-Revised (SCL90R) and the Eating Disorder Inventory-2 (EDI). Patients were assessed at the start and end of treatment by means of commonly used diagnostic criteria and clinical questionnaires.

**Results:** The AN patients presented more sleep disturbances and poorer overall sleep quality than did the healthy controls ($p=0.026$) but there were no global differences between groups in plasma orexin-A concentrations ($p=.071$). In the AN sample, orexin-A concen-trations were associated with greater sleep disturbances ($|r|=.30$), sleep inefficiency ($|r|=.22$) and poorer overall sleep ($|r|=.22$). Structural Equation Modeling (SEM) showed that both elevated orexin-A concentrations and inadequate sleep predicted poorer treatment outcome.

**Conclusion:** Plasma orexin-A concen-trations contribute to poor sleep quality in AN, and both OF these variables are associated with therapy response.

## 1. Introduction

Anorexia Nervosa (AN) is a severe eating disorder (ED) particularly prevalent in adolescent girls and young women (Lucas et al., 1991, 1999; Hoek and van Hoeken, 2003; Hudson et al., 2007). AN is characterized by inappropriate eating behaviour, an extreme pursuit of thinness, an intense fear of weight gain and a disturbance in body image (American Psychiatric Association, 2013). The syndrome yields numerous critical medical complications (Winston and Stafford, 2000; do Carmo et al., 2007; Misra and Klibanski, 2014). Some studies report that, compared to healthy controls (HC), AN patients display sleep disturbances (Lauer and Krieg, 2004; Pieters et al., 2004; Kim et al., 2010), including reduced slow wave sleep (SWS) and REM sleep, shorter sleep duration and poor sleep efficiency (Benca et al., 1992; Nobili et al., 1999; Marca et al., 2004; Kim et al., 2010). Other studies have failed to find sleep-related disturbances in AN (Lauer et al., 1988; Lauer, Krieg, Riemann, Zulley and Berger, 1990). Inconsistencies may be due to heterogeneity of patient samples and methodological differences across studies (Lauer and Krieg, 2004).

Some studies have linked sleep disturbances in AN to low body mass index (BMI) (Della Marca et al., 2004) and malnutrition (Delvenne et al., 1996). Malnutrition is known to have damaging effects in the functioning of several neurological networks, including some

involved in sleep behavior. For example, reduced connectivity strength and an increase in the characteristic path length of the thalamus have been identified in AN (Geisler et al., 2015). Additionally, thalamocortical circuitry is believed to play an important role in the regulation of sleep oscillations (Tsai et al., 2010). Another factor that might be implicated in the sleep disturbances reported by AN regards the clinical characteristics of eating disorders (e.g. drive for thinness, bulimic episodes and impulse regulation). Patients with ED who have sleep disturbances present more severe ED symptoms, such as drive for thinness and impulse regulation (Kim et al., 2010). Similarly, college students identified as having severely disturbed eating habits have also been found to sleep less than those with a more realistic body image (Makino et al., 2006).

Animal studies exploring the link between food deprivation and sleep have implicated nutrition-linked changes in plasma orexin (OX) concentrations (Lauer and Krieg, 2004; Ohno and Sakurai, 2008). OXs/hypocretins, consisting of Orexin-A (OXA) and -B (OXB), are 33- and 28- amino acid neuropeptides expressed in the lateral hypothalamic area (Sakurai et al., 1998). Situated downstream from the leptin regulatory pathway, OXs are believed to act as orexigenic peptides that signal hunger in response to limited food availability (Sakurai et al., 1998). Food restriction has been found to augment OXA expression in rodents (Pankevich et al., 2011). Fasting in non-obese humans results in a gradual increase in serum OXA, which normalizes with re-feeding (Komaki et al., 2001). In addition, OXs seem to be involved in the sleep/wake cycle, promoting wakefulness and arousal (Tsujino and Sakurai, 2013). Injection of OX has an overall stimulatory effect in the physical activity of rodents (Teske and Mavanji, 2012). Concurrently, OX deficiency/neuronal loss has been linked to narcolepsy, a sleep disorder characterized by the sudden intrusion of sleep and/or cataplexy and sleep attacks (Nishino et al., 2000; Ohno and Sakurai, 2008; Sellayah and Sikder, 2013; Tsujino and Sakurai, 2013).

Few studies have examined relationships among plasma OXA concentrations, nutritional status, and sleep processes in AN. Bronsky et al. (2011) found baseline plasma OXA concentrations to be elevated in AN compared to those of HC, while (Janas-Kozik et al., 2011) reported lower concentrations in their AN sample. Both studies showed plasma concentrations to decrease with re-feeding (Bronsky et al., 2011; Janas-Kozik et al., 2011). To our knowledge, no study to date has examined whether or not OXA is associated with the sleep disturbances in AN. In a related vein, the bearing of OXA concentrations upon outcome has not been previously studied.
The objectives of this study were to explore the relationship between OXA concentrations and sleep behaviour in AN and normal weight controls (HC), and to examine how OXA concentrations and sleep may be related to treatment outcome. Based on the available literature, we anticipated that higher plasma OXA concentrations would be associated with poorer sleep quality in both AN and HC, although we expected

sleep disturbances to be more pronounced in AN participants. Furthermore, we expected OXA concentrations to be associated with poorer sleep quality, and both to have a negative effect on treatment outcome.

## 2. Method

### 2.1. Participants

Participants in this study included 48 women with AN (BMI<18.5, kg/m$^2$) and 98 female HC (BMI=18.5-24.9, kg/m$^2$). The AN participants were diagnosed using DSM-IV-TR criteria (American Psychiatric Association, 2000) and were consecutively admitted female patients to the Day Hospital Treatment Program at the ED Unit of the University Hospital of Bellvitge (Barcelona, Spain). ED diagnoses were established via the face-to-face semi-structured clinical interview (SCID-I) (First et al., 1997). Mean age of all participants was 27.5 years (SD=8.2). Clinical and control groups did not differ as to age (control mean= 27.5 $\pm$7.9, AN mean= 27.2 $\pm$8.7, p=.84). A total of 15 AN patients were taking antidepressants and 14 benzodiazepine- anxiolytics /hypnotics.

Participants were excluded if their age was below 18 or above 60 years. Males were excluded from the study given the low prevalence of male AN patients and inclusion of this group might confound results. To be eligible for the HC group, participants had to be free of any ED history and to have a BMI between 18.5 and 30 kg/m$^2$. The physical and mental health of the HC participants was evaluated by means of the General Health Questionnaire-28 (GHQ-28) (Goldberg, 1978). HC participants were recruited via word-of-mouth and advertisements posted around university and hospital areas (CIBERobn Spanish Research Network). All participants gave written informed consent to participate in this Institutional Research Ethics Committee approved study, conducted in accordance with the Declaration of Helsinki.

### 2.2. Treatment protocol

After an initial assessment, the AN patients received treatment-as-usual, consisting of a 12-week manualized Day Hospital Program as previously described (Fernández-Aranda and Turón Gil, 1998; Custal et al., 2014), throughout which the patients participated in group therapy sessions covering both nutritional and symptom-related topics. Upon termination of the programme, the patients continue with outpatient follow-up sessions. Based on the DSM-IV-TR criteria, expert clinicians defined the outcome of the day hospital treatment as “full remission”, “partial remission” or “non-remission”. “Full remission” was ascertained when the patient reached a BMI above 18.5, did not present bingeing/purging behaviour nor anorexic symptoms (e.g. intense fear of weight gain or distorted body perception) for a continuous period of time (4 weeks) and showed an amelioration in the psychological state as measured by clinical questionnaires. “Partial remission” was established when there was a notable improvement in the ED symptoms, but residual symptoms were still present. Finally, “non-remission” or “poor outcome” was used to indicate patients who showed little or no

improvement in ED symptoms at the end of the treatment program or abandoned before its termination.

2.3. Measures

**2.3.1. Sleep.** The Pittsburgh Sleep Quality Index (PSQI: Buysse et al., 1989) was administered to explore subjective sleep quality and disturbances. This is a self-rated, 19-item questionnaire from which seven "components" of sleep are obtained: 1) subjective sleep quality; 2) sleep latency; 3) sleep duration; 4) habitual sleep efficiency; 5) sleep disturbances; 6) use of sleeping medication; 7) daytime dysfunction. Scores in each component range from 0 (no difficulty) to 3 (severe difficulty). The sum of the subscale scores yields a PSQI global score ranging from 0 to 21, with higher scores indicating poorer sleep quality. A global score over 5 is indicative of sleep disturbance. Test-retest reliability for the global score was of 0.85, and comparison between patients and control participants at the cut-off score of 5 shows a sensitivity of 89.6% and a specificity of 86.5% (Buysse et al., 1989). Internal consistency of the Spanish version of the PSQI was of (Chrombach alpha) 0.81 (Royuela and Macías, 1997).

**2.3.2 Orexin-A plasma concentrations.** Blood samples were collected from all participants between 0800h and 0900h after an overnight fast. Blood was drawn from an antecubital vein using a 10mL ethylenediaminetetraacetic acid (EDTA) containing BD Vacutainer® tubeSamples were centrifuged at 3130 g for 15 min at 4 °C. Plasma and serum were distributed in aliquots and stored at 80 °C until analysis. Several plasma biochemical variables were measured in duplicate. OXA/ Hypocretin-1 concentrations were measured with the EIA kit (Phoenix Pharmaceuticals, Inc., Burlingame, California, USA).

**2.3.3. Body Mass Index and Body Composition.** These parameters were assessed with the weighting instrument Tanita Multi-Frequency Body Composition Analyzer MC-180MA (Tanita Corporation, Tokyo, Japan), which calculates body fat and composition by means of bioelectrical impedance analysis. This device is repeatedly revised to meet the reference standards dual-energy X-ray absorptiometry (DEXA) (http://www.bl-biologica.es/tanita_tbf.htm) and has been validated against other weighing methods (Strain et al., 2008). Height was obtained with a stadiometer.

**2.3.4. Physical Activity.** The activity monitor Actiwatch AW7 (Actiwatch AW7®; CamNtech Ltd, Cambridge Neurotechnology, Cambridge, UK) was used to evaluate daytime physical activity intensity and the time spent in moderate-to-vigorous physical activity (MVPA), a potential confounding factor in this study. A detailed description of the instrument is available in Fernandez-Aranda et al. (2014). The Actiwatch AW4, an earlier version of the instrument, has been shown to be a reliable measure of PA (Routen et al., 2012).

**2.3.5. General psychopathology.** The Symptom Checklist-revised (SCL-90-R) (Derogatis, 1990) was used to explore psychological problems and symptoms of

psychopathology. This is a 90 item self-reported questionnaire answered in a 5-point Likert scale. The items are grouped into 9 primary symptom dimensions: 1) Somatization; 2) Obsession-Compulsion; 3) Interpersonal Sensitivity; 4) Depression; 5) Anxiety; 6) Hostility; 7) Phobic Anxiety; 8) Paranoid Ideation; and 9) Psychoticism as well as three global indexes: 1) the Global severity index (GSI) to indicate overall distress; 2) the positive symptom distress index (PSDI) to evaluate the intensity of the symptoms; 3) the positive symptom total (PST) to assess self-reported symptoms. The test has been validated in a Spanish population (Derogatis, 2002), with a mean internal consistency of 0.75 (Coefficient alpha). Given their well-known relatedness to sleep behavior (Mason and Harvey, 2014), in this study we focused on the depression and anxiety scales.

**2.3.6. Eating Disorder Psychopathology.** To examine eating disorder characteristics, the Eating Disorder Inventory-2 (EDI-2) (Garner, 1991) was used. This test, composed of 91 items, each rated on a six-point Likert scale, evaluates 11 dimensions of the cognitive and behavioral characteristics seen in people with EDs: 1) Drive for thinness; 2) Bulimia; 3) Body dissatisfaction; 4) Ineffectiveness; 5) Perfectionism; 6) Interpersonal distrust; 7) Interoceptive awareness; 8) Maturity fears; 9) Ascetism; 10) Impulse regulation; and 11) Social insecurity. This instrument was validated in a Spanish population (Garner, 1998) with a moderate mean internal consistency of 0.63 (Coefficient alpha).

## 2.4. Procedure

The AN patients received clinical and physical evaluations by experienced psychologists and psychiatrists during two structured face-to-face interviews. Self-report questionnaires providing information on general health were applied upon first evaluation. After the evaluation process, at the start of treatment, the PSQI was administered and the extraction of blood samples took place. All HC completed the self-reported questionnaires, including the PSQI, coinciding with the blood sample extraction.

## 2.5. Statistical Analysis

Stata 13 for Windows was used to conduct the analyses. First, sleep measures, plasma OXA concentrations and clinical characteristics (depression and anxiety symptoms, eating disorder levels) in AN and the HC were compared via the use of analysis of variance, with covariates adjusting for age and medication use. Cohen's *d* coefficient assessed the effect size for mean differences (a moderate effect size was considered for $|d|>0.50$ and high for $|d|>0.80$). Second, partial correlations ($r$) adjusted by the patient's age and use of medication were used to evaluate linear associations among OXA concentrations, PSQ sleep measures, EDI-total score and SCL-90-R depression and anxiety scores. A partial correlation between OXA concentrations and both daytime physical activity intensity and MVPA was also conducted to discard elevated physical activity as a possible confounding variable. Given the large simple size and

resulting high statistical power, small correlations tended to achieve statistical significance. For this reason, the interpretations of *r*-coefficients were based on the sizes : |r|<.20 for a poor relationship, .20<|r|<.30 for a moderate relationship and |r|>.30 as indicative of a good association. Structural Equation Models (SEM) were adjusted to evaluate the mediational pathway between OXA concentrations, sleep measures and treatment outcome (controlling for age and medication). The overall goodness-of-fit was evaluated via the $\chi^2$ test, the Root Mean Squared Error of Approximation (RMSEA), baseline comparison indexes (Comparative Fit Index CFI and Tucker-Lewis Index TLI) and the residuals' size (Standardized Mean Squared Residual SMSR). A good fit was considered for (Kline, 2010): a non-significant result ($p$>.05) in the $\chi^2$ procedure, RMSEA<0.10, CFI>0.90, TI>0.90 and SRMR<0.08.

## 3. Results

### 3.1. Comparison between AN patients and the HC on OXA concentrations, sleep quality parameters and relevant clinical measures

Table 1 shows the results of the ANOVA, adjusted according to the patients' age and use of medication, which compares OXA concentrations, sleep measures (PSQ scores), eating (EDI-2 total score) and depression/anxiety levels (SCL-90-R scores) between the diagnostic subtypes (AN versus HC). The results indicate that AN patients obtained statistically higher mean scores than the HC in depression, anxiety and total EDI-2, as well as sleep disturbances. Effect size for these mean differences ranged between moderate (|*d*|= 0.52 for PSQ-disturbances) and high (|*d*|= 1.41 for EDI-2 total). A statistically higher mean score was also achieved in the PSQ-total score by AN patients compared to HC, but the effect size for this mean difference was in the low range (*d*=0.42). AN patients and HC did not differ in terms of plasma OXA concentrations (*p*=.07, *d*=0.38).

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Table 1

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### 3.2. Association between OXA concentrations and sleep parameters.

Table 2 contains the partial correlations adjusted according to age and use of medication, evaluating the association between OXA and PSQ scores, stratified according to diagnostic subtype. In the AN group, OXA concentrations were strongly related to sleep disturbances (*r*=.303, the higher the OXA level the poorer the sleep quality), and moderately associated with sleep efficiency and total sleep quality. For the HC participants, a moderate correlation was found between OXA concentrations and the sleep latency scale. In both groups, OXA concentrations were not found to be related to time in MVPA (AN: p=.11; HC: p=.09) nor daytime physical activity intensity (AN: p=.11; HC: p=.46).

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Table 2

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3.3. Association between global sleep quality, depression and anxiety symptoms and eating disorder psychopathology.

Partial correlations, adjusted for age and medication show that among the AN patients higher scores on global sleep quality corresponded to elevated depression (r=.325), anxiety (r=.231) and eating-disorder symptoms (r=.207). Differently, in the HC, a high correlation was only found between global sleep quality and the SCL-90 depression score (the poorer the sleep measure the higher the depression score; r=.388).

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Table 3
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3.4. Pathway for the orexin level, sleep quality and treatment outcome

The treatment outcome distribution in the AN sample was: 39.6% total remission, 20.8% partial remission, 27.1% no-remission and 12.5% dropout. "No remission" and "dropout" categories were combined to produce a single "poor outcome" category. Figure 1 shows the pathway-diagram of the SEM evaluating the role of OXA concentrations and sleep measures on treatment outcome. The path-diagram in this study is composed of boxes (observed variables) and arrows (called paths, which connect some of the boxes). The connection of an arrow to another means that the first variable affects the second ($s \rightarrow d$ involves adding $\beta_{ks}$ to the linear equation for d, with $\beta_k$ called the path coefficient). A curved arrow-path states that there is a correlation to be estimated between the variables that are connected. Therefore, the path-diagram in Figure 1 provides the graphical image of the relationship pattern tested in the study. Fitting indices were into the good range ($\chi^2$=0.52; p=.470), RMSEA=.001; CFI=.999; TLI=.999, SRMR=.025) and the global predictive capacity was .149. Results show that the PSQ sleep efficiency is a mediator of the relationship between OXA concentrations and a poor therapy remission: high concentrations of OXA were associated with high scores in PSQ efficiency, and predicted a higher risk of dropout or no-remission. In the global model, OXA did not modify the scores in the PSQ duration-dysfunction scales, and did not have a direct effect on the risk of poor treatment outcome.

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Figure 1
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**4. Discussion**

The current study aimed to compare the relationships between OXA and sleep in AN patients to that in healthy normal-weight controls and to explore how these variables may be related to treatment outcome.

Differences between AN and the healthy controls were found in the self-reported experience of sleep quality; AN patients indicated overall poorer sleep quality and greater sleep disturbances, including waking up at night, feeling too hot, too cold or in pain during sleep. Global sleep complaints in AN have been reported in past studies ( Pieters et al., 2004; Kim et al., 2010), and may be partly linked to the ED symptoms (Makino et al., 2006; Kim

et al., 2010). The high comorbidity with depression and elevated anxiety present in ED (Blinder et al., 2006) which have been linked to impaired sleep (Taylor et al., 2005), might also be implicated. Corroborating this notion, compared to the controls, our AN patients evidenced greater depression, anxiety and eating-disorder symptoms, all of which are associated with an overall poorer sleep quality.

In our study, AN patients were found to have similar plasma OXA concentrations to those of the controls. This finding seems counter-intuitive, as fasting produces increases in plasma OXA concentrations in both animals (Pankevich et al., 2011) and healthy-weight humans (Komaki et al., 2001). With regard to the studies in AN, findings are incongruent; one study identified lower plasma OXA concentrations in the AN group compared to that of controls (Janas-Kozik et al., 2011), while a second study found plasma concentrations to be elevated in the AN group (Bronsky et al., 2011). To explain these discrepant findings, de Rijke et al. (2005) proposed that there may be other important factors rather than energy balance *per se* that are implicated in the regulation of OXA in AN. In fact, plasma OXA concentrations do not seem to be linked to BMI, or percentage of body fat (Bronsky et al., 2011). We also believe that impaired expression of OXA receptors may have played a major role in the greater sleep disruption of the AN group. It has been noted that OXA secretion may be regulated by energy status. Thus, OXA neurons are sensitive to both glucose and leptin; elevated circulating glucose levels lead to reduced firing of orexin neurons, suggesting that orexins are part of a negative feedback loop, and leptin inhibits food intake by suppressing orexin neuronal activity (Fernø et al., 2015). A possible hypothesis is that AN patients may have greater sleep impairment due to the dysregulation of OXA receptors secondary to chronic malnutrition.

In a similar manner to controls, where greater plasma OXA concentrations were associated with longer sleep latency, OXA concentrations and overall sleep quality of our AN patients were found to be related. In particular, the higher the OXA concentrations, the greater the sleep disturbances and poorer sleep efficiency. This was independent of the link between OXA and physical activity, evidenced to be related (Teske and Mavanji, 2012), given that OXA levels were not found to be associated with both time in MVPA and daytime physical activity intensity. With respect to treatment outcome, both elevated OXA concentrations and sleep inefficiency were also predictors of a poor outcome. The role of these factors is not yet clear, however, reduced SWS (Stage 3 and 4) has elsewhere been found to predict a longer time to recover from AN (Pieters et al., 2004). Pieters and colleagues (2004)proposed that this might be related to the changes in growth hormone (GH) release in AN. Another possible hypothesis is that inadequate sleep, which has been linked to daytime degraded emotional and constructive thinking skills (Killgore et al., 2008) might also hinder treatment responsiveness. Results obtained in the current study further suggest that OXA does not have a direct effect on treatment

outcome but might, instead, have an unfavorable influence through its association with diminished sleep efficiency. Further work is required to clarify such questions.

As far as known, ours is the first study to explore the relationship between sleep and OXA in AN patients. Elevated plasma OXA concentrations were found to be associated with poorer sleep, and OXA concentrations and poor sleep both seemed to have negative effects on the outcome of a Day Hospital Program. Despite its novelty, there are limitations in the current study that must be taken into account. Firstly, sleep was assessed via the use of the self-reported PSQI questionnaire. Secondly, the cross-sectional analysis of sleep and OXA does not permit directionality to be established. Finally, recent studies have shown that OXA has a dual origin. It was initially thought to be only synthesized by neurons located primarily in the perifornical area of the posterolateral hypothalamus, yet there is now evidence for the production of OXA in various human peripheral tissues (ganglion cells of the thoracic sympathetic trunk, myenteric plexuses and endocrine cells of the gastrointestinal tract, islet cells of the pancreas and syncytiotrophoblasts and decidual cells of the placenta) (Nakabayashi et al., 2003). In the case of this study, only peripheral OXA concentrations were measured but both sleep and appetite centers are in the brain and there is no direct proof that orexin enters the BBB in humans. However, we infer that it does on the basis of indirect data from animal studies (Kastin AJ, Akerstrom, 1999; Nobunaga et al., 2014; Volkoff, 2014). Until now, whenever a given peptide was shown to cross the BBB in rodents, the result was fully replicated, without any exception, in humans. Hence, we feel that our interpretation relies on a solid basis. Obviously the only way to demonstrate this is by performing an extraction of CSF to measure OX. Yet such invasive procedure was not in our protocol and Ethics Committee authorization. Despite these limitations, the findings obtained in this study suggest that both plasma OXA concentrations and sleep quality need to be taken into consideration in the treatment of AN.

## 5. Acknowledgment

Financial support was received from Fondo de Investigación Sanitaria -FIS (PI14/00290) and co-funded by FEDER funds/European Regional Development Fund (ERDF) a way to build Europe. CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn) and CIBER Salud Mental (CIBERSAM), are both initiatives of Instituto de Salud Carlos III (ISCIII), organization assigned to the Ministerio de Economía y Competitividad. Sarah Sauchelli is recipient of a pre-doctoral Grant (2013–17) by IDIBELL. José C. Fernández-García is recipient of a research contract from Servicio Andaluz de Salud (SAS) (B-0033-2014). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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**Table 1.** Comparison between the healthy-weight controls and AN patients in poor sleep quality, OXA levels and relevant clinical variables. (Higher scores in the PSQ sleep measures indicate poorer sleep).

| | Adjusted means (SD) | | | | Factor group | | | |
|---|---|---|---|---|---|---|---|---|
| | HC; (*n=98*) | | AN; (*n=48*) | | $F_{1;142}$ | *p* | MD | \|*d*\| |
| *SCL-90-scale:* | | | | | | | | |
| Depression symptoms | 0.63 | 0.44 | 1.05 | 0.87 | 12.05 | **.001*** | 0.42 | **0.616†** |
| Anxiety symptoms | 0.80 | 0.56 | 1.73 | 0.92 | 41.74 | **<.001*** | 0.93 | **1.226†** |
| *EDI-2 scale:* | | | | | | | | |
| EDI-2: total score | 27.50 | 20.54 | 74.29 | 42.25 | 56.37 | **<.001*** | 46.78 | **1.408†** |
| *Sleep measures:* | | | | | | | | |
| PSQ-quality | 0.87 | 0.77 | 1.20 | 0.99 | 3.367 | .069 | -0.33 | 0.375 |
| PSQ-latency | 1.17 | 0.88 | 1.16 | 1.07 | 0.000 | .983 | 0.01 | 0.004 |
| PSQ-duration | 0.42 | 0.67 | 0.68 | 0.83 | 2.576 | .111 | -0.25 | 0.335 |
| PSQ-disturbances | 1.10 | 0.36 | 1.37 | 0.62 | 7.393 | **.007*** | -0.27 | **0.523†** |
| PSQ-medication | 0.26 | 0.45 | 0.55 | 1.32 | 3.280 | .072 | -0.29 | 0.294 |
| PSQ-dysfunction | 0.91 | 0.68 | 1.17 | 0.91 | 3.002 | .085 | -0.26 | 0.328 |
| PSQ-efficiency | 0.31 | 0.58 | 0.38 | 0.78 | 0.290 | .591 | -0.07 | 0.109 |
| PSQ_total | 5.01 | 2.73 | 6.47 | 4.04 | 5.044 | **.026*** | -1.46 | 0.423 |
| OXA concentrations | 2.88 | 0.76 | 2.57 | 0.84 | 3.317 | .071 | 0.30 | 0.378 |

OXA: orexin- A; AN: anorexia nervosa; HC: healthy controls; PSQ: Pittsburgh Sleep Questionnaire. SD: standard deviation. MD: mean difference.
*Bold: significant mean difference comparison (.05 level).
†Bold: moderate (|*d*|>0.5) to high effect size (|*d*|>0.8).

**Table 2.** Correlations between orexin levels and sleep parameters in AN patients and HC. (Higher scores indicate poorer sleep).

| | HC | AN |
|---|---|---|
| PSQ-quality | .043 | .049 |
| PSQ-latency | **.273†** | .162 |
| PSQ-duration | .063 | .071 |
| PSQ-disturbances | .000 | **.303†** |
| PSQ-medication | .032 | .034 |
| PSQ-dysfunction | .024 | -.056 |
| PSQ-efficiency | .076 | **.221†** |
| PSQ_total | .142 | **.219†** |

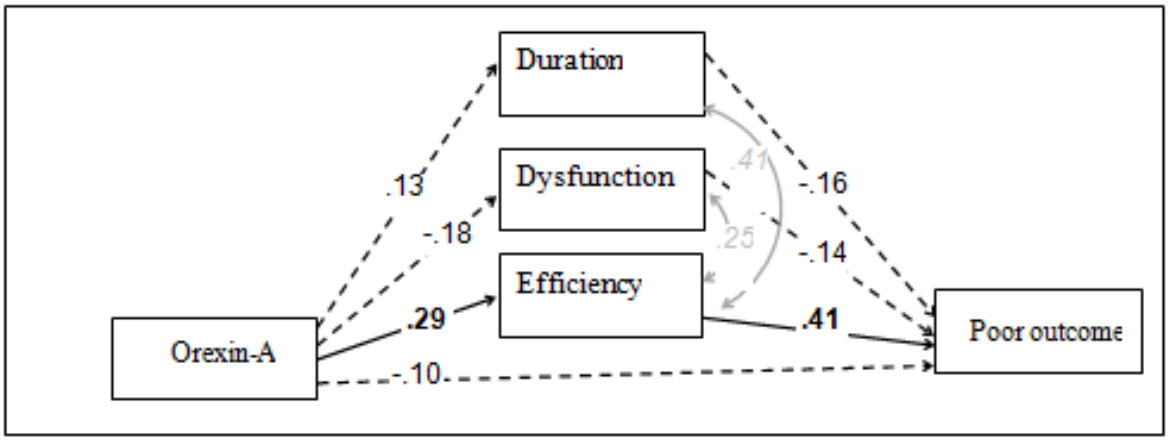
HC: healthy controls. AN: anorexia; OXA: orexin-A
†Bold: moderate (|*r*|>0.20) to high effect size (|*r*|>0.30).

**Table 3.** Correlations between clinical measures and global sleep quality (Higher scores indicate poorer sleep).

| | HC | AN |
|---|---|---|
| SCL-90: Depressive | **.388** | **.325** |
| SCL-90: Anxiety | .167 | **.231** |
| EDI: Total score | .085 | **.207** |

HC: healthy controls. AN: anorexia.
Bold: moderate ($|r|>0.20$) to high effect size ($|r|>0.30$).

**Figure 1.** Structural Equation Model (SEM) of OXA levels and sleep quality (duration/ dysfunction/efficiency) on a poor treatment outcome. (Higher scores in the sleep measures indicate poorer sleep). PSQ sleep efficiency is a mediator of the relationship between OXA concentrations and a poor therapy remission: high concentrations of OXA were associated with high scores in PSQ efficiency, and predicted a higher risk of dropout or no-remission.




Continuous line: significant parameter

# CHAPTER 5: GENERAL DISCUSSION

## 5.1. PHYSICAL ACTIVITY AND SLEEP IN EXTREME WEIGHT CONDITIONS

### 5.1.1. PHYSICAL ACTIVITY

Extensive multidisciplinary evidence has emphasized the importance of PA in weight control [287,288]. Furthermore, professionals and practitioners have pointed out the presence of problematic PA in some patients with AN [296]. As described in *Section 1.3.2.* studies vary in the definition of problematic PA employed, and there are several methodological shortcomings. One of the primary goals of **Studies 1 to 4** was therefore to explore different types of PA in individuals with OB and AN.

**Study 1** and **Study 2** revealed that there were no distinctions in the daytime PA of individuals with OB and MOB compared to a healthy-weight comparison group. Disparity was found between groups in the amount of MVPA; the participants with OB and MOB spent significantly less time in MVPA. These findings are consistent with those of previous studies whereby interventions promoting regular aerobic or concurrent training to facilitated weight loss in individuals with overweight or OB [288,290,401]. Furthermore, studies that have distinguished between PA levels have shown that the effects of PA on BMI are mostly due to PA of moderate to vigorous intensity whereas the relevance of constant light PA has been questioned, especially in women [6,402].

Differently, the PA profile of individuals with AN, as portrayed by the findings of **Study 3** and **Study 4**, seems to be more complex. Alike the case of OB, daytime PA was similar between the patients with AN and healthy participants. Yet, mean time in MVPA also did not significantly differ between groups. Of interest, a wide and uneven spread was found in the amount of MVPA of the patients. Although the majority of patients with AN presented slightly less MVPA in comparison to healthy individuals, 37% showed notably high levels of MVPA. Some studies have not found a difference in exercise patterns between AN and healthy controls [322,323], while others, alike in **Study 3**, have shown the existence of high exercise levels in some of these patients [306,308].

When the CET was administered in **Study 4** to evaluate the nature of exercise in EDs, results suggested that in AN, PA is less motivated by a compulsive urge to exercise than in bulimia nervosa (BN) and EDs not-otherwise-specified (EDNOS), but was highly similar to that of the heathy participants. The only discrepancy was that exercise for mood improvement was more relevant to the healthy controls than the patients. These findings are in line with the work by Bratland-Sanda and colleagues [403], who observed that MVPA when measured objectively via the use of an accelerometer was greater than that reported subjectively by the participants. The authors proposed that individuals with AN have a different understanding of MVPA [403], which implies that these patients may struggle to adequately recognise the compulsive cognitions underlying their exercise behaviour.

**Study 3** and **Study 4** also emphasize the need to develop an appropriate definition of problematic PA in AN. In **Study 3** several measures of were explored PA from a quantitative approach and using an objective instrument, whereas in **Study 4** a multidimensional and qualitative understanding of PA was examined by administering a self-reported instrument that focused on the compulsive nature of exercise. Depending on the definition employed, differences were obtained in results. When patients with AN and healthy participants are compared in the amounts of daytime PA and MVPA, findings suggested that patients engage in a modest amount of PA. Upon establishing a set threshold to identify high *versus* low exercise levels, over one third of patients were found to be excessively active. Nonetheless, the results obtained from the CET, indicated that exercise is not more compulsive in AN patients compared to a nonclinical population.

### 5.1.2. SLEEP

Sleep is a crucial biological process implicated in body energy restoration, metabolism, memory consolidation, and cognitive function. **Study 5** and **Study 6** demonstrated that sleep is disrupted in individuals with OB and AN.

An increasing number of studies are showing that short sleep and poor sleep efficiency are vulnerability factors for weight gain and adiposity [362,404]. Accordingly, the participants with OB and MOB in **Study 5** reported poorer sleep quality (e.g. sleep

alterations and inefficient sleep) compared to the healthy-weight comparison group. However, longer sleep duration was also found in the OB group compared to the MOB and control groups. This discrepancy from the literature may be due to the use of only a female sample. Sex differences have been identified in the relationship between sleep duration and OB [405,406]. In one study, sleep duration was found to be a predictor of BMI and the risk of overweight in males, but not in females [406], and other authors reported similar findings, adding that struggling to fall and/or maintain sleep indicative of diminished sleep was associated with BMI only in females [405]. These results suggest that sleep may influence weight in separate manners according to sex; in males sleep duration may be more important, while in females, as seen in **Study 5**, sleep quality may play a greater role on weight status. Two putative explanations have been proposed [406]. Firstly, the discrepancy may be associated with sex-specific hormones that are secreted during puberty. In males, an increase in muscle mass and a decrease in body fat mass occur due to the secretion of testosterone, growth hormone and insulin-like growth factor-1, whereas, puberty causes an augmentation of fat mass and circulating estrogen in females [407]. The hormonal changes could be influencing, in a gender-specific manner the effects of sleep on endocrine function, BMI and adiposity [406]. Alternatively, it is gender differences in sleep patterns (outside hormone change) that may be causing inter-sex variation in endocrine function. In line with the findings from **Study 5**, women have been found to have greater difficulties in falling asleep, more SWS, less non-REM (nREM) stage 1 and stage 2 sleep, day to day sleepiness, and are at greater risk of developing a sleep disorder [408]. Endocrine hormones regulated by sleep (see *Section 5.2.2.*) may thus be modulated differently in women versus men [406] and in turn also affects sleep. It must be noted, nonetheless, that in **Study 5** a male sample was not present for comparison, and in all three groups mean sleep times were below the recommended eight hours.

Regarding sleep in AN, consistent with previous findings [13,14,365,368], **Study 6** demonstrated that patients with AN experienced poorer sleep quality compared to their healthy counterparts, particularly reporting sleep disturbances (uncomfortableness and waking up at night). Studies with animals [409] and healthy humans [410] have shown an increase in arousal and a deterioration of sleep after a continuous period of food restriction. Although not examined in the current thesis, refeeding and weight gain seem

to improve sleep [368,411]. Early models suggested that arousal is an evolutionary response to conditions of food scarcity given that a state of arousal enables the animal to search for food despite low energy stores [412]. In support, complex endocrine processes have been identified that are highly implicated in the coordination of the energy balance/feeding and sleep-wake systems (e.g. orexin system [118]; see *Section 5.2.2.*). It was later evidenced that sleep disturbances may also be due to other cognitive-behavioural characteristics inherent of the pathology (see below).

### 5.1.3. CLINICAL CORRELATES

As portrayed by **Studies 3** to **6**, a connection exists between the characteristic patterns of PA and sleep in OB and AN and their profile in psychopathology. In conformity with the existing literature [308,309], the patients with AN who had a compulsive urge to exercise presented greater ED severity, specifically elevated body dissatisfaction, greater drive for thinness, poorer interoceptive awareness and more social insecurity (**Study 4**). Individuals with AN often report difficulties in social interactions and a heightened sensitivity to perceived negative criticism, particularly that concerning their body weight/shape [413]. Exercise becomes the means to control body weight/shape in order to achieve the "ideal body" promoted by the media. However, if the individual internalizes that physical appearance is the principal manner to obtain social approval, the individual might experience fear that an increase in weight gain from not exercising could result in criticism from others. As a consequence, exercise may turn into a ritualistic and compulsive behaviour. This unrealistic cognitive schema is particularly relevant in the initial phases of the disorder, when the individual is able to engage in exercise and PA can be used as an additional strategy to facilitate weight loss [1010]. However, it might become less important as the disorder progresses given that the weakening effect of prolonged energy deficiency on the body restrains the individual from being able to engage in PA. Both **Study 3** and other authors [330] have shown duration of the disorder to correlate inversely with PA. Furthermore, a recent study demonstrated that weight gain results in an increase in PA to the extent that after discharge patients become more active than healthy individuals [414].

Increasingly, PA is also considered to have a mood regulatory function in AN [222,307,403,415,416]. The antidepressant and anti-anxiety effects of PA have been observed in

studies with both healthy [274,417] and psychiatric [418] samples. **Study 4** demonstrated that those patients who had a stronger urge to exercise (as measured by the CET) also presented more severe depressive and anxiety symptoms. In **Study 3,** the patients who spent more time in MVPA had less severe depressive symptoms. Exercise may influence mood by serving the function of distracting the individual from unfavourable thoughts/stimuli, it can improve the subjective perception of self-efficacy, and it provides the opportunity to interact with others [419]. In AN, PA may therefore aid the individual escape from the obsessive thoughts related to the ED (e.g. food, body weight/shape concerns and inappropiate eating behaviour) and lower social insecurity. Exercise also has an effect on the synaptic transmission of monoamines [420] that play a critical role in depression [421,422], and provokes the release of endogenous opioids (i.e. endorphins) that influence the mood-related response to exercise [423,424]. However, the extent to which PA can be considered as a strategy to regulate mood in AN is unclear. As seen in **Study 4** and has been reported by other authors [318], exercise for mood improvement may be less important for patients with AN compared to healthy individuals. It has been argued that exercise in individuals with ED does not influence affective state by inducing positive feelings, rather it facilitates the inhibition of negative emotions [322,416]. Alternatively, it may be hypothized that individuals with AN rely principally on other behaviours inherent to the ED pathology (e.g. self-induced starvation) to regulate their emotions [425,426]; PA to regulate mood is a less-adopted strategy.

In OB, however, the symptoms of depression and anxiety (**Study 5**) [38,39] appear to be directly related to the insufficient MVPA seen in these individuals (**Study 1** and **Study 2**), which predisposes the individual to develop OB. When in OB, stigmatization and the internalization of a negative perspective of self can result in negative affect, low-self esteem, poor body image, psychological stress and depression [52,54]. Consequentially, the individual may become more inhibited and less willing to engage in PA, thus perpetuating the obese condition.

**Study 5** also demonstrated the involvement of general psychopathology in the sleep patterns observed in OB. The poorer sleep quality reported by the participants with OB and MOB was associated with increased depression, anxiety and somatization

symptoms. This relationship, however, is likely to be bi-directional [427]. Individuals with mood and anxiety disorders are 40% more likely to suffer from insomnia [428]. This might be attributed to the worry and rumination associated with the disorder that could be interfering with the individual's ability to enter a resting state. Alterations in biological systems could also be involved. For example, serotonin is strongly implicated in both the sleep/wake cycle [429] and depression [422]. In parallel, sleep problems appear to trigger an increase in the development of somatic complaints and symptoms of depression and anxiety [350].

The relationship between psychopathology and sleep can also be seen in AN (**Study 6**). In **Study 6**, the patients presented greater depressive, anxiety and ED symptom severity, and these were associated with poorer global sleep quality. Once again, a vicious cycle is probable, where greater symptoms of depression and anxiety are both primary and secondary to the reported sleep difficulties. Impaired sleep may be attributed to the strong comorbidity between AN and affective disorders, especially major depression [13]. Indviduals with AN may also experience sleep difficulties because of their ED psychopathology [13]. In line with the results obtained in **Study 5**, Kim and colleagues [13] found that those patients who reported sleep disturbances also presented more severe ED symptoms such as drive for thinness, bulimia, ineffectiveness, interpersonal distrust and social insecurity compared to those who did not. Rumination on topics associated with the disorder might impoverish the individual's ability to fall and maintain efficient sleep. This can become problematic because it might enhance the maladaptive and unrealistic thoughts [13], and could result in ED behaviours such as night time exercising.

### 5.1.4. TEMPERAMENT

It is widely acknowledged that personality plays an important role in PA habits [430]. This may be particularly relevant to OB. **Study 1** showed that individuals with OB and MOB have a temperament profile characterized by low novelty-seeking and significantly high harm avoidance and reward dependence. These findings are in agreement with those of previous studies. [141,143]. In **Study 1** high novelty seeking was found to be correlated with increased MVPA, wheras harm avoidance was linked to less MVPA. Individuals who are novelty seekers tend to be more sociable, open to new experiences, proactive, and are therefore more likely to be physically active [380,381,431]. As was seen in **Study 1**,

MVPA is associated with the activation of the endocannabinoid system, which plays an important role in the experience of reward [432]. In fact, the activation of this system with exercise may generate exercise-induced analgesia (where the physical effort of exercising is no longer noticed) and the described "runner's high": the experience of elation and happiness when exercising [378]. Therefore individuals with high novelty seeking might engage in PA to achieve this gratifying state. On the contrary, those individuals with traits of harm avoidance tend to be adverse to uncertainty and are highly sensitive to possible harm [139]. Hence, they may be less willing to participate in sports (especially those that are of high risk or physical contact). In conclusion, an individual who has a temperament profile characterized by low novelty seeking and high harm avoidance (as seen in OB) is less likely to engage in MVPA, and they could have a preference for more
sedentary activities that can lead to an increased likelihood of developing OB.

### 5.1.5. COGNITIVE PROFILE

Providing further evidence to the literature [147,148], **Study 2** showed that individuals with OB struggle with the decision-making capacity; they choose options from which to obtain immediate benefits despite future losses. PA is considered as an enhancer of cognitive function across all ages [433,434]. Physical fitness and aerobic exercise have been associated with superior functional brain connectivity and an increased volume in brain regions that are highly implicated in cognitive control (e.g. bilateral hippocampus, basal ganglia and PFC) [435], as well as better spatial memory and improved performance in several tasks requiring executive function [435,436]. Additionally, PA has beneficial effects on the preservation of function against age-related cognitive decay [437,438] and in conditions of mild cognitive impairment [434]. This led to the hypothesis that PA could also be related to the difficulties in cognitive performance tasks experienced by individuals with OB and MOB.

**Study 2** demonstrated that the association between low MVPA and difficulties in executive function is somewhat present in OB. In support, a correlation has been found between OB and PA in school-aged children that is linked to academic achievement [439]. Engaging in MVPA may be beneficial for improving executive control as well as

depressive and anxiety symptoms. However, the relationship between PA and cognition is reciprocal. Self-regulation is a key factor in the maintanence of continued PA [440]. Executive functions such as working memory, inhibitatory control (both in terms of behaviour inhibition and selective attention), planning, scheduling and cognitive flexibility facilitate self-regulation, which enables the individual to set goals (e.g. a PA program) and adapt to changing environments in order to sustain those goals over a long period of time [440,441]. Accordingly, individual differences in executive skills appear to influence the adherence to a long-term exercise intervention [442]. Given the difficulties in decision making observed in participants with OB and MOB in **Study 2**, it may be hypothized that these individuals have poor self-regulatory skills [147], thus struggling to engage in the recommended amount of weekly MVPA.

As seen from **Study 2**, irisin may be one of the factors influencing the cognitive profile of individuals with OB; an inverse association was found between plasma concentrations of irisin and the performance of individuals with OB in decision making. This may occur due to its actions on neurotransmitters such as gamma-aminobutyric acid (GABA) and BDNF. Evidence has shown the presence of irisin in GABAergic cells [387], and alterations in the GABAergic system may result in cognitive impairment [443]. Additionally, the role of irisin in the expression of the UCP1 may be regulated by the peroxisome proliferator-activated receptors (PPAR)γ coactivator-1 α (PGC1α) [132], where an increased expression of PGC1α correlates with augmented circulating levels of irisin. PGC1α is a transcriptional regulator of mitochondrial function that has been linked to neurological defecits and cerebral anomalies [444,445]. In **Study 2** we found that circulating levels of irisin were elevated in the obese groups in comparison to the lean control group. Hence, a possible hypothesis could be that altered function of irisin might be contributing to the poorer decision making abilities of individuals with OB.

## 5.2. NEUROENDOCRINE MECHANISMS UNDERLYING PHYSICAL ACTIVITY AND SLEEP IN EXTREME WEIGHT CONDITIONS

### 5.2.1. ENDOCANNABINOIDS

Given its involvement in energy metabolism and the physiological responses to exercise [377,378,446], the endocannabinoid system appears to play an important role in the relationship between PA and BMI. **Study 1** provides support for this notion by showing that MVPA is directly linked to increased plasma concentrations of AEA and OEA, which in turn are associated with fluctuations in BMI. Both AEA and OEA are N-acylethanolamides (NAEs) that are synthesized from the hydrolysis of N-arachidonoyl phosphatidylethanolamine (NAPE) and are degraded by the fatty acid amide hydrolase (FAAH). An augmented activity of FAAH in the lymphocytes has been observed in physically active adults [447]. The effects of these chemical compounds on BMI however seem to be contradictory; AEA was found to correlate positively with BMI, whereas an inverse association was observed between OEA and BMI. Mimicking the effects of the marijuana plant on the experience of reward, endocannabinoids such as AEA stimulate mesolimbic dopamine transmission in the nucleus accumbens, which plays a key role in the experience of reward and addiction [448,449]. Via this mechanism, endocannabinoids enhance the rewarding properties of food (i.e. increasing palatability), thus augmenting the individual's vulnerability to continued food intake and the development of OB [126]. Differently, OEA (as an endocannabinoid-related compound) has an anorectic effect; it stimulates vagal signals of satiety to encourage the termination of food intake [450,451].

The involvement of the endocannabinoid system in energy balance has led to the development of several first generation CB1 receptor antagonists (e.g. Rimonabant) as potential pharmacological treatments for OB by inhibiting appetite [452]. However, although a few clinical trials have reported CB1 receptor blockers as effective anti-OB drugs [453–455], these were later retrieved from the market due to evidence of psychiatric side effects such as depression and anxiety [456]. MVPA may be an alternative strategy to promote the beneficial actions of the endocannabinoid system for the management of OB.

## 5.2.2. METABOLIC HORMONES AND OREXIN-A

Further than their involvement in energy homeostasis [118], research has also emphasized the role of orexins in the sleep/wake cycle [119,457], suggesting an interplay between altered orexin function, impaired sleep and fluctuations in BMI. This was explored in **Study 5** and **Study 6**.

The circadian process is a biological system believed to be regulated by the superchiasmatic nucleus (SCN) of the hypothalamus, which regulates sleep timing over 24 hour periods via the activation and deactivation of processes that regulate sleeping behaviour. It has been shown that orexins, being released in the hypothalamus, may contribute to the activation of wake-promoting systems in order to inhibit sleep-promoting systems and prevent sudden sleep [457]. Continued analysis of orexin activity in squirrel monkeys has demonstrated that plasma orexin concentrations are low upon awakening, elevated throughout the day (reaching peak at 18:00 hr) and decrease drastically at night time [458,459]. The most dense projections of orexin neurons are in the locus coerouleus, ventral tegmental area, laterodosal tegmental nucleus, pedunculopontine tegmental nucleaus, arcuate nucleus, dorsal raphe and tubermammilary nucleus, thus influencing the noradrenalinergic, serotoninergic, dopominergic, histaminergic and aetylcholine pathways [460–463] (see *Figure 6*). Anomalies in these systems result in altered sleep and increased arousal [462]. Orexin neurons therefore must be "switched-off" in order to achieve consolidated nREM sleep and the muscle atonia seen during REM sleep, accompanied by some bust discharges occurring during phasic REM sleep [463]. This may occur via several mechanisms.

In their role in homeostatic signalling, orexins in the lateral hypothalamic area receive input from peripheral metabolic cues such as ghrelin, leptin and glucose [462–464](see *Section 1.1.6.5*.). The decrese of serum concentrations of glucose have been found to depolarize and increase the frequency of orexin neuron action potential, while the opposite occurs when concentrations are augmented [465]. Differently, leptin inhibits the signalling of orexin neurons [412,466], and fasting results in a decrease in circulating leptin followed by the activation of orexin neurons [467]. GABAergic projections from the preoptic area to the lateral hypothalamic area also oppose and inhibit orexin neuron action [462,463]. Finally, despite receiving excitatory signals from orexins, both

noradrenalin, dopamine and serotonin hyperpolarize to inhibit orexin neurons [412,468,469]. In support, two-hour sleep deprivation leads to changes in the effects of noradrenalin from the excitation to inhibition of orexin activity, possibly to induce the experience of sleepiness when there has been a lack of sleep [470].

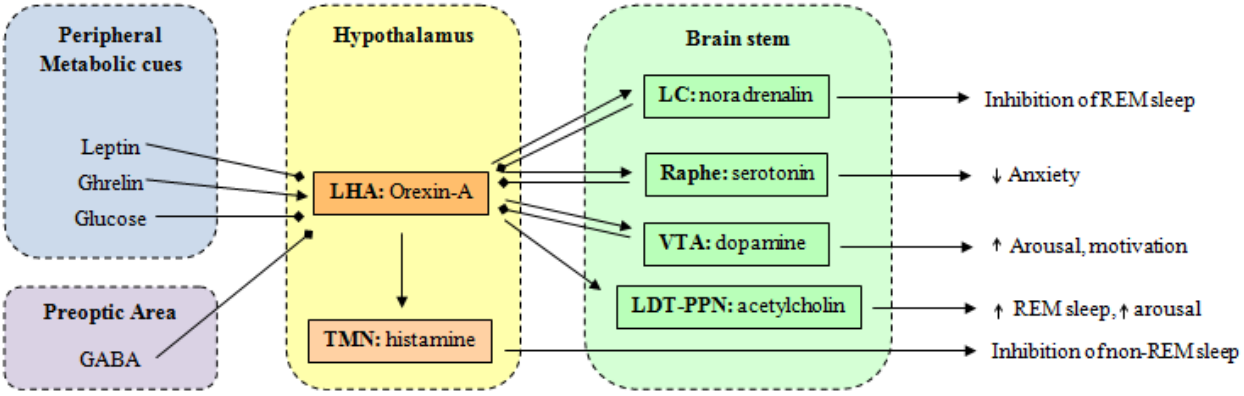



**Figure 6.** *The role of orexin-A in the regulation of sleep; adapted from Sakurai [462]. Arrows are indicative of excitatory pathways, whereas lines ending with a square represent inhibitatory effect. GABA: Gamma-aminobutyric acid; TMN: Tuberomammillary nucleus; LC: Locus coerolus; VTA: Ventral tegmental area; LDT-PPN: Laterodorsal tegmental and pedunculopontine nuclei.*

As demonstrated by **Study 5**, circulating orexin-A seems to be elevated in OB. Additionally, high orexin-A concentrations were associated with poor sleep quality. These findings are in line with those of previous studies that have examined individuals with narcolepsy or the effects of genetically ablated orexin neurons in mice [118]. Following the model developed by Sakurai [462] (*see Figure 6*), the augmented levels of orexin-A in OB might be over-activating the "wake-promoting" systems, and this is resulting in the sleep disturbances reported by these individuals. In support, basal dopamine levels are increased in severe OB, and individuals with overweight and mild OB display higher levels of phasic dopamine concentrations compared to lean individuals [471].

Furthermore, **Study 5** shows that the sleep disturbances observed in OB may be mediating the role of orexin-A in the development of OB. A possible mechanism is that

orexin-induced changes in sleep patterns might be influencing the function of hormones responsible for signaling energy homeostasis. The endocrine system is another biological process that is regulated by the SCN of the hypothalamus and is believed to be aligned with the sleep/wake cycle [472]. Circulating levels of endocrine hormones also oscillate throughout the circadian period in a time-dependent manner. For example, an increase in plasma concentrations of glucose and/or insulin occurs in the early morning before wakening, which contributes to the experience of morning hunger [473–476]. Differently, serum levels of leptin are higher throughout the night in order to inhibit hunger and facilitate rest [477]. Studies, however, have found that disturbances in habitual sleep patterns have a negative impact on endocrine function, which consequentially can impair metabolism [472]. A modification of habitual sleep/wake patterns was found to result in a decrease in leptin levels [478]. Similarly, Spiegel and collegues [479] showed that when sleep was reduced to 4 hours daily, leptin levels were inhibited and there was a simultaneous increase in ghrelin. An impaired function of these peptides results in alterations in metabolism and the experience of hunger and appetite for high-carbohydrate-containing foods despite homeostatic satiety [479–481]. An overconsumption of food (including binge eating) may occur as a consequence, thus inducing weight gain. Therefore, the increase in circulating orexin-A could be associated with impaired sleep, which in turn negatively alters endocrine function and induces weight gain.

However, animal studies whereby the effects of orexin-A administration have been examined in relation to OB have obtained highly incongruent results. On the one hand, consistent with the role of orexin-A in the stimulation of hunger (possibly mediated by sleep disturbances, **Study 5**), studies have found that the intraventricular injection of orexin-A in the hypothalamus generates an increase in food intake [118,482]. On the other hand, the administration of orexin-A has also been found to increase energy expenditure (both via more spontaneous PA (SPA) and nonexercise activity thermogenesis (NEAT), thus suggesting that orexin is a protective peptide against the development of OB [118,482]. Interestingly, however, Novak and colleagues [483] examined the effects of microinjections of orexin-A within the paraventricular nucleus of the hypothalamus on SPA and NEAT in both diet-induced obese versus OB-resistant rats. These authors found that after a high-fat diet, there was a significant increase in SPA and NEAT in the OB-resistant mice that compensated for the excess fat intake, whereas in the diet-

induced obese rats NEAT remained the same and SPA decreased [483]. Obese and OB-prone rats may have an altered endogenous orexin-A tone due to a chronic elevated release of orexin [484], which may result in a reduced sensitivity to the SPA-activating effects of brain orexin-A. In support, the ability of orexin-1 receptor antagonist to reduce food intake is elevated in OB-prone rodents [484], and in **Study 5** the basal plasma concentrations of orexin-A in the participants with OB were higher compared to those in the lean controls. The mechanisms underlying the regulating role of orexin-A on SPA and food intake are highly complex, and the effects on these functions may be differential and possibly dependant of the brain region [483]. In addition, the extent to which animal findings can be applied to humans is questionable, and thus far human studies examining the implication of orexins in food intake and weight gain have primarily assessed cases of orexin neuron deficiency such as narcolepsy. Individuals with narcolepsy have presented an increased likelihood of OB, reduced food intake and a fall in PA [118]. Finally, in **Study 6**, we did not find plasma concentrations of orexin-A to be associated with MVPA or daytime PA intensity in neither the patients with AN nor healthy controls, implying that the actions of orexin on human PA may be different to those observed in animals.

The interactions between orexin-A, sleep and BMI have important implications for the management of OB. Given the effect of orexin function on sleep, orexin receptor antagonists have been developed as pharmacological treatments for sleep disorders. Among these are Almorexan and Suvorexant, which have completed phase III of clinical trials [485,486] and are considered as effective in the treatment of insomnia [397,487,488]. More recently, Suvorexant has been proposed as a drug that might aid the treatment of metabolic diseases, as the administration of Suvorexant in mice at resting phase ameliorated hepatic glucose metabolism [489], although, as the author points out, these findings cannot be directly extrapolated to humans. Nonetheless, by promoting sleep, orexin-A receptor antagonists could inhibit appetite and facilitate weight control in individuals with OB. Research in humans is lacking.

Alike in OB, plasma concentrations of orexin-A also appear to be related to the sleep difficulties reported by individuals with AN (**Study 6**); higher plasma concentrations of orexin-A were associated with poorer sleep-efficiency. Interestingly, however, in **Study**

**6** the plasma concentrations of orexin-A in the patients with AN did not differ from those of a healthy weight/eating comparison group. This seems paradoxical given the extreme energy deficiency characteristic of this disorder, and fasting generally seems to result in an increase in circulating orexin-A in both animals [490] and healthy weight humans [491]. While the literature and the findings obtained in **Study 5** suggest that circulating orexin-A are closely associated with energy homeostasis, this does not seem to be applicable to AN. In fact, De Rijke and colleagues [492] proposed that energy balance *per se* is not as relevant in AN as might be expected, rather it is likely that there are other biological processes regulating orexin-A in this ED. In support, an earlier study that examined plasma orexin-A in AN found that the circulating concentrations of this peptide did not fluctuate according to BMI nor percentage body fat [399]. There may be an impaired expression of orexin receptors in AN, and a dysregulation of metabolic signals due to malnutrition could be altering expected actions of orexin on food intake. This, however, is only a tentative hypothesis given the lack of research examining orexin-A in AN.

## 5.3. PHYSICAL ACTIVITY AND SLEEP IN A MODEL FOR BODY MASS INDEX

Numerous models have been developed to demonstrate the complexity of OB and AN that integrate social, psychological and biological factors. Based on the findings obtained from the studies in this thesis, the model below is proposed (*Figure 7*) that attempts to bring together some of these factors in order to present the mechanisms underlying the role of PA and sleep in BMI from a multifaceted perspective.

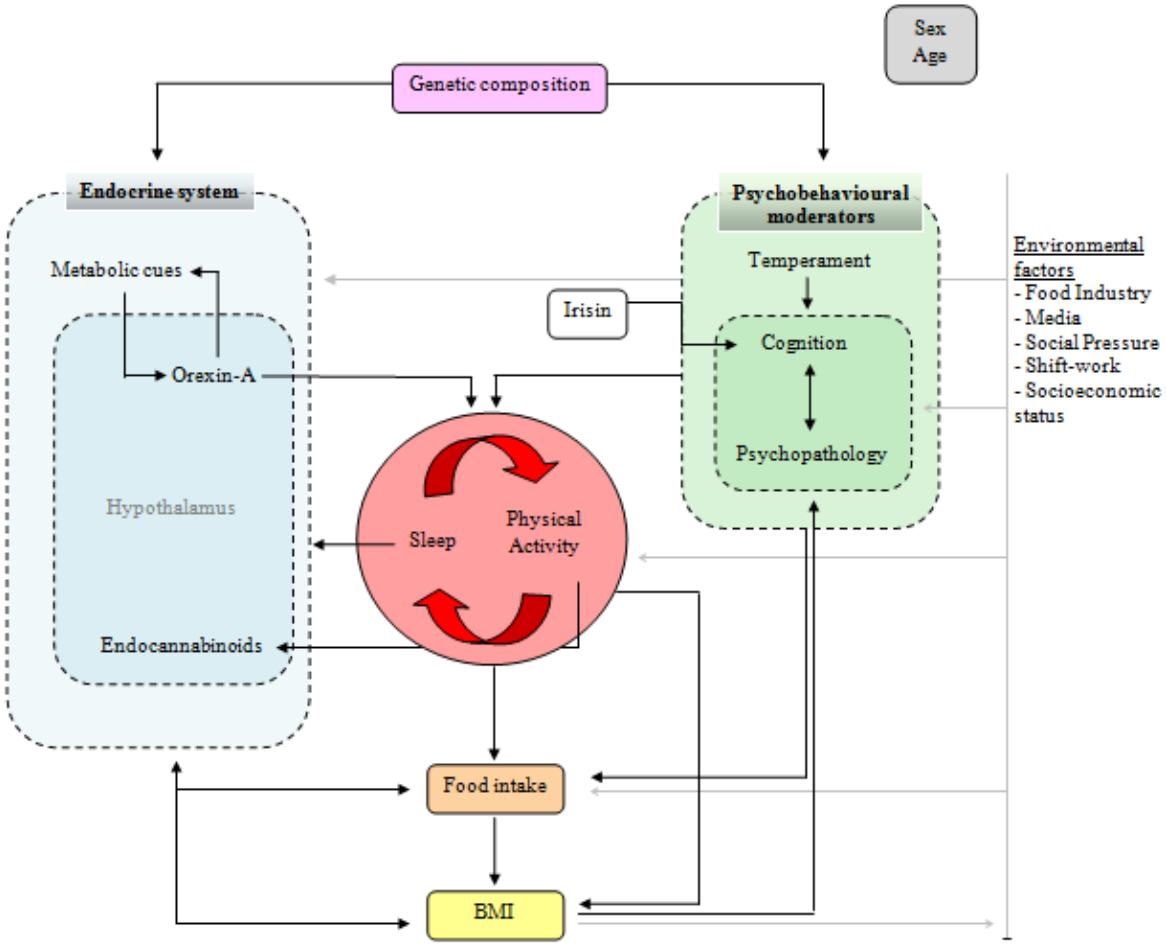



***Figure 7.*** *Psychobiological model of PA and sleep in BMI*

### 5.3.1. CIRCULAR INTERACTION BETWEEN PHYSICAL ACTIVITY AND SLEEP

A close interaction is visible between PA and sleep behaviours [493]. In adults, acute exercise seems to have a positive influence in total sleep time, SWS, sleep onset latency, sleep efficiency and wake time after sleep onset, and it reduces both REM sleep

and overall sleep disturbances [493]. Furthermore, engaging in regular exercise has been found to have moderate-to-large beneficial effects on sleep quality across all ages [493,494], and small-to-moderate effects on total sleep time, sleep efficiency and sleep onset latency [493]. For this reason PA may be useful in the treatment of sleep disorders such as insomnia [495]. Differently, a delay in habitual sleep timing has been found to increase the time spent in sedentary behaviour and decrease time in MVPA [496]. Reducing sleep time by 2.3 hours has been reported to result in a 31% decrease in total PA, a 24% fall in MVPA, reduced vigor, and an increase in sedentary behaviour [497]. The effect of sleep restriction appears to be stronger in regular exercisers than non-exercisers [497]. Accordingly, the participants with OB in the studies presented in this thesis demonstrated both low MVPA (**Study 1** and **Study 2**) and poorer sleep quality (**Study 5**).

There are several pathways through which MVPA may influence BMI. As is well known, PA is the main source of energy expenditure; engaging in sufficient MVPA can compensate for energy intake in order to maintain energy balance. Furthermore, PA uses fat rather than carbohydrates as fuel, which delays the development of abdominal OB. In support, it has been shown that physically active adults are able to maintain energy balance in an *ad libitum* diet comprising 40% fat intake, but when sedentary, their consumption of fat cannot exceed 20% to maintain energy balance [498]. Long-term exercising also results in an increase in glucose metabolisation [499]. As stated previously, after its discovery in the skeletal muscle, irisin was proposed to have a mediatory role in the link between PA and BMI due to its actions in the browning of white adipose tissue [131,132]. However, later studies did not find the expected association between irisin and PA, rather a correlation was identified with resting energy expenditure [135,500]. In active individuals PA also acts as a regulator of appetite (enhanced appetite sensitivity) in order to maintain energy balance; an incrementation of food intake takes place proportional to the increase in PA [501,502]. Yet, low PA appears to have a paradoxical effect; instead of inhibiting appetite, it promotes energy consumption and a significant increase in fat mass [502]. It has therefore been postulated that physical inactivity might also contribute to OB by dysregulating appetite and as a consequence facilitating energy intake and generating energy imbalance [502,503]. Finally, as demonstrated in **Study 1**, MVPA may augment circulating concentrations of AEA and OEA, which in turn

influence BMI; AEA appears to promote weight gain, while OEA seems to have the opposite effect.

Regarding sleep, poor sleep may result in inappropriate food intake and body weight through its influence on the peripheral metabolic hormones themselves. Sleep deprivation has been found to lead to the suppression of circulating leptin levels (hindering satiety signalling) and increasing ghrelin levels (stimulating hunger) [481,504]. As expected, these changes in metabolic signalling result in an raised appetite for food high in fat and carbohydrates [480,481]. Sleep disturbances may also influence the individual's ability to follow a healthy lifestyle [505]. A national poll found that individuals with sleep difficulties report greater irritability, tiredness and stress [506]. Poor daily function may make it more difficult for the individual to follow the recommended diet and amount of regular MVPA.

In a bi-directional manner, diet may in itself influence sleep, contributing to the sleep difficulties reported by both individuals with OB and AN. The consumption of a diet high in fibre and protein has been associated with reduced stage 1 sleep, fewer wake episodes and arousal and greater SWS, a diet high in carbohydrate has been linked to a shorter sleep latency, whereas a diet consisting of food with high saturated fat and sugar content has been found to induce a decrease in SWS and more frequent nighttime arousals [507,508]. Thus, the preference for foods high in saturated fat, carbohydrates and sugars resulting from impoverished sleep, may in turn contribute to further sleep disturbances and weight gain.

### 5.3.2. ENDOCRINE SYSTEM

Genetic anomalies may affect the biosynthesis of several neuropeptides and prohormones in endocrine tissue. An example is the proprotein convertase 1 (PCSK1) gene. GWAS have demonstrated common variants of the PCSK1 gene that are associated with OB [509]. PCSK1 encodes the prohormone convertase 1/3 (PC1/3), which plays a key role in activating cleavae of peptide hormone precursors that are involved in the regulation of food consumption and energy homeostasis [510,511]. The expression of PC1/3 is especially strong in the arcuate nucleus of the hypothalamus, specifically in the

POMC-expressing neurons and both NPY- and AgRP-expressing neurons that are highly sensitive to the actions of leptin [512,513]. It is also involved in the biosynthesis of orexin within the hypothalamus [514]. Alike orexin, NPY and AgRP are hunger-stimulating neuropeptides that increase appetite and simultaneously inhibit energy expenditure [515,516]. In addition, PC1/3 is the sole prohormone convertase implicated in the cleaving of proghrelin and proinsulin within the stomach [517] and pancreas [518,519] respectively. Given the role of PC1/3 in the synthesis of multiple endocrine hormones [510], polymorphism in the PCSK1 gene could influence endocrine function and promote weight gain. In support, human studies have found important increases in circulating proinsulin when there is a PCSK1 deficiency [520,521]. Similarly, leptin gene polymorphism (LEP c.-2548 G>A) has been associated with elevated plasma levels of leptin and BMI in individuals with OB [522].

Impared function of peripheral metabolic signalling alters the activation/inhibition of orexin neurons and their actions on energy balance via the effects of sleep, arousal and PA. Elevated concentrations of orexin-A in plasma results in sleep difficulties (**Study 5** and **Study 6**), possibly due to its effects on monoamine neurotransmitter pathways such as the serotonergic and dopaminergic pathways [462,463]. In a feedback manner, abnormal sleep patterns desynchronize the circadian regulation of metabolic signalling [472]. An increase in food intake and BMI due to poor sleep induces further alterations in peripheral hormone function: hormone sensitivity to changes in energy homeostasis is inhibited (hormone resistance) resulting in damaged vagal signalling [62,104,108]. This signifies that the individual will not receive signals of satiety even though further energy intake is no longer required. Furthermore, although it is unclear the extent to which orexin-A may affect PA, the findings obtained in **Study 5** suggest that humans with OB have an impaired sensitivity to the claimed effects of orexin-A on SPA, thus resulting in an an increased energy intake that is not compensated by sufficient MVPA.

### 5.3.3. PSYCHOBEHAVIOURAL MODERATORS

Attempts have been made to identify some of the genetic variants that might be responsible for differences in temperament. For example, polymorphism in the BDNF gene (BDNF Val66Met) has been associated with novelty seeking and harm avoidance [523]. Specifically, individuals with the 66Met+/A1+ variant have been found to score

lower in novelty seeking and higher in harm avoidance compared to non-carriers [523]. As demonstrated in **Study 1**, this temperament profile is characteristic of OB, and the Val66Me polymorphism has also been detected in these individuals [524]. However, meta-analyses of GWAS indicate that the data is inconsistent and it is too early to attribute differences in temperament to specific genetic variants [525,526]. Nonetheless, consistent with Cloninger's [527] psychobiological model of personality, twin studies have shown that there is a genetic contribution to an individual's temperament [528,529].

The temperament profile presented by the individuals with OB in **Study 1** may be having a direct negative impact on the individual's attitude towards MVPA, delineated by an adversity towards participating in sports or following a daily exercise schedule (see *Section 5.1.4.*). Furthermore, the temperament trait reward dependence has been associated with grey-matter volume in parts of the lateral PFC [530], a brain region implicated in value-based decision-making [531]. Similarly, greater activation of the subgenual ACC during a response-inhibition task is required by individuals with increased scores in harm avoidance [532]. The ACC plays a key role in the ability to make optimal decisions by integrating both costs and rewards and learning from previous errors [533,534]. Given this evidence, a relationship between temperament and executive function may be proposed, where variations in harm avoidance and reward dependence are associated with an individual's ability to make appropriate decisions. Impaired decision making, where the individual with OB struggles to delay reward even though this action would be beneficial in the long-run (**Study 2**), could influence the individual's ability to follow healthy sleep, diet and MVPA patterns (see *Section 5.1.5.*).

Both temperament and cognition are risk and maintenance factors of psychopathology. For example, high harm avoidance is linked with elevated symptoms of depression, anxiety and somatization [535–537]. Similarly, a maladaptive loop has been identified between decision making and psychopathology, where individuals in a depressed or anxious state tend to make biased decisions that are followed by actions that may increase the severity of the symptoms [538]. In a study examining temperament and eating styles across extreme eating/weight conditions (AN, healthy controls, OB), harm avoidance was found to be linked to emotional and restrained eating, novelty seeking correlated with external eating, and persistence was also associated with restrained

eating [539]. Emotional eating was more frequent in the obese group compared to the other groups (associated with BED), whereas restrained eating was characteristic of the AN group [539]. Therefore, a specific temperament profile and impaired executive functioning interact with elevated symptoms of psychopathology that, as described in *Section 5.1.3.*, and seen in **Studies 3** to **5**, can alter both sleep and time in MVPA. Psychopathology also has an effect on BMI due to its influence on eating behaviour. Weight gain often provokes an increase in both general symptoms of psychopathology (e.g. depression and anxiety) and ED-related symptoms (e.g. body dissatisfaction), which can be largely attributed to the emphasis of the media on the "thin ideal" and social pressure from surrounding others. Another maladaptive behavioural circle is therefore generated.

# 5.4. PHYSICAL ACTIVITY AND SLEEP ON THE OUTCOME OF A COGNITIVE-BEHAVIOURAL TREATMENT FOR ANOREXIA NERVOSA

## 5.4.1. PHYSICAL ACTIVITY

PA in the treatment of AN has been traditionally viewed skeptically given the influence of exercise in the aetiopathology of the disorder and the medical complications that often emerge. Treatments have therefore primarily focused on limiting PA. However, in **Study 3** we observed that patients with AN spent a similar amount of time in MVPA to a nonclinical population, and our findings suggest that a certain amount of MVPA may have a positive effect in reducing the likelihood of poor treatment outcome. This is in line with authors that have promoted the use of supervised exercise for weight gain [295].

There are several ways through which the incorporation of a structured exercise program in current treatments may be beneficial. First, supervised PA may facilitate the recovery from physiological damage that occurs as a consequence of chronic starvation (e.g. reduced bone mineral density and bone fractures) [394]. Second, research has shown that starvation in AN is often used as a strategy for emotion regulation (see *Section 5.1.3*). Healthy PA could become an alternative method for affect regulation. Third, PA also improves cognitive capacity [435,436], which has been found to be altered in AN (see *Section 1.2.5.5.*). Amelioration in reasoning abilities might help patients challenge their maladaptive cognitions. Finally, difficulties in social interactions are frequently reported by individuals in the acute stage of AN [413]. Adjusted group activities that promote teamwork can aid patients improve social skills such as communication, adaptability and trust.

Despite the potential usefulness of supervised PA in the treatment of AN care needs to be taken. PA programs must be adapted to the individual's singular psychological and physiological condition. Accordingly, centres who have introduced PA as part of the intervention, authorize patients to engage in PA when they reach an established body weight, and modify the permitted duration and intensity of exercise according to the patient's body weight [295].

### 5.4.2. SLEEP

As shown in **Study 6**, poor sleep efficiency may be particularly problematic in the treatment for AN. The involvement of sleep in the outcome has been previously reported [368]; SWS is a predictor of a faster time needed for weight recovery. It has been proposed that this may be through the effects of sleep on the growth hormone [368]. Growth hormone release occurs immediately after sleep onset and continues to increase for the following four hours, reaching its peaks during non-REM sleep [339]. In AN, elevated growth hormone levels in the acute stage of the disorder have been associated with remission [540]. Sleep disturbances in AN could be inhibiting growth hormone release, which is then influencing treatment. Alternatively, sleep disturbances might be influencing treatment outcome via its effects on cognitive function. Inadequate sleep can result in weakened attention abilities, poor interpersonal functioning and emotional instability [541,542], which could hinder treatment progress.

# CHAPTER 6: LIMITATIONS

Despite the novelty of the studies presented in the thesis, and the usefulness of the findings to obtain a greater understanding of the role of PA and sleep in extreme weight conditions and the benefits it may provide to improve existing interventions, some limitations need to be considered.

1) The studies were conducted with female samples. Males have been found to show distinct PA [543] and sleep [408] patterns. Future studies should therefore evaluate the interactions presented in male participants.

2) Assessment was primarily cross-sectional in nature. Although this presents associations between examined variables, it does not permit causality to be determined.

3) Some of the factors explored were self-reported (e.g. sleep quality, compulsive exercise cognitions, temperament), which may be vulnerable to bias. Objective techniques should be considered in the future. The use of the accelerometer Actiwatch provided the opportunity to evaluate additonal measures of sleep (e.g. sleep time) and PA (daytime PA, MVPA, PA profile) in an objective manner, although it may not be sufficient to place the instrument in the non-dominant wrist as lower-body movement may not have been accurately captured.

4) Blood sampling was peripheral and not central. Although animal studies have shown the presence of both irsin [387] and orexin-A in the cerebellum [118], it is unknown whether these peptides can cross the blood-brain barrier in humans. However, thus far, when a given peptide has been found to cross the blood-brain barrier in animals, the mechanism has been fully replicated in humans. It must be noted that the only way to determine if this applies to orexin-A and irisin is through an extraction of celebrospitnal fluid, however, for ethical considerations this was not carried out.

5) The participants with AN and OB were individuals actively seeking treatment. The results obtained may not reflect the PA and sleep patterns in non-treatment seeking individuals.

# CHAPTER 7: MAIN FINDINGS AND CONCLUSIONS

Given the rapidly increasing worldwide prevalence of OB and AN, and the medical complications and economic costs associated with these conditions, there is an urgent need to understand the underlying mechanisms in order to develop efficient intervention strategies. The current thesis aimed to understand the role of PA and sleep. Below are the main findings and conclusions.

1) Individuals with OB and MOB do not present differences in daytime PA compared to lean individuals, but spend a considerably less amount of time in MVPA (**Study 1** and **Study 2**). Differently, there is a large variability in the PA patterns of patients with AN **(Study 3)**. Daytime PA and MVPA is similar to that of healthy eating/weight individuals in the majority of patients. However, a third of patients with AN appear to be remarkably active. The Compulsive Exercise Test also seems to indicate that exercise in patients with AN is less compulsive compared to that of patients with other EDs, and alike that of healthy individuals **(Study 4)**. Yet, it is unclear whether compulsive exercise is truly less prevalent in patients of AN, or these patients are unaware of the underlying nature of their exercise behaviour.

2) Patients with AN report impoverished sleep qualtity, especially sleep disturbances **(Study 6)**. Individuals with OB also indicate several sleep difficulties, although sleep duration measured with a sleep monitor is longest in those individuals with OB compared to individuals with healthy weight or MOB **(Study 5).**

3) Both ED severity and elevated symptoms of general psychopathology are associated with poorer sleep quality (**Study 5**), less MVPA (**Study 1** and **Study 3**), and a reduced urge to exercise (**Study 4**). Additionally, in OB symptoms of somatisation appear to be related to an increased BMI (**Study 5**). In AN, the duration of the disorder, associated with deteriorated physical fitness, could be hindering the individual's ability to engage in intense exercise (**Study 3**).

4) The personality profile of individuals with OB, characterized by elevated harm avoidance (inhibition) and low novelty-seeking (decreased impulsivity), may contribute to the difficulties faced by these individuals in engaging in exercise and

achieving the recommended weekly amount of MVPA (**Study 1**). Furthermore, individuals with OB present deficient decision-making abilities, whereby they tend to select options that generate immediate rewards despite future losses (non-optimal decision-making) (**Study 2**). This was found to be inversely associated with plasma irisin concentrations and time spent in MVPA.

5) Activation of the endocannabinoid system may be one of the processes via which MVPA influences BMI. Alterations in the plasma concentrations of certain endocannabinoids have been identified in OB. Exercise may therefore promote the effects of endocannabinoids on energy balance (**Study 1**).

6) A clear association between augmented serum orexin-A and sleep difficulties seems to be present in both individuals with OB and AN (**Study 5** and **Study 6**). The augmented serum orexin-A detected in individuals with OB suggests that there is a resistance to metabolic changes in conditions of excess adipose tissue and weight (**Study 5**). This may be altering sleep patterns, which in turn promotes food intake and weight gain despite energy homeostasis. Regarding AN, the absence of a difference in plasma orexin-A levels between these patients and healthy individuals indicates an anomaly in the function of orexin-A in AN (**Study 6**). A tentative explanation is that the secretion of orexin-A may be altered by the chronic malnutrition, but research is needed to confirm this hypothesis.

7) In AN, a supervised program of MVPA adapted according to the physiological and psychological status of each patient may be a useful additional intervention for improving the outcome of a standard treatment for AN (**Study 3**). Promoting healthy sleep habits may also have beneficial effects on the patients' responsiveness during psychotherapy sessions (**Study 6**).

Alterations in PA habits and sleep are highly relevant features of OB and AN. They seem to contribute to the aetiology, development and maintenance of these conditions by interacting with impaired endocrinological function and a distinctive psychobehavioural profile. Further research is therefore essential to deepen our understanding of the mechanisms underpinning PA and sleep patterns in order to develop and/or improve strategies for the prevention and treatment of these EWC.

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# APPENDIX I: COMPULSIVE EXERCISE TEST - Spanish translation

**Instrucciones**

A continuación hay una serie de afirmaciones relacionadas con hacer ejercicio. Por favor lea atentamente cada afirmación y redondee el número que mejor indique con cuál se identifica más. Por favor responda **todas** las preguntas con la mayor honestidad que le sea posible.

| Nunca | Raramente | A veces | A menudo | Normalmente | Siempre |
|---|---|---|---|---|---|
| **0** | **1** | **2** | **3** | **4** | **5** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1) | Me siento más feliz y/o más positivo/a después de hacer ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |
| 2) | Realizo ejercicio para mejorar mi apariencia. | 0 | 1 | 2 | 3 | 4 | 5 |
| 3) | Me gusta que mis días estén organizados y estructurados, en los que el ejercicio es sólo una parte. | 0 | 1 | 2 | 3 | 4 | 5 |
| 4) | Me siento menos ansioso/a después de hacer ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |
| 5) | Encuentro que el ejercicio es como una obligación. | 0 | 1 | 2 | 3 | 4 | 5 |
| 6) | Si siento que he comido demasiado, hago más ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |
| 7) | Mi patrón semanal de ejercicio es repetitivo. | 0 | 1 | 2 | 3 | 4 | 5 |
| 8) | No realizo ejercicio para estar delgado/a. | 0 | 1 | 2 | 3 | 4 | 5 |
| 9) | Si no puedo hacer ejercicio me siento bajo/a de ánimo o deprimido/a. | 0 | 1 | 2 | 3 | 4 | 5 |
| 10) | Me siento extremadamente culpable si me pierdo una sesión de ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |
| 11) | Normalmente continúo realizando ejercicio a pesar de una lesión o enfermedad, a menos que esté muy enfermo/a o demasiado lesionado/a. | 0 | 1 | 2 | 3 | 4 | 5 |
| 12) | Disfruto haciendo ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |
| 13) | Realizo ejercicio para quemar calorías y perder peso. | 0 | 1 | 2 | 3 | 4 | 5 |
| 14) | Me siento menos estresado/a y/o menos tenso/a después de hacer ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |
| 15) | Si me pierdo una sesión de ejercicio, trato de compensarlo la próxima vez que haga ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |
| 16) | Si no puedo realizar ejercicio me siento intranquilo /a y/o irritable. | 0 | 1 | 2 | 3 | 4 | 5 |
| 17) | El ejercicio mejora mi humor. | 0 | 1 | 2 | 3 | 4 | 5 |
| 18) | Si no puedo realizar ejercicio, me preocupa que pueda ganar peso. | 0 | 1 | 2 | 3 | 4 | 5 |
| 19) | Sigo una rutina fija durante mis sesiones de ejercicio, p.ej. caminar o correr la misma ruta, ejercicios concretos, la misma cantidad de tiempo, etc… | 0 | 1 | 2 | 3 | 4 | 5 |
| 20) | Si no puedo realizar ejercicio, me siento enfadado/a y/o frustrado/a. | 0 | 1 | 2 | 3 | 4 | 5 |
| 21) | No disfruto haciendo ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |
| 22) | Siento como si me dejara/abandonara si me pierdo una sesión de ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |
| 23) | Si no puedo hacer ejercicio me siento ansioso/a. | 0 | 1 | 2 | 3 | 4 | 5 |
| 24) | Me siento menos deprimido/a o bajo de ánimo después de hacer ejercicio. | 0 | 1 | 2 | 3 | 4 | 5 |

# APPENDIX II: CURRICULUM VITAE

**Sarah SAUCHELLI TORAN, Msc.**

**Address:** Carrer Saragossa, 95, 4º 11º, 08006, Barcelona, Spain
**Birth date:** June 14, 1990
**Nationality**: Spanish/Italian
**E-mail:** sarah.sauchellit@bellvitgehospital.cat

Current Position

| | |
|---|---|
| 2013- | **University Hospital of Bellvitge**, Barcelona, Spain.<br>Associate researcher<br>Field: Eating disorders and obesity. Gambling disorder. |

Education

| | |
|---|---|
| 2013- | **University of Barcelona**, Barcelona, Spain<br>PhD, (expected termination December 2016)<br>Dissertation: Physical activity and sleep in Extreme Weight Conditions: the involvement of endocrinological and psychopathological and their effect on treatment outcome. |
| 2012-2013 | **University of Barcelona**, Barcelona, Spain.<br>Msc. Research on Cognition and Behavior<br>Grade: Excellent |
| 2011-2012 | **University Ramon Llull**, Barcelona, Spain.<br>Msc. Health Psychology and Psychotherapy<br>Grade: Excellent |
| 2008-2011 | **University of Bristol**, Bristol, United Kingdom<br>Bsc. Experimental Psychology<br>Grade: First Class |

Professional Experience

| | |
|---|---|
| 01-04/2016 | **University of Tübingen**, Tübingen, Germany<br>*Collaborating international researcher*<br>Field: Review of relapse prevention treatments for anorexia nervosa. |
| 2013 - | **University Hospital of Bellvitge**, Barcelona, Spain<br>*Assistant psychologist:* Psychometric evaluation and clinical assessment of patients with a possible diagnosis of an eating disorder. |
| 2012- 2016 | **Fundaciò Vidal I Barraquer**, Barcelona, Spain.<br>*Collaborating researcher* |

Field: Treatment outcome of a psychoanalytic therapy with psychiatry patients and domestic violence victims.

2019-2011 **University of Bristol**, Bristol, United Kingdom
*Research assistant to a visually impaired Msc student*
Field: Effects of visual impairment in counseling.

Grants

2013- 2017 **Bellvitge Biomedical Research Institute**, Barcelona, Spain
Predoctoral internship grant

2016 **CIBER Fisiopatología de la Obesidad y Nutrición – CB06/03 (CIBERobn).** Madrid (Spain)
Research travelling grant

Publications

**Sauchelli S.**, Arcelus, J., Granero R., Jiménez-Murcia S., Aguera Z., Fernandez-Aranda F. (2016). Dimensions of compulsive exercise across eating disorder diagnostic subtypes and the validation of the spanish version of the Compulsive Exercise Test. Frontiers in Psychology, 7:1852 **(IF:2.46; Q1)**.

**Sauchelli S.,** Jiménez-Murcia S., Fernández-García JC; Garrido-Sánchez, L., Tinahones, FJ., Casanueva FF., Baños R., Botella C., Crujeiras AB., de la Torre R., Fernández-Real JM; Frühbeck G., Granero R., Ortega FJ., Rodríguez A., Zipfel S., Giel KE., Menchón JM., Fernández-Aranda F**.** (2016) Interaction between orexin-A and sleep quality in females in extreme weight conditions. European Eating Disorders Review, 24(6):510-517 (**IF:2.9; Q1)**.

Fagundo AB., Jiménez-Murcia S., Giner-Bartolomé C., Agüera Z., **Sauchelli S.,** Pardo M., Crujeras AB., Granero R., Baños R., Botella C., de la Torre R., Fernández-Real JM., Fernández-García JC., Frühbeck G., Rodríguez A., Mallorquí-Bagué N., Tárrega S., Tinahones FJ:, Rodríguez R-., Ortega F., Menchón JM., Casanueva FF., Fernández-Aranda F. (2016) Modulation of itisin and physical activity on executive functions in obesity and morbid obesity. Scientific Reports, 6: 30820. **(IF:5.23; Q1)**

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Oral Communications

**Sauchelli S**., Jiménez-Murcia S., Sánchez I., Custal N., Tinahones FJ., Baños RM., de la Torre R., Granero R., Tarrega S. "Orexin-a and sleep in anorexia nervosa: clinical relevance and influence on treatment" 14th meeting of the European Council on Eating Disorders (ECED) 2015 Meeting. 20-22 Novembre 2015. Heidelberg (Germany).

**Sauchelli S**., Arcelus J., Sánchez I., Riesco N., Giner-Bartolomé C., Islam M., Woltz I., Jiménez-Murcia S., Menchón JM., Fernández-Aranda F. *"Involvement of physical activity in anorexia nervosa and treatment outcome: Analysis of prediction"* XXX Event of Behavioural Therapy and Conduct Medicine in the Clinical Practice. 26 March 2015. Barcelona (Spain).

**Sauchelli S**, Arcelus J, Sánchez I, Riesco N, Jiménez-Murcia S, Granero R, Gunnard K, Baños R; Botella C; de la Torre R; Fernández-García JC; Fernández-Real JM; Frühbeck G; Gómez-Ambrosi J; Tinahones FJ; Casanueva FF; Menchón JM, Fernandez-Aranda F *"Physical activity in Anorexia Nervosa: How relevant is it to therapy response?"* X Congress of the Spanish Association for the study of Eating Disorders (AEETCA)

Poster Presentations

**Sauchelli S.,** Jiménez-Murcia S., Sánchez I., Riesco N., Custal N., Fernández-García JC., Garrido-Sánchez L., Tinahones FJ., Steiger H., Israel M., Baños RM., Botella C., de la Torre R., Fernández-Real JM., Ortega FJ., Frühbeck G., Granero R., Tárrega S., Crujeiras AB., Rodríguez A., Estivill X., Beckmann JS., Casanueva FF., Menchón JM., Fernández-Aranda F. *"Orexin-a and sleep in anorexia nervosa: clinical relevance and influence on treatment"*. International Congress on Eating Disorders, 4-7 May 2016. San Francisco (U.S.A).

**Sauchelli S**., Arcelus J., Sánchez I., Jiménez-Murcia S., Granero R., Riesco N., Menchón JM., Fernandez-Aranda F. *"Physical activity and depression in anorexia nervosa: relevance for treatment outcome"*. International Congress on Eating Disorders, 4-7 May 2016. San Francisco (U.S.A).

**Sauchelli S**., Arcelus J., Riesgo N., Jiménez-Murcia S., Granero R., Botella C., Fernández-Real JM., Frühbeck G., Casanueva FF. *"Physical activity and depression in anorexia nervosa: relevance for treatment outcome"*. 14th meeting of the European Council on Eating Disorders (ECED) 2015 Meeting. 20-22 November 2015. Heidelberg (Germany).

Fernandez-Aranda F., **Sauchelli S.,** Arcelus J., Sanchez I., Jimenez-Murcia S., Granero R., Riesco N., Menchon JM. *"Physical activity and depression in anorexia nervosa: relevance for treatment outcome"*. XXIst Annual Meeting of the Eating Disorders Research Society. 17-19 September 2015. Taormina (Italy)

**Sauchelli S**., Fernández-Aranda F., Pastor A., González ML., de la Torre R., Granero R., Jiménez-Murcia S., Baños R., Botella C., Fernández-Real JM., Fernández-García JC., Frübeck G., Gómez-Ambrosi J., Rodríguez R., Tinahones FJ., Arcelus J., Fagundo AB., Agüera Z., Miró J., Casanueva FF. *Moderate-Vigorous Physical Activity across Body Mass Index in Females: Moderating Effect of Endocannabinoids and Temperament.* Biomedical Network Research Centre in Physiopathology of Obesity and Nutrition - Symposium CIBEROBN- 2014, 20-22 November 2014, El Escorial, Madrid (Spain).

Research Projects

**Research Project (FIS), Instituto de Salud Carlos III.** Cortico-limbic connectivity in patients with altered emotion regulation: the modulatory and development factors of personalized therapies based on functional neuroimaging (FIS: PI13/01958). Centre: Department of Psychiatry, University Hospital of Bellvitge, Barcelona. Support period: 2014-2017. IP: Carles Soriano-Mas.

**Research Project (FIS), Instituto de Salud Carlos III.** Neurocognition and emotion regulation in extreme weight conditions: Study of cerebral activity and changes associated to an intervention based on a therapeutic videogame (PI14/00290). Centre: Department of Psychiatry, University Hospital of Bellvitge, Barcelona. Support period 2014-2017. IP: Fernando Fernández-Aranda.

**CIBER Fisiopatología de la Obesidad y Nutrición- CB06/03 (CIBERobn)**. Instituto Carlos III - Ministry of Health. Center: Department of Psychiatry, University Hospital of Bellvitge, Barcelona. Support period: 01.2007- . IP: F. Fernández Aranda. (http://www.ciberobn.es/)

**Predimed-Plus.** Effect of an intensive lifestyle intervention with an energy-restricted mediterranean diet, increased physical activity, and behavioural treatment on the

primary prevention of cardiovascular diseases: The predimed-plus randomized controlled trial (Instituto Carlos III). Centre: University Hospital of Bellvitge, Barcelona. Support period: 2013-. PI: Jordi Salas-Salvadór, MD. (http://www.predimedplus.com/)

Teaching Experience

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| 2016- | **University of Barcelona**, Barcelona, Spain<br>Subject: Psychology Lab.<br>Degree: Bachelor in Molecular Medicine |
| 2014-2015 | **University of Barcelona**, Barcelona, Spain<br>Subject: Psychology Lab.<br>Degree: Bachelor in Podology |
| 2014-2015 | **University of Barcelona**, Barcelona, Spain<br>Subject: Psychology Lab.<br>Degree: Bachelor in Molecular Medicine. |
| 2013-2014 | **University of Barcelona**, Barcelona, Spain<br>Subject: Psychology Lab.<br>Degree: Bachelor in Podology |
| 2012-2013 | **University of Barcelona**, Barcelona, Spain<br>Subject: Psychology Lab.<br>Degree: Master in Behavior and cognition research. |

Other skills

Languages: English, Italian, Spanish, Catalan (all Native-equivalent level)
Advanced use of statistical analysis software (SPSS)
Proficiency in cognitive/psychological assessment procedures

Memberships

Member of the Academy of Eating Disorders

Other scientific activities

External reviewer for the European Eating Disorders Review and PlosOne