

# Inflammatory Proteins and Muscle Strength in Adolescents

## The AVENA Study

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**Objectives:** To examine the associations between inflammatory proteins and muscle strength and to determine whether this association varies between overweight and nonoverweight adolescents.

**Design:** Cross-sectional study.

**Participants:** A total of 416 Spanish adolescents (230 boys and 186 girls) aged 13 to 18½ years.

**Main Exposures:** Muscle strength score was computed as the mean of the handgrip and standing broad jump standardized values. The adolescents were categorized as overweight (including obese) or nonoverweight according to body mass index. Body fat and fat-free mass were derived from skinfold thickness.

**Outcome Measures:** C-reactive protein, complement factors C3 and C4, ceruloplasmin, and prealbumin levels.

**Results:** The results of the regression analysis showed that C-reactive protein, C3, and ceruloplasmin were negatively associated with muscle strength after controlling for sex, age, pubertal status, weight, height, socioeconomic status, and cardiorespiratory fitness. Moreover, C-reactive protein and prealbumin levels were associated with muscle strength in overweight adolescents after controlling for potential confounders, including body fat and fat-free mass.

**Conclusions:** Low-grade inflammation is negatively associated with muscle strength in adolescents. The patterns of these associations seem more relevant in overweight adolescents, suggesting that having high levels of muscle strength may counteract the negative consequences ascribed to body fat.

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**L**OW-GRADE INFLAMMATION seems to play a role in the development of cardiovascular disease from an early age.<sup>1,2</sup> It is negatively associated with cardiorespiratory fitness and positively associated with body fat in young people and adults.<sup>3-7</sup> Recent findings have shown a higher prevalence of having high C-reactive protein levels in overweight and unfit Spanish adolescents compared with their overweight and fit counterparts.<sup>8</sup> Inflammatory proteins have also been negatively associated with muscle strength in adults.<sup>9-11</sup> The causal pathway leading from inflammation to loss of muscle strength has not been fully explained, but it has been suggested that low-grade inflammation may cause a decline of physical functioning through its catabolic effects on skeletal muscle.<sup>12</sup>

The role of muscle strength in the performance of exercise and activities of daily living as well as in preventing disease has become increasingly recognized.<sup>13,14</sup> Resis-

tance exercise training increases muscle strength, and it is currently prescribed by the major health organizations for improving health and fitness.<sup>15</sup> Cardiovascular disease risk factors have also been associated with aerobic exercise and cardiorespiratory fitness, not only in adults but also in young people.<sup>16,17</sup> Whether low-grade inflammation is associated with muscle strength in adolescents is unknown. The aim of our study was to examine the associations between inflammatory proteins and muscle strength in adolescents and to determine whether these associations vary in overweight and nonoverweight adolescents.

## METHODS

### PARTICIPANTS

The study participants were Spanish adolescents aged 13 to 18½ years from the *Alimentación y Valoración del Estado Nutricional de los Adolescentes Españoles* (Food and Assessment of the Nutritional Status of Spanish Adolescents)

(AVENA) Study. The AVENA Study was designed to assess the health and nutritional status of Spanish adolescents. Study design and sampling procedure have been reported in detail elsewhere.<sup>18-20</sup> After exclusion of 9 adolescents with concentrations of C-reactive protein greater than 10 mg/L (to convert to nanomoles per liter, multiply by 9.524), the study sample comprised 416 adolescents (230 boys and 186 girls) who had a complete set of inflammatory protein, muscle strength, and cardiorespiratory fitness measurements. A comprehensive verbal description of the nature and purpose of the study was given to both the adolescents and their teachers. Written consent was requested from parents and adolescents, and all adolescents gave verbal assent. Adolescents who had a history of cardiovascular disease, were taking medication at the time of the study, or were pregnant were excluded after completion of the field work. The study protocol was performed in accordance with the ethical standards established in the 1961 Declaration of Helsinki (as revised in Hong Kong in 1989 and in Edinburgh in 2000) and was approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla, Santander, Spain.

The parents completed a questionnaire that addressed the adolescents' previous and current health status. Socioeconomic status was also assessed in the questionnaire and was defined by the educational achievement and occupation of the father. According to this information, and following the recommendation of the Spanish Society for Epidemiology, the adolescents were classified into 5 categories: low, medium-low, medium, medium-high, and high socioeconomic status.

### PHYSICAL EXAMINATION

Anthropometric measurements were obtained as described elsewhere.<sup>21,22</sup> Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. The BMI categories (nonoverweight and overweight [including obesity]) were computed according to the proposed sex- and age-adjusted BMI cutoff points derived from adult values associated with health risk.<sup>23</sup> Skinfold thickness was measured at the biceps, triceps, subscapular, suprailiac, thigh, and calf on the left side of the body to the nearest 0.2 mm using a Holtain skinfold caliper. The sum of 6 skinfolds was used as an indicator of total body fat.<sup>22</sup> Body fat percentage was calculated from skinfold thicknesses (triceps and subscapular) using equations from Slaughter et al.<sup>24</sup> Fat-free mass (in kilograms) was derived by subtracting fat mass from total body weight.

Identification of pubertal status (stages I-V) was assessed according to Tanner and Whitehouse.<sup>25</sup> The standard staging of pubertal maturity describes breast and pubic hair development in girls and genital and pubic hair development in boys.

### BLOOD SAMPLING

After overnight fasting, blood samples were collected between 8 and 9:30 AM by venipuncture. High sensitive C-reactive protein, complement factors C3 and C4, and ceruloplasmin were measured by immunoturbidimetry (AU2700 Biochemistry Analyzer; Olympus, Rungis, France). Prealbumin was measured by immunoturbidimetry (Roche/Hitachi 912; Roche Diagnostics, Indianapolis, Indiana). Quality control of the assays was assured by the regional health authority. A detailed description of the blood analysis has been reported already.<sup>20,26</sup>

### MUSCLE STRENGTH

Upper body strength was assessed by handgrip strength test, and lower body strength was assessed by the standing broad

jump test. The handgrip strength test was performed on both hands with a hand dynamometer (Takei TTK 5101 Grip-D; Takei, Tokyo, Japan), standing and with the arm completely extended. The standing broad jump test was performed in an indoor rubber-floored gymnasium. The participants were instructed to push off vigorously and jump as far forward as possible, trying to land on both feet. The distance from the take-off line to the point where the back of the heel closest to the take-off line landed on the floor was scored.

A muscle strength score was computed by combining the standardized values of handgrip strength and standing broad jump. Each of these variables was standardized as follows: standardized value = (value - mean)/SD. The standardized values of the handgrip strength obtained with the right and left hands were averaged. The muscle strength score was calculated as the mean of the 2 standardized scores (handgrip strength and standing broad jump). The score was calculated separately for boys and girls and for each age group (13, 14, 15, 16, and 17-18½ years).

### CARDIORESPIRATORY FITNESS

Cardiorespiratory fitness was assessed by the 20-m shuttle run test as previously described.<sup>27</sup> In brief, participants were required to run between 2 lines 20 m apart in time with an audio signal. The initial speed of the signal was 8.5 km/h and was increased by 0.5 km/h/min (1 minute equal to 1 step). The test was finished when the participant failed to reach the end lines concurrent with the audio signals on 2 consecutive occasions. Otherwise, the test ended when the participant stopped because of fatigue. Cardiorespiratory fitness was considered to be the number of steps completed. Detailed methodology of these fitness tests have been reported elsewhere.<sup>28</sup>

### STATISTICAL ANALYSES

All the residuals showed a satisfactory pattern after skinfold thickness and C-reactive protein, C3, C4, ceruloplasmin, and prealbumin values were normalized by natural logarithm transformation. Sex differences were analyzed by 1-way analysis of variance and adjusted for mass significance as described by Holm.<sup>29</sup> Nominal data were analyzed using  $\chi^2$  tests. Partial correlations were used to examine bivariate correlations between cardiorespiratory fitness and muscle strength after controlling for sex. Mean values of inflammatory proteins, skinfold thickness, body fat percentage, and fat-free mass in overweight and nonoverweight adolescents were compared by 1-way analysis of variance.

Multiple regression was used to study the association between inflammatory proteins and muscle strength after controlling for sex, age, pubertal status, weight, height, socioeconomic status, and cardiorespiratory fitness. A separate regression model was performed for every outcome variable (ie, C-reactive protein, C3, C4, ceruloplasmin, and prealbumin levels). Because no significant interaction was found with sex, all the analyses were performed with the male and female adolescents together to have a stronger statistical power.

The associations between inflammatory proteins and muscle strength were also examined separately for overweight and nonoverweight adolescents owing to the strong differences observed in inflammatory proteins and body composition-related variables. Moreover, a significant interaction was observed between BMI categories and muscle strength. The regression analyses were controlled for sex, age, pubertal status, socioeconomic status, and cardiorespiratory fitness. Further analyses were performed additionally controlling for skinfold thickness (or body fat percentage) and fat-free mass. The

**Table 1. Physical Characteristics of Study Sample**

Characteristic	Mean (SD)		
	Total Sample (N=416)	Adolescent Boys (n=230)	Adolescent Girls (n=186)
Age, y	15.4 (1.4)	15.4 (1.4)	15.4 (1.4)
Weight, kg	61.3 (12.8)	64.9 (13.4)	56.8 (10.5) <sup>a</sup>
Height, cm	166.6 (8.7)	171.0 (8.1)	161.4 (6.2) <sup>a</sup>
Body mass index <sup>b</sup>	22.0 (3.7)	22.1 (3.9)	21.8 (3.6)
Overweight (including obese), %	26	31	20
Pubertal status, %			
I	0	0	0
II	3	4	1
III	12	15	9
IV	47	41	55
V	38	40	35
Sum of 6 skinfolds, mm	44.5 (5.8)	43.0 (6.1)	44.1 (5.3)
Body fat, %	23.1 (6.8)	22.8 (8.2)	23.5 (4.3)
Fat-free mass, kg <sup>c</sup>	46.6 (7.2)	49.3 (6.9)	43.1 (6.0) <sup>a</sup>
C-reactive protein, mg/L	1.44 (3.1)	1.56 (2.6)	1.28 (3.7)
Complement factor C3, µg/mL	1350 (240)	1360 (240)	1330 (220)
Complement factor C4, µg/mL	270 (100)	270 (90)	270 (100)
Ceruloplasmin, mg/dL	21 (5)	20 (4)	22 (5) <sup>a</sup>
Prealbumin, mg/dL	23.76 (6.6)	24.30 (6.9)	23.02 (6.0)
Cardiorespiratory fitness, No. of steps	5.8 (2.8)	7.1 (2.6)	4.1 (1.9) <sup>a</sup>
Handgrip strength, kg	31.8 (8.0)	35.5 (7.6)	25.4 (4.0) <sup>a</sup>
Standing broad jump, cm	173.9 (32.5)	191.0 (29.1)	152.7 (22.2) <sup>a</sup>
Socioeconomic status, %			
Low	5	4	6
Medium low	26	27	25
Medium	40	43	37
Medium high	23	21	25
High	6	5	7

SI conversion factors: To convert ceruloplasmin to milligrams per liter, multiply by 10; complement factors C3 and C4 to grams per liter, multiply by 0.001; C-reactive protein to nanomoles per liter, multiply by 9.524; and prealbumin to milligrams per liter, multiply by 10.

<sup>a</sup>Significantly different from boys,  $P < .001$  (analysis of variance), adjusted for mass significance.

<sup>b</sup>Calculated as weight in kilograms divided by height in meters squared.

<sup>c</sup>Derived by subtracting fat mass from total body weight.

SPSS, version 15.0 (SPSS Inc, Chicago, Illinois), was used to perform all the analyses.  $P \leq .05$  was considered significant.

## RESULTS

All adolescents (N=416) had a complete set of data, with the exception of pubertal status and socioeconomic status data, which were not available in 37 (9%) and 83 (20%) adolescents, respectively. The descriptive characteristics of the study sample are presented in **Table 1**. The analysis of variance showed that adolescent boys had significantly higher levels of ceruloplasmin than adolescent girls as well as higher fat-free mass, cardiorespiratory fitness, handgrip strength, and standing broad jump. The results of the partial correlation showed that cardiorespiratory fitness was significantly associated with both handgrip strength ( $r=0.148$ ,  $P < .01$ ) and standing broad jump ( $r=0.746$ ,  $P < .001$ )

**Table 2. Regression Coefficients, SEM, and Standardized Coefficient of Determination Showing the Association Between Inflammatory Proteins and Muscle Strength<sup>a</sup>**

Measure	$\beta$	SEM	$R^2$	P Value
C-reactive protein	- 0.208	0.077	0.10	.007
Complement factor C3	- 0.115	0.013	0.20	.047
Complement factor C4	- 0.033	0.029	0.06	.26
Ceruloplasmin	- 0.142	0.004	0.10	.03
Prealbumin	0.054	0.090	0.01	.55

<sup>a</sup>Controlling for sex, age, pubertal status, weight, height, socioeconomic status, and cardiorespiratory fitness.

**Table 3. Inflammatory Proteins and Body Composition Variables by Body Mass Index Categories**

Measure	Mean (SD)	
	Nonoverweight Adolescent (n=308)	Overweight Adolescent <sup>a</sup> (n=108)
C-reactive protein, mg/L	1.26 (3.16)	1.99 (3.09) <sup>b</sup>
Complement factor C3, µg/mL	1330 (200)	1490 (270) <sup>c</sup>
Complement factor C4, µg/mL	250 (90)	320 (100) <sup>c</sup>
Ceruloplasmin, mg/dL	20 (4)	22 (5) <sup>d</sup>
Prealbumin, mg/dL	23.46 (6.61)	24.43 (6.52)
Sum of 6 skinfolds, mm	41.0 (3.5)	50.7 (5.1) <sup>c</sup>
Body fat, %	20.3 (4.7)	31.3 (5.2) <sup>c</sup>
Fat-free mass, kg <sup>e</sup>	44.8 (6.3)	51.9 (6.9) <sup>c</sup>

SI conversion factors: To convert ceruloplasmin to milligrams per liter, multiply by 10; complement factors C3 and C4 to grams per liter, multiply by 0.001; C-reactive protein to nanomoles per liter, multiply by 9.524; and prealbumin to milligrams per liter, multiply by 10.

<sup>a</sup>Including obese adolescents.

<sup>b</sup> $P < .05$ .

<sup>c</sup> $P < .001$  (analysis of variance) for comparison between nonoverweight and overweight (including obese) adolescents.

<sup>d</sup> $P < .01$ .

<sup>e</sup>Derived by subtracting fat mass from total body weight.

as well as with muscle strength score ( $r=0.339$ ,  $P < .001$ ) after controlling for sex.

The statistics of the regression analysis showing the association between inflammatory proteins and muscle strength after controlling for sex, age, pubertal status, weight, height, socioeconomic status, and cardiorespiratory fitness are presented in **Table 2**. C-reactive protein, C3, and ceruloplasmin levels were negatively associated with muscle strength, whereas C4 and prealbumin were not significantly associated with muscle strength. Inflammatory proteins were not significantly associated with cardiorespiratory fitness. The results did not change when the analyses were controlled for BMI or body fat (expressed as either skinfold thickness or body fat percentage) instead of weight and height.

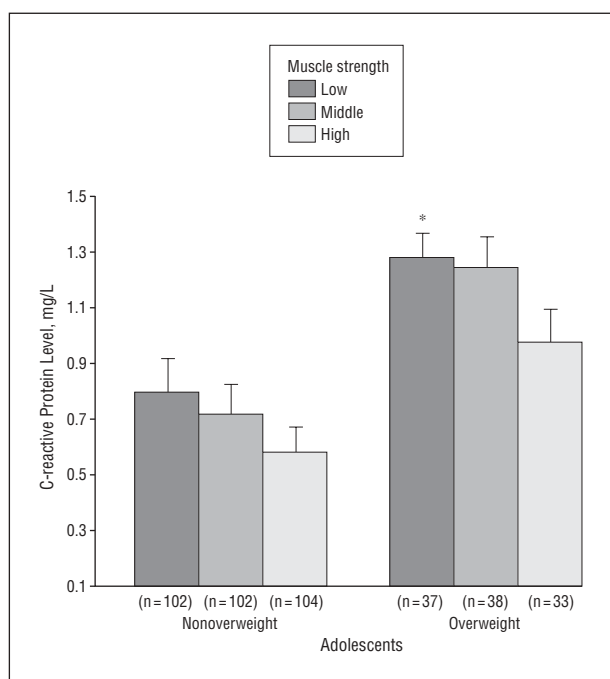
Mean values of inflammatory proteins and body composition variables by BMI categories are presented in **Table 3**. The results of the analysis of variance showed that overweight adolescents had significantly higher levels of C-reactive protein, C3, C4, and ceruloplasmin than nonoverweight adolescents. Moreover, overweight ado-

**Table 4. Regression Coefficients, SEM, and Standardized Coefficient of Determination Showing the Association Between Inflammatory Proteins and Muscle Strength in Overweight and Nonoverweight Adolescents<sup>a</sup>**

Measure	Nonoverweight Adolescent (n=308)				Overweight Adolescent <sup>b</sup> (n=108)			
	$\beta$	SEM	R <sup>2</sup>	P Value	$\beta$	SEM	R <sup>2</sup>	P Value
C-reactive protein	-0.155	0.090	0.01	.09	-0.327	0.132	0.19	.02
Complement factor C3	-0.013	0.014	0.05	.25	-0.033	0.028	0.09	.24
Complement factor C4	-0.024	0.032	0.01	.46	-0.023	0.059	0.02	.69
Ceruloplasmin	-0.019	0.020	0.06	.33	-0.048	0.035	0.10	.18
Prealbumin	-0.085	0.105	0.02	.42	0.497	0.156	0.11	.02

<sup>a</sup>Controlling for sex, age, pubertal status, socioeconomic status, and cardiorespiratory fitness.

<sup>b</sup>Including obese adolescents.



**Figure 1.** Mean values of C-reactive protein (log transformed) stratified in tertiles by muscle strength (low, middle, and high) in overweight (including obese) and nonoverweight adolescents. Error bars represent standard errors of the mean. \*Indicates a significant difference ( $P=.04$ ) was observed between overweight adolescents with high muscle strength (third tertile) and overweight adolescents with low muscle strength (first tertile). To convert C-reactive protein to nanomoles per liter, multiply by 9.524.

lescents had significantly higher skinfold thickness, body fat percentage, and fat-free mass than their nonoverweight counterparts.

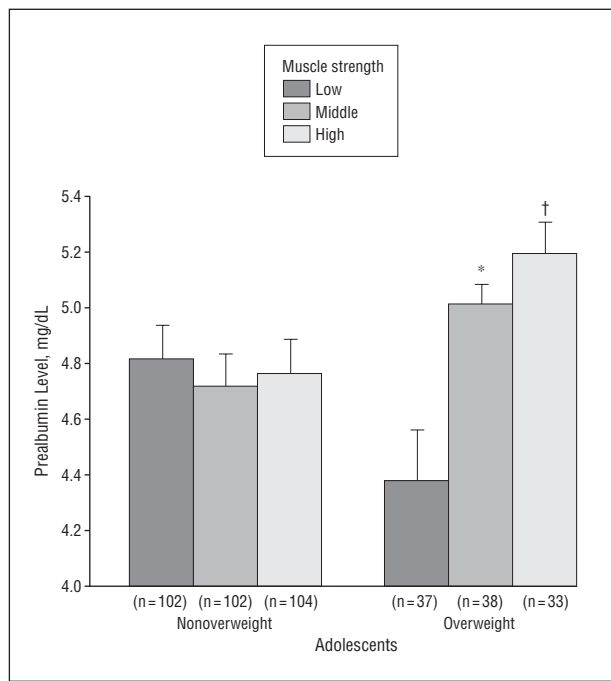
The results of the regression analysis showing the association between inflammatory proteins and muscle strength in overweight and nonoverweight adolescents after controlling for sex, age, pubertal status, socioeconomic status, and cardiorespiratory fitness are presented in **Table 4**. C-reactive protein and prealbumin were associated with muscle strength in overweight adolescents, whereas no association was found in the nonoverweight group. Ceruloplasmin, C3, and C4 levels were not significantly associated with muscle strength in either overweight or nonoverweight adolescents. The results did not change when the analyses were additionally controlled for skinfold thickness or body fat percentage. Like-

wise, the inclusion of fat-free mass in the regression model did not alter the results. No significant effect of pubertal status on the outcome was observed in any of the analyses performed (data not shown).

Both overweight and nonoverweight adolescents were divided into 3 groups (tertiles) of muscle strength (low, middle, and high). The analysis of covariance with Bonferroni adjustment for sex, age, pubertal status, socioeconomic status, and cardiorespiratory fitness showed that overweight adolescents with high muscle strength (third tertile) had significantly lower values of C-reactive protein than overweight adolescents with low muscle strength (first tertile) (**Figure 1**). Moreover, overweight adolescents with middle or high muscle strength (second and third tertile, respectively) had significantly higher levels of prealbumin than overweight adolescents with low muscle strength (first tertile) (**Figure 2**). Overweight adolescents with high muscle strength had significantly lower skinfold thickness and body fat percentage than overweight adolescents with low muscle strength (skinfold thickness, 49.3 vs 53.4 mm, respectively,  $P=.03$ ; body fat percentage, 30.1% vs 33.8%, respectively,  $P=.001$ ). Furthermore, overweight adolescents with high muscle strength also had significantly higher fat-free mass levels than overweight adolescents with low muscle strength (52.6 vs 51.4 kg, respectively,  $P=.001$ ).

#### COMMENT

The primary findings of this study show that (1) C-reactive protein, C3, and ceruloplasmin are negatively associated with muscle strength in adolescence and (2) C-reactive protein and prealbumin are associated with muscle strength in overweight adolescents after controlling for different confounders including cardiorespiratory fitness. Moreover, we observed an increased low-grade inflammation in overweight adolescents compared with their nonoverweight counterparts, which confirms previous findings.<sup>3,4,26</sup> Taken together, these findings suggest that interventions designed to prevent low-grade inflammation and related metabolic disorders should focus not only on reducing fatness but also on improving muscle fitness. To the best of our knowledge, this is the first population-based study showing the association between low-grade inflammation and muscle strength in adolescents.



**Figure 2.** Mean values of prealbumin (log transformed) stratified in tertiles by muscle strength (low, middle, and high) in overweight (including obese) and nonoverweight adolescents. Error bars represent standard errors of the mean. \*Indicates a significant difference ( $P=.048$ ) was observed between overweight adolescents with middle muscle strength (second tertile) and overweight adolescents with low muscle strength (first tertile); †, a significant difference ( $P=.004$ ) was observed between overweight adolescents with high muscle strength (third tertile) and overweight adolescents with low muscle strength (first tertile). To convert prealbumin to milligrams per liter, multiply by 10.

The association between inflammatory proteins and muscle strength has only been examined in a few studies in adults. Two cross-sectional studies have shown a negative association of C-reactive protein, IL-6 (interleukin-6), and tumor necrosis factor- $\alpha$  with muscle strength.<sup>9,11</sup> Additionally, 1 prospective study found that higher levels of IL-6 and C-reactive protein were associated with loss of muscle strength in older persons.<sup>10</sup>

Features of metabolic syndrome have also been negatively associated with muscle strength in men.<sup>30</sup> Likewise, findings from a prospective study in men suggested that muscle strength may additively protect against the incidence of metabolic syndrome beyond that attributed to cardiorespiratory fitness and that overweight men may benefit more than their nonoverweight counterparts.<sup>31</sup> These results concur with those obtained in the present study. That low-grade inflammation was negatively associated with muscle strength after controlling for cardiorespiratory fitness may indicate that muscle strength in young people confer additional benefits beyond those attributed to cardiorespiratory fitness.<sup>4,6,8,16,17,32</sup> We did not find a significant association between inflammatory markers and cardiorespiratory fitness. Findings from the European Youth Heart Study showed that C-reactive protein and C3 were negatively associated with cardiorespiratory fitness and positively associated with body fat (expressed as the sum of 5 skinfolds) in Swedish children aged 9 to 10 years.<sup>33</sup> Further analyses revealed that, for most of the variables, body fat was slightly more predictive than cardiorespiratory fitness. In our study, the

associations between inflammatory proteins and muscle strength persisted after (separately) controlling for weight and height, BMI, and body fat (expressed as either the sum of 6 skinfolds or as body fat percentage). These findings add supportive evidence to the body of knowledge that suggests that muscle strength is important for health in young people, as it has been shown in adults.<sup>9-11,30,31</sup>

It is also noteworthy that the observed associations between C-reactive protein, prealbumin, and muscle strength in overweight adolescents remained significant after controlling for body fat and fat-free mass. This suggests that other mechanisms are involved in these associations. The key role of muscle mass in a number of metabolic processes as well as in the prevention of many pathologic conditions and chronic diseases has recently been highlighted.<sup>13,14</sup> The group of overweight adolescents in the present study have higher values of body fat and fat-free mass than the nonoverweight group, which is in agreement with another study in obese children.<sup>34</sup> In addition, the group of overweight adolescents with high muscle strength have lower body fat and higher fat-free mass than the overweight group with low muscle strength. Taken together, these results may partially explain why the group of overweight adolescents with high muscle strength are also those with the lowest levels of C-reactive protein and the highest levels of prealbumin. The findings suggest that the deleterious consequences ascribed to high fatness could be counteracted by having high levels of fitness. The role of both body fat and fat-free mass in the association between low-grade inflammation and muscle strength warrants further investigation. The use of more advanced approaches to better estimate both body fat and fat-free mass<sup>35</sup> may help to elucidate the role of these 2 tissues in the association between low-grade inflammation and muscle strength.

A high concentration of C-reactive protein is considered a major cardiovascular risk factor,<sup>1-3</sup> and evidence also links C3 and C4 with vascular disease.<sup>37</sup> Body fat is known to promote a state of low-grade inflammation,<sup>38</sup> which lends credibility to the results obtained in our study. Furthermore, high concentrations of inflammatory proteins have been hypothesized to play a role in the functional decline of older persons through its catabolic effects on skeletal muscle.<sup>12,39,40</sup> Collectively, these mechanisms may explain the observed association between C-reactive protein and muscle strength in overweight adolescents. Prealbumin, also known as transthyretin, is a negative acute-phase protein that declines in response to inflammation.<sup>41</sup> Other factors, such as starvation and decreased skeletal muscle function, are also known to affect prealbumin concentrations.<sup>42</sup> Therefore, the association between prealbumin levels and muscle strength observed in our study in overweight adolescents could be explained by prealbumin's putative association to muscular weakness and the state of increased low-grade inflammation seen in the overweight adolescents.

In light of these results, special efforts should focus on subgroups of adolescents at increased risk of early cardiovascular disease, such as overweight or obese adolescents. As a first step, promotion of regular participation in strength activities may be helpful, since this mode of

exercise may be easier and better tolerated than aerobic training for overweight or obese youth.<sup>43</sup> Strength training may have a number of beneficial effects for overweight or obese individuals, including increased muscle mass, decreased total and central fat mass, and enhanced insulin sensitivity.<sup>15,44-47</sup> A recent study in obese men has shown that a 12-week resistance training program (3 d/wk) induces an improvement of whole body insulin sensitivity independent of changes in body composition.<sup>44</sup> Moreover, plasma levels of C-reactive protein, adiponectin, IL-1 $\beta$ , IL-6, and tumor necrosis factor- $\alpha$  as well as gene expression of adipokines in abdominal adipose tissue were not changed after the training period. Interventional studies have failed to show significant changes on low-grade inflammation (fibrinogen, plasminogen activator inhibitor 1, and C-reactive protein) in overweight or obese children after completion of an aerobic physical training program.<sup>48,49</sup> Nevertheless, further studies are required to show whether resistance exercise can effectively attenuate the moderately increased resting levels of inflammatory proteins as well as reduce the fat mass in overweight adolescents. Increases in muscle strength may also positively influence the levels of cardiorespiratory fitness, since both variables are closely related.

The observations of our study are limited by its cross-sectional design. Prospective and intervention studies are required to draw more robust conclusions on the determining effect of inflammatory proteins on muscle strength. We used a single blood measurement of inflammation that may not accurately reflect long-term inflammatory status. Although we did not include any adolescent with a known underlying cause of infection, we cannot be sure that elevated concentrations were not due to the onset of an infection. However, to minimize the confounding effect of an ongoing infection, adolescents with high concentrations of C-reactive protein (> 10 mg/L) were not included in this study. We should therefore consider the whole sample as healthy, and the modest but significant associations presented here may imply a chronic state of inflammation clinically relevant in the long-term rather than as a short-term diagnostic risk marker.

Rapid and dynamic changes in various metabolic systems, including hormonal regulation, changes in body composition, as well as transient changes in insulin resistance are known to occur during puberty.<sup>50</sup> However, we did not measure either sex hormones or insulin, which hamper further study of hormone- and insulin-inflammatory protein relationships in our population. All the analyses were controlled for pubertal status as a measure of biological age. We have shown that pubertal status influences serum lipid and lipoprotein levels in the AVENA Study population,<sup>51</sup> whereas, we observed neither an influence on low-grade inflammation nor an interaction with muscle strength.

In conclusion, the results presented in this study indicate that low-grade inflammation is negatively associated with muscle strength in adolescents. The patterns of these associations seem more relevant in overweight adolescents, suggesting that having high levels of muscle strength may counteract the negative consequences ascribed to body fat. More studies are needed to elucidate

the role of body fat, fat-free mass, and muscle strength on low-grade inflammation. Intervention studies examining the effect of strength training on muscle mass and inflammatory markers in adolescents are warranted.

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**Author Contributions:** Dr Ruiz had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Ruiz, Moreno, Gonzalez-Gross, Carrero, and Marcos. **Acquisition of data:** Ruiz, Ortega, Wärnberg, Moreno, Gonzalez-Gross, Marcos, and Gutierrez. **Analysis and interpretation of data:** Ruiz, Ortega, Wärnberg, Moreno, Carrero, Marcos, Gutierrez, and Sjöström. **Drafting of the manuscript:** Ruiz. **Critical revision of the manuscript for important intellectual content:** Ruiz, Ortega, Wärnberg, Moreno, Carrero, Gonzalez-Gross, Marcos, Gutierrez, and Sjöström. **Statistical analysis:** Ruiz. **Obtained funding:** Moreno, Carrero, Gonzalez-Gross, Marcos, Gutierrez, Ruiz, and Wärnberg. **Administrative, technical, or material support:** Ruiz, Moreno, Gonzalez-Gross, Marcos, and Gutierrez. **Study supervision:** Ruiz, Moreno, Gonzalez-Gross, Marcos, Gutierrez, and Sjöström.

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