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Abstract
Background and aims: Orexins/hypocretins are orexigenic peptides implicated in the regulation of feedingbehavior and the sleep/wake cycle. Little is known about the functioning of these peptides in anorexianervosa (AN). The aims of the current study were to evaluate the extent to which orexin-A might belinked 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 dayhospital treatment and in 98 normal-eater/healthy-weight controls. The Pittsburgh Sleep Quality Indexwas administered at the beginning of the treatment as a measure of sleep quality. Other psychopatho-logical variables were evaluated with the Symptom Checklist-Revised (SCL90R) and the Eating DisorderInventory-2 (EDI). Patients were assessed at the start and end of treatment by means of commonly useddiagnostic criteria and clinical questionnaires.Results: The AN patients presented more sleep disturbances and poorer overall sleep quality than didthe healthy controls ($p = .026$) but there were no global differences between groups in plasma orexin-Aconcentrations ($p = .071$). In the AN sample, orexin-A concentrations were associated with greater sleepdisturbances ($|r| = .30$), sleep inefficiency ($|r| = .22$) and poorer overall sleep ($|r| = .22$). Structural EquationModeling (SEM) showed that both elevated orexin-A concentrations and inadequate sleep predictedpoorer treatment outcome.contribute to poor sleep quality in AN, and both of these variables are associated with therapy response.

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

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, Theys, Vandereycken, Leroy, & Peuskens, 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, Zulley, Krieg, Riemann and Berger, 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 individuals with AN and in 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 normal weight female controls (HC) (BMI=18.5-24.9, kg/m^2). The AN participants were diagnosed using DSM-IV-TR criteria (APA, 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, 1981). 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, Reynolds, Monk, Berman, and Kupfer, 1989) was administered to explore 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 8 and 9 am 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

Clinical and physical evaluations were conducted by experienced psychologists and psychiatrists during two structured face-to-face interviews. The PSQI and other self-report questionnaires from which to obtain information on general health were administered upon the first evaluation. Blood samples were extracted after the second interview.

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 χ^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 χ^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).

Insert Table 1 around here

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).

Insert Table 2 around here

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).

Insert Table 3 around here

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 by 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→d involves to add β_{ks} to the linear equation for d and β_k is called the path coefficient). A

curved arrow-path states that there is a correlation to be estimated between the variables it connects. So, 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 (χ^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.

Insert Figure 1 around here

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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, Cumella, and Sanathara, 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 (Panherick et al., 2001) 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, 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).

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 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. Nonetheless, 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.

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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).

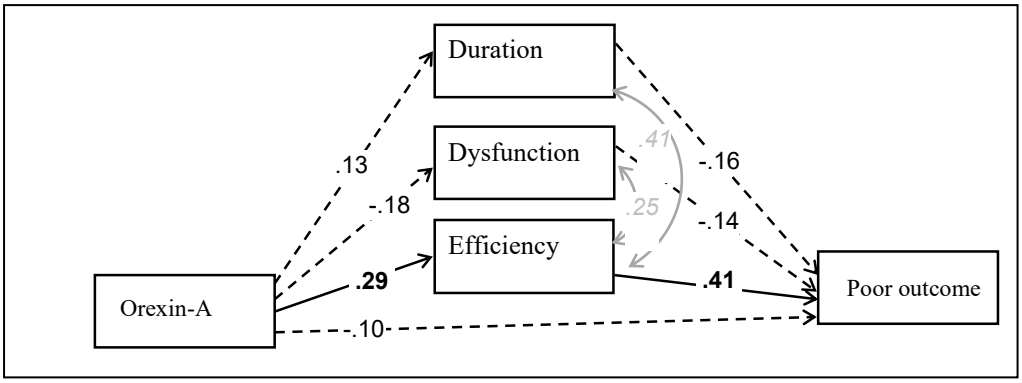

Figure 1. Structural Equation Model (SEM) of OXA levels and sleep quality (duration/ dysfunction/efficiency) on a poor treatment outcome. (Higher scores in the sleep measures indicate poorer sleep).

Continuous line: significant parameter