# FITNESS AND ACADEMIC PERFORMANCE IN ADOLESCENTS. THE MEDIATING ROLE OF LEPTIN: DADOS STUDY

**What is known:**

- Academic performance is associated with physical fitness and leptin concentration in children and adolescents.
- Fitness modulates leptin concentration levels, regardless of physical activity and adiposity.

**What is new:**

- Leptin concentration mediates the association of cardiorespiratory fitness and muscular strength with academic performance in adolescents.
- Improvements in cardiorespiratory fitness and muscular strength might reduce leptin concentration levels enhancing academic performance.


## Abstract

We tested the mediating effect of leptin on the association between physical fitness (PF) components and academic performance indicators in healthy adolescents. A total of 263 adolescents (13.9 ± 0.3 years; 47.5% girls; 12.5% overweight) from the DADOS (Deporte, ADOlescencia y Salud) Study were included in the analysis. PF components were assessed by the handgrip test for upper-limb muscular strength, standing long jump test for lower-limb muscular strength, 4 x 10-m shuttle run test for speed-agility, and 20-m shuttle run test for cardiorespiratory fitness. Plasma leptin concentration was assessed from antecubital vein blood after an overnight fast using a sensitive ELISA kit. Academic performance indicators were assessed through final school grades and through the Spanish version of the SRA Test of Educational Ability. Body composition was assessed by body mass index and skinfold thicknesses. Boot-strapped mediation procedures were performed and indirect effects with confidence intervals not including zero were interpreted as statistically significant. Our findings suggest that the positive associations observed between cardiorespiratory fitness and lower-limb muscular strength with academic performance indicators were mediated by leptin concentration after adjusting for sex, pubertal stage, socioeconomic status and adiposity (percentage of mediation ranging from 54.61% to 82.02%). *Conclusions*: Improvements on PF components, particularly in cardiorespiratory fitness and lower-limb

muscular strength, might reduce leptin concentration with potential benefits on academic performance in adolescents, independently of adiposity.

**Key words**: cognition, health, school grades, adolescence, leptin.

**Abbreviations:** CRF: Cardiorespiratory Fitness; GPA: grade point average; MS: Muscular Strength; PF: physical fitness; SES: socioeconomic status; SRT: shuttle run test.

## INTRODUCTION

In the last decades, the role of physical fitness (PF) as a key determinant of current and future health status has been demonstrated in childhood and adolescence [1, 2]. Cardiorespiratory fitness (CRF), muscular strength (MS) and speed-agility have been proposed as the components of PF more closely related with health status [2, 3]. European children and adolescents, on average, meet the standards for healthy CRF (78% of boys and 83% of girls), however, these percentages decrease yearly [4].

Empirical evidence and systematic reviews have suggested a positive association between PF components and academic performance in children and adolescents [5–9]. For instance, Marques et al. [10] have shown that there is a strong evidence for the association between CRF and academic performance which may be related to the positive effects of CRF on brain structure and cognitive functions [11]. In addition, Esteban-Cornejo et al. [12] showed that CRF and speed-agility, but not MS, positively influenced academic performance in youths independently of adiposity. By contrast, in a study conducted by Olivares et al. [13], MS was the fitness component most associated with academic performance, while no association was found between CRF and academic performance when adiposity was considered. The divergent results found in the scientific literature may be related to the fact that some of the studies analyzing the associations between PF and academic performance used measures scaled by body mass (i.e., $VO_2$ in ml/min/kg), without recognizing the influence that adiposity could have in these associations [1].

Leptin is a 167-amino-acids adipokine classically involved in the regulation of energy homeostasis and appetite by sending signals to receptors within the hypothalamus [14]. Physiologically normal leptin levels, based on healthy European adolescents reference values, have been established at <12.41 ng/mL in males and <38.61 ng/mL in females [15]. Leptin has been associated with enhanced cognitive processes at normal concentrations, while at high levels it has been related with several cardiovascular disease risk factors [16], impaired cognitive function [17] and poor academic performance

[18, 19]. In parallel, an inverse association between PF and circulating leptin concentration levels has been reported in youth [20–22]. Although prior research assessing the association between CRF and leptin in adolescents has shown an inverse relationship independently of adiposity [20, 21], Hosick et al. [22] found that this association was explained by adiposity, suggesting that not considering adiposity could lead to misleading conclusions.

Despite Haapala et al. identified a mediating role of motor performance on the association between leptin and reading fluency in a sample of 106 boys aged 6–8 years [19], no previous studies have examined the mediating role of leptin concentration on the association between PF and academic performance. Given the growing body of research highlighting the key role of PF on academic performance, and the previously argued independent inverse associations of leptin with PF and academic performance, we hypothesized that leptin concentration could act as an underlying mechanism for the influence of PF on academic performance. Thus, the objective of the current study was to test the mediating role of leptin concentration levels on the association between PF components and academic performance indicators in healthy adolescents, adjusting by several potential confounders including adiposity.

## METHODS

### *Participants*

The present study is part of the DADOS (Deporte, ADOlescencia y Salud) study, a 3-year longitudinal research project (from 2015 to 2017) aimed to analyze the influence of competitive sport practice on health, academic performance and psychological wellness through adolescence. All participants were recruited from secondary schools and sport clubs of Castellon (Spain), and met the general DADOS inclusion criteria; born in 2001, enrolled in 2nd grade of secondary school and free of any chronic disease. The results presented in this study belong to baseline data obtained between February and May of 2015. From the total DADOS study sample (n=274), a subsample of 263 adolescents (125 girls) with valid data for at least PF, academic performance and leptin concentration were included in the analysis.

### *Physical fitness*

PF was assessed using the ALPHA (Assessing Levels of Physical Activity) health-related fitness test battery for adolescents [23]. In brief, CRF was assessed using the 20-m Shuttle Run Test. The final

number of completed stages was used in the analyses. Speed-agility was assessed using the 4x10m Shuttle Run Test. The test was performed twice and the best time (s) was used in the analyses. Because speed-agility is inversely related to high physical fitness, it was first multiplied by -1. Upper-limb MS was assessed using a hand dynamometer with adjustable grip (TKK 5401 Grip D; Takey Scientific Instruments, Tokyo, Japan). The test was performed twice for each hand alternatively, using the optimal grip-span. The handgrip score (kg) was calculated as the average of the best score for each hand. Lower-limb MS was assessed using the standing long jump test. The test was performed twice and the longest distance (cm) was used in the analyses.

*Plasma Leptin concentrations*

After an overnight fast of at least 10h, blood samples were drawn from the antecubital vein while subjects remained in seated position. Blood samples in EDTA tubes (Greiner bio-one, Kremsmünster, Austria) were used for plasma collection after centrifugation (3500 rpm×10 min at 4ºC). Plasma leptin concentrations were measured using a sensitive ELISA kit (EIA-2395, Enzyme-Linked Immunosorbent Assay; DRG Instruments GmbH, Marburg, Germany). The sensitivity of the leptin concentration assay was 0.7 ng/mL, with intra-assay and inter-assay coefficients of variation of <10% and <15% respectively.

*Academic performance indicators*

Academic performance was assessed using school grades and an academic questionnaire. 1) School grades: the final school grades from the 1st course of secondary school on a ten-point scale were provided by the participants. Four indicators were included in the analyses: math, language, the average of math and language, and grade point average (GPA). Language is the grade of Catalan; the official teaching language at school. GPA was defined as the single average of the grades of Geography and History, Natural Science, Math, Spanish, Catalan, English and Physical Education. 2) Academic questionnaire: the Spanish version of the Science Research Associates Test of Educational Abilities was completed by the participants. This test measures three basic abilities: verbal ability (command of language), numeric ability (speed and precision in performing operations with numbers and quantitative concepts) and reasoning ability (the aptitude to find logical ordination criteria in sets of numbers, figures or letters). Scores for the three abilities were obtained by adding positive answers. Overall academic ability was calculated by adding the three abilities' scores (verbal + numeric + reasoning). As previously published from DADOS study [9], this work used the level 3 of the TEA test.

115 *Covariates*

116 Measures were assessed in duplicate by trained members of the DADOS research group
117 following standardized procedures [24], and average measures were used for the analysis. Briefly, body
118 weight was measured to the nearest 0.1 kg using an electronic scale (SECA 861, Hamburg, Germany).
119 Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (SECA 213, Hamburg,
120 Germany). Body mass index (BMI) was calculated as weight/height squared ($kg/m^2$). Participants were
121 classified into normal weight and overweight or obese, according to the BMI cutoffs proposed by Cole et
122 al. [25]. The BMI values were transformed into standard deviation scores (SDS) according to WHO
123 reference population for sex and age [26]. In addition, skinfold thicknesses were measured at the left side
124 of the body to the nearest 0.2 mm using a Holtain skinfold caliper at 2 sites (triceps and subscapular).
125 Sum of both skinfolds was used for data analyses as indicator of adiposity.

126 Pubertal stage was self-reported according to the 5 stages described by Tanner and Whitehouse
127 [27] based on external primary and secondary sex characteristics. Standardized pictures were used to
128 assess two components: pubic hair growth for boys and girls, plus breast development in girls and genital
129 development in boys. A 5-point maturity rating was used where stage 1 corresponds to the prepubertal
130 state and stage 5 corresponds to mature state. The highest rating of the 2 components was used for data
131 analyses.

132 The Family Affluence Scale (FAS) developed by Currie et al. [28] was used as a proxy of
133 socioeconomic status (SES), which is based on material conditions in the family such as car ownership,
134 bedroom occupancy, computer ownership and home internet access.

135 *Statistical analyses*

136 Characteristics of the sample are presented as mean ± standard deviation (SD) unless otherwise
137 stated. Differences between sexes were assessed by *t* test for continuous variables and chi-square test for
138 nominal variables. All variables were checked for normality using both graphical (normal probability
139 plots) and statistical (Kolmogorov-Smirnov test) procedures. Due to its skewed distribution, leptin
140 concentrations were log-transformed before the analysis. As preliminary analyses did not show significant
141 interactions of sex with leptin and PF variables in relation to academic performance indicators (all
142 $P>0.05$), all analyses were performed for boys and girls together.

Partial correlation analysis controlling for sex, pubertal stage and SES were performed to examine the relationships between PF components, leptin concentration, adiposity and academic performance indicators. Additionally, linear regression analyses were performed to clarify the potential role of leptin concentration as mediator variable in the association between PF components (independent variables) and academic performance indicators (dependent variables) using 2 separate models. Model 1 included sex, pubertal stage and SES; and Model 2 included Model 1 plus leptin concentration. Finally, separate mediation analyses were performed in order to elucidate whether the associations between PF and academic performance were mediated by leptin concentration. The PROCESS macro version 2.16.3, model four, with 5.000 bias-corrected bootstrap samples and 95% confidence intervals was used for these analyses. Mediation is assessed by the indirect effect of the PF (independent variable) on academic performance (dependent variable) through leptin concentration (mediator). The total (c path), direct (c′ path) and indirect effects (a*b paths) are presented (**figure I**). Indirect effects (ab) with confidence intervals not including zero were interpreted as statistically significant [29], which can be so regardless of the significance of the total effect (the effect of PF on academic performance) and the direct effect (the effect on academic performance when both PF and leptin concentration are included as independent variables). Percentage of mediation ($P_M$) was calculated as '(indirect effect / total effect) x 100' to know how much of the total effect was explained by the mediation when the following assumptions were achieved; the total effect is larger than the indirect effect and of the same sign. These analyses were adjusted by sex, pubertal stage, SES and adiposity. All the analyses were performed using the IBM SPSS Statistics for Windows version 22.0 (Armonk, NY: IBM Corp), and the level of significance was set to $P<0.05$.

## RESULTS

The baseline characteristics of the final sample included in the analyses are shown in **Table I**. Girls had significantly higher SES (4.4 vs. 4.0; $P<0.05$), sum of skinfold thicknesses (30.3 vs. 21.7; $P<0.001$) and leptin concentration levels (6.1 vs. 1.6; $P<0.001$) than boys. Meanwhile boys were taller and showed higher levels for all PF components than girls (all $P<0.001$). No differences were reported for academic performance, except for numeric ability that was higher in boys (14.7 vs. 12.0; all $P<0.001$).

Partial correlations between PF components, leptin concentration, adiposity and academic performance indicators, after adjustment for sex, pubertal stage and SES are presented at **Table II**. CRF

was positively associated with math, math and language, GPA and numeric ability (all $P<0.05$). Lower-limb MS was positively associated with verbal ability ($P<0.05$). Leptin concentration was negatively associated with all the PF components, adiposity, school grades and numeric ability (all $P<0.05$).

Linear regression analyses examining the associations between PF components and academic indicators showed positive associations of CRF and lower-limb MS with academic performance, which disappeared after including leptin concentration as a confounder (data not shown).

The results of the separated mediation models after controlling for sex, pubertal stage, SES and adiposity are in **Tables III** and **IV**. Mediation analyses were not significant for the association of speed-agility (4x10m test) and upper-limb MS (handgrip test) with academic performance indicators (data not shown). According to the mediation analyses, CRF was positively associated with numeric ability (total effect, c; $P<0.05$) and negatively associated with leptin concentration (path a = -1.101; $P<0.001$). In path b, leptin concentration was negatively associated with academic performance, although the associations were not statistically significant. The direct effect (path c') of CRF on academic performance when leptin was included in the model was not significant. There was a significant mediating effect of leptin in the relationship of CRF with GPA, reasoning ability and overall academic ability ($P_M$ ranged from 52.9 to 58.2%). Regarding the analysis of leptin concentration as a potential mediator of the relationship between lower-limb MS and academic performance, lower-limb MS was positively associated with verbal ability ($P<0.01$) and negatively associated with leptin concentration (path a = -1.867; $P<0.001$). In path b, leptin concentration was negatively associated with language, math and language, and GPA ($P<0.05$) and the direct effect (path c') of lower-limb MS on academic performance was not statistically significant. These results suggest a significant mediating effect of leptin concentration in the relationship of lower-limb MS with all the school grades and with numeric, reasoning and overall academic abilities ($P_M$ ranged from 42.2 to 75.1%).

## DISCUSSION

The main finding of the present study revealed a mediating effect of leptin concentration on the relationship between PF components (CRF and lower-limb MS) and academic performance indicators after controlling for sex, pubertal stage, SES and adiposity. These results contribute to the current scientific knowledge by suggesting that leptin concentration could partially explain the positive association between PF and cognition in adolescents.

In consonance with prior research, the present study showed a positive association between PF, particularly CRF and lower-limb MS, and some academic performance indicators in adolescents [5–9, 30, 31], which disappeared after further adjustment for leptin concentration. The association of CRF with academic performance could be explained by the positive effects that this fitness component has on cognition and brain. For instance, Chaddock et al. found greater bilateral hippocampal volumes and memory performance in those preadolescents children with higher levels of CRF [11]. In another study, Buck et al. analysed a sample of 74 preadolescents showing a positive association between aerobic fitness and interference control [32]. Therefore, it is plausible that CRF produces structural and functional brain changes with potential improvements in cognitive processes [33] which may lead to better academic performance in adolescents. The mechanisms by which MS influences academic performance have been less explored showing uncertain results [3]. However, we speculate that better MS, as a result of strength training, may positively influence academic performance by inducing the expression of neurotrophic factors [34] and synaptogenesis [35].

Our results showed that the association between PF and academic performance disappeared when including leptin as a confounder. This fact could be related to the strong link between leptin and adipose tissue [14] which could indirectly affect academic performance. In fact, overweight and obese adolescents, who have shown lower PF levels than their normal-weight peers [36], have also reported several psychological and behavioural disturbances [37, 38], such as weight-related teasing and low self-confidence, which could impair school functioning and academic outcomes. In addition, leptin has shown to induce brain-derived neurotrophic factor, which also plays a key role on control of body weight, and promotes neuron survival and neural plasticity [39], which in turn, might improve academic performance.

In our sample of physically active and mainly normal weight adolescents, leptin was revealed as an underlying mechanism of the association between PF and academic performance, after adjustment of sex, pubertal stage, SES and adiposity. Similarly to previous studies, we found an inverse association between PF and leptin concentration [20, 21, 40], which could significantly influence academic performance [18, 19]. According to prior research, high levels of CRF and MS might stimulate insulin and leptin sensitivity which translates into lower leptin circulating levels [20, 21]. For instance, Olmedillas et al. found higher MS and hypertrophy in the dominant arm of professional tennis players, with an increased expression of leptin receptors and signalling compared to the contralateral arm [41]. Moreover, a previous study in rodents showed that aerobic training increased leptin receptor in

hippocampus and prefrontal cortex, enhancing leptin signaling in the hypothalamus of mice through the phosphorylation of signal transducer and activator of transcription 3 (STAT3) [42]. Therefore, higher CRF and MS seem to be related with lower leptin concentration, which may improve synaptic plasticity, cognitive processes (e.g., memory) and mood [17], enhancing academic performance.

*Limitations and strengths*

The results of the present study should be interpreted with caution. First, the cross-sectional design of our analyses prevents us from inferring causal relationships; however, our mediation analysis strategy allows us to provide data supporting the importance of leptin concentration in the fitness-cognition relationship. Second, despite some methodological limitations [43], the 20m shuttle run test was used as indirect measure of cardiorespiratory fitness. Third, pubertal stage was self-reported. However, the strengths of the study comprise the use of objective and standardized measures of PF, leptin concentration and academic indicators, as well as a relatively large and age-matched sample of healthy adolescents (13.9 ± 0.3 yrs) with no academic performance differences. In addition, as suggested by previous research [5, 22], our statistical analyses were controlled for sex, pubertal stage, SES and adiposity, which are relevant given their association with PF and cognition.

*Conclusions*

In conclusion, the present study reveals a mediating effect of leptin in the associatión between PF and academic performance in adolescents. Therefore, if confirm prospectively, improvements on PF components, specifically in CRF and MS, may reduce leptin concentration with potential benefits on academic performance. Health and education professionals could benefit from collaborating to achieve both enhanced health status, and academic performance. Our findings extend prior knowledge about the positive influence of PF on cognition by suggesting a novel physiological mechanism. Further research is needed to clarify the pathway by which leptin is closely linked to PF and cognition.

**Author's contribution:** MAR was involved in the data collection and analysis, drafting of the initial manuscript. MRBV was involved in the data collection and critical revision of the manuscript. DJP, JGPG and OCB were involved in the critical revision of the manuscript. DMU was involved in the study design and data collection, manuscript preparation, and critical revision. All authors have read and approved the final manuscript.

## COMPLIANCE WITH ETHICAL STATEMENTS

**Conflict of interest.** The authors declare that they have no conflict of interest.

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**Informed consent.** Adolescents and their parents or guardians were informed of the nature and characteristics of the study, and all signed an informed written consent. The DADOS study protocol was designed in accordance with the ethical guidelines of the Declaration of Helsinki 1961 (last revision of Fortaleza, Brazil, 2013) and approved by the Research Ethics Committee of the University Jaume I of Castellon (Spain).

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409 **Table I.** Characteristics of the study population by sex

| | All | Boys | Girls | P |
|---|---|---|---|---|
| n (%) | 263 (100) | 138 (52.5) | 125 (47.5) | |
| Demographics | | | | |
| Age (y) | 13.9 ± 0.3 | 13.9 ± 0.3 | 13.9 ± 0.3 | 0.797 |
| Pubertal stage (I-V) (%) [a] | 0/8/33/49/10 | 0/10/32/44/14 | 0/6/35/54/5 | |
| SES score (0-8) | 4.2 ± 1.4 | 4.0 ± 1.3 | 4.4 ± 1.4 | **0.031** |
| Anthropometry | | | | |
| Height (cm) | 163.1 ± 7.9 | 164.6 ± 8.6 | 161.4 ± 6.7 | **<0.001** |
| Weight (kg) | 54.2 ± 9.2 | 54.5 ± 9.6 | 53.9 ± 8.8 | 0.600 |
| Body mass index (kg/m$^2$) | 20.3 ± 2.7 | 20.0 ± 2.6 | 20.6 ± 2.9 | 0.054 |
| Overweight (%) [b] | 12.5 | 11.6 | 13.7 | 0.710 |
| Body mass index SDS [c] | 0.29 ± 0.89 | 0.30 ± 0.91 | 0.28 ± 0.88 | 0.838 |
| Triceps skinfold (mm) | 16.0 ± 6.8 | 13.1 ± 6.1 | 19.2 ± 6.2 | **<0.001** |
| Subscapular skinfold (mm) | 9.9 ± 3.5 | 8.7 ± 2.9 | 11.3 ± 3.5 | **<0.001** |
| Sum of skinfold thickness (mm) | 25.8 ± 9.7 | 21.7 ± 8.5 | 30.3 ± 9.0 | **<0.001** |
| PF Components | | | | |
| 20-m SRT (stages) | 7.7 ± 2.5 | 9.0 ± 2.0 | 6.2 ± 2.2 | **<0.001** |
| 4 x 10-m SRT (s) | 12.6 ± 1.0 | 12.0 ± 0.7 | 13.1 ± 1.0 | **<0.001** |
| Handgrip strength (kg) | 28.9 ± 5.9 | 30.8 ± 6.7 | 26.8 ± 4.0 | **<0.001** |
| Standing Long Jump (cm) | 171.1 ± 26.3 | 180.1 ± 24.2 | 161.2 ± 25.0 | **<0.001** |
| School grades (0-10) | | | | |
| Math | 6.9 ± 1.6 | 7.0 ± 1.6 | 6.8 ± 1.6 | 0.281 |
| Language | 6.8 ± 1.5 | 6.7 ± 1.5 | 6.9 ± 1.5 | 0.147 |
| Math and Language | 6.8 ± 1.4 | 6.8 ± 1.5 | 6.8 ± 1.4 | 0.859 |
| GPA | 7.1 ± 1.3 | 7.1 ± 1.3 | 7.2 ± 1.3 | 0.346 |
| Academic abilities | | | | |
| Verbal (0-50) | 18.7 ± 5.3 | 19.1 ± 5.8 | 18.2 ± 4.6 | 0.167 |
| Numeric (0-30) | 13.4 ± 4.7 | 14.7 ± 4.5 | 12.0 ± 4.5 | **<0.001** |
| Reasoning (0-30) | 16.5 ± 5.9 | 16.1 ± 5.7 | 17.0 ± 6.1 | 0.214 |
| Overall (0-110) | 48.6 ± 12.6 | 49.9 ± 12.8 | 47.2 ± 12.3 | 0.074 |
| Leptin concentration (ng/mL) | 3.7 ± 4.5 | 1.6 ± 2.8 | 6.1 ± 4.9 | **<0.001** |

Data are presented as mean ± standard deviation or percentages. Differences between sexes were examined by *t* tests. Statically significant values are in bold.

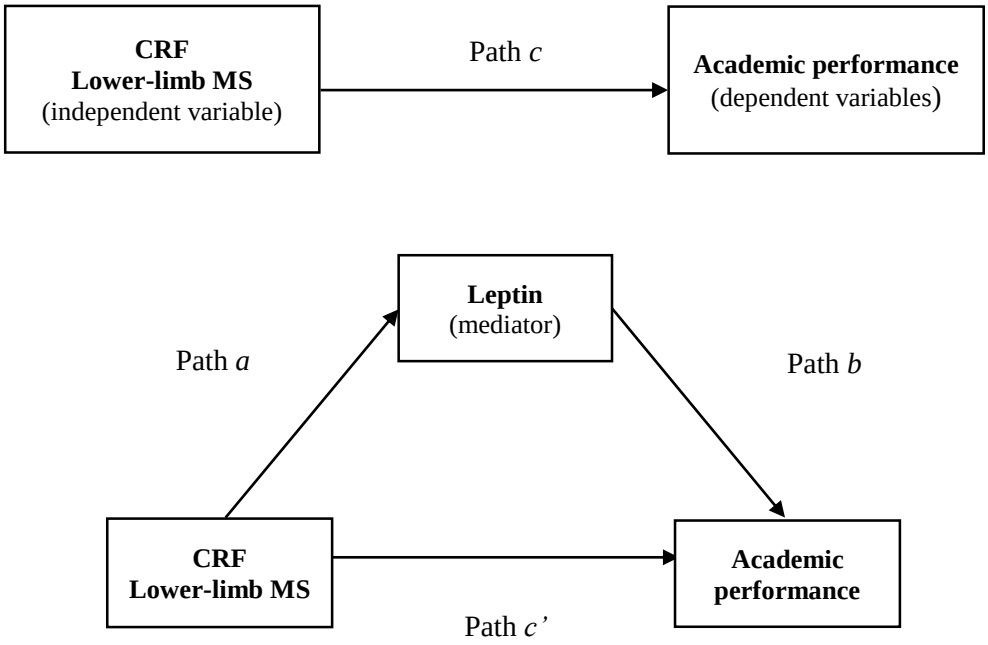
Pubertal stage described by Tanner and Whitehouse [27] was based on pubic hair growth for boys and girls, plus breast development in girls and genital development in boys. Overweight includes obese adolescents according to the age- and sex-specific BMI cutoffs proposed by Cole et al. [25]. Body mass index SDS was calculated using the WHO 2007 reference values [26]. Skinfold thicknesses indicates the sum of two skinfolds (triceps and subscapular). Overall indicates the sum of the three abilities scores.

SES: socioeconomic status; SDS: standard deviations scores; PF: physical fitness; SRT: shuttle run test; GPA: grade point average;

410 **Table II.** Partial correlations of PF components, leptin concentration and academic performance indicators controlling for sex, pubertal stage and SES.

| | | School grades | | | | Academic abilities | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leptin | Math | Language | Math and Language | GPA | Verbal | Numeric | Reasoning | Overall |
| PF Components | | | | | | | | | |
| 20-m SRT (stages) | -0.552*** | 0.153* | 0.102 | 0.138* | 0.146* | 0.098 | 0.144* | 0.022 | 0.103 |
| 4 x 10-m SRT (s) | -0.491*** | 0.109 | 0.066 | 0.094 | 0.101 | 0.103 | 0.042 | -0.033 | 0.043 |
| Handgrip strength (kg) | -0.126* | -0.117 | -0.107 | -0.121 | -0.112 | -0.007 | 0.002 | -0.035 | -0.019 |
| Standing long jump (cm) | -0.452*** | 0.119 | 0.048 | 0.091 | 0.106 | 0.176* | 0.111 | 0.014 | 0.121 |
| Leptin (ng/mL) | - | -0.137* | -0.136* | -0.147* | -0.167** | -0.072 | -0.127* | -0.047 | -0.099 |
| Adiposity (mm) | 0.688*** | -0.093 | -0.092 | -0.097 | -0.114 | -0.023 | -0.002 | -0.065 | -0.144 |

Adiposity indicates the sum of two skinfolds (triceps and subscapular). Overall indicates the sum of the three abilities scores. SRT: shuttle run test; GPA: grade point average. ***P≤0.001, **P<0.01 and *P<0.05.

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412 **Figure I.** Causal diagram reflecting the study simple mediation analyses. Path c shows the association between
413 independent and dependent variables. Arrows a x b show the natural indirect effect pathway, and c' shows the
414 natural direct effect pathway. CRF: Cardiorespiratory fitness; MS: Muscular strength.

**Table III.** Total, direct and indirect effects of the simple mediation analyses investigating leptin concentration as a mediator between cardiorespiratory fitness and academic performance (N=263).

| Outcome | Total effect (*c*) | Direct effect (*c'*) | Path *a* | Path *b* | Indirect effect (*ab*) | BC 95% CI Lower; Upper | $P_M$ (%) |
|---|---|---|---|---|---|---|---|
| ***School grades*** | | | | | | | |
| **Math** | 0.097 (0.052) | 0.058 (0.057) | -1.101 (0.144)*** | -0.036 (0.023) | 0.039 (0.022) | -0.006; 0.084 | - |
| **Language** | 0.077 (0.053) | 0.035 (0.058) | -1.101 (0.144)*** | -0.039 (0.023) | 0.042 (0.027) | -0.009; 0.098 | - |
| **Math and Language** | 0.083 (0.047) | 0.043 (0.052) | -1.101 (0.144)*** | -0.036 (0.021) | 0.040 (0.022) | -0.003; 0.084 | - |
| **GPA** | 1.137 (0.649) | 0.536 (0.716) | -1.101 (0.144)*** | -0.546 (0.281) | **0.602 (0.312)** | 0.030; 1.241 | 52.90 |
| ***Academic abilities*** | | | | | | | |
| **Verbal** | 0.080 (0.066) | 0.039 (0.073) | -1.101 (0.144)*** | -0.037 (0.0.29) | 0.041 (0.033) | -0.024; 0.107 | - |
| **Numeric** | 0.221 (0.090)** | 0.154 (0.099) | -1.101 (0.144)*** | -0.060 (0.039) | 0.066 (0.038) | -0.007; 0.1411 | - |
| **Reasoning** | 0.063 (0.108) | -0.023 (0.120) | -1.101 (0.144)*** | -0.078 (0.047) | **0.086 (0.046)** | 0.004; 0.183 | N/A |
| **Overall** | 0.106 (0.062) | 0.044 (0.068) | -1.101 (0.144)*** | -0.056 (0.027) | **0.062 (0.028)** | 0.011; 0.121 | 58.21 |

Results showed as unstandardized coefficients (Standard Error, SE) and BC 95%CI based on 5000 bootstraps. All analyses were adjusted for sex, pubertal stage, SES and adiposity.

BC: Bias corrected; CI: confidence interval; $P_M$: percentage of mediation; GPA: grade point average score; N/A: non-applicable according to statistical assumptions specified previously.

Statistical significant indirect effects indicating that 0 is not in the 95% confidence interval (CI) of the indirect effect are presented in bold.

*P<0.05, **P<0.01, ***P<0.001.

417 **Table IV.** Total, direct and indirect effects of the simple mediation analyses investigating leptin concentration as

418 a mediator between lower-limb muscular strength and academic performance (N=263).

| Outcome | Total effect (*c*) | Direct effect (*c'*) | Path *a* | Path *b* | Indirect effect (*ab*) | BC 95% CI Lower; Upper | $P_M$ (%) |
|---|---|---|---|---|---|---|---|
| ***School grades*** | | | | | | | |
| **Math** | 0.160 (0.105) | 0.087 (0.112) | -1.867 (0.302)*** | -0.039 (0.022) | **0.073 (0.039)** | 0.005; 0.161 | 45.80 |
| **Language** | 0.044 (0.108) | -0.045 (0.114) | -1.867 (0.302)*** | -0.048 (0.022)* | **0.089 (0.047)** | 0.008; 0.197 | N/A |
| **Math and Language** | 0.104 (0.097) | 0.026 (0.103) | -1.867 (0.302)*** | -0.042 (0.020)* | **0.078 (0.039)** | 0.010; 0.160 | 75.08 |
| **GPA** | 0.105 (0.081) | 0.037 (0.086) | -1.867 (0.302)*** | -0.036 (0.017)* | **0.068 (0.032)** | 0.011; 0.136 | 64.54 |
| ***Academic abilities*** | | | | | | | |
| **Verbal** | 0.323 (0.132)** | 0.277 (0.141) | -1.867 (0.302)*** | -0.024 (0.027) | 0.046 (0.051) | -0.052; 0.155 | - |
| **Numeric** | 0.325 (0.183) | 0.188 (0.195) | -1.867 (0.302)*** | -0.073 (0.038) | **0.137 (0.064)** | 0.022; 0.279 | 42.15 |
| **Reasoning** | 0.069 (0.220) | -0.079 (0.235) | -1.867 (0.302)*** | -0.079 (0.045) | 0.148 (0.079) | 0.012; 0.328 | N/A |
| **Overall** | 0.221 (0.126) | 0.118 (0.134) | -1.867 (0.302)*** | -0.055 (0.026)* | 0.103 (0.047) | 0.022; 0.210 | 46.48 |

Results showed as unstandardized coefficients (Standard Error, SE) and BC 95%CI based on 5000 bootstraps. All analyses were adjusted for sex, pubertal stage, SES and adiposity.

BC: Bias corrected; CI: confidence interval; $P_M$: percentage of mediation; GPA: grade point average score; N/A: non-applicable according to statistical assumptions specified previously.

Statistical significant indirect effects indicating that 0 is not in the 95% confidence interval (CI) of the indirect effect are presented in bold.

*P<0.05, **P<0.01, ***P<0.001.