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Association of fasting Orexin-A levels with energy intake at breakfast and subsequent snack in Chilean adolescents

Fabian Lanuza Rilling [a,b], Marcela Reyes [c], Estela Blanco [d,e], Raquel Burrows [c], Patricio Peirano [f], Cecilia Algarín [f], Tomás Meroño [a], Sheila Gahagan [d,*]

[a] Biomarkers and Nutrimetabolomics Laboratory, Department of Nutrition, Food Sciences and Gastronomy, Food Technology Reference Net (XIA), Nutrition and Food Safety Research Institute (INSA), Faculty of Pharmacy and Food Sciences, University of Barcelona, 08028 Barcelona, Spain
[b] Centro de Epidemiología Cardiovascular y Nutricional (EPICYN), Facultad de Medicina, Universidad de La Frontera, 4781218 Temuco, Chile
[c] Department of Public Health Nutrition, Institute of Nutrition and Food Technology (INTA), University of Chile, 7830489 Santiago, Chile
[d] Child Development and Community Health Division, University of California San Diego, 92093–0927 La Jolla, CA, USA
[e] Department of Public Health, Faculty of Medicine, Pontificia Universidad Católica, Santiago, Chile
[f] Department of Human Nutrition, Institute of Nutrition and Food Technology (INTA), University of Chile, 7830489 Santiago, Chile

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ABSTRACT

Orexin-A, a hormone secreted by orexin neurons, is involved in caloric-intake regulation. Current understanding is based primarily on animal studies. Studies of orexin in humans are scarce, and to our knowledge there are no prior studies in adolescents. We studied fasting Orexin-A levels related to energy intake at breakfast and a subsequent snack in adolescents (n = 668) from a longitudinal study in Chile. Body-Mass Index (BMI), components of the metabolic syndrome and fasting blood levels of leptin, insulin, ghrelin, and orexin-A were measured. Energy intake was calculated based on food weights before and after the standardized breakfast and subsequent snack. High energy intake was defined as ≥ 75th percentile. We assessed the relationship between orexin-A and high energy intake, adjusting for confounders. Higher orexin levels were associated with high breakfast energy intake (OR: 1.21; 95%CI: 0.98–1.49). Conversely, those with higher orexin levels showed a non-significant trend for lower odds of high energy intake for the snack (OR: 0.87; 95%CI: 0.70–1.07). There was a significant interaction between high breakfast energy intake and orexin levels. Those who ate more calories at breakfast displayed a lower inhibitory effect of orexin on eating at the snack ($p < 0.05$). There was no significant interaction between weight status and orexin. In conclusion, orexin-A levels were associated with breakfast energy intake and inversely related with subsequent snack energy intake in participants whose caloric intake at breakfast was within the normal range. Based on these findings, it appears that the association of orexin-A with energy intake depends on eating behavior.

1. Introduction

The growing prevalence of childhood obesity has led to higher rates of type 2 diabetes and cardiovascular diseases at younger ages (Caprio et al., 2020). These conditions may be preceded by the metabolic syndrome (MetS)(Koskinen et al., 2017). Adolescence is a critical life stage for the development and establishment of adult eating habits (Lanuza et al., 2020). Therefore, mechanisms and modulators of appetite regulation and eating behaviors are of major interest in this age group. Many of the hormones involved in the regulation of food intake are secreted by the gastrointestinal system. However, orexins (also called hypocretins) are mainly secreted by orexin neurons in the lateral area of the hypothalamus. In particular, orexin-A, which can be measured in the peripheral circulation (Hussain and Bloom, 2013; Messina. et al., 2014), is the hypocretin most directly associated with food intake (Messina. et al., 2014).

Orexin-A promotes food intake and energy expenditure and stimulates insulin secretion and sensitivity in peripheral organs (Rani et al., 2018). The multi-tasking orexin neuronal system is involved in the regulation of motivated behaviors, sleep/wake states, neuroendocrine

Abbreviations: BMI, Body mass index; OR, Odds ratio; MetS, Metabolic Syndrome; OW, overweight; OB, obese.

* Correspondence to: Department of Pediatrics, Division of Child Development and Community Health, University of California, San Diego, 4305 University Avenue, Suite 590, San Diego, CA 9215, USA.
E-mail address: sgahagan@ucsd.edu (S. Gahagan).

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and locomotor functions, cardio-vascular system, energy homeostasis, reward system and cognitive functions (Goodrick, 2015; Polito et al., 2020; Sperandeo et al., 2018). The orexin effect on behavioral and physiological processes, such as eating behaviors, may differ under specific circumstances, and thus, studies should control for external (e.g. stress) and internal (e.g. blood glucose) factors and interactions with other hormones (García-García et al., 2014; Khanh et al., 2014; Mahler et al., 2014).

Based on animal studies, it is expected that orexin-A levels promote food intake (Shiuchi et al., 2009; Yamada et al., 2000). However, it has been suggested that this function may be altered by obesity due to the inhibitory effect of eating on the orexin system (González et al., 2016). Obese mice fed a high fat diet showed weakened reward seeking behaviors and altered stress responses associated with impairments of the orexin/hypocretin system (Tan et al., 2020). Some human studies have shown that orexin-A levels are reduced in individuals with obesity (Adam et al., 2002; Mishra et al., 2019). To our knowledge, there are no prior observational studies of the association between peripheral orexin-A and energy intake in adolescents. Overall, the role of orexin-A in human eating behavior remains largely unknown. Obesity, impaired satiety responses and increased subsequent snacking were observed in patients with Orexin deficiency, a genetic disease known as Narcolepsy Type 1 (Van Holst et al., 2016). Snacking is relevant because it has been associated with unhealthy diets and obesity in adolescents (Lanuza et al., 2020; Tripicchio et al., 2019). To our knowledge, the association between orexin levels and snacking has not been studied in the general population.

We aimed to study the association of fasting orexin-A levels with energy intake at a standardized breakfast and with energy intake during a snack which took place twenty minutes after the breakfast, among adolescent participants from a birth cohort, the Santiago Longitudinal Study. Our hypothesis was that fasting orexin-A levels would be positively associated with energy intake at breakfast and inversely associated with energy intake at the snack which followed the breakfast. Exploratory analyses for the effects of overweight/ obesity, number of MetS components and breakfast energy intake and their interactions with orexin levels were also conducted.

2. Methods

Adolescents who had been part of an infancy iron-deficiency anemia preventive trial and follow-up study, the Santiago Longitudinal Study, were included in this study. They had been recruited, as 4-month-old infants, from low- to middle-income communities near the Institute for Nutrition and Food Technology, University of Chile in Santiago, Chile (Lozoff et al., 2003). The (IDA) preventive trial took place when infants were 6–12 months old. Non-anemic infants were randomized to high-iron or low-iron supplementation or no added iron. Infants found to be anemic were treated with therapeutic doses of iron and did not enter the trial. Instead, they were enrolled in a study of neurofunctional development, along with the next non-anemic control (Roncagliolo et al., 1998). In total, 1657 infants completed the trial. All participants were assessed for developmental functioning at 12 months and invited to participate in follow up studies at 5 and 10 years, multiple time points during adolescence and at 21 years. Sample sizes varied at each wave of testing with the smallest group at 5 years, when only two of the three groups were recruited (n = 888) due to a limited funding from NIH. The adolescents were representative of the original cohort (sex, birth weight, 1-year anthropometry, age at first bottle, maternal education, and maternal depressive symptomatology). At 16–17 years, those assessed at 5 years were invited to participate in a study related to obesity and cardiovascular risk, and 679 were assessed from 2009 to 2012 (Vasquez et al., 2019). The study was approved by the Ethics Committees at Institute of Nutrition and Food Technology, University of Chile, the University of California, San Diego, and the University of Michigan. Parents gave informed consent and participants signed informed assent. For this study, only participants with complete data for energy intake serum hormones in adolescence and with z-score for body mass index (BMI) were included (n = 668), see Fig. 1.

Supplementary Fig. 1 provides an overview of procedures, detailed below, conducted during the adolescent follow-up.

2.1. Anthropometry

Trained physicians assessed participant height and weight in the Frankfurt position, without shoes, wearing underwear. Weights and heights were measured twice, with Precision Hispana scales (SECA 703, Seca GmbH & co. Hamburg, Germany) and a stadiometer (Holtain Ltd, UK) to 0.1 kg and 0.1 cm, respectively. Using the average of the two measurements, BMI was computed as weight [kg]/ height [m]2; BMI z-score was determined for sex and age using World Health Organization standards. The participants were classified as underweight/normal weight (z-score ≥ -2 to < 1), OW/ OB (z-score ≥ 1).

2.2. Measurement of Leptin, Insulin, Ghrelin, and Orexin-A

Blood samples were obtained after an overnight fast (time elapsed since the evening meal was not recorded) and processed within two hours. Serum aliquots were kept at − 80 °C until analysis. The enzyme-linked immunosorbent assay was used to determine serum leptin levels (DRG International, Inc., New Jersey, NJ, USA) and the radioimmunoassay (RIA) technique was used for a quantitative measurement of insulin (DCP Diagnostic Products Corporation LA, USA), ghrelin (Phoenix Pharmaceuticals, INC. Burlingame CA, USA.) and orexin-A (Phoenix Pharmaceuticals, INC. Burlingame CA, USA.).

2.3. Breakfast intake

Following anthropometry and the blood draw, participants were offered a standardized breakfast that included juice, canned fruit, sandwiches (ham and cheese or jam and butter), flavored milk (chocolate or strawberry) and tea or coffee (with or without sugar). Each participant ate breakfast, in the presence of a researcher who encouraged them to eat as much as they wanted. No other participants were present. Each participant confirmed verbally that they had eaten until satisfied. They were unaware that their breakfast intake was being recorded. After the meal was complete and the participant had left the room, a dietitian calculated energy intake based on the nutritional information on the labels of each product and the difference between the original weight of each food and what was left after consumption (Reyes et al., 2014).

2.4. Subsequent snack intake

Twenty minutes after the meal, participants were individually invited into a room furnished with teen magazines and a variety of snacks and beverages (soft drinks, cookies, crackers, potato chips, chocolate, ice cream and candy). Participants had not been told that they would be offered snacks after breakfast. The research staff member invited participants to help themselves to a magazine or something to eat or drink, as they wished. After 20 min (time recorded), another member of the research team escorted the participant from the waiting room to complete the assessment. The foods and beverages were weighed before and after the snack in order to determine energy intake consumed (Reyes et al., 2014).

2.5. Metabolic syndrome

MetS was diagnosed using the joint IDF/AHA/NHLBI phenotype, which includes the presence of 3 of 5 of the following conditions: waist circumference (WC; $\geq$ 80 cm and $\geq$ 90 cm in females and males, respectively); high blood pressure (SBP $\geq$ 130 mmHg and/or DBP $\geq$

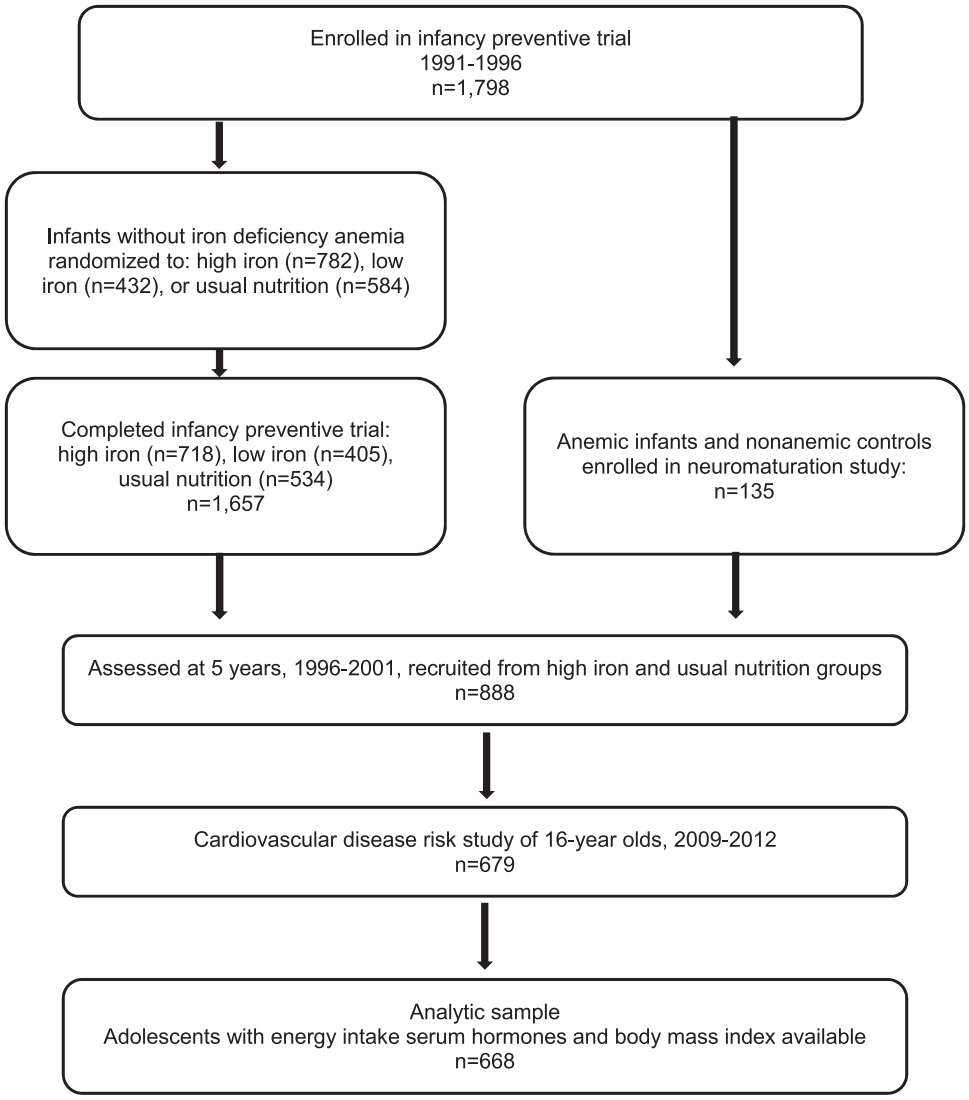

Fig. 1. Flow chart of participants in the current study of the relationship between fasting orexin-A levels and energy intake among adolescents in Santiago, Chile 2009–2012. Participants were drawn from a larger birth cohort, the Santiago Longitudinal Study.

85 mmHg); hypertriglyceridemia (TG $\geq$ 150 mg/dl); low HDL (HDL $\leq$ 50 and $\leq$40 mg/dl in females and males, respectively); and fasting hyperglycemia (Gli $\geq$ 100 mg/dl (Alberti et al., 2009; Magge et al., 2017).

2.6. Other covariates

Start time of the evaluation, which ranged from 08:07–11:50 AM, was transformed to hours elapsed since midnight of the prior night and used as a covariate in multivariate models. Other covariates included nighttime sleep duration (hours) of the previous night (self-reported as bedtime and waking time).

2.7. Statistical analysis

Normal distribution of the variables was evaluated through visual analysis of histograms and the Shapiro Wilk test. Descriptive statistics included mean and standard deviation for normally distributed variables, or median and quartile (Q1-Q3), for non-normally distributed variables or frequency for categorical variables. Descriptive statistics by nutritional status (normal vs OW/OB) were compared using appropriate parametric and non-parametric analyses (Student t-test, Mann-Whitney U test, or chi-square test).

We categorized breakfast energy intake as 'regular breakfast intake' (<75th percentile) or 'high breakfast intake' ($\geq$ 75th percentile). The 75th percentile cutoff corresponded to 748 Kcal, which represents approximately $\geq$ 30% total daily energy expenditure (TEE) in this age group (i.e., 2510 kcal, according to average for males and females with light physical activity (FAO/WHO/UNU)). We also determined two energy intake groups for the snacking period –'regular subsequent snack intake' or 'high subsequent snack intake'-according to the 75th percentile of the population, corresponding to 158 kcals.

Using logistic regression models, we assessed the association between fasting orexin-A and high energy intake at breakfast and/or the subsequent snack. All analyses were adjusted for potential confounders using three statistical models: model 1 – adjusted for sex; model 2 – adjusted for sex, start time of the evaluation, nighttime sleep duration, weight status (BMI categorized normal weight and overweight/obesity); and model 3 – adjusted for sex, start time of the evaluation, nighttime sleep duration, weight status (BMI categorized normal weight and, overweight/obesity), hormones: leptin, insulin and ghrelin (log transformed and standardized, z-score) and number of MetS components. In the logistic regression models with high subsequent snack energy intake as the dependent variable, high breakfast energy intake was included as a covariate. Interactions between orexin-A and weight status, Mets components and breakfast intake category (when subsequent snack intake was considered as the outcome) were tested in the models. When

interactions were significant (p value <0.1), stratified analyses were performed. IBM SPSS 27.0 was used for statistical analysis.

3. Results

The sample consisted of 668 adolescents (48% female). General characteristics are shown in Table 1. The total sample showed clinical and biochemical values within the reference range. While 258 (38.6%) individuals had weights in the overweight or obese range, 78.9% of the participants had ≥ 1 MetS component and 9.5% met diagnostic criteria for the MetS.

As expected, participants with overweight/ obesity showed higher adiposity measures and central obesity. These participants had lower HDL-cholesterol and higher total and LDL-cholesterol, as well higher triglycerides. MetS components were also more prevalent among adolescents with overweight/ obesity than their peers with normal weight (Table 1).

The overweight/ obesity group had lower ghrelin and higher insulin and leptin serum levels compared with the normal weight group. However, orexin levels were not different between groups (Table 1). Furthermore, there were no differences in energy intake at breakfast or subsequent snack (as continuous or categorical variables) between the groups.

The association between orexin levels and high breakfast energy intake or high subsequent snack energy intake, using logistic regression models, are shown in Table 2. In model 1 and model 2, higher orexin levels were associated with increased odds for high breakfast energy intake. In model 3 (Figs. 2 and 3), the association between orexin and breakfast energy intake did not quite reach significance (OR: 1.21; 95% CI: 0.98–1.49; p = 0.066). In contrast, when high subsequent snack energy intake was used as a dependent variable, fasting orexin levels showed a non-significant negative association with the outcome (OR: 0.87; 95%CI: 0.70–1.07). Presence of OW/OB was inversely associated with high subsequent snack energy intake (OR: 0.60; 95%CI: 0.40–0.88), while high breakfast energy intake (OR: 1.80; 95%CI: 1.12–2.90) and higher number of Mets components showed a direct association with high subsequent snack energy intake (OR: 1.34; 95%CI: 1.01–1.77).

There were no significant interactions between BMI categories and orexin-A for any of the outcomes (all p > 0.1). However, we observed a significant interaction between orexin and high breakfast energy intake for models testing high subsequent snack energy intake. Thus, analyses were stratified by category of breakfast energy intake (Table 3). Among participants who did not have high breakfast energy intake, higher fasting orexin levels were inversely associated with high subsequent energy intake in model 2 (OR: 0.74; 95%CI: 0.57–0.95). On the other hand, the association of orexin levels and energy intake during the snack, among participants with high breakfast energy intake, did not reach significance (OR: 1.24; 95%CI: 0.84, 1.84).

4. Discussion

The results of the present study showed that higher orexin-A levels were positively associated with higher breakfast energy intake. However, fasting orexin-A levels were negatively related to subsequent snack energy intake. Among participants who did not have high energy intake at breakfast, this inverse association was statistically significant. To our knowledge, there are no other observational studies reporting the associations between peripheral orexin-A levels and energy intake in humans.

The significant interaction between breakfast energy intake and orexin related to subsequent snacking, suggesting that eating behavior

Table 1
General characteristics of the participants according to normal or overweight/ obese nutritional status.

	All	Normal	Overweight/ Obese	P-value
Participants (n, %)	668	410 (61.4)	258 (38.6)	
Age (years)	16.8 ± 0.3	16.8 ± 0.2	16.8 ± 0.2	0.908[a]
Female sex (n, %)	318 (48)	184 (45)	134 (52)	0.08[b]
Weight (kg)	65.5 ± 13.9	58.0 ± 7.6	77.7 ± 13.2	< 0.001[c]
Height (m)	1.65 ± 0.1	1.66 ± 0.1	1.65 ± 0.1	0.122[a]
BMI z-score	0.65 ± 1.1	-0.09 ± 0.7	1.83 ± 0.6	< 0.001[a]
Waist circumference (cm)	81.3 ± 11.3	74.8 ± 5.8	91.6 ± 10.3	< 0.001[c]
High waist circumference (n, %)	203 (30.4)	42 (10.2)	161 (62.4)	< 0.001[b]
Energy intake (kcal)				
Breakfast (kcal)	635 ± 242	644 ± 240	621 ± 244	0.234[a]
High breakfast intake (n, %)*	166 (24.8)	112 (27.3)	54 (20.9)	0.066[b]
Snack intake (kcal)	0 (0–158)	0 (0–172)	0 (0–157)	0.320[c]
High snack intake (n, %)	167 (25.0)	105 (25.6)	62 (24.0)	0.715[b]
Clinical and biochemical measures				
Orexin-A (pg/mL)	16.1 (13.7–18.5)	16.2 (5.1)	16.1 (4.7)	0.263[a]
Leptin (ng/mL)	7.0 (1.2–18.5)	3.4 (1.0–11.9)	13.2 (5.3–25.1)	< 0.001[c]
Insulin (μUl/ dL)	6.7 (4.6–9.8)	5.6 (4.0–7.8)	9.0 (6.1–12.5)	< 0.001[c]
Ghrelin (pg/mL)	200.7 (142.3–290.8)	210.2 (149.6–314.7)	181.9 (128.7–260.8)	< 0.001[c]
Total cholesterol (mg/dl)	146.9 (133.2–166.0)	145.4 (132.4–162.1)	150.7 (136.0–173.4)	< 0.01[a]
HDL cholesterol (mg/dl)	39.4 (32.6–46.4)	40.5 (34.2–48.3)	37.0 (30.1–43.6)	< 0.001[a]
LDL cholesterol (mg/dl)	92.0 (78.0–107.6)	90.8 (76.3–104.9)	95.7 (81.7–112.0)	< 0.01[a]
Glucose (mg/dl)	88.5 (82.8–94.7)	88.0 (82.5–94.3)	89.1 (82.9–95.1)	0.244[a]
Triglycerides (mg/dl)	81.0 (57.0–101.2)	67.0 (4.5–91.8)	86.4 (66.7–122.9)	< 0.001[c]
SBP (mm Hg)	110 (104–120)	109 (101–115)	114 (108–124)	< 0.001[c]
DBP (mm Hg)	70 (64.2–74.0)	67.5 (62–71)	70 (68–75)	< 0.001[a]
MetS (n, %)	64 (9.6)	6 (1.5)	58 (22.5)	< 0.001[b]
MetS count (n, %)				x
0	141 (21.1)	124 (30.2)	17 (6.6)	
1	285 (42.7)	228 (55.6)	57 (22.1)	
2	178 (26.6)	52 (12.7)	126 (48.8)	
3	50 (7.5)	6 (1.5)	44 (17.1)	
4	11 (1.6)	–	11 (4.3)	
5	3 (0.4)	–	3 (1.2)	

Values are either frequencies, means ± SD or p25-p75; BMI: Body mass index;; SBP: systolic blood pressure; DBP: diastolic blood pressure; MetS: metabolic syndrome; *high breakfast intake: ≥ 75 Percentile (748 Kcal). High subsequent snack intake: ≥ 75 Percentile (158 Kcal). a Student's t test for independent samples, b chi square, ($\chi 2$) and c Mann Whitney / Wilcoxon Rank Sum.

Table 2
Odds ratio for breakfast and subsequent snack energy intake on orexin logistic regression models.

	High breakfast OR (95% CI)	p[2,3]	High snack[1] OR (95% CI)	p[2,3]
Model 1				
Orexin-A (z-score)	1.38 (1.15–1.66)	< 0.001	0.83 (0.69–1.00)	< 0.05
Female sex	0.48 (0.33–0.70)	< 0.001	1.04 (0.72–1.50)	0.797
High breakfast energy	–		1.61 (1.07–2.41)	< 0.05
Model 2				
Orexin-A (z-score)	1.31 (1.07–1.60)	< 0.01	0.86 (0.70–1.05)	0.145
Female sex	0.45 (0.29–0.69)	< 0.001	1.00 (0.67–1.51)	0.969
Star time (h)	0.97 (0.75–1.26)	0.848	0.87 (0.67–1.12)	0.294
Nighttime Sleep (h)	0.97 (0.87–1.08)	0.625	1.01 (0.91–1.12)	0.827
BMI cat	0.76 (0.58–1.01)	0.065	0.84 (0.64–1.10)	0.215
High breakfast energy	–		1.54 (0.98–2.41)	0.060
Model 3				
Orexin-A (z-score)	1.21 (0.98–1.49)	0.066	0.87 (0.70–1.07)	0.190
Female sex	0.34 (0.19–0.59)	< 0.001	1.17 (0.66–2.04)	0.584
Start time (h)	0.97 (0.74–1.27)	0.839	0.86 (0.66–1.13)	0.289
Nighttime Sleep (h)	0.97 (0.87–1.09)	0.713	0.99 (0.89–1.10)	0. 911
Leptin (z-score)	1.22 (0.90–1.64)	0.189	0.84 (0.61–1.14)	0.272
Insulin (z-score)	0.59 (0.45–0.77)	< 0.001	1.33 (1.03–1.71)	< 0.05
Ghrelin (z-score)	1.31 (1.04–1.65)	< 0.05	0.94 (0.75–1.18)	0.650
BMI cat	0.91 (0.61–1.36)	0.661	0.60 (0.40–0.88)	< 0.05
High breakfast energy	–		1.80 (1.12–2.90)	< 0.05
MetS components (per unit)	1.12 (0.82–1.51)	0.46	1.34 (1.01–1.77)	< 0.05

Dependent variable in high: High breakfast energy intake.
Dependent variable in high: High subsequent snack energy intake.
Model 1: Sex.
Model2: Sex, start time of the evaluation, nighttime sleep duration, BMI categorized normal and overweight/ obese.
Model 3: Sex, start time of the evaluation, nighttime sleep duration, BMI categorized normal and overweight/ obese, hormones (log transformed and z-score), MetS components.

or energy intake at breakfast may modulate the association between orexin levels and subsequent snacking. Indeed, in the stratified analysis, the association between orexin and energy intake at breakfast was negative in those whose breakfast energy intake was in the normal range and positive in the group with high breakfast energy intake. Therefore, we conclude that there could be a threshold in which the physiological role of orexin would have different effects according to eating behavior or energy intake. Prior research in mice showed different feeding behaviors given different intracortical doses of orexin-A (Rodgers et al., 2000). Yet, it appears that in humans eating behavior relates not only to orexin-A levels, but also to energy intake. This association could be related to a lack of inhibitory signaling due to lower orexin-A or a depressed orexin system. Indeed, reduced inhibition and impaired satiety signaling have been proposed to be involved in some feeding characteristics observed in patients with obesity (Chieffi et al., 2017). In our study, participants who did not have high caloric intake at breakfast had lower levels of orexin-A and increased risk for higher subsequent snack energy intake. This may suggest that these participants have dysfunctional cortico-limbic connectivity during reward processing and may be prone to snacking (Monteleone et al., 2012; Tan et al., 2020). In this context, a second measurement of orexin levels after breakfast may have been informative of the kinetics of orexin response to feeding. In another study, patients with type 1 narcolepsy characterized by orexin deficiency, had increased energy intake compared to healthy controls (Van Holst et al., 2016). Moreover, it was also suggested that this deficiency could lead to impaired homeostatic signaling and lower satiety signaling in response to food (Van Holst et al., 2016). In addition, these patients showed increased activation of the ventral medial prefrontal cortex in response to food words relative to neutral words, indicating enhanced activity to food cues suggesting a potential mechanism for future weight gain (van Holst et al., 2018).

Previous studies in adult patients with morbid obesity or MetS found reduced levels of orexin-A compared to healthy individuals (Adam et al., 2002; Bronský et al., 2007; Mishra et al., 2019). Moreover, orexin-A levels, were inversely associated with some components of MetS, like waist circumference and triglycerides (Mishra et al., 2019). In patients following bariatric surgery, increased orexin-A by day five was an early marker predicting greater improvement of metabolic risk factors over the following year (Gupta et al., 2015). Moreover, several studies have observed that the level of orexin-A increased after weight-reduction therapy (Bronský et al., 2007; Valenzano et al., 2019). In our cohort, there were no significant differences in orexin-A levels according to weight status. Surprisingly, participants with overweight or obesity showed lower odds for high subsequent snack energy intake, disregarding their energy intake at breakfast (Table 3). There was no significant interaction between BMI categories and orexin levels. Moreover, another study of this cohort found that adolescents with obesity consumed fewer calories during this assessment compared to those without obesity and the relationship between eating behavior and obesity did not vary by sex (Blanco et al., 2019). Despite these results, it might be possible that lower fasting orexin levels may indicate an early alteration in the effect of orexin on energy intake regardless of body weight. In animal models, the act of eating itself led to a drop of orexin levels within milliseconds, which was related to a signal to stop eating (González et al., 2016). In individuals with an already depressed orexin system due to overeating, this inhibition signal might be impaired. Other possible mechanisms behind our results have been mentioned previously such as effects of the cortico-limbic system and hedonic eating (Rossi and Stuber, 2018). Indeed, the action and interaction of peptides, such as orexin-A and the opioid peptide, dynorphin, and their receptors in the brain, and brain-site specific effects on hedonic intake may be affected as in individuals with obesity. (Ghule et al., 2020; Mattar et al., 2020).

Previous studies assessing the relationship between appetite-related hormones and food intake had similar findings.(Buss et al., 2014; He et al., 2011; Yannakoulia et al., 2003). As expected, there were positive associations between breakfast energy intake with orexin and ghrelin while the association with insulin was negative. However, no association was found between breakfast energy intake and leptin levels. These results suggest that peripheral leptin levels may not accurately represent the hormone action at the central nervous system. Leptin resistance may be one possible mechanism behind this result (Sáinz et al., 2015). Another plausible explanation is related to particularities of the studied cohort in which adolescents with overweight/ obesity (and higher leptin levels) had lower energy intakes than their normal-weight peers (Blanco et al., 2019). Other variables that could have influenced this association are physiological stage, and genetics, among others (Andreoli et al., 2019; Kroll et al., 2019). On the other hand, increasing cardiometabolic risk factors showed a progressively negative effect on subsequent snacking regulation, independently of the measured hormones.

Several factors, not considered in our study, could also be associated with intake at breakfast and subsequent snacking, such as behavioral, emotional, hedonic and sociocultural influences. This study has some limitations. First, the sample of Chilean adolescents was not

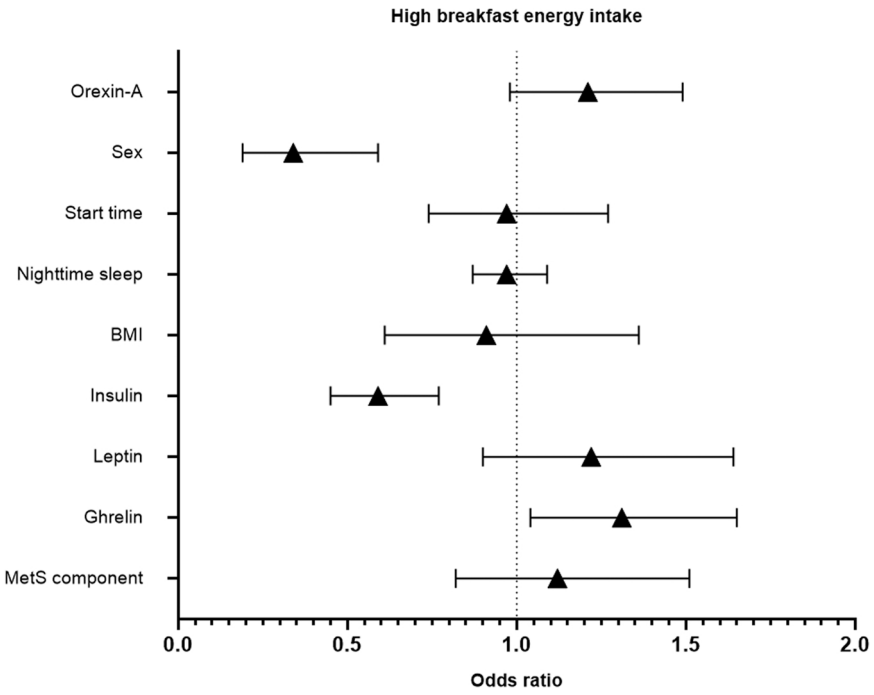

Fig. 2. Model 3 with dependent variable: High breakfast energy intake (≥75th percentile). Independent variable: orexin levels (z-score). Confounders: Sex, start time of the evaluation and nighttime sleep (per hour), BMI categorized normal and overweight/ obese, hormones (log transformed and z- score) and MetS components count (per unit). EI: Energy intake; BMI: Body mass index; MetS: Metabolic syndrome.

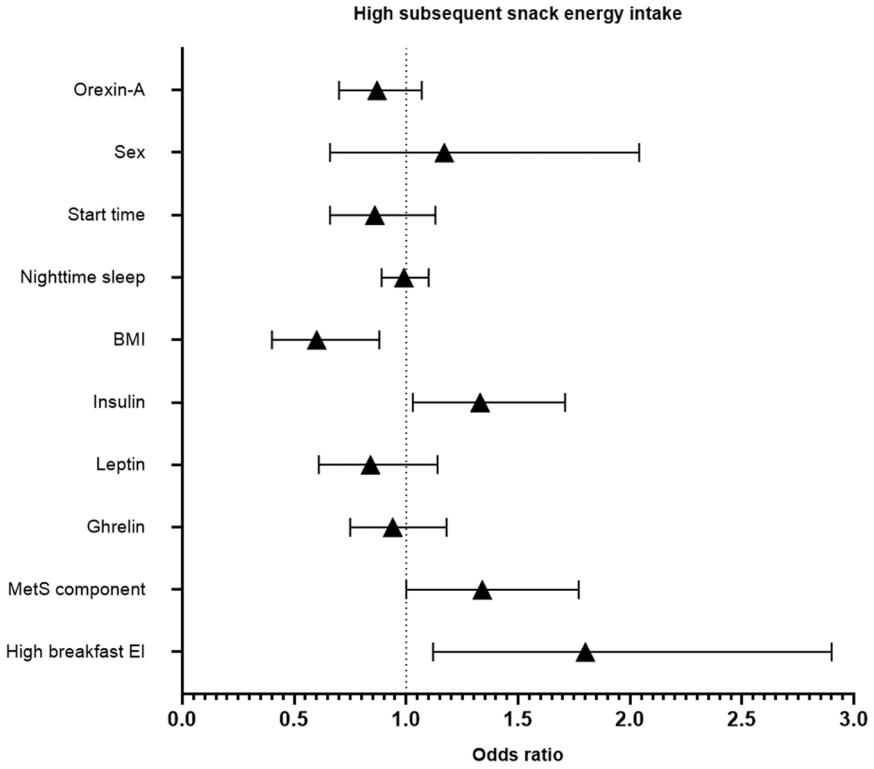

Fig. 3. Model 3 with dependent variable: High subsequent energy intake (≥75th percentile). Independent variable: orexin levels (z-score). Confounders: Sex, start time of the evaluation and nighttime sleep (per hour), BMI categorized normal and overweight/ obese, hormones (log transformed and z- score) and MetS components count (per unit). EI: Energy intake; BMI: Body mass index; MetS: Metabolic syndrome.

representative of the Chilean adolescent population, as most were from low- to middle-income backgrounds from one area of Santiago. Other studies suggest that the adolescent period, genetics, adiposity and others characteristics of the cohort could have influenced the results (Andreoli et al., 2019; Kroll et al., 2019). Second, the assessments were performed at a nutrition research institute, which may have influenced the participants eating behavior; however, information or comments to participants related to the eating behavior, or weight status were avoided.

Table 3
High subsequent snack energy Intake on orexin regression models according to breakfast energy intake categories (≥P75).

	BKF <P75 (n = 502) OR (95% CI)	P	BKF ≥P75 (n = 166) OR (95% CI)	P
Model 1				
Orexin-A (z-score)	0.71 (0.56–0.89)	< 0.01	1.16 (0.84–1.61)	0.340
Model 2				
Orexin-A (z-score)	0.74 (0.57–0.95)	< 0.05	1.24 (0.84–1.84)	0.268
Female sex	1.50 (0.76–2.94)	0.238	0.68 (0.23–2.01)	0.489
Start time (per hour)	0.85 (0.61–1.18)	0.337	0.83 (0.50–1.37)	0.482
Nighttime sleep (per hour)	1.01 (0.89–1.15)	0.795	0.95 (0.76–1.19)	0.710
BMI cat	0.62 (0.39–0.98)	< 0.05	0.48 (0.22–1.06)	0.071
Leptin (z-score)	0.76 (0.51–1.12)	0.170	0.92 (0.53–1.59)	0.782
Insulin (z-score)	1.27 (0.94–1.70)	0.109	1.48 (0.88–2.49)	0.134
Ghrelin (z-score)	0.92 (0.70–1.21)	0.572	1.01 (0.65–1.56)	0.963
MetS compounds (per unit)	1.44 (1.02–2.03)	< 0.05	1.32 (0.77–2.26)	0.298

Dependent variable: High subsequent snack energy intake
Model 1: Unadjusted model
Model 3: Fully adjusted model: sex, start time of the evaluation, nighttime sleep duration, BMI categorized normal and overweight/ obese, hormones (log transformed and z- score) and MetS components.

Third, plasma levels of orexin-A may not precisely reflect its hypothalamic expression (Bronský et al., 2007). Fourth, it is possible that some participants chose not to eat at the subsequent snacking because they did not find the snacks appetizing. Future studies might consider requiring all foods to be tasted and palatability rated by each participant (Reyes et al., 2014). Last, participants were not assessed for eating disorders, which could also be a source of bias (García-Luna et al., 2010). This study also has important strengths including the precise measurement of hormone levels, food intake and anthropometric measures at a nutrition research institute. More studies are needed to assess the role of other factors related to appetite and orexin-A levels including sleep-wake patterns (Barateau et al., 2020) and physical activity (Monda et al., 2019). It would also be interesting to assess day-to-day variability of fasting hormone levels in free-living individuals. Furthermore, as sociocultural factors and genetics may influence results, studies from other settings should be conducted for comparative purposes.

In conclusion, orexin-A levels were directly associated with breakfast energy intake. However, we found an inverse trend for subsequent snack energy intake in participants whose caloric intake at breakfast was in the average range. There was a significant interaction between high breakfast energy intake and orexin levels suggesting a bidirectional interplay between caloric intake and the orexigenic system in appetite regulation. Future studies are needed to explore the important functional role of orexin in energy intake related to human eating behavior.

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CRediT authorship contribution statement

Fabian Lanuza: Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Marcela Reyes:** Supervision, Writing – review & editing. **Raquel Burrow:** Resources, Writing – review & editing. **Estela Blanco, Patricio Peirano, Cecilia Algarín:** Writing – review & editing. **Tomas Meroño:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Sheila Gahagan:** Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105718.

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