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Leptin status in adolescence is associated with academic performance in high school students

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ABSTRACT

Objective: Leptin is a pleiotropic hormone associated with learning and memory via brain receptors. Because individual differences in memory performance affects students' ability to learn, we hypothesized that leptin resistance (LR) would compromise the ability to perform well in school.

Design and setting: Retrospective study in 568 16 years-old adolescents who were part of a follow-up study.

Primary and secondary outcome measures: We measured serum leptin concentration using an enzyme-linked immunoabsorbent assay. Cutoffs from the HELENA Study for 16-years-olds were used for diagnosis of LR. Academic performance was measured using high-school grades and grade-point average (GPA). Weight status, dietary and physical activity habits, sex, maternal education as a proxy for socioeconomic background, and type of secondary education were uses as covariates. Data were collected in 2009-2012, and data analysis was performed in 2014.

Results: Prevalence of LR was 14.6%. Leptin resistant students had significantly lower school grades and GPA compared to leptin sensitive participants (e.g. GPA mean difference= 34.6 points. 95% CI: 12.7-55.4. *d*= -0.38). With a Cohen's *d* of -0.38, there is a 39% chance that a person picked at random from the leptin resistant group will have a higher GPA than a person picked at random from the leptin sensitive group. After controlling health, sociodemographic and education confounders, the odds of performing ≥50th percentile in leptin resistant adolescents were 56% (95% CI: 0.32-0.95) that of their leptin sensitive peers. Likewise, the chances of having a performance ≥75th percentile in students classified as leptin resistant were 42% (95% CI: 0.19-0.89) that of students classified as leptin sensitive.

Conclusions: In high-schoolers, abnormally high levels of leptin were associated with poorer academic performance. These findings support the idea of a relationship between leptin and learning. Further research is needed on the cognitive effects of leptin in younger populations.

Keywords: leptin, leptin resistance, cognition, academic performance, adolescents.

Strengths and Limitations of this Study

- Our results supports the notion of leptin as a cognitive enhancer in addition to its role in energy balance regulation.
- This paper is the first to link leptin status in a healthy younger-age human population with scholastic measures of cognition (high school grades), aiming at examine this relation in the 'real' world.
- Our sample is not representative of the Chilean adolescent population, as it consisted of adolescents from middle to low SES. However, the prevalence of risk unhealthy dietary habits and obesity, both of which may lead to abnormally high leptin levels, is higher in these groups.
- We used cut-offs for leptin resistance diagnosis based on statistical criteria, which are the only values described for healthy adolescents. Future studies should use cut-offs based on biological risk.
- We did not consider the mediating effect of other important influences, such as: maternal diet, BMI or smoke/alcohol consumption before and during pregnancy, and potential learning and cognitive disorders.
- Because association does not imply causation, future studies should replicate this analysis in other young populations.

1. INTRODUCTION

Leptin, the protein hormone produced in fat tissue which regulates the amount of fat stored in the body, was originally thought to be involved only in the regulation of food intake and energy balance. Recent evidence shows that leptin also plays a role in physiological courses other than eating behavior; in fact it can influence several developmental processes in the immature brain[1-3].

Leptin receptors are expressed throughout the brain, especially in the hippocampus and various cortical regions. Numerous evidence supports its cognitive enhancer properties, particularly the ability to boost physiological events underlying hippocampal-dependent learning and memory[4]. In the hippocampus, leptin facilitates the induction of synaptic plasticity by converting short-term potentiation of synaptic transmission into long-term potentiation (LPT), a process regarded as part of the neurophysiological basis of learning and memory formation[5]. Impairment of this process has been associated with cognitive deficits[1,6,7]. In the prefrontal cortex, which plays an important role in higher cognitive functions, leptin has been associated with increased brain-derived neurotrophic factor expression and neurogenesis[8,9].

The cognitive effects of leptin depend on its ability to cross the blood-brain-barrier (BBB) and the functionality of leptin receptors within the hippocampus and other brain regions[10]. The inability of peripheral leptin to reach the brain is called peripheral leptin resistance (LR), while a diminished leptin receptor quantity and impaired signal transduction is known as central LR. In humans, both defects coexist. Aging, disease such as diabetes and neurodegenerative disorders, and excessive exposure to saturated fats and refined sugars (e.g., triglycerides and fructose) have been associated with dysfunctional leptin transport into the brain[11-14]. On the other hand, obesity has been initially associated with a period of central leptin hypersensitivity, followed by a phase of central LR[11,15]. Moreover, chronic exposure to central LR, causes a LR that reduces leptin receptors, diminishes signaling, and impairs responsiveness to exogenous leptin[11].

LR is associated with poorer cognitive outcomes in the middle-aged and elderly population as well as in certain diseases, including diabetes and Alzheimer's[3]. Although adolescence is an important period for shaping learning and memory, very few studies have approached this topic in younger age groups, and they have mostly used animal models[16-19]. We aimed to assess the association between leptin status and learning in youths by using scholastic measures such as school grades and grade point average (GPA). Because leptin modulates the cellular processes underlying hippocampal-dependent learning and memory, and because memory skills are good predictors of learning outcomes[20,21], we hypothesized that LR would compromise the ability of adolescents to perform well in school.

2. METHODS

Study sample

We studied 568 16-year-old adolescents living in Santiago, from low to middle SES who were part of a follow-up study beginning in infancy. Participants, recruited at 4 months, were healthy, full-term singleton infants weighing ≥3 kg at birth. Enrolment is described in detail elsewhere[22]. They were assessed for developmental outcomes in infancy, 5, 10 and 16 years[22]. At 16 years, they were also assessed for obesity risk and the presence of cardiovascular risk factors in a half-day evaluation that included a fasting blood draw[24]. This study was approved by the institutional review boards of the University of Michigan, Institute of Nutrition and Food Technology (University of Chile), and the University of California, San Diego. Participants and their primary caregiver provided informed and written consent, which was designed following the norms for Human Experimentation, Code of Ethics of the World Medical Association (Declaration of Helsinki, 1995).

Measures

Leptin status at age 16

A fasting venous blood sample was collected in the morning (8-9 am). Serum specimens were separated by centrifugation and stored at −70 °C. Serum leptin was measured by a sensitive enzyme-linked immunosorbent assay (Active Human Leptin ELISA, DSL-10-23100, Diagnostic System, Webster, TX, USA). The minimum detectable concentration was 0.05 µg/l. The intra- and interassay coefficients of variation were 4.8% and 4.3%, respectively. Due to the lack of cut-off values for LR diagnosis in adolescents and because high circulating leptin may be a strong indicator of LR[12,24], reference values from the HELENA Study for 16-year-old adolescents were used for LR diagnosis in our sample: serum leptin values >75th percentile for sex (8.99 µg/l in males and 39.56 µg/l in females)[25]. These are the only descriptive values for establishing leptin levels in apparently healthy adolescents.

Academic performance

Academic performance (AP) was assessed using the student's grades in high-school (9th to 12th) and final GPA. Data were collected from administrative records of the Curriculum and Assessment Unit, Ministry of Education (Chile). Since schools may have differed in grading policies, grades (on a scale of 1-7) were transformed into scores (ranging 210-825), following the Ministry of Education criteria. The arithmetic average of each subject taken during each academic year was calculated and the result was compared in the conversion table provided by the Department of Assessment, Measurement and Educational Record, University of Chile, which complies specifications on behalf of the Ministry of Education[27]. The same procedure was used to convert the GPA into a score. School grades were used as continuous variables, whereas GPA was used as a continuous and categorical variable (GPA scores ≥50th and 75th percentile in our sample).

Nutritional status at age 16

Body-mass index (BMI=Kg/m2) at age 16 was evaluated by standardized methods, and z-scores were estimated according to the 2007 World Health Organization references. Nutritional status was defined as follows: underweight (BMI z-score <-1 SD), normal weight (BMI z-score from ≥-1 SD to ≤1 SD), overweight (BMI z-score >1 SD to 2 SD), and obesity (BMI z-score >2 SD).

Eating habits at age 16

Diet has been associated with academic achievement in numerous studies[27-30], therefore, it could be a confounder for the association between leptin status and academic performance. Diet was evaluated using validated and standardized self-report questionnaires, scored from 0 to 10, with higher scores denoting healthier habits. The questionnaire was administered by a researcher during the assessment at 16 years. The quality of diet was measured by the amount of saturated fat, fiber, sugars and salt in the food items consumed during breakfast, lunch, supper, snacks at school and at home. Meals were considered to be unhealthy (foods high in fat, sugar, salt, and calories), acceptable or satisfactory (highly processed items although low in fat) and healthy (nutrient rich items and protective foods). Cut-offs for the Chilean adolescent population were applied to classify the overall eating habits of participants into three groups: unhealthy, fair and healthy[31].

Physical activity patterns at age 16

A physical activity (PA) questionnaire that was validated in a previous study using accelerometry-based activity monitors was administered by a researcher during the half-day assessment[32]. PA was measured including: number of weekly hours devoted to physical education and extracurricular sports, number of

daily hours of sedentary activities, number of daily hours of active play, and daily active commuting to school by means of walking. Average time allocation for each PA category was estimated. Overall PA scores ranged from 0-10, with higher scores denoting healthier habits. We used cut-offs for the Chilean adolescent population to classify participants into three groups: physically inactive, moderately active, and physically active[31].

Socioeconomic background

Mother's education acted as indirect indicator of the socioeconomic status (SES) of the family. Maternal education was reported as the highest completed level on three categories: (1) complete elementary education, (2) complete secondary education, (3) complete vocational training or associate degree. In our analysis we merged these categories into two: incomplete secondary education (1), and complete secondary education or higher (2+3), denoting low- and middle- SES, respectively.

Type of secondary education

In Chile, secondary education includes academic high-schools, which provide theoretical education in languages, mathematics, history and sciences; vocational school, a combination of theoretical education and vocational training; and adult school, for students who in the past did not receive their secondary education certificate. Data on the type of secondary education attended by participants was retrieved from publicly available records at the Curriculum and Assessment Unit (Ministry of Education).

Statistical Analysis

Statistical analysis included Chi-square test for categorical variables and Student's *t* test for continuous variables. We tested for effect measure modification (interaction) by sex, weight status and diet at 16

years in the association between LR and academic performance by using two-way analysis of variance. The interactions were non-significant (data not shown) and, therefore, we did not stratify the analysis. Because school grades and GPA scores may not have an intrinsic meaning, the effect size for difference was estimated using Cohen's *d,* along with common language effect size statistics (Cohen's U_3 Index and probability of superiority). Next, by using multivariate analysis, we tested the association of leptin status (main exposure) with performing $\geq 50^{th}$ and 75^{th} percentile in our sample (outcome). For each outcome, three models were estimated. The first one included health-related variables as covariates: weight status, dietary and PA habits as independent variables. A second model added sociodemographic covariates: sex and SES as measured by maternal educational attainment. Finally, a fully adjusted model contained all mentioned covariates with the addition of the type of secondary education. Data were analyzed using Stata for Windows version 12.0 (Lakeway Drive College Station, TX, US).

RESULTS

The mean age of participants during the clinical assessments was 16.8 (0.3 SD) years. Males accounted for 51% of the sample. Prevalence of LR was 14.6% and prevalence of obesity and overweight were 24.6% and 13.5%, respectively. The mean leptin concentration was 6.0 µg/l in males and 19.4 µg/l in females. The mean GPA score was 481.1 points (range 269-795).

Table 1 shows the descriptive statistics by leptin status at 16 years. Mean serum leptin in youths with LR was 21.4 µg/l in males and 51.3 µg/l in females. In leptin sensitive participants, levels were 2.2 µg/l and 16.0 µg/l for males and females, respectively. Mean BMI z-score at 16 years was significantly higher in participants with LR ($P<0.001$) and, thus, the proportion of obese adolescents was significantly higher in this group (43%; $P<0.001$). Yet, 28% of participant with LR were normal weight. Other factors did not differ between leptin sensitive and leptin resistant participants. Leptin status was also significantly related to sex.

Table 1 Descriptive statistics of participants in the sample

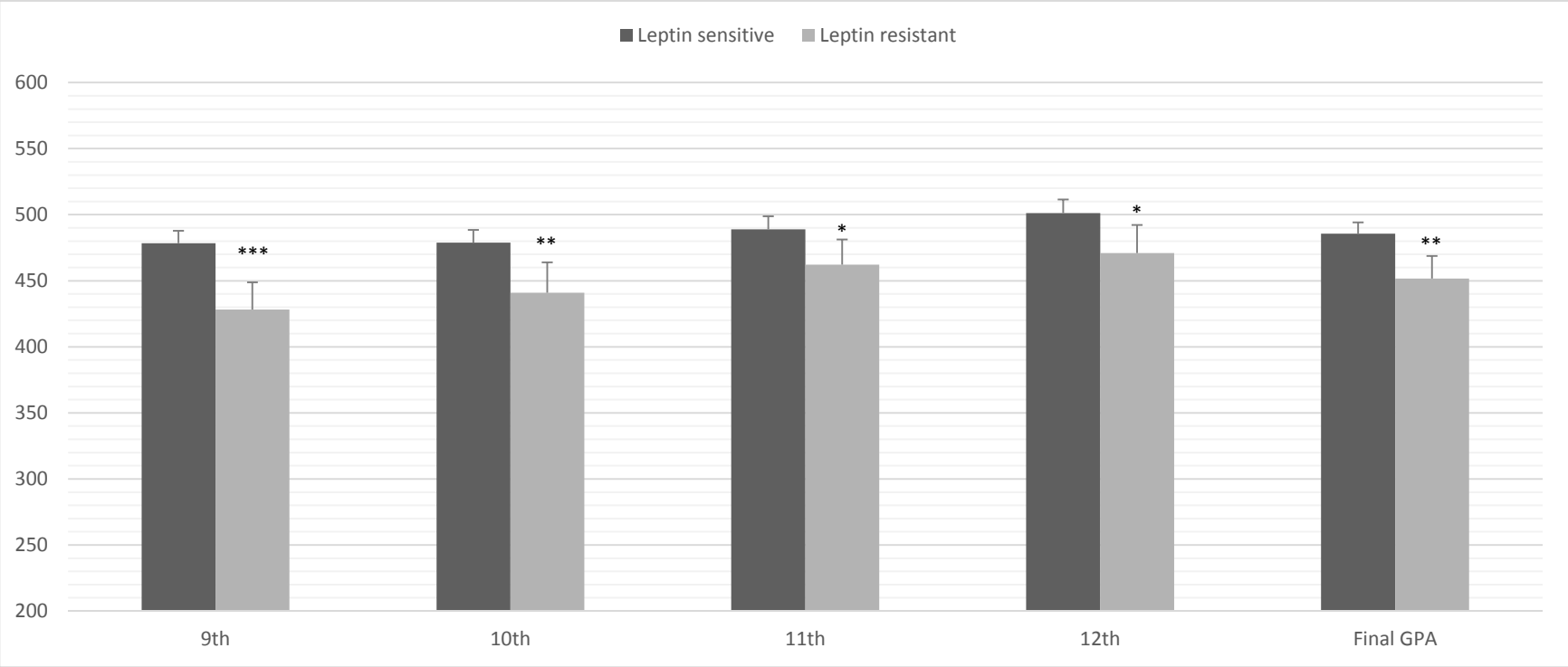
	Total (n=568)		Leptin sensitive (n= 485)		Leptin resistant (n=83)		*P* value*
	Mean or number	SD or Percentage	Mean or number	SD or Percentage	Mean or number	SD or Percentage	
Chronological age							
Age (years)	16.8	0.3	16.8	0.3	16.8	0.3	N.S.
Sex							
Male	287	50.5	231	47.6	56	67.5	0.001†
Female	281	49.5	254	52.4	27	32.5	
Serum leptin concentration							
Males (µg/l)	6.0	9.6	2.2	1.9	21.40	13.1	<0.0001
Females (µg/l)	19.4	14.4	16.0	9.7	51.32	12.7	<0.0001
Weight status at 16 y							
BMI at 16y (z score)	0.63	1.2	0.47	1.1	1.58	1.3	<0.0001
Normal weight	352	61.9	329	67.8	23	27.7	<0.0001
Overweight	139	24.5	115	23.7	24	28.9	
Obesity	77	13.6	41	8.5	26	43.4	
PA patterns at 16y							
Physically active	104	18.3	90	18.5	14	16.8	N.S†
Moderately active	238	41.9	205	42.3	33	39.8	
Physically inactive	226	39.8	190	39.2	36	43.4	
Diet habits at 16y							
Healthy diet	112	19.8	97	20.0	15	18.1	N.S†
Intermediate diet	293	51.7	250	51.7	43	51.8	

Unhealthy diet	162	28.5	137	28.3	25	30.1	
Maternal education (proxy for SES)							
Complete secondary education (Mid-SES)	377	66.4	319	65.8	58	69.9	N.S[†]
Incomplete secondary education (Low SES)	191	33.6	166	34.2	25	30.1	
Type of secondary education							
Academic high school	154	27.1	133	27.4	21	25.3	N.S[†]
Vocational high school	316	55.6	273	56.3	43	51.8	
Adult school	98	17.3	79	16.3	19	22.9	

[*]Student's *t* test, except as indicated. [†]Chi^2 test (Pearson). Normal weight: BMI z ≤ 1 SD. Overweight: BMI z > 1SD and ≤ 2 SD. Obesity: BMI z > 2 SD. Reference values from the HELENA Study for16 year-old adolescents were used for leptin resistance diagnosis in males (8.99 µg/l) and females (39.56 µg/l).

As for academic outcomes, leptin resistant youths had significantly lower school grades and GPA compared to leptin sensitive participants (P<0.05) (Table 2 and Figure 1). The grades mean difference varied from 49 points to 27 points, whereas GPA mean difference was 34.6 points. When the effect size for difference was estimated, Cohen's *d* was -0.38 for GPA and varied from -0.48 to -0.27 for school grades. With a Cohen's *d* of -0.38, 65% of the leptin resistant group will be below the mean score of the leptin sensitive group (based on Cohen's U_3 Index), and there is a 39% chance that a person picked at random from the leptin resistant group will have a higher score than a person picked at random from the leptin sensitive group (probability of superiority).

Figure 1 High School grades and final Grade-Point Average (GPA) in Chilean youths by leptin status at age 16 (n=568)



Grades and GPA expressed as standardized scores.

Error bars are 95% confidence interval.

* $P<0.05$. ** $P<0.01$. *** $P<0.001$

Reference values from the HELENA Study for16 years-old adolescents were used for leptin resistance diagnosis in males (8.99 µg/l) and females (39.56 µg/l).

Table 2 Association of academic performance across high school grades with leptin status at 16y (n=568)

	Leptin sensitive (n=485)	Leptin resistant (n=83)			Effect size for difference		
	Mean (SD)	Mean (SD)	Mean difference	95% CI	*d* ǂ	U_3 Index	Probability of Superiority
9th	478.7 (103.9)	429.7 (93.0)	49.0***	24.9-73.1	-0.48	0.32	0.36
10th	479.0 (103.0)	441.0 (99.5)	38.0**	13.3-62.7	-0.37	0.36	0.40
11th	487.5 (102.2)	460.5 (85.5)	27.0*	2.4-51.5	-0.27	0.39	0.42
12th	498.4 (102.7)	470.0 (93.0)	28.4*	3.2-53.6	-0.28	0.39	0.42
Final GPA	486.3 (93.3)	451.7 (78.8)	34.6**	13.2-55.4	-0.38	0.35	0.39

Grades and GPA expressed as standardized scores.

Reference values from the HELENA Study for16 years-old adolescents were used for leptin resistance diagnosis in males (8.99 µg/l) and females (39.56 µg/l).

ǂAll *d* coefficients accounted for the effect of different sample size.

Table 3 contains the estimated association between having a final GPA ≥50th p in the sample and leptin status at 16 years after controlling for confounders. After full adjustments (Model 3), the odds of performing ≥50th p in leptin resistant youths were 56% (95% CI: 0.32-0.95) that of their leptin sensitive peers. We likewise found that lower academic performance was significantly associated with unhealthy dietary habits (OR: 0.48; 95% CI: 0.28-0.80), being male (OR: 0.64; 95% CI: 0.44-0.94), and attending vocational (OR: 0.42; 95% CI: 0.28-0.64) or adult schools (OR: 0.23; 95% CI: 0.13-0.39). Association of academic results with weight status at 16y was non-significant at an alpha level of 0.05.

Table 3 Relationship between having a GPA >50th percentile and leptin resistance in Chilean youths after controlling relevant confounders (n=568)

	Model 1		Model 2		Model 3	
	OR	95%CI	OR	95%CI	OR	95%CI
Leptin resistance	0.49**	0.29-0.83	0.56*	0.33-0.94	0.56*	0.32-0.95
Overweight	0.69	0.38-1.26	0.70	0.38-1.27	0.65	0.35-1.22
Obesity	0.81	0.47-1.41	0.86	0.49-1.50	0.81	0.45-1.33
Fair diet	0.70	0.47-1.03	0.73	0.49-1.09	0.78	0.55-1.18
Unhealthy diet	0.44**	0.27-0.72	0.44**	0.27-0.73	0.48**	0.28-0.80
Physically inactive	0.97	0.69-1.36	0.80	0.54-1-16	0.86	0.58-1.26
Male sex	(…)		0.60*	0.42-0.87	0.64*	0.44-0.94
Low SES	(…)		0.90	0.77-1.57	0.93	0.89-1.26
Vocational high school	(…)		(…)		0.42***	0.28-0.64
Adult school	(…)		(…)		0.23***	0.13-0.39

OR [95%CI]: Odd Ratio [95% Confidence Interval]. (…) Non-observed. Significance level: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Leptin resistance: males (serum leptin concentration >8.99) female (serum leptin concentration > 39.56). Overweight: BMI-Z >1 SD and ≥2SD. Obesity: BMI-Z ≥ 2 SD. Fair diet: diet high in simple carbohydrates. Unhealthy diet: diet high in simple carbohydrates and saturated fats. Low SES: Mothers with incomplete secondary education.Vocational school: vocational training with theoretical education in language, mathematics, history, and sciences. Adult school: education for students who in the past were unable to receive their diploma.

Table 4 shows the association between having a final GPA ≥75th p in the sample and leptin status at 16 years after controlling for a number of confounders. In a fully adjusted model (Model 3), the likelihood of performing ≥75th p in leptin resistant youths were 42% (95% CI: 0.19-0.89) that of leptin sensitive students. We likewise found that lower academic performance was significantly related to having unhealthy dietary habits (OR: 0.36; 95% CI: 0.19-0.69), being male (OR: 0.49; 95% CI: 0.31-0.76), and the type of secondary education. Again, weight status was unrelated to academic performance.

Table 4 Relationship between having a GPA >75th percentile and leptin resistance in Chilean youths after controlling a number of confounders (n=568)

	Model 1		Model 2		Model 3	
	OR	95%CI	OR	95%CI	OR	95%CI
Leptin resistance	0.35**	0.17-0.73	0.41*	0.20-0.87	0.42*	0.19-0.89
Overweight	0.67	0.41-1.10	0.62	0.30-1.03	0.62	0.37-1.03
Obesity	0.97	0.51-1.82	0.89	0.46-1.71	0.96	0.50-1.83
Fair diet	0.63*	0.41-0.95	0.69	0.44-1.06	0.73	0.46-1.13
Unhealthy diet	0.32**	0.17-0.58	0.33**	0.18-0.63	0.36**	0.19-0.69
Physically inactive	0.97	0.65-1.45	0.72	0.47-1-12	0.77	0.49-1.21
Male sex	(…)		0.45***	0.29-0.70	0.49**	0.31-0.76
Low SES	(…)		0.88	0.58-1.34	0.96	0.69-1.63
Vocational high school	(…)		(…)		0.45***	0.29-0.69
Adult school	(…)		(…)		0.21***	0.10-0.44

OR [95%CI]: Odd Ratio [95% Confidence Interval]. (…) Non-observed. Significance level: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Leptin resistance: males (serum leptin concentration >8.99) female (serum leptin concentration > 39.56). Overweight: BMI-Z >1 SD and ≥2SD. Obesity: BMI-Z ≥ 2 SD. Fair diet: diet high in simple carbohydrates. Unhealthy diet: diet high in simple carbohydrates and saturated fats. Low SES: Mothers with incomplete secondary education. Vocational school: vocational training with theoretical education in language, mathematics, history, and sciences. Adult school: education for students who in the past were unable to receive their diploma.

DISCUSSION

In a sample of high-school graduates, we examined the relationship between LR and learning by using high-school grades and GPA. To our best knowledge this is the first study addressing the link between leptin status and this domain of cognition in a younger-age human population. Compared with leptin sensitive students, those who were categorized as leptin resistant had lower school grades and GPA. Similarly, they had lowers odds of performing at or above the 50th and 75th percentile of the sample. Even after controlling relevant confounders, the association between leptin status in adolescence and academic performance remained significant.

Our results are of importance for several reasons. Leptin plays a key role in memory processing through induction of hippocampal and prefrontal cortex synaptic plasticity. A growing body of evidence suggests that LR, either peripheral or central, can limit the potential for synaptic plasticity and could partially explain some cognitive deficits[3,7,16-19]. Likewise, links are strong between memory performance and learning. In children, working memory and particularly the ability to retrieve and manipulate information from long-term memory has been found to predict math, reading and spelling outcomes, even after controlling for IQ[20]. Dysfunctions in this domain can lead to learning difficulties in activities that involve storing and processing information[20,21]. Furthermore, high-school grades are a predictor of performance on college admission tests, higher education outcomes and subsequent job status and income[33].

Very few studies have explored the link between leptin and cognition in younger animal populations, and their findings are in line with ours. Oomura *et al.* showed that leptin modulates higher neural functions in mice 4-8 weeks-old[16]. While infusion of low doses of leptin enhanced learning and memory performance and hippocampal LPT, high doses impaired them. The notion that abnormally high levels of leptin could be responsible for some learning deficits in adolescent mice is supported by Valladolid-Acebes and colleagues[17-19]. In age-matched mice, short-term exposure to high fat diet compromised hippocampal dependent learning and memory. Moreover, in adolescent mice, the behavioral

impairment was accompanied by changes in hippocampal morphology and functionality of leptin receptors within de hippocampus.

Leptin levels in this Chilean sample were lower than those reported for European adolescents[27], but higher than those reported for adolescents in Latin-America and Asia[34-35]. Population differences in the epidemiologic and nutrition transition may in part explain the disparity in levels of circulating leptin. Increased intake of fat, sugar and processed foods, reduced PA, and increased risk of non-communicable disease, including obesity, are more prevalent in the last stages of the transition[36].

Although an association between weight status and academic results was not observed in this sample, obesity in pediatric populations has been related with impaired cognitive function[37,38] and the ability to perform well in school[39,40]. Some authors have postulated that cognitive impairment may actually precede excessive weight gain[17,41-43]. Two main ideas are behind this view: first, impairment of learning is observed before other metabolic alterations; second, while the adverse effects of consuming high fat/high sugar diet on cognitive function is a good predictor of subsequent weight gain, the effect of those nutrients on weight gain does not reliably predict subsequent cognitive deficit. Our results suggest that leptin might mediate, in part, the effect of weight status on cognitive skills and academic outcomes.

It is very likely that LR in most individuals in our sample may be both central and peripheral, which entails under-responsiveness to exogenous leptin and impairment of signal transduction in target neurons. Although obesity has been traditionally associated with LR in the non-elderly population, new data indicate that fructose, sucrose and triglycerides may induce LR regardless of the amount of body fat[14]. In our sample, less than a half of leptin resistant participants were obese, and 28% were normal weight. Likewise, almost 80% had a diet in the intermediate or unhealthy zone, which means excessive intake of saturated fats and refined sugars, mostly from sweet snacks and sugary drinks. Administration of even small quantities of triglycerides to normal weight rats immediately resulted in resistance to leptin transport across the BBB[44,45]. Similarly, rats exposed to high fructose diet demonstrated an impaired response to peripheral leptin injection and central leptin infusion. Unlike triglycerides, fructose-induced

LR may be reversible; switching to a sugar-free diet reversed the LR in rats[46]. In both cases, chronic fructose and triglycerides consumption led to LR prior to body weight.

Our findings also confirm the role of nutrition in academic outcomes. Participants having an unhealthy diet had significantly lower odds of performing well in high-school, independent of leptin status. Numerous studies report that diet relates to specific outcomes that are important for the educational attainment of children and adolescents[27-30]. The consumption of refined carbohydrates and saturated fats is linked to impaired cognitive performance. Exposure to these macronutrients interferes with hippocampal functioning by cutting down the production of neurotrophins, increasing neuroinflammatory markers, and altering the BBB[42,47].

Implications from these results

The Chilean adolescent population is highly exposed to risk factors for LR. As reported by the 2014 Chilean National Food Consumption Survey[48], dietary habits of poor nutritional quality are wide spread. Adolescents aged 14-18 rank first in the consumption of refined sugar (121 g/day) and second in the consumption of saturated fats. According to this survey, the prevalence of overweight and obesity in adolescents is 38% and 13%, respectively.

A further implication from this study, relates the fact that exposure to abnormally high levels of leptin would be starting very early in life. A non-modifiable condition such as ageing is associated with LR, but exposure to high fat/high sugar diet as well as to overweight and obesity are avoidable. While schools should serve as a point of entry for the promotion of healthy lifestyles, the food industry uses schools as a means of reaching young consumers. Despite the efforts to limit the availability of unhealthy foods in schools, in the Latin-American Southern Cone 70% of adolescents drink sugar-sweetened beverages on a daily basis[49]. Frequent (1-2 times/week) and very frequent (≥3 times/week) fast-food

consumption among Latin-American adolescents range from 40% in Uruguay to 70% in Bolivia, according to a recent international study[50].

The following points should be considered in the design and application of preventive strategies. Fructose-induced LR may be reversible, which means there is a possibility for improvement. Second, LR may be triggered by exposure to even small amounts of saturated fats and refined sugars, which are major components of the Western diet. Moreover, the effects on leptin levels appear soon after exposure to these macronutrients. Finally, central LR may be induced by chronic exposure to hyperleptinemia; at this point it might be late for adolescents to reach their full cognitive and academic potential.

Study limitations and strengths

Our results supports the notion of leptin as a cognitive enhancer in addition to its role in energy balance regulation. A further strength is that we approached this topic in a group younger than previous research on leptin and cognition. Third, we used a scholastic measure of cognition: standardized academic performance in high-school, aiming at examine this relation in the 'real' world. In spite of these strengths, the study has limitations. Our sample is not representative of the Chilean adolescent population, as it consisted of adolescents from low to middle SES. However, the SES level may be especially important. The prevalence of unhealthy dietary habits and obesity, both of which may lead to LR, is higher among adolescents from middle to low SES compared to adolescents of high SES[48]. Second, we used cut-offs for LR diagnosis based on statistical criteria, which are the only values described for healthy adolescents. Future studies should use cut-offs based on biological risk. Third, we did not consider the mediating effect of other important influences, such as: maternal diet, BMI or smoke/alcohol consumption before and during pregnancy; and potential learning and cognitive disorders which may impact student's academic functioning. Because association is not enough to prove causation, future studies should replicate and

extend this analysis in other young populations, and further investigate how early health-related behaviors influence subsequent cognitive and academic outcomes.

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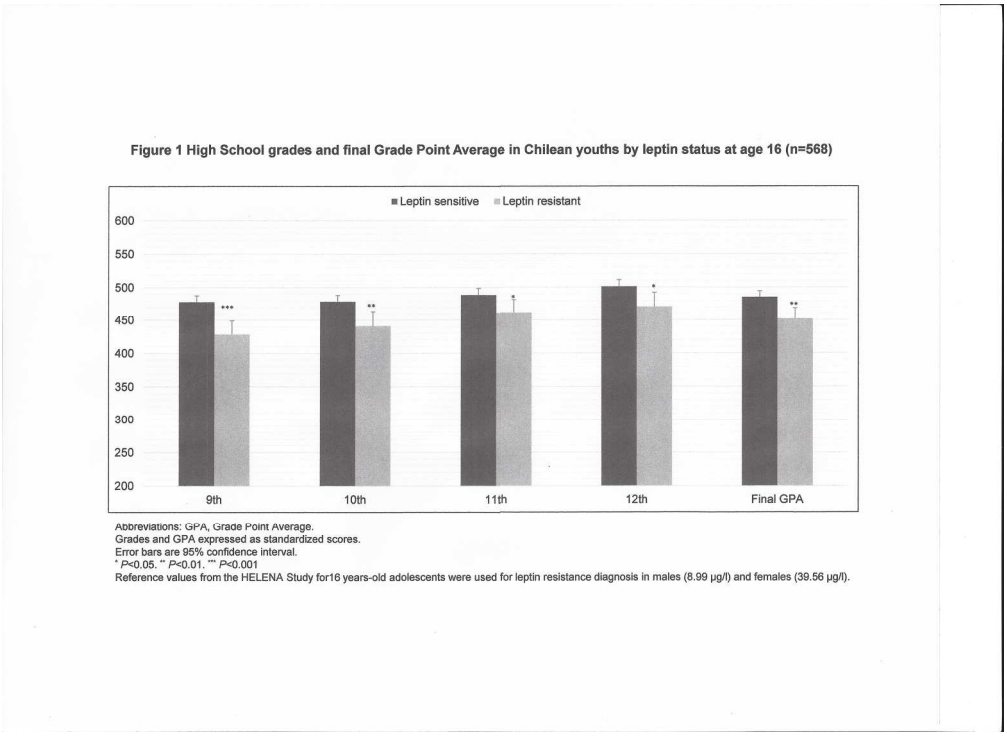

High School grades and final Grade Point Average in Chilean youths by leptin status at age 16 (n=568)

215x156mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	*Recommendation*	*Status*	*Page No*
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	✓	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	✓	2
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓	5-6
Objectives	3	State specific objectives, including any pre specified hypotheses	✓	6
Methods				
Study design	4	Present key elements of study design early in the paper	✓	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	✓	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	✓	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓	7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓	7-9
Bias	9	Describe any efforts to address potential sources of bias	✓	4,23‡
Study size	10	Explain how the study size was arrived at	✓	6§
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓	7-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	✓	9
		(*b*) Describe any methods used to examine subgroups and	✓	9

		interactions		
		(*c*) Explain how missing data were addressed	n.a.	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	✓	6§
		(*e*) Describe any sensitivity analyses	n.a.	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓	10
		(b) Give reasons for non-participation at each stage	✓	6§
		(c) Consider use of a flow diagram		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓	10-11
		(b) Indicate number of participants with missing data for each variable of interest	n.a	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	✓	6§
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	✓	6§
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures	✓	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓	13-19
		(*b*) Report category boundaries when continuous variables were categorized	✓	13-19
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	✓	16-19
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓	7
Discussion				
Key results	18	Summarise key results with reference to study objectives	✓	20-21
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓	23
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓	20-21
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓	20-23

Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓	25

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

§Enrolment, assessments and data from previous waves are described in detail in previous works by the authors'. Reference for these works are provided in the Methods section (Study sample).

ǂ This was reckoned as a limitation.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Leptin status in adolescence is associated with academic performance in high school students from a Chilean birth cohort

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Leptin status in adolescence is associated with academic performance in high school students from a Chilean birth cohort.

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Leptin status in adolescence is associated with academic performance in high-school students from a Chilean birth cohort.

ABSTRACT (250)

Objective: Leptin is a pleiotropic hormone associated with learning and memory via brain receptors. However, elevated plasma leptin levels may impair cognitive and memory functions. Because individual differences in memory performance affects students' ability to learn, we aimed to study the relation between leptin status in adolescence and school performance.

Design and setting: We studied 568 16-17 years-old adolescents from Santiago, Chile, who were part of a follow-up study beginning in infancy.

Primary and secondary outcome measures: We measured serum leptin concentration using an enzyme-linked immunoabsorbent assay. Cutoffs from the HELENA Study for 16-years-olds were used to define abnormally high leptin levels (hyperleptinemia). Academic performance was measured using high-school grades and grade-point average (GPA). Data were collected in 2009-2012; data analysis was performed in 2014.

Results: Fifteen percent of participants had hyperleptinemia. They had significantly lower school grades and GPA compared to participants with normal leptin levels (e.g. GPA mean difference= 33.8 points). After controlling for health, sociodemographic and education confounders, the odds of performing $\geq 50^{th}$ percentile in adolescents with hyperleptinemia were 56% (95% CI: 0.32-0.95) that of adolescents with normal leptin levels. Likewise, the chances of having a performance $\geq 75^{th}$ percentile in students classified as having hyperleptinemia were 32% (95% CI: 0.19-0.89) that of students having normal serum leptin concentration.

Conclusions: In high-schoolers, abnormally high levels of leptin were associated with poorer academic performance. These findings support the idea of a relationship between leptin and cognition. Further research is needed on the cognitive effects of leptin in younger populations.

Keywords: leptin, hyperleptinemia, cognition, academic performance, adolescents.

Strengths and Limitations of this Study

- Our results support the role of leptin in cognitive function.
- This paper is the first to link leptin status in a healthy younger-age human population with functional measures of cognition (high school grades), aiming at examine this relation in the 'real' world.
- Our sample is not representative of the Chilean adolescent population, as it consisted of adolescents from middle to low SES. However, the prevalence of risk unhealthy dietary habits and obesity, both of which may lead to abnormally high leptin levels, is higher in these groups.
- We used cut-offs for hyperleptinemia definition based on statistical criteria, which are the only values described for healthy adolescents. Future studies should use cut-offs based on biological risk.
- We did not consider the mediating effect of other important influences, like the prevalence of neuropsychiatric conditions and learning disorders which may impact student's academic functioning. Also, information on family structure is lacking in the analysis.
- Because association does not imply causation, future studies should replicate this analysis in other young populations.

1. INTRODUCTION

Leptin, the protein hormone produced in fat tissue which regulates the amount of fat stored in the body, was originally thought to be involved only in the regulation of food intake and energy balance. Recent evidence shows that leptin also plays a role in physiological courses other than eating behavior; in fact it can influence several developmental processes in the immature brain[1-3].

Leptin receptors are expressed throughout the brain, especially in the hippocampus and various cortical regions. Numerous evidence supports the role of leptin in higher cognitive functions, particularly the ability to boost physiological events underlying hippocampal-dependent learning and memory[4]. In the hippocampus, leptin facilitates the induction of synaptic plasticity by converting short-term potentiation of synaptic transmission into long-term potentiation (LTP), a process regarded as part of the neurophysiological basis of learning and memory formation[5]. Impairment of this process is associated with cognitive deficits[1,6,7]. In the prefrontal cortex, leptin is associated with increased brain-derived neurotrophic factor expression and neurogenesis[8,9].

If leptin in the physiological range may serve as a cognitive enhancer, elevated plasma leptin levels or hyperleptinemia may act as a pathophysiological marker for impaired cognitive function due to tissue leptin resistance (LR). The cognitive effects of leptin depend on its ability to cross the blood-brain-barrier (BBB) and the functionality of leptin receptors within the hippocampus and other brain regions[10]. The inability of peripheral leptin to reach the brain is called peripheral LR, while a diminished leptin receptor quantity and impaired signal transduction is known as central LR. In humans, both defects coexist. Also, hyperleptinemia is a strong indicator of LR[11]. Aging, disease such as diabetes and neurodegenerative disorders, and excessive exposure to saturated fats and refined sugars (e.g., triglycerides and fructose) have been associated with dysfunctional leptin transport into the brain[12-15]. On the other hand, obesity has been initially associated with a period of central leptin hypersensitivity, followed by a phase of central LR[12,16].

Elevated plasma leptin levels have been associated with poorer cognitive outcomes in the middle-aged and elderly population as well as in certain diseases, including diabetes and Alzheimer's[3]. Although adolescence is an important period for shaping memory, very few studies have approached this topic in younger age groups, and they have mostly used animal models[17-20]. Aiming to translate knowledge from research to practice and policy, the objective of this study was to assess the association between leptin and cognition in youths in the 'real' world by using functional cognitive measures such as school grades and grade point average (GPA). Because leptin modulates the cellular processes underlying hippocampal-dependent learning and memory, and because memory skills are good predictors of academic outcomes[21,22], we hypothesized that abnormally high circulating leptin would affect the ability of adolescents to perform well in school.

2. METHODS

Study sample

We studied 16-17 year-old adolescents living in Santiago, Chile, from low-to-middle SES, who were part of a birth cohort. Participants were recruited at 4 mo from public healthcare facilities in the southeast area of Santiago (n=1,791). They were born at term of uncomplicated vaginal births, weighed >3.0 kg, and were free of acute or chronic health problems. At 6 mo, infants free of IDA (n=1,657) were randomly assigned to receive iron supplementation or no added iron (ages 6-12 mo). They were assessed for developmental outcomes in infancy, 5, 10 and 15 years[23]. At 16-17 years, those with complete data in each wave (n=678) were also assessed for obesity risk and the presence of cardiovascular risk factors in a half-day evaluation that included a fasting blood draw[24]. Of them, n=568 (84% of those participating in the obesity/cardiovascular study) had completed high school by mid-2014 and met the criteria for this study. The institutional review boards of the University of Michigan, Institute of Nutrition and Food Technology (University of Chile), and the University of California, San Diego, approved this research. Participants and their primary caregiver provided informed and written consent, which was designed following the

norms for Human Experimentation, Code of Ethics of the World Medical Association (Declaration of Helsinki, 1995).

Measures

A) Data collected in the 16-17y wave

Leptin status in adolescence

After a 12-h overnight fast, a fasting venous blood sample was collected (8-9 am). Serum specimens were separated by centrifugation at 3,000 rpm for 10 minutes at 4°C, and stored at −70 °C. Serum leptin was measured by a sensitive enzyme-linked immunosorbent assay (Active Human Leptin ELISA, DSL-10-23100, Diagnostic System, Webster, TX, USA). The minimum detectable concentration was 0.05 µg/l. The intra- and interassay coefficients of variation were 4.8% and 4.3%, respectively. Hyperleptinemia was defined according to age- and sex-specific serum leptin reference for healthy adolescents as serum leptin >75th percentile on the HELENA Study (8.99 µg/l in males and 39.56 µg/l in females)[25]. These are the only descriptive values for establishing leptin levels in apparently healthy adolescents.

Academic performance

Academic performance (AP) was assessed using the student's grades in high-school (9th to 12th) and final GPA. Data were collected from administrative records of the Curriculum and Assessment Unit, Ministry of Education (Chile). Since schools may have differed in grading policies, grades (on a scale of 1-7) were transformed into scores (ranging 210-825), following the Ministry of Education criteria. The arithmetic average of each subject taken during each academic year was calculated. Then the result was converted into a score by consulting a conversion table provided by the Department of Assessment, Measurement and Educational Record, University of Chile, which provides specifications on behalf of the Ministry of

Education[26]. The same procedure was used to convert the GPA into a standard score. School grades (9th to 12th) were used as continuous variables, whereas GPA was used as a continuous and categorical variable (GPA scores ≥50th and ≥75th percentile in our sample).

Anthropometric assessment and weight status at age 16

A trained physician obtained all anthropometric measurements. Weight (Kg) and height (m) were assessed with a Seca scale (SECA 703, Seca GmbH & co. Hamburg, Germany) and a Holtain stadiometer (Harpeden 602 VR, Holtain Ltd., Wales, UK) accurate to 0.1 kg and 0.1 cm, respectively. Participants were measured without shoes, wearing underwear, in the Frankfurt position. Body-mass index (BMI= [weight (Kg)/height (m^2)]) at 16-17y was calculated, and z-scores were estimated according to the 2007 World Health Organization references[27]. Weight status was defined as: normal weight (BMI z-score from ≥-1 SD to ≤1 SD), overweight (BMI z-score >1 SD to 2 SD), and obesity (BMI z-score >2 SD).

Insulin sensitivity

Metabolic and hormonal factors, such as glucose and insulin, influence the synthesis and secretion of leptin in the body[28]. Fasting serum total glucose and insulin levels were performed after a 12-hour overnight fast. Radioimmunoassay (RIA DCP Diagnostic Products Corporation LA, USA. Intra-assay variation ≤5.1%, interassay variation ≤7.1%) was used for insulin determination. Glucose was measured with enzymatic-colorimetric test (QCA S.A. Amposta, Spain). HOMA-IR was calculated as HOMA-IR= [Glucose x Insulin]/405. HOMA-IR values ≥2.6 were considered insulin resistance (IR), according to national references for healthy adolescents[29].

Diet assessment

Diet has been associated with academic achievement[30-33], therefore, it could be a confounder for the association between leptin status and academic performance. The nutritional quality of items consumed during meals at 16-17y was measured accounting for the amount of saturated fat, fiber, sugar and salt in the food. We used a validated food frequency questionnaire used in previous studies to assess the usual diet during breakfast, lunch, dinner, snacks at school and snacks at home[34]. A list of 110 foods and beverages was used. The frequency of food consumption was assessed by a multiple response grid; participants were asked to estimate how often a particular food/beverage was consumed. Categories ranged from 'never' to 'seven times a week'. A software based on the Chilean Food Composition Tables 2010 calculated nutrient intake[35]. Each meal was considered to be unhealthy (poor nutritional value items, high in fat, sugar, salt, and calories), satisfactory (highly processed items although low in fat) or healthy (nutrient rich foods). A score ranging from 0-2 was assigned to each meal category, with higher scores representing healthier habits. To estimate the overall quality of diet, scores were summed as a raw score (range 0-10). We applied cut-offs for the Chilean adolescent population to classify the overall diet of participants into three groups: unhealthy (0-4.3), fair (4.4-5.9) and healthy (6-10)[34].

Physical activity habits

We approached physical activity (PA) habits with scheduled, repetitive and planned PA, accounting for the number of weekly hours devoted to school-based Physical Education (PE), and extracurricular sports. To measure this, we used a questionnaire that was validated in a previous study using accelerometry-based activity monitors in both elementary and high school children[36]. The questionnaire was administered by a researcher to all students at the time they attended the anthropometric examination. Participants were asked: (1) On average, over the past week, how often did you engage in PE?; (2) On average, over the past week, how often did you engage in extracurricular sports, either school- or non-school-organized?; (3) On those days, on average, how long did you engage in such PAs? With this information, we estimated

the average hours per week of scheduled PA. Participants having ≤ 90 minutes of weekly scheduled PA were considered to be physically inactive.

Type of secondary education

In Chile, secondary education includes academic high-schools, which provide theoretical education in languages, mathematics, history and sciences; vocational school, a combination of theoretical education and vocational training; and adult school, for students who in the past did not receive their secondary education certificate. Data on the type of secondary education attended by participants was retrieved from publicly available records at the Curriculum and Assessment Unit (Ministry of Education).

B) Data from previous waves

Parental education

Parental educational attainment provides an important measure of human capital level among populations and, also, is an important predictor of children's educational outcomes[37]. In infancy, participant's mother and father were ask to report the highest schooling level they have been enrolled in, as well as the highest grade they completed at that level. In our analysis, five standard hierarchic levels were defined according to the 2011 International Standard Classification of Education (ISCED): (1) no education completed, (2) first level (primary school or 1st-8th), (3) secondary level (first phase or 9th-10th), (4) secondary level (second phase or 11th-12th), and (5) and post-secondary non-tertiary educations or short-cycle tertiary education[38]. Then, we merged these categories into two: incomplete secondary education (1+2+3), and complete secondary education or higher (4+5). In health research, parental education has been often used as proxy for socioeconomic background[39].

Iron supplementation in infancy

To control potential design biases, we used a categorical variable denoting whether the participant had received iron supplementation or no-added iron at 6-12 mo.

Statistical Analysis

Statistical analysis included Chi-square test for categorical variables and Student's *t* test for continuous variables. We tested for effect measure modification (interaction) by sex, weight status and diet in the association between hyperleptinemia and academic performance by using two-way analysis of variance. The interactions were non-significant (data not shown) and, therefore, we did not stratify the analysis. Analysis of covariance was used in testing differences in serum leptin concentrations stratified by weight status (normal weight, overweight, obesity). Bonferroni's adjustments for multiple comparisons were used to examine the contrasts between groups. The same technique was used in testing differences in transformed school grades (9th to 12th) and GPA by leptin status (normal serum leptin and hyperleptinemia). Adjustments were made for sex, weight status, and IR status. Next, by using multivariate analysis, we tested the association of leptin status (main exposure) with performing $\geq 50^{th}$ and $\geq 75^{th}$ percentile in our sample (outcome). For each outcome, three logistic models were estimated. The first one included health-related variables as covariates: weight status, IR, dietary and PA habits as independent variables. A second model added sex, parental education and type of secondary education in which participants' completed HS. Finally, a fully adjusted model control for the potential effect of iron supplementation in infancy. Odds ratios were estimated along with 95% CI. Data were analyzed using Stata for Windows version 12.0 (Lakeway Drive College Station, TX, US).

RESULTS

The mean age of participants during the clinical assessments was 16.8 (0.3 SD) years. Males accounted for 51% of the sample. Prevalence of hyperleptinemia was 14.6% and the mean leptin concentration was 6.0 µg/l in males and 19.4 µg/l in females. Overall, the prevalence of obesity and overweight were 24.6% and 13.5%, respectively. Likewise, 17% of participants had IR. As for school performance, the mean GPA score was 481.1 points (range 269-795), whereas school grades score varied from 471.4 to 494.2 points.

Table 1 shows the descriptive statistics by leptin status at 16-17 years. Mean serum leptin in youths with high leptin levels was 21.4 µg/l in males and 51.3 µg/l in females. In participants with normal serum leptin, levels were 2.2 µg/l and 16.0 µg/l for males and females, respectively. Mean BMI z-score at 16-17 years was significantly higher in participants with hyperleptinemia (P<0.001) and, thus, the proportion of obese adolescents was significantly higher in this group (43%; P<0.001). Yet, 28% of participant with hyperleptinemia were normal weight. Other factors did not differ between participants having hyperleptinemia and those having normal leptin levels. Leptin status was also significantly related to sex.

Table 1 Descriptive statistics of participants in the sample

	Total (n=568)		Normal leptin levels (n= 485)		Hyperleptinemia (n=83)		*P* value*
	Mean or number	(SD) or Percentage	Mean or number	(SD) or Percentage	Mean or number	(SD) or Percentage	
Chronological age							
Age (years)	16.8♠	(0.3)	16.8♠	(0.3)	16.8♠	(0.3)	N.S.
Sex							
Male	287	50.5	231	47.6	56	67.5	0.001†
Female	281	49.5	254	52.4	27	32.5	
Serum leptin							
Males (µg/l)	6.0♠	(9.6)	2.2♠	(1.9)	21.40♠	(13.1)	<0.0001
Females (µg/l)	19.4♠	(14.4)	16.0♠	(9.7)	51.32♠	(12.7)	<0.0001
Weight status							
BMI at 16y (z-score)	0.63♠	(1.2)	0.47♠	(1.1)	1.58♠	(1.3)	<0.0001
Normal weight	352	61.9	329	67.8	23	27.7	<0.0001
Overweight	139	24.5	115	23.7	24	28.9	
Obesity	77	13.6	41	8.5	26	43.4	
Insulin sensitivity							
HOMA-IR	1.77♠	(1.2)	1.64♠	(1.0)	2.39♠	(1.9)	<0.0001
IR	83	14.6	59	12.5	24	25.3	<0.0001†
PA patterns							
Weekly scheduled PA ≤ 90 min	322	56.7	275	56.7	47	56.6	N.S†
Diet habits							
Unhealthy diet	162	28.5	137	28.3	25	30.1	N.S†

Parental education							
Mother's schooling: incomplete secondary	377	34.0	168	34.6	25	30.1	N.S[†]
Father's schooling: incomplete secondary	159	28.0	140	28.9	19	22.9	N.S[†]
Type of secondary education							
Adult school	98	17.3	79	16.3	19	22.9	N.S[†]
Iron supplementation (infancy)							
Non-added Fe	238	41.9	205	42.3	33	39.8	N.S[†]

[♠] Values expressed as Mean and (SD). Otherwise, values are number of observations and percentage. [*]Student's *t* test, except as indicated. [†]Chi^2 test (Pearson). Normal weight: BMI z ≤ 1 SD. Overweight: BMI z > 1SD and ≤ 2 SD. Obesity: BMI z > 2 SD. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al.

Overall in the sample, significant contrasts in serum leptin concentrations were seen when comparing normal weight participants with overweight (P<0.001) and obese (P<0.001) ones. Also when comparing overweight adolescents with those being obese (P<0.001). In males, serum leptin levels were significantly higher in obese participants compared to their normal weight peers (P=0.01). Last, differences in serum leptin levels were significant when comparing females with healthy weight with females having overweight (P<0.001) and obesity (P<0.001). Also, significant contrasts in serum leptin concentrations were found when comparing overweight with obese females (P<0.001) (Figure 1).

As for academic outcomes, youths with hyperleptinemia had significantly lower school grades and GPA compared to participants with normal serum leptin (Table 2 and Figure 2). After adjusting sex, weight status and insulin sensitivity, ANCOVA showed that the grades mean difference varied from 28.1 points, in 11th grade (P=0.038), to 38.6 points, in 9th grade (P=0.002), whereas GPA mean difference was 33.8 points (P=0.004).

Table 2 Association of academic performance across high school grades with leptin status at 16y (n=568)

	Hyperleptinemia (n=83)	Normal leptin levels (n=485)				
HS Grade level	Mean score	Mean score	Mean score difference	[95% CI]	*t*	*P*-value
9th	437.6	476.2	-38.6	[-63.6 ; -13.6]	-3.04	0.002
10th	446.7	478.1	-31.4	[-57.5 ; -5.32]	-2.36	0.018
11th	456.3	484.4	-28.1	[-54.6 ; -1.52]	-2.08	0.038
12th	470.3	500.1	-29.8	[-57.2 ; -2.48]	-2.14	0.033
Final GPA	454.9	488.7	-33.8	[-56.9 ; -10.7]	-2.88	0.004

Grades (9th to 12th) and final GPA expressed as scores, according to the Chilean Ministry of Education. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al. Adjustments were made for weight status and insulin sensitivity at 16-17y.

Table 3 contains the estimated association between having a final GPA ≥50th p in the sample and leptin status at 16 years after controlling for other influences. After full adjustments (Model 3), the odds of performing ≥50th p in youths with hyperleptinemia were 56% (95% CI: 0.32-0.95) that of their peers with normal serum leptin. We likewise found that performing ≥50th percentile was negative and significantly associated with unhealthy dietary habits (OR: 0.52; 95% CI: 0.33-0.81), being male (OR: 0.63; 95% CI: 0.28-0.80), and attending adult schools (OR: 0.38; 95% CI: 0.23-0.62). Association of academic results with weight status, IR and parental schooling was non-significant at an alpha level of 0.05. Iron supplementation in infancy was not associated with GPA later in adolescence.

Table 3 Relationship between having a GPA >50th percentile and leptin resistance in Chilean youths after controlling other health, sociodemographic and educational influences (n=568)

	Model 1		Model 2		Model 3	
	OR	95%CI	OR	95%CI	OR	95%CI
Hyperleptinemia	0.50**	0.30-0.84	0.50*	0.33-0.94	0.56*	0.32-0.95
Overweight	0.87	0.57-1.30	0.70	0.38-1.27	0.65	0.35-1.22
Obesity	0.99	0.86-1.18	0.86	0.49-1.50	0.81	0.45-1.33
Insulin resistance	0.94	0.80-1.11	0.93	0.49-1.09	0.78	0.55-1.18
Unhealthy diet	(…)		0.53	0.34-0.81	0.52**	0.33-0.81
Physically inactive	(…)		0.89	0.59-1.34	0.99	0.64-1.53
Male sex	(…)		(…)		0.63**	0.28-0.80
Maternal education: incomplete HS	(…)		(…)		1.09	0.82-1.74
Paternal education: incomplete HS	(…)		(…)		0.96	0.65-1.45
Adult high school	(…)		(…)		0.38***	0.23-0.62
No-Fe suppl. (infancy)	(…)		(…)		0.91	0.64-1.28

OR [95%CI]: Odd Ratio [95% Confidence Interval]. (…) Non-observed. Significance level: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al.. Overweight: BMI-Z >1 SD and ≥2SD. Obesity: BMI-Z ≥ 2 SD. IR: HOMA-IR 2.6. Unhealthy diet: diet high in simple carbohydrates and saturated fats. Physically inactive: scheduled PA ≤90 min/week. Adult high school: education for students who in the past were unable to receive their diploma.

Table 4 shows the association between having a final GPA ≥75th p in the sample and leptin status at 16 years after controlling for a number of confounders. In a fully adjusted model (Model 3), the likelihood of performing ≥75th p in leptin resistant youths were 42% (95% CI: 0.19-0.89) that of students with normal leptin levels. We likewise found that school performance was negatively related to being male (OR: 0.43; 95% CI: 0.28-0.71), having unhealthy dietary habits (OR: 0.41; 95% CI: 0.24-0.75), father's schooling (incomplete secondary education) (OR: 0.57; 95% CI: 0.35-0.93) and the type of secondary education (OR: 0.35; 95% CI: 0.18-0.69). Again, weight status, IR and maternal educational level were unrelated to school performance. Iron supplementation in infancy was not related to HS performance as measured by having a GPA in the highest quartile.

Table 4 Relationship between having a GPA >75th percentile and leptin resistance in Chilean youths after controlling other health, sociodemographic and educational influences (n=568)

	Model 1		Model 2		Model 3	
	OR	95%CI	OR	95%CI	OR	95%CI
Hyperleptinemia	0.35***	0.17-0.72	0.35***	0.17-0.73	0.42*	0.19-0.89
Overweight	0.66	0.40-1.10	0.67	0.42-1.13	0.62	0.37-1.05
Obesity	0.83	0.42-1.64	0.83	0.42-1.64	0.74	0.36-1.54
Insulin resistance	0.79	0.41-0.95	0.69	0.44-1.06	0.73	0.46-1.13
Unhealthy diet	(…)		0.43***	0.26-0.78	0.41***	0.24-0-75
Physically inactive	(…)		1.01	0.63-1.59	1.00	0.66-1.54
Male sex	(…)		(…)		0.43***	0.28-0.71
Maternal education: incomplete HS	(…)		(…)		1.07	0.68-1.67
Paternal education: incomplete HS	(…)		(…)		0.57*	0.35-0.93
Adult high school	(…)		(…)		0.35***	0.18-0.69
No-Fe suppl. (infancy)	(…)		(…)		0.71	0.48-1.06

OR [95%CI]: Odd Ratio [95% Confidence Interval]. (…) Non-observed. Significance level: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al. Overweight: BMI-Z >1 SD and ≥2SD. Obesity: BMI-Z ≥ 2 SD. IR: HOMA-IR 2.6. Unhealthy diet: diet high in simple carbohydrates and saturated fats. Physically inactive: scheduled PA ≤90 min/week. Adult high school: education for students who in the past were unable to receive their diploma.

DISCUSSION

In a sample of high-school graduates, we examined the relationship between leptin status and cognition by using functional measures such as high-school grades and GPA. To our best knowledge this is the first study addressing the link between leptin status and this domain of cognition in a younger-age human population. Compared with students having normal serum leptin, those having hyperleptinemia had lower school grades and GPA. Similarly, they had lowers odds of performing ≥50th and ≥75th percentile of the sample. Even after controlling relevant confounders, the association between leptin status in adolescence and academic performance remained significant.

Our results are of importance for several reasons. Leptin plays a key role in memory processing through induction of hippocampal and prefrontal cortex synaptic plasticity. However, a growing body of evidence suggests elevated plasma leptin levels may limit the potential for synaptic plasticity and could partially explain some cognitive deficits[3,7,17-20]. Likewise, links are strong between memory performance and academic outcomes. In children, working memory and particularly the ability to retrieve and manipulate information from long-term memory has been found to predict math, reading and spelling outcomes, even after controlling for IQ[21]. Dysfunctions in this domain can lead to learning difficulties in activities that involve storing and processing information[21,22]. Furthermore, high-school grades predict higher education outcomes and subsequent job status and income[40].

Very few studies have explored the link between leptin and cognition in younger animal populations, and their findings are in line with ours. Oomura *et al.* showed that leptin modulates higher neural functions in mice 4-8 weeks-old[17]. While infusion of low doses of leptin enhanced learning and memory performance and hippocampal LTP, high doses impaired them. The notion that hyperleptinemia could be responsible for some cognitive deficits in adolescent mice is supported by Valladolid-Acebes and colleagues[18-20]. In age-matched mice, short-term exposure to high fat diet compromised hippocampal dependent learning and memory. Moreover, in adolescent mice, the behavioral impairment was

accompanied by changes in hippocampal morphology and functionality of leptin receptors within de hippocampus.

Leptin levels in this Chilean sample were lower than those reported for European adolescents[27], but higher than those reported for healthy adolescents in other Latin-America countries and Asia[41,42]. Population differences in the epidemiologic and nutrition transition may in part explain the disparity in levels of circulating leptin. Increased intake of fat, sugar and processed foods, reduced PA, and increased risk of non-communicable disease, including obesity, are more prevalent in the last stages of the transition, which is the case of Chile and the European countries[43].

Although an association between weight status and academic results was not observed in this sample, obesity in pediatric populations has been related with impaired cognitive function[44,45] and the ability to perform well in school[46,47]. Some authors have postulated that cognitive impairment may actually precede excessive weight gain[18,48-50]. Two main ideas are behind this view: first, impairment of learning is observed before other metabolic alterations; second, while the adverse effects of consuming high fat/high sugar diet on cognitive function is a good predictor of subsequent weight gain, the effect of those nutrients on weight gain does not reliably predict subsequent cognitive deficit. Our results suggest that leptin might mediate, in part, the effect of weight status on cognition and academic outcomes.

It is very likely that hyperleptinemia in most individuals in our sample may lead to both under-responsiveness to exogenous leptin and impairment of signal transduction in target neurons. Although obesity has been traditionally associated with hyperleptinemia in the non-elderly population, new data indicate that fructose, sucrose and triglycerides may induce hyperleptinemia regardless of the amount of body fat[15]. In our sample, less than a half of participants having hyperleptinemia were obese, and 28% were normal weight. Likewise, almost 80% had a diet in the intermediate or unhealthy zone, which means excessive intake of refined sugars and fats. Administration of even small quantities of triglycerides to normal weight rats immediately resulted in dysfunctional leptin transport across the BBB[51,52]. Similarly, rats exposed to high fructose diet demonstrated an impaired response to peripheral leptin injection and

central leptin infusion. However, switching to a sugar-free diet improved leptin sensitivity [53]. In both cases, chronic fructose and triglycerides consumption led to impaired responsiveness to exogenous leptin prior to body weight.

Our findings also confirm the role of nutrition in academic outcomes. Participants having an unhealthy diet had significantly lower odds of performing well in high-school, independent of leptin status. Numerous studies report that diet relates to specific outcomes that are important for the educational attainment of children and adolescents[30-33]. The consumption of refined carbohydrates and saturated fats is linked to impaired cognitive performance. Exposure to these macronutrients interferes directly with hippocampal functioning by cutting down the production of neurotrophins, increasing neuroinflammatory markers, and altering the BBB[49,54].

Last, in our sample, the share of participants with hyperlpetinemia was significantly higher in males compared to females. Despite that leptin is produced in the fat tissue, serum leptin concentration is also dependent on determinants such as insulin sensitivity and overconsumption of sucrose and fructose. Male and females participants in our study had similar prevalence of excess weight and IR, but the proportion of adolescents having a diet high in simple sugars, mostly sucrose and fructose from candies and sugar sweetened beverages (a dietary pattern regarded as a fair diet in our questionnaire) was significantly higher among males compared to females. Population surveys in the Chilean adolescent population confirm that adolescent males have excess consumption of simple carbohidrates[55,56].

Implications from these results

The Chilean adolescent population is highly exposed to risk factors for hyperleptinemia. As reported by the 2014 Chilean National Food Consumption Survey[55], dietary habits of poor nutritional quality are wide spread. Adolescents aged 14-18 rank first in the consumption of refined sugar (121 g/day) and second in

the consumption of saturated fats. According to this survey, the prevalence of overweight and obesity in adolescents is 38% and 13%, respectively.

A further implication from this study relates the fact that exposure to abnormally high levels of leptin would be starting early in life. A non-modifiable condition such as ageing is associated with hyperleptinemia, but exposure to high fat/high sugar diet as well as to overweight and obesity are avoidable. While schools should serve as a point of entry for the promotion of healthy lifestyles, the food industry uses schools as a means of reaching young consumers. Despite the efforts to limit the availability of unhealthy foods in schools, in the Latin-American Southern Cone 70% of adolescents drink sugar-sweetened beverages on a daily basis[56]. Frequent (1-2 times/week) and very frequent (≥3 times/week) fast-food consumption among Latin-American adolescents range from 40% in Uruguay to 70% in Bolivia, according to a recent international study[57].

The following points should be considered in the design and application of preventive strategies. Fructose-induced hyperleptinemia may be reversible, which means there is a possibility for improvement. Second, hyperleptinemia may be triggered by exposure to even small amounts of saturated fats and refined sugars, which are major components of the Western diet. Moreover, the effects on leptin levels appear soon after exposure to these macronutrients. Finally, hyperleptinemia is a precondition for LR; at this point it might be late for adolescents to reach their full cognitive and academic potential.

Study limitations and strengths

Our results supports the notion that leptin has an important role in cognitive function in addition to its role in energy balance regulation. A further strength is that we approached this topic in a group younger than previous research on leptin and cognition. Third, we used functional measures of cognition (e.g. academic performance in high-school), aiming to examine this relation in the 'real' world. In spite of these

strengths, the study has limitations that should be considered when interpreting the findings. First, our sample is not representative of the Chilean adolescent population, as it consists of adolescents from low to middle SES. However, SES level may be especially important. The prevalence of unhealthy dietary habits and obesity, both of which may lead to hyperleptinemia, is higher among adolescents from middle to low SES compared to adolescents of high SES[55]. Second, we used cut-offs for hyperleptinemia definition based on statistical criteria in a European cohort, which are the only values described for healthy adolescents. It is worth noting that Chile, like Western European countries, is in the last stage of the epidemiological transition. Yet, future studies should use cut-offs based on biological risk. Third, due to data constraint the prevalence of neuropsychiatric conditions was not included in the study. Leptin effects on memory and cognition might be confounded by the co-existence of neuropsychiatric disorders such as depression, schizophrenia and substance-related disorders. Fourth, we did not consider the mediating effect of potential learning and cognitive disorders which may impact student's academic functioning. Also, information on family structure is lacking in the analysis. Yet, we considered the impact of parental education. Because association is not enough to prove causation, future studies should replicate and extend this analysis in other young populations, and further investigate how leptin influence cognitive and academic outcomes.

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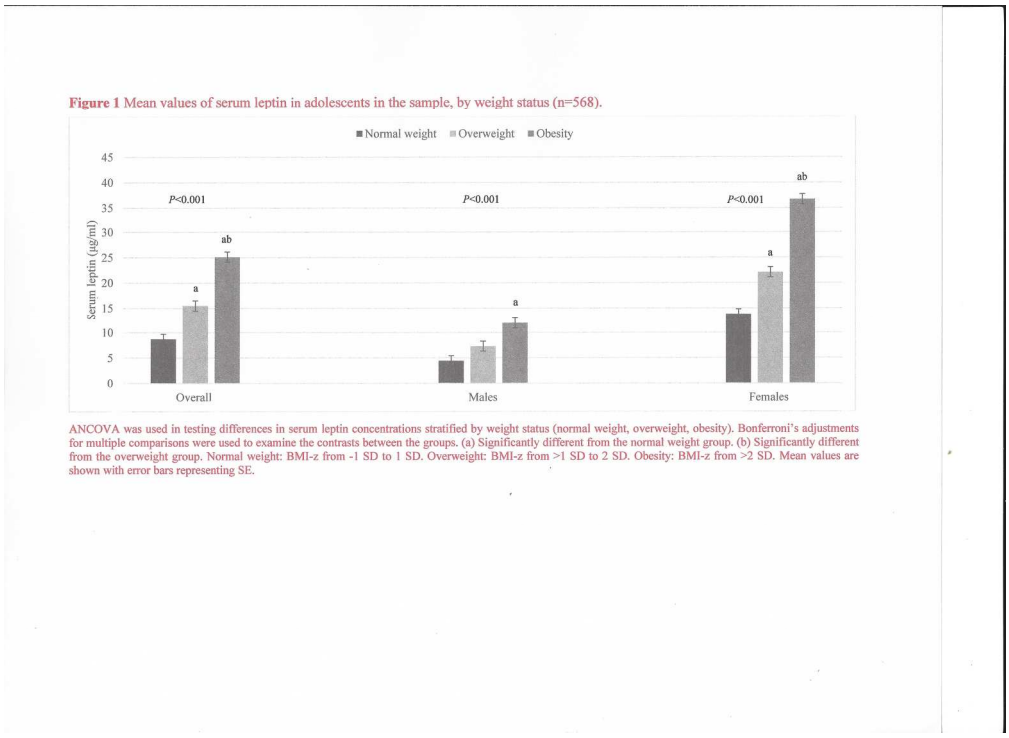

Figure 1 Mean values of serum leptin in adolescents in the sample, by weight status (n=568).

ANCOVA was used in testing differences in serum leptin concentrations stratified by weight status (normal weight, overweight, obesity). Bonferroni's adjustments for multiple comparisons were used to examine the contrasts between the groups. (a) Significantly different from the normal weight group. (b) Significantly different from the overweight group. Normal weight: BMI-z from -1 SD to 1 SD. Overweight: BMI-z from >1 SD to 2 SD. Obesity: BMI-z from >2 SD. Mean values are shown with error bars representing SE.

215x156mm (300 x 300 DPI)

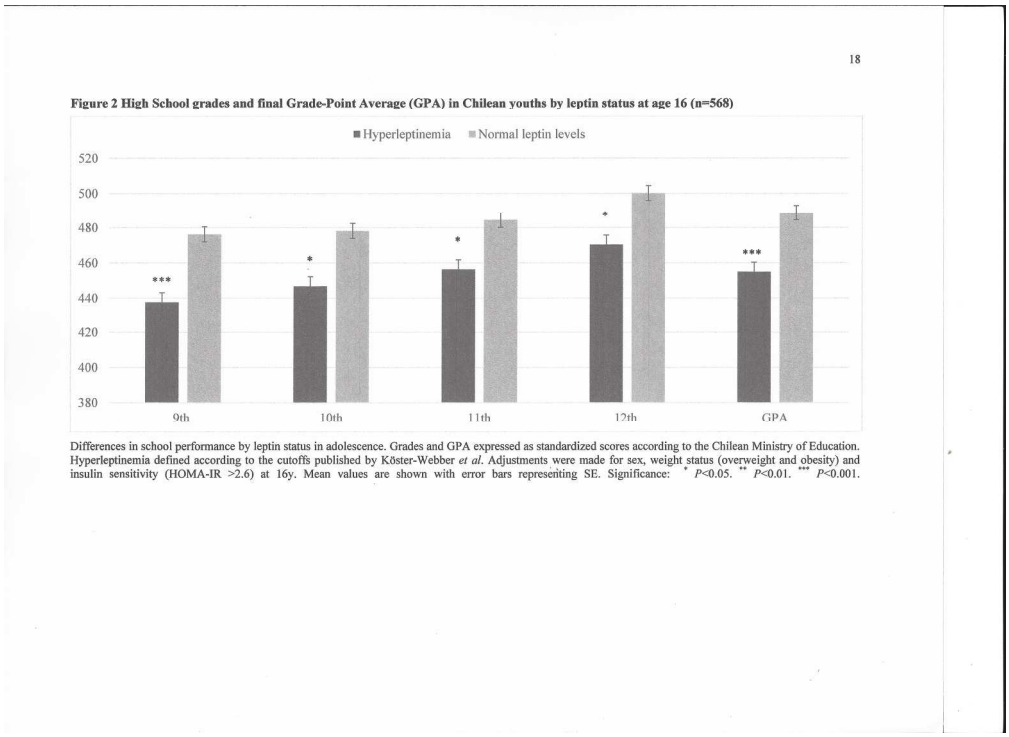

Figure 2 High School grades and final Grade-Point Average (GPA) in Chilean youths by leptin status at age 16 (n=568)

Differences in school performance by leptin status in adolescence. Grades and GPA expressed as standardized scores according to the Chilean Ministry of Education. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al. Adjustments were made for sex, weight status (overweight and obesity) and insulin sensitivity (HOMA-IR >2.6) at 16y. Mean values are shown with error bars representing SE. Significance: * P<0.05. ** P<0.01. *** P<0.001.

215x156mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	*Recommendation*	*Status*	*Page No*
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	✓	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	✓	2
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓	5-6
Objectives	3	State specific objectives, including any pre specified hypotheses	✓	6
Methods				
Study design	4	Present key elements of study design early in the paper	✓	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	✓	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	✓	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓	7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓	7-9
Bias	9	Describe any efforts to address potential sources of bias	✓	4,23‡
Study size	10	Explain how the study size was arrived at	✓	6§
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓	7-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	✓	9
		(*b*) Describe any methods used to examine subgroups and	✓	9

		interactions		
		(*c*) Explain how missing data were addressed	n.a.	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	✓	6§
		(*e*) Describe any sensitivity analyses	n.a.	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓	10
		(b) Give reasons for non-participation at each stage	✓	6§
		(c) Consider use of a flow diagram		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓	10-11
		(b) Indicate number of participants with missing data for each variable of interest	n.a	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	✓	6§
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	✓	6§
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures	✓	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓	13-19
		(*b*) Report category boundaries when continuous variables were categorized	✓	13-19
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	✓	16-19
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓	7
Discussion				
Key results	18	Summarise key results with reference to study objectives	✓	20-21
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓	23
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓	20-21
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓	20-23

Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓	25

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

§Enrolment, assessments and data from previous waves are described in detail in previous works by the authors'. Reference for these works are provided in the Methods section (Study sample).

ǂ This was reckoned as a limitation.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Leptin status in adolescence is associated with academic performance in high school: A cross-sectional study in a Chilean birth cohort

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Leptin status in adolescence is associated with academic performance in high school: A cross-sectional study in a Chilean birth cohort.

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Leptin status in adolescence is associated with academic performance in high school: A cross-sectional study in a Chilean birth cohort.

ABSTRACT (249)

Objective: Leptin is a pleiotropic hormone associated with learning and memory via brain receptors. However, elevated plasma leptin levels may impair cognitive and memory functions. Because individual differences in memory performance affects students' ability to learn, we aimed to study the relation between leptin status in adolescence and school performance.

Design and setting: We studied 568 16-17 years-old adolescents from Santiago. A cross-sectional analysis was carried out into a birth cohort conducted in Santiago (Chile).

Primary and secondary outcome measures: We measured serum leptin concentration using an enzyme-linked immunoabsorbent assay. Cutoffs from the HELENA Study for 16-years-olds were used to define abnormally high leptin levels (hyperleptinemia). Academic performance was measured using high-school grades and grade-point average (GPA). Data were collected in 2009-2012; data analysis was performed in 2014.

Results: Fifteen percent of participants had hyperleptinemia. They had significantly lower school grades and GPA compared to participants with normal leptin levels (e.g. GPA mean difference= 33.8 points). Leptin levels were negative and significantly correlated with school grades in 9th, 10th and 12th. Similarly, it was negatively correlated with high school GPA. After controlling for health, sociodemographic and education confounders, the chances of having a performance ≥75th percentile in students having hyperleptinemia were 32% (95% CI: 0.19-0.89) that of students having normal serum leptin concentration.

Conclusions: In high-schoolers, abnormally high levels of leptin were associated with poorer academic performance. These findings support the idea of a relationship between leptin and cognition. Further research is needed on the cognitive effects of leptin in younger populations.

Keywords: leptin, hyperleptinemia, cognition, academic performance, adolescents.

Strengths and Limitations of this Study

- Our results support the role of leptin in cognitive function.
- This paper is the first to link leptin status in a healthy younger-age human population with functional measures of cognition (high school grades), aiming at examine this relation in the 'real' world.
- Our sample is not representative of the Chilean adolescent population, as it consisted of adolescents from middle to low SES. However, the prevalence of risk unhealthy dietary habits and obesity, both of which may lead to abnormally high leptin levels, is higher in these groups.
- We used cut-offs for hyperleptinemia definition based on statistical criteria, which are the only values described for healthy adolescents. Future studies should use cut-offs based on biological risk.
- We did not consider the mediating effect of other important influences, like the prevalence of neuropsychiatric conditions and learning disorders which may impact student's academic functioning. Also, information on family structure is lacking in the analysis.
- Because association does not imply causation, future studies should replicate this analysis in other young populations.

1. INTRODUCTION

Leptin, the protein hormone produced in fat tissue which regulates the amount of fat stored in the body, was originally thought to be involved only in the regulation of food intake and energy balance. Recent evidence shows that leptin also plays a role in physiological courses other than eating behavior; in fact it can influence several developmental processes in the immature brain[1-3].

Leptin receptors are expressed throughout the brain, especially in the hippocampus and various cortical regions. Numerous evidence supports the role of leptin in higher cognitive functions, particularly the ability to boost physiological events underlying hippocampal-dependent learning and memory[4]. In the hippocampus, leptin facilitates the induction of synaptic plasticity by converting short-term potentiation of synaptic transmission into long-term potentiation (LTP), a process regarded as part of the neurophysiological basis of learning and memory formation[5]. Impairment of this process is associated with cognitive deficits[1,6,7]. In the prefrontal cortex, leptin is associated with increased brain-derived neurotrophic factor expression and neurogenesis[8,9].

If leptin in the physiological range may serve as a cognitive enhancer, elevated plasma leptin levels or hyperleptinemia may act as a pathophysiological marker for impaired cognitive function due to tissue leptin resistance (LR). The cognitive effects of leptin depend on its ability to cross the blood-brain-barrier (BBB) and the functionality of leptin receptors within the hippocampus and other brain regions[10]. The inability of peripheral leptin to reach the brain is called peripheral LR, while a diminished leptin receptor quantity and impaired signal transduction is known as central LR. In humans, both defects coexist. Also, hyperleptinemia is a strong indicator of LR[11]. Aging, disease such as diabetes and neurodegenerative disorders, and excessive exposure to saturated fats and refined sugars (e.g., triglycerides and fructose) have been associated with dysfunctional leptin transport into the brain[12-15]. On the other hand, obesity has been initially associated with a period of central leptin hypersensitivity, followed by a phase of central LR[12,16].

Elevated plasma leptin levels have been associated with poorer cognitive outcomes in the middle-aged and elderly population as well as in certain diseases, including diabetes and Alzheimer's[3]. Although adolescence is an important period for shaping memory, very few studies have approached this topic in younger age groups, and they have mostly used animal models[17-20]. Aiming to translate knowledge from research to practice and policy, the objective of this study was to assess the association between leptin and cognition in youths in the 'real' world by using functional cognitive measures such as school grades and grade point average (GPA). Because leptin modulates the cellular processes underlying hippocampal-dependent learning and memory, and because memory skills are good predictors of academic outcomes[21,22], we hypothesized that abnormally high circulating leptin would affect the ability of adolescents to perform well in school.

2. METHODS

Study sample

We studied 16-17 year-old adolescents living in Santiago, Chile, from low-to-middle SES, who were part of a birth cohort. Participants were recruited at 4 mo from public healthcare facilities in the southeast area of Santiago (n=1,791). They were born at term of uncomplicated vaginal births, weighed >3.0 kg, and were free of acute or chronic health problems. At 6 mo, infants free of IDA (n=1,657) were randomly assigned to receive iron supplementation or no added iron (ages 6-12 mo). They were assessed for developmental outcomes in infancy, 5, 10 and 15 years[23]. At 16-17 years, those with complete data in each wave (n=678) were also assessed for obesity risk and the presence of cardiovascular risk factors in a half-day evaluation that included a fasting blood draw[24]. Of them, n=568 (84% of those participating in the obesity/cardiovascular study) had completed high school by mid-2014 and met the criteria for this study. The institutional review boards of the University of Michigan, Institute of Nutrition and Food Technology (University of Chile), and the University of California, San Diego, approved this research. Participants and their primary caregiver provided informed and written consent, which was designed following the

norms for Human Experimentation, Code of Ethics of the World Medical Association (Declaration of Helsinki, 1995).

Measures

A) Data collected in the 16-17y wave

Leptin status in adolescence

After a 12-h overnight fast, a fasting venous blood sample was collected (8-9 am). Serum specimens were separated by centrifugation at 3,000 rpm for 10 minutes at 4°C, and stored at −70 °C. Serum leptin was measured by a sensitive enzyme-linked immunosorbent assay (Active Human Leptin ELISA, DSL-10-23100, Diagnostic System, Webster, TX, USA). The minimum detectable concentration was 0.05 µg/l. The intra- and interassay coefficients of variation were 4.8% and 4.3%, respectively. Hyperleptinemia was defined according to age- and sex-specific serum leptin reference for healthy adolescents as serum leptin >75th percentile on the HELENA Study (8.99 µg/l in males and 39.56 µg/l in females)[25]. These are the only descriptive values for establishing leptin levels in apparently healthy adolescents.

Academic performance

Academic performance (AP) was assessed using the student's grades in high-school (9th to 12th) and final GPA. Data were collected from administrative records of the Curriculum and Assessment Unit, Ministry of Education (Chile). Since schools may have differed in grading policies, grades (on a scale of 1-7) were transformed into scores (ranging 210-825), following the Ministry of Education criteria. The arithmetic average of each subject taken during each academic year was calculated. Then the result was converted into a score by consulting a conversion table provided by the Department of Assessment, Measurement and Educational Record, University of Chile, which provides specifications on behalf of the Ministry of

Education[26]. The same procedure was used to convert the GPA into a standard score. School grades (9th to 12th) were used as continuous variables, whereas GPA was used as a continuous and categorical variable (GPA scores ≥50th and ≥75th percentile in our sample).

Anthropometric assessment and weight status at age 16

A trained physician obtained all anthropometric measurements. Weight (Kg) and height (m) were assessed with a Seca scale (SECA 703, Seca GmbH & co. Hamburg, Germany) and a Holtain stadiometer (Harpeden 602 VR, Holtain Ltd., Wales, UK) accurate to 0.1 kg and 0.1 cm, respectively. Participants were measured without shoes, wearing underwear, in the Frankfurt position. Body-mass index (BMI= [weight (Kg)/height (m^2)]) at 16-17y was calculated, and z-scores and percentiles were estimated according to the 2007 World Health Organization[27] and Centers for Disease Control and Prevention references, respectively[28]. Z-scores and percentiles for height at 16-17y were also calculated. Weight status was defined as: normal weight (BMI z-score from ≥-1 SD to ≤1 SD), overweight (BMI z-score >1 SD to 2 SD), and obesity (BMI z-score >2 SD). Total fat mass (TFM) was determined on dual X-ray absorptiometry (DXA) (Lunar Prodigy Corp., Madison, WI, USA. Software, Lunar iDXA ENCORE 2011, Version 13.60.033 Copyright © 1998-2010).

Insulin sensitivity

Metabolic and hormonal factors, such as glucose and insulin, influence the synthesis and secretion of leptin in the body[29]. Fasting serum total glucose and insulin levels were performed after a 12-hour overnight fast. Radioimmunoassay (RIA DCP Diagnostic Products Corporation LA, USA. Intra-assay variation ≤5.1%, interassay variation ≤7.1%) was used for insulin determination. Glucose was measured with enzymatic-colorimetric test (QCA S.A. Amposta, Spain). HOMA-IR was calculated as HOMA-IR=

[Glucose x Insulin]/405. HOMA-IR values ≥2.6 were considered insulin resistance (IR), according to national references for healthy adolescents[30].

Diet assessment

Diet has been associated with academic achievement[31-34], therefore, it could be a confounder for the association between leptin status and academic performance. The nutritional quality of items consumed during meals at 16-17y was measured accounting for the amount of saturated fat, fiber, sugar and salt in the food. We used a validated food frequency questionnaire used in previous studies to assess the usual diet during breakfast, lunch, dinner, snacks at school and snacks at home[35]. A list of 110 foods and beverages was used. The frequency of food consumption was assessed by a multiple response grid; participants were asked to estimate how often a particular food/beverage was consumed. Categories ranged from 'never' to 'seven times a week'. A software based on the Chilean Food Composition Tables 2010 calculated nutrient intake[36]. Each meal was considered to be unhealthy (poor nutritional value items, high in fat, sugar, salt, and calories), satisfactory (highly processed items although low in fat) or healthy (nutrient rich foods). A score ranging from 0-2 was assigned to each meal category, with higher scores representing healthier habits. To estimate the overall quality of diet, scores were summed as a raw score (range 0-10). We applied cut-offs for the Chilean adolescent population to classify the overall diet of participants into three groups: unhealthy (0-4.3), fair (4.4-5.9) and healthy (6-10)[35].

Physical activity habits

We approached physical activity (PA) habits with scheduled, repetitive and planned PA, accounting for the number of weekly hours devoted to school-based Physical Education (PE), and extracurricular sports. To measure this, we used a questionnaire that was validated in a previous study using accelerometry-based

activity monitors in both elementary and high school children[37]. The questionnaire was administered by a researcher to all students at the time they attended the anthropometric examination. Participants were asked: (1) On average, over the past week, how often did you engage in PE?; (2) On average, over the past week, how often did you engage in extracurricular sports, either school- or non-school-organized?; (3) On those days, on average, how long did you engage in such PAs? With this information, we estimated the average hours per week of scheduled PA. Participants having ≤ 90 minutes of weekly scheduled PA were considered to be physically inactive.

Type of secondary education

In Chile, secondary education includes academic high-schools, which provide theoretical education in languages, mathematics, history and sciences; vocational school, a combination of theoretical education and vocational training; and adult school, for students who in the past did not receive their secondary education certificate. Data on the type of secondary education attended by participants was retrieved from publicly available records at the Curriculum and Assessment Unit (Ministry of Education).

B) Data from previous waves

Parental education

Parental educational attainment provides an important measure of human capital level among populations and, also, is an important predictor of children's educational outcomes[38]. In infancy, participant's mother and father were ask to report the highest schooling level they have been enrolled in, as well as the highest grade they completed at that level. In our analysis, five standard hierarchic levels were defined according to the 2011 International Standard Classification of Education (ISCED): (1) no education completed, (2)

first level (primary school or 1st-8th), (3) secondary level (first phase or 9th-10th), (4) secondary level (second phase or 11th-12th), and (5) and post-secondary non-tertiary educations or short-cycle tertiary education[39]. Then, we merged these categories into two: incomplete secondary education (1+2+3), and complete secondary education or higher (4+5). In health research, parental education has been often used as proxy for socioeconomic background[40].

Iron supplementation in infancy

To control potential design biases, we used a categorical variable denoting whether the participant had received iron supplementation or no-added iron at 6-12 mo.

Statistical Analysis

Statistical analysis included Chi-square test for categorical variables and Student's *t* test for continuous variables. We tested for effect measure modification (interaction) by sex, weight status and diet in the association between hyperleptinemia and academic performance by using two-way analysis of variance. The interactions were non-significant (data not shown) and, therefore, we did not stratify the analysis. Analysis of covariance was used in testing differences in serum leptin concentrations stratified by weight status (normal weight, overweight, obesity). Bonferroni's adjustments for multiple comparisons were used to examine the contrasts between groups. The same technique was used in testing differences in transformed school grades (9th to 12th) and GPA by leptin status (normal serum leptin and hyperleptinemia). Adjustments were made for sex, weight status, and IR status. To examine the association of leptin with school performance, we first conducted linear regression analysis. Serum leptin levels were tested against school grades (9th to 12th and GPA), using two models. Model 1 was adjusted for sex, fat mass and parental education. Model 2 added IR, nutritional quality of diet, PA status, type of secondary education (vocational and adult school), and iron supplementation in infancy (no-added iron) to

the covariates in Model 1. A further analysis was conducted with logistic regressions to estimate the odds of performing ≥50th and ≥75th percentile in our sample (outcome) in participants with hyperleptinemia. For each outcome, three logistic models were estimated. The first one included health-related variables as covariates: weight status, IR, dietary and PA habits as independent variables. A second model added sex, parental education and type of secondary education in which participants' completed HS. Finally, a fully adjusted model control for the potential effect of iron supplementation in infancy. Odds ratios were estimated along with 95% CI. Data were analyzed using Stata for Windows version 12.0 (Lakeway Drive College Station, TX, US).

RESULTS

The mean age of participants during the clinical assessments was 16.8 (0.3 SD) years. Males accounted for 51% of the sample. Prevalence of hyperleptinemia was 14.6% and the mean leptin concentration was 6.0 µg/l in males and 19.4 µg/l in females. Overall, the prevalence of obesity and overweight were 24.6% and 13.5%, respectively. Likewise, 17% of participants had IR. As for school performance, the mean GPA score was 481.1 points (range 269-795), whereas school grades score varied from 471.4 to 494.2 points.

Table 1 shows the descriptive statistics by leptin status at 16-17 years. Mean serum leptin in youths with high leptin levels was 21.4 µg/l in males and 51.3 µg/l in females. In participants with normal serum leptin, levels were 2.2 µg/l and 16.0 µg/l for males and females, respectively. Mean BMI z-score and BMI percentile at 16-17 years were significantly higher in participants with hyperleptinemia (P<0.001) and, thus, the proportion of obese adolescents was significantly higher in this group (43%; P<0.001). Yet, 28% of participant with hyperleptinemia were normal weight. Other factors did not differ between participants having hyperleptinemia and those having normal leptin levels. Leptin status was also significantly related to sex.

Table 1 Descriptive statistics of participants in the sample

	Total (n=568)		Normal leptin levels (n= 485)		Hyperleptinemia (n=83)		*P* value*
	Mean or number	(SD) or Percentage	Mean or number	(SD) or Percentage	Mean or number	(SD) or Percentage	
Chronological age							
Age (years)	16.8♠	(0.3)	16.8♠	(0.3)	16.8♠	(0.3)	N.S.
Sex							
Male	287	50.5	231	47.6	56	67.5	0.001†
Female	281	49.5	254	52.4	27	32.5	
Serum leptin							
Males (µg/l)	6.0♠	(9.6)	2.2♠	(1.9)	21.40♠	(13.1)	<0.0001
Females (µg/l)	19.4♠	(14.4)	16.0♠	(9.7)	51.32♠	(12.7)	<0.0001
Anthropometrics							
Height at 16y (WHO z-score)	-0.45♠	0.8	-0.47♠	0.8	-0.35♠	0.8	N.S
Height at 16y (CDC percentile)	35.0♠	24.7	34.5♠	24.7	37.8♠	24.2	N.S.
BMI at 16y (WHO z-score)	0.63♠	(1.2)	0.47♠	(1.1)	1.58♠	(1.3)	<0.0001
BMI at 16y (CDC percentile)	64.4♠	28.0	61.4♠	27.4	81.4♠	25.3	<0.0001
Weight status							
Normal weight	352	61.9	329	67.8	23	27.7	<0.0001
Overweight	139	24.5	115	23.7	24	28.9	
Obesity	77	13.6	41	8.5	26	43.4	
Insulin sensitivity							
HOMA-IR	1.77♠	(1.2)	1.64♠	(1.0)	2.39♠	(1.9)	<0.0001
IR	83	14.6	59	12.5	24	25.3	<0.0001†

PA patterns							
Weekly scheduled PA ≤ 90 min	322	56.7	275	56.7	47	56.6	N.S[†]
Diet habits							
Unhealthy diet	162	28.5	137	28.3	25	30.1	N.S[†]
Parental education							
Mother's schooling: incomplete secondary	377	34.0	168	34.6	25	30.1	N.S[†]
Father's schooling: incomplete secondary	159	28.0	140	28.9	19	22.9	N.S[†]
Type of secondary education							
Adult school	98	17.3	79	16.3	19	22.9	N.S[†]
Iron supplementation (infancy)							
Non-added Fe	238	41.9	205	42.3	33	39.8	N.S[†]

♠ Values expressed as Mean and (SD). Otherwise, values are number of observations and percentage. [*]Student's *t* test, except as indicated. [†]Chi^2 test (Pearson). Normal weight: BMI z ≤ 1 SD. Overweight: BMI z > 1SD and ≤ 2 SD. Obesity: BMI z > 2 SD. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al.

Overall in the sample, significant contrasts in serum leptin concentrations were seen when comparing normal weight participants with overweight (P<0.001) and obese (P<0.001) ones. Also when comparing overweight adolescents with those being obese (P<0.001). In males, serum leptin levels were significantly higher in obese participants compared to their normal weight peers (P=0.01). Last, differences in serum leptin levels were significant when comparing females with healthy weight with females having overweight (P<0.001) and obesity (P<0.001). Also, significant contrasts in serum leptin concentrations were found when comparing overweight with obese females (P<0.001) (Figure 1).

As for academic outcomes, youths with hyperleptinemia had significantly lower school grades and GPA compared to participants with normal serum leptin (Table 2 and Figure 2). After adjusting sex, weight status and insulin sensitivity, ANCOVA showed that the grades mean difference varied from 28.1 points, in 11th grade (P=0.038), to 38.6 points, in 9th grade (P=0.002), whereas GPA mean difference was 33.8 points (P=0.004).

Table 2 Association of academic performance across high school grades with leptin status at 16y (n=568)

	Hyperleptinemia (n=83)	Normal leptin levels (n=485)				
HS Grade level	Mean score	Mean score	Mean score difference	[95% CI]	*t*	*P*-value
9th	437.6	476.2	-38.6	[-63.6 ; -13.6]	-3.04	0.002
10th	446.7	478.1	-31.4	[-57.5 ; -5.32]	-2.36	0.018
11th	456.3	484.4	-28.1	[-54.6 ; -1.52]	-2.08	0.038
12th	470.3	500.1	-29.8	[-57.2 ; -2.48]	-2.14	0.033
Final GPA	454.9	488.7	-33.8	[-56.9 ; -10.7]	-2.88	0.004

Grades (9th to 12th) and final GPA expressed as scores, according to the Chilean Ministry of Education. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al. Adjustments were made for weight status and insulin sensitivity at 16-17y.

We observed a negative and significant association of serum leptin levels at 16-17y with academic performance, as measured by transformed school grades (Table 3). Serum leptin was negatively correlated with school performance in 9th, 10th and 12th, and, similarly, it was negatively correlated with high school GPA. For instance, for a one-unit increase in leptin levels, GPA decreased -0.80 points. School grades in 11th also decreased with leptin rise, but association was non-significant in our sample.

Table 3 Regression coefficients of the association between serum leptin levels at 16-17y and academic performance in high school

	Intercept	Coef.	Robust SE	R^2
9th grade				
Model 1	491.2***	-0.56	0.32	0.04
Model 2	501.9***	-0.62*	0.30	0.09
10th grade				
Model 1	495.6***	-0.76	0.43	0.02
Model 2	508.3***	-0.82*	0.37	0.14
11th grade				
Model 1	511.7***	-0.47	0.35	0.04
Model 2	522.0***	-0.57	0.34	0.10
12th grade				
Model 1	527.9***	-0.87*	0.37	0.04
Model 2	540.5***	-0.98***	0.37	0.13
HS GPA				
Model 1	511.2***	-0.71*	0.31	0.04
Model 2	521.3***	-0.80***	0.30	0.16

HS GPA: High school grade-point average. Standard errors (SE) are robust to heteroscedasticity. *P<0.05. **P<0.01. ***P<0.001. Model 1: adjusted for sex, fat mass and parental education. Model 2: also included IR, quality of diet, PA status, type of secondary education and iron supplementation in infancy.

Table 4 contains the estimated association between having a final GPA ≥50th p in the sample and leptin status at 16 years after controlling for other influences. After full adjustments (Model 3), the odds of performing ≥50th p in youths with hyperleptinemia were 56% (95% CI: 0.32-0.95) that of their peers with normal serum leptin. We likewise found that performing ≥50th percentile was negative and significantly associated with unhealthy dietary habits (OR: 0.52; 95% CI: 0.33-0.81), being male (OR: 0.63; 95% CI: 0.28-0.80), and attending adult schools (OR: 0.38; 95% CI: 0.23-0.62). Association of academic results with weight status, IR and parental schooling was non-significant at an alpha level of 0.05. Iron supplementation in infancy was not associated with GPA later in adolescence.

Table 4 Relationship between having a GPA >50th percentile and leptin resistance in Chilean youths after controlling other health, sociodemographic and educational influences (n=568)

	Model 1		Model 2		Model 3	
	OR	95%CI	OR	95%CI	OR	95%CI
Hyperleptinemia	0.50**	0.30-0.84	0.50*	0.33-0.94	0.56*	0.32-0.95
Overweight	0.87	0.57-1.30	0.70	0.38-1.27	0.65	0.35-1.22
Obesity	0.99	0.86-1.18	0.86	0.49-1.50	0.81	0.45-1.33
Insulin resistance	0.94	0.80-1.11	0.93	0.49-1.09	0.78	0.55-1.18
Unhealthy diet	(…)		0.53	0.34-0.81	0.52**	0.33-0.81
Physically inactive	(…)		0.89	0.59-1.34	0.99	0.64-1.53
Male sex	(…)		(…)		0.63**	0.28-0.80
Maternal education: incomplete HS	(…)		(…)		1.09	0.82-1.74
Paternal education: incomplete HS	(…)		(…)		0.96	0.65-1.45
Adult high school	(…)		(…)		0.38***	0.23-0.62
No-Fe suppl. (infancy)	(…)		(…)		0.91	0.64-1.28

OR [95%CI]: Odd Ratio [95% Confidence Interval]. (…) Non-observed. Significance level: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al.. Overweight: BMI-Z >1 SD and ≥2SD. Obesity: BMI-Z ≥ 2 SD. IR: HOMA-IR 2.6. Unhealthy diet: diet high in simple carbohydrates and saturated fats. Physically inactive: scheduled PA ≤90 min/week. Adult high school: education for students who in the past were unable to receive their diploma.

Table 5 shows the association between having a final GPA ≥75th p in the sample and leptin status at 16 years after controlling for a number of confounders. In a fully adjusted model (Model 3), the likelihood of performing ≥75th p in leptin resistant youths were 42% (95% CI: 0.19-0.89) that of students with normal leptin levels. We likewise found that school performance was negatively related to being male (OR: 0.43; 95% CI: 0.28-0.71), having unhealthy dietary habits (OR: 0.41; 95% CI: 0.24-0.75), father's schooling (incomplete secondary education) (OR: 0.57; 95% CI: 0.35-0.93) and the type of secondary education (OR: 0.35; 95% CI: 0.18-0.69). Again, weight status, IR and maternal educational level were unrelated to school performance. Iron supplementation in infancy was not related to HS performance as measured by having a GPA in the highest quartile.

Table 5 Relationship between having a GPA >75th percentile and leptin resistance in Chilean youths after controlling other health, sociodemographic and educational influences (n=568)

	Model 1		Model 2		Model 3	
	OR	95%CI	OR	95%CI	OR	95%CI
Hyperleptinemia	0.35***	0.17-0.72	0.35***	0.17-0.73	0.42*	0.19-0.89
Overweight	0.66	0.40-1.10	0.67	0.42-1.13	0.62	0.37-1.05
Obesity	0.83	0.42-1.64	0.83	0.42-1.64	0.74	0.36-1.54
Insulin resistance	0.79	0.41-0.95	0.69	0.44-1.06	0.73	0.46-1.13
Unhealthy diet	(…)		0.43***	0.26-0.78	0.41***	0.24-0-75
Physically inactive	(…)		1.01	0.63-1.59	1.00	0.66-1.54
Male sex	(…)		(…)		0.43***	0.28-0.71
Maternal education: incomplete HS	(…)		(…)		1.07	0.68-1.67
Paternal education: incomplete HS	(…)		(…)		0.57*	0.35-0.93
Adult high school	(…)		(…)		0.35***	0.18-0.69
No-Fe suppl. (infancy)	(…)		(…)		0.71	0.48-1.06

OR [95%CI]: Odd Ratio [95% Confidence Interval]. (…) Non-observed. Significance level: *$P<0.05$; **$P<0.01$; ***$P<0.001$. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al. Overweight: BMI-Z >1 SD and ≥2SD. Obesity: BMI-Z ≥ 2 SD. IR: HOMA-IR 2.6. Unhealthy diet: diet high in simple carbohydrates and saturated fats. Physically inactive: scheduled PA ≤90 min/week. Adult high school: education for students who in the past were unable to receive their diploma.

DISCUSSION

In a sample of high-school graduates, we examined the relationship between leptin status and cognition by using functional measures such as high-school grades and GPA. To our best knowledge this is the first study addressing the link between leptin status and this domain of cognition in a younger-age human population. Compared with students having normal serum leptin, those having hyperleptinemia had lower school grades and GPA. Similarly, they had lowers odds of performing ≥50th and ≥75th percentile of the sample. Even after controlling relevant confounders, the association between leptin status in adolescence and academic performance remained significant.

Our results are of importance for several reasons. Leptin plays a key role in memory processing through induction of hippocampal and prefrontal cortex synaptic plasticity. However, a growing body of evidence suggests elevated plasma leptin levels may limit the potential for synaptic plasticity and could partially explain some cognitive deficits[3,7,17-20]. Likewise, links are strong between memory performance and academic outcomes. In children, working memory and particularly the ability to retrieve and manipulate information from long-term memory has been found to predict math, reading and spelling outcomes, even after controlling for IQ[21]. Dysfunctions in this domain can lead to learning difficulties in activities that involve storing and processing information[21,22]. Furthermore, high-school grades predict higher education outcomes and subsequent job status and income[41].

Very few studies have explored the link between leptin and cognition in younger animal populations, and their findings are in line with ours. Oomura *et al.* showed that leptin modulates higher neural functions in mice 4-8 weeks-old[17]. While infusion of low doses of leptin enhanced learning and memory performance and hippocampal LTP, high doses impaired them. The notion that hyperleptinemia could be responsible for some cognitive deficits in adolescent mice is supported by Valladolid-Acebes and colleagues[18-20]. In age-matched mice, short-term exposure to high fat diet compromised hippocampal dependent learning and memory. Moreover, in adolescent mice, the behavioral impairment was

accompanied by changes in hippocampal morphology and functionality of leptin receptors within de hippocampus.

Leptin levels in this Chilean sample were lower than those reported for European adolescents[25], but higher than those reported for healthy adolescents in other Latin-America countries and Asia[42,43]. Population differences in the epidemiologic and nutrition transition may in part explain the disparity in levels of circulating leptin. Increased intake of fat, sugar and processed foods, reduced PA, and increased risk of non-communicable disease, including obesity, are more prevalent in the last stages of the transition, which is the case of Chile and the European countries[44].

Although an association between weight status and academic results was not observed in this sample, obesity in pediatric populations has been related with impaired cognitive function[45,46] and the ability to perform well in school[47,48]. Some authors have postulated that cognitive impairment may actually precede excessive weight gain[18,49-51]. Two main ideas are behind this view: first, impairment of learning is observed before other metabolic alterations; second, while the adverse effects of consuming high fat/high sugar diet on cognitive function is a good predictor of subsequent weight gain, the effect of those nutrients on weight gain does not reliably predict subsequent cognitive deficit. Our results suggest that leptin might mediate, in part, the effect of weight status on cognition and academic outcomes.

It is very likely that hyperleptinemia in most individuals in our sample may lead to both under-responsiveness to exogenous leptin and impairment of signal transduction in target neurons. Although obesity has been traditionally associated with hyperleptinemia in the non-elderly population, new data indicate that fructose, sucrose and triglycerides may induce hyperleptinemia regardless of the amount of body fat[15]. In our sample, less than a half of participants having hyperleptinemia were obese, and 28% were normal weight. Likewise, almost 80% had a diet in the intermediate or unhealthy zone, which means excessive intake of refined sugars and fats. Administration of even small quantities of triglycerides to normal weight rats immediately resulted in dysfunctional leptin transport across the BBB[52,53]. Similarly, rats exposed to high fructose diet demonstrated an impaired response to peripheral leptin injection and

central leptin infusion. However, switching to a sugar-free diet improved leptin sensitivity [54]. In both cases, chronic fructose and triglycerides consumption led to impaired responsiveness to exogenous leptin prior to body weight.

Our findings also confirm the role of nutrition in academic outcomes. Participants having an unhealthy diet had significantly lower odds of performing well in high-school, independent of leptin status. Numerous studies report that diet relates to specific outcomes that are important for the educational attainment of children and adolescents[31-34]. The consumption of refined carbohydrates and saturated fats is linked to impaired cognitive performance. Exposure to these macronutrients interferes directly with hippocampal functioning by cutting down the production of neurotrophins, increasing neuroinflammatory markers, and altering the BBB[50,55]. In addition, indirect adverse effects of diet on cognitive functioning, including reduced potential for synaptic plasticity and trafficking of neurotransmitter receptors in the hippocampus, have been related to diet induced leptin and insulin resistance[18-20,49,50].

Last, in our sample, the share of participants with hyperlpetinemia was significantly higher in males compared to females. Despite that leptin is produced in the fat tissue, serum leptin concentration is also dependent on determinants such as insulin sensitivity and overconsumption of sucrose and fructose. Male and females participants in our study had similar prevalence of excess weight and IR, but the proportion of adolescents having a diet high in simple sugars, mostly sucrose and fructose from candies and sugar sweetened beverages (a dietary pattern regarded as a fair diet in our questionnaire) was significantly higher among males compared to females. Population surveys in the Chilean adolescent population confirm that adolescent males have excess consumption of simple carbohidrates[56,57].

Implications from these results

The Chilean adolescent population is highly exposed to risk factors for hyperleptinemia. As reported by the 2014 Chilean National Food Consumption Survey[56], dietary habits of poor nutritional quality are wide spread. Adolescents aged 14-18 rank first in the consumption of refined sugar (121 g/day) and second in the consumption of saturated fats. According to this survey, the prevalence of overweight and obesity in adolescents is 38% and 13%, respectively.

A further implication from this study relates the fact that exposure to abnormally high levels of leptin would be starting early in life. A non-modifiable condition such as ageing is associated with hyperleptinemia, but exposure to high fat/high sugar diet as well as to overweight and obesity are avoidable. While schools should serve as a point of entry for the promotion of healthy lifestyles, the food industry uses schools as a means of reaching young consumers. Despite the efforts to limit the availability of unhealthy foods in schools, in the Latin-American Southern Cone 70% of adolescents drink sugar-sweetened beverages on a daily basis[57]. Frequent (1-2 times/week) and very frequent (≥3 times/week) fast-food consumption among Latin-American adolescents range from 40% in Uruguay to 70% in Bolivia, according to a recent international study[58].

The following points should be considered in the design and application of preventive strategies. Fructose-induced hyperleptinemia may be reversible, which means there is a possibility for improvement. Second, hyperleptinemia may be triggered by exposure to even small amounts of saturated fats and refined sugars, which are major components of the Western diet. Moreover, the effects on leptin levels appear soon after exposure to these macronutrients. Finally, hyperleptinemia is a precondition for LR; at this point it might be late for adolescents to reach their full cognitive and academic potential.

Study limitations and strengths

Our results supports the notion that leptin has an important role in cognitive function in addition to its role in energy balance regulation. A further strength is that we approached this topic in a group younger than previous research on leptin and cognition. Third, we used functional measures of cognition (e.g. academic performance in high-school), aiming to examine this relation in the 'real' world. In spite of these strengths, the study has limitations that should be considered when interpreting the findings. First, our sample is not representative of the Chilean adolescent population, as it consists of adolescents from low to middle SES. However, SES level may be especially important. The prevalence of unhealthy dietary habits and obesity, both of which may lead to hyperleptinemia, is higher among adolescents from middle to low SES compared to adolescents of high SES[56]. Second, we used cut-offs for hyperleptinemia definition based on statistical criteria in a European cohort, which are the only values described for healthy adolescents. It is worth noting that Chile, like Western European countries, is in the last stage of the epidemiological transition. Yet, future studies should use cut-offs based on biological risk. Third, due to data constraint the prevalence of neuropsychiatric conditions was not included in the study. Leptin effects on memory and cognition might be confounded by the co-existence of neuropsychiatric disorders such as depression, schizophrenia and substance-related disorders. Fourth, we did not consider the mediating effect of potential learning and cognitive disorders which may impact student's academic functioning. Also, information on family structure is lacking in the analysis. Yet, we considered the impact of parental education. Because association is not enough to prove causation, future studies should replicate and extend this analysis in other young populations, and further investigate how leptin influence cognitive and academic outcomes.

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the final study design, interpretation of data and added intellectual content during manuscript preparation. All authors read and approved the final manuscript.

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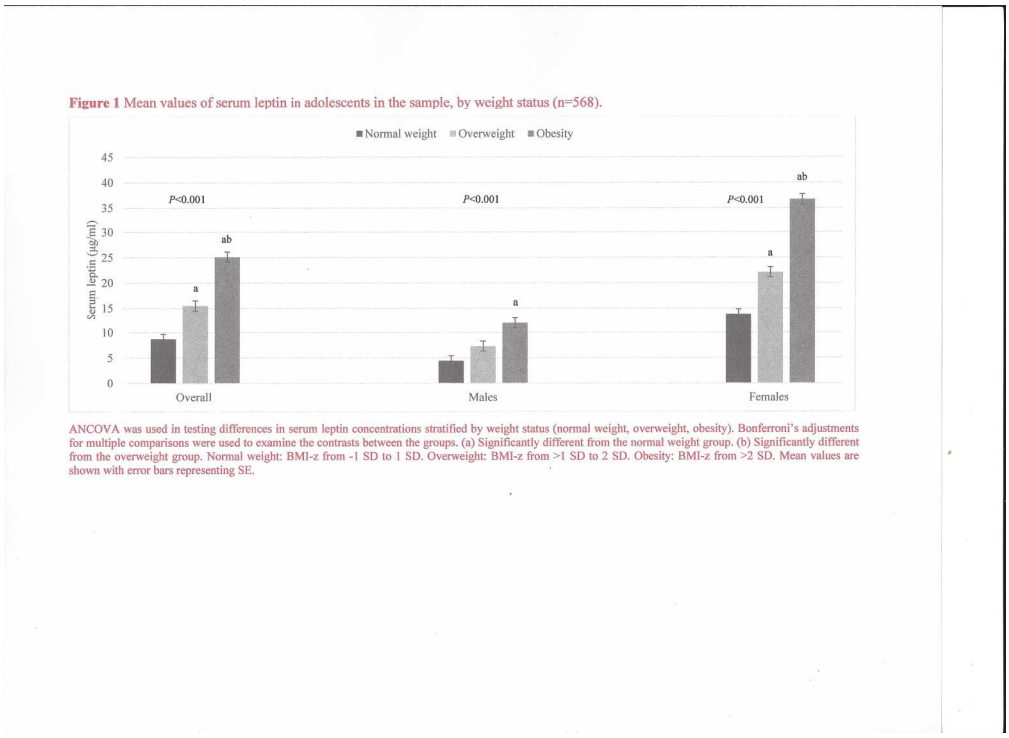

Figure 1 Mean values of serum leptin in adolescents in the sample, by weight status (n=568).

ANCOVA was used in testing differences in serum leptin concentrations stratified by weight status (normal weight, overweight, obesity). Bonferroni's adjustments for multiple comparisons were used to examine the contrasts between the groups. (a) Significantly different from the normal weight group. (b) Significantly different from the overweight group. Normal weight: BMI-z from -1 SD to 1 SD. Overweight: BMI-z from >1 SD to 2 SD. Obesity: BMI-z from >2 SD. Mean values are shown with error bars representing SE.

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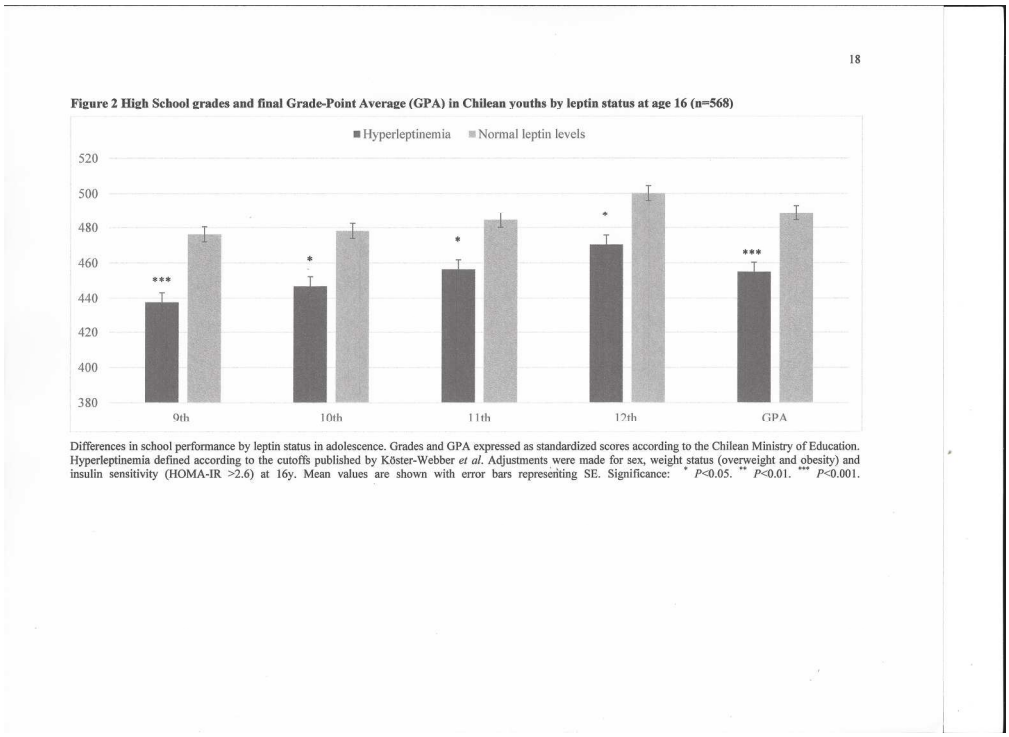

Figure 2 High School grades and final Grade-Point Average (GPA) in Chilean youths by leptin status at age 16 (n=568)

Differences in school performance by leptin status in adolescence. Grades and GPA expressed as standardized scores according to the Chilean Ministry of Education. Hyperleptinemia defined according to the cutoffs published by Köster-Webber et al. Adjustments were made for sex, weight status (overweight and obesity) and insulin sensitivity (HOMA-IR >2.6) at 16y. Mean values are shown with error bars representing SE. Significance: * P<0.05. ** P<0.01. *** P<0.001.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	*Recommendation*	*Status*	*Page No*
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	✓	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	✓	2
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✓	5-6
Objectives	3	State specific objectives, including any pre specified hypotheses	✓	6
Methods				
Study design	4	Present key elements of study design early in the paper	✓	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✓	6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	✓	6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	✓	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✓	7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✓	7-9
Bias	9	Describe any efforts to address potential sources of bias	✓	4,23‡
Study size	10	Explain how the study size was arrived at	✓	6§
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✓	7-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	✓	9
		(*b*) Describe any methods used to examine subgroups and	✓	9

		interactions		
		(*c*) Explain how missing data were addressed	n.a.	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	✓	6§
		(*e*) Describe any sensitivity analyses	n.a.	
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✓	10
		(b) Give reasons for non-participation at each stage	✓	6§
		(c) Consider use of a flow diagram		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✓	10-11
		(b) Indicate number of participants with missing data for each variable of interest	n.a	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	✓	6§
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	✓	6§
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures	✓	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✓	13-19
		(*b*) Report category boundaries when continuous variables were categorized	✓	13-19
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	✓	16-19
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✓	7
Discussion				
Key results	18	Summarise key results with reference to study objectives	✓	20-21
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✓	23
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✓	20-21
Generalisability	21	Discuss the generalisability (external validity) of the study results	✓	20-23

Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✓	25

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

§Enrolment, assessments and data from previous waves are described in detail in previous works by the authors'. Reference for these works are provided in the Methods section (Study sample).

ǂ This was reckoned as a limitation.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.