# Nonobese, exercising children diagnosed with dyslipidemia have normal C-reactive protein



Miguel Arturo Salazar Vázquez[1]
Beatriz Yadira Salazar Vázquez[2,3]
M Intaglietta[3]
Pedro Cabrales[4]

[1]Facultad de Ciencias Químicas Gómez Palacio, Universidad Juárez del Estado de Durango, Dgo., México; [2]Faculty of Medicine, Universidad Juárez del Estado de Durango, Victoria de Durango Dgo., México; [3]Department of Bioengineering, University of California, San Diego, La Jolla, CA, USA; [4]La Jolla Bioengineering Institute,La Jolla, CA, USA

**Abstract:** Nonobese children age 10.4 ± 1.1 years diagnosed with dyslipidemia (n = 51) were compared to normal children age 10.8 ± 1.1 years (n = 38). Affected individuals had increased total cholesterol: 223 ± 23 vs 152 ± 17 mg/dl, $p < 0.001$; and decreased high-density lipoprotein-cholesterol: 41.9 ± 4.1 vs 57.6 ± 5.7 mg/dl, $p < 0.001$ and triglycerides: 90.8 ± 40.5 vs 65.7 ± 25.0 mg/dl, $p < 0.002$. Fasting glucose was also significantly elevated ($p < 0.02$). All other parameters, including blood pressure, were not statistically different between groups. The concentration of C-reactive protein was not statistically different between groups. Analysis of medical records showed that this anomaly may be related to this group (as well as the control group) performing regular, daily exercise. This activity was quantified via a self administered questionnaire, and found to be statistically identical in controls and dyslipidemic individuals. Exercise is associated with the release of antiinflammatory cytokines, therefore our results support the contention that it is a significant factor in promoting health conditions from an early stage in life.

**Keywords:** dyslipidemia, C-reactive protein, children, exercise, inflammation, diabetes, obesity, metabolic syndrome, hispanics

## Introduction

Dyslipidemia is a metabolic condition associated with abdominal obesity, hypertension, insulin resistance (glucose intolerance), prothrombotic conditions and the presence of pro-inflammatory factors, even tough this latter condition maybe at a low level. It presents different forms whose common denominator is the alteration of lipid metabolism, manifested by changes in lipids and lipoproteins in blood. A generalized pro-inflammatory condition is presently recognized to be the precursor for arthrosclerosis,[1,2] type 2 diabetes, and associated long range risk factor for vascular and cardiovascular disease.[3,4]

The incidence of inflammatory conditions and dyslipidemia is relatively high in the adult population. Conversely, its prevalence in children is low, but, increases significantly in overweight children.[5] However, circumscribing clinical evaluation for identification of proinflammatory conditions solely to obese children and adolescents would limit the opportunities of diagnosis and prevention in lean young individuals.[6] Recent studies show that children from Hispanic origin (Mexican-American) present a higher risk to develop this syndrome than their Anglo-Saxon counterpart.[7,8]

The present study was conducted to determine the association between the diagnosis of dyslipidemia in lean children who perform regular exercise and the incidence of plasma C-reactive protein (CRP), to test the hypothesis that dyslipidemia is associated with the presence of inflammatory conditions, that may pose a risk even in the absence of overweight. Results were compared to those from a nondysplipidemic group.

## Methods

Nonobese children in the age range of 8 to 12 years residing in the city of Durango were enrolled in this study. They were recruited in the Unidad de Medicina del Deporte de la

Correspondence: Pedro Cabrales
La Jolla Bioengineering Institute, 505 Coast Boulevard South Suite #405, La Jolla, CA 92037, USA
Tel +1 858 5342315
Email pcabrales@ucsd.edu

Facultad de Medicina Sports Medicine Unit of the Faculty of Medicine) UJED by open invitation. They were entered into the study following individual and parental consent, according to regulations governing the study and treatment of children. Individuals were divided into a group consisting of nonobese children with dyslipidemia, and a control group of nonobese children without dyslipidemia. Exclusion criteria were the use of any medicament taken daily, children with previous diagnosis of renal, renovascular and endocrine disease, and hypertension.

For the purpose of this study we defined a control group selected on the basis of not being dyslipidemic. Anthropometric parameters recorded were height, weight and body mass index.

Fasting glucose (10–12 hours after food ingestion) was measured in blood simples by the glucose oxidase enzymatic method. Hyperglycemia was defined when fasting glucose was equal to or greater than 100 mg/dL. Dyslipidemia was diagnosed following determination of elevated cholesterol (>200 mg/dL) and triglycerides (>150 mg/dL) and reduced high-density lipoprotein-cholesterol (HDL-C) (<50 mg/dL), when 2 out 3 of these parameters were exceeded. Concentrations were measured from 5 ml blood samples (no anticoagulants), incubated at 37 °C for 5 minutes, centrifuged at 3000 RPM for 5 minutes and processed by an autoanalyzer (Data Pro Plus, Thermo Fisher Scientific, Inc. Waltham, MA, USA). C-reactive protein was determined from 3 ml venous blood samples (no anticoagulant) processed using the DPC IMMULITE High Sensitivity CRP instrument (Diagnostic Products Corp., Los Angeles, CA, USA).

Systolic and diastolic pressures were recorded and mean arterial blood pressure (MAP) was determined using the relationship:

$$MAP = P_{diastolic} + 1/3(P_{systolic} - P_{diastolic})$$

The level of physical activity is increasingly recognized as a factor in promoting the metabolic syndrome therefore it is important to establish the relative level of activity in both groups. Daily activity was measured by means of a self-report questionnaire, designed following the scheme of Aadahl and Jørgensen[9] partially modified to reflect the younger age of the tested population and the difference in environment. The test was administered to 20 individuals in each group chosen at random by blind selection.

The data was analyzed to determine statistically significant differences according to the Student's t test. Statistical significance is assumed for $p < 0.05$.

## Results

Fifty one dyslipidemic nonobese children age 10.4 ± 1.1 years and 38 controls age 10.8 ± 1.1 were entered into the study. Their characteristics are given in Table 1. Table 2 summarizes our results showing that affected individual had triglycerides 90.0 ± 40.5 vs 65.7 ± 25.0 mg/dl, $p < 0.002$; increased total cholesterol: 223 ± 23 vs 152 ± 17 mg/dl, $p < 0.001$; and diminished HDL-C: 41.9 ± 4.1 vs 57.6 ± 5.7 mg/dl, $p < 0.001$. Fasting glucose were also significantly elevated ($p < 0.02$). All other parameters, including blood pressure and the concentration of CRP were not statistically different between groups. Analysis of medical records showed that this anomaly was related to this group (as well as the control group) performing regular, daily exercise for a minimum period of one hour.

Twenty individuals were selected at random for each group to determine the respective levels of physical activity. This was found to be 45.7 ± 3.1 for controls and 45.9 ± 2.9 h MET-time/day (MET: metabolic equivalents or estimation of energy expenditure for specific physical activity) for the sample of study population, where the differences were not statistically significant. As a comparison Aadahl and Jørgensen[9] report a range of results for young adults including both leisure time and work activity ranging from 41.0 ± 9.9 to 61.7 ± 14.5 24 h MET-time/day. An independently sampled equivalent sampled group of normal children with comparatively low physical measured 38.5 ± 1.2 h MET-time/day using the same

**Table 1** Characteristics of the sample populations

| | Children with dyslipidemia n = 51 | Controls n = 38 | Significance (p, Student's t test) |
|---|---|---|---|
| Age, years | 10.3 ± 1.1 | 10.7 ± 1.1 | n.s. |
| Males, n (%) | 30 (59%) | 16 (42%) | – |
| Females, n (%) | 21 (41%) | 22 (58%) | – |
| History of diabetes in family, n (%) | 29 (57%) | 19 (50%) | n.a. |
| History of hypertension in family, n | 31 (61%) | 17(45%) | n.a. |
| Weight, kg | 37.4 ± 6.7 | 41.3 ± 9.5 | <0.05 |
| BMI, kg/m$^2$ | 18.4 ± 2.4 | 19.1 ± 2.6 | n.s. |

**Abbreviations:** BMI, body mass index; n.s., not significant, $p > 0.05$; n.a., not applicable because data was mostly anecdotical.

**Table 2** Differences between children with dyslipidemia and controls

| | Children with dyslipidemia | Controls | Significance p, student's t test |
|---|---|---|---|
| C-reactive protein, mg/L | 2.5 ± 1.3 | 2.3 ± 1.4 | 0.40 (n.s.) |
| | Range: 0.3–4.5 | Range: 0.7–4.5 | |
| Systolic pressure, mmHg | 97.7 ± 13.4 | 96.6 ± 9.5 | n.s. |
| Diastolic pressure, mmHg | 56.5 ± 9.7 | 57.6 ± 10.5 | n.s. |
| Fasting glucose, mg/dl | 90.3 ± 7.4 | 85.4 ± 9.8 | <0.02 |
| Triglycerides, mg/dl | 90.9 ± 40.5 | 65.7 ± 25.0 | <0.002 |
| Total cholesterol, mg/dl | 223 ± 23 | 152 ± 17 | <0.001 |
| HDL-cholesterol, mg/dl | 41.9 ± 4.1 | 57.6 ± 5.7 | <0.001 |

**Abbreviations:** HDL, high-density lipoprotein; n.s. not significant, $p > 0.05$.

self evaluation questionnaire, the difference being statistically significant from the measurement in control active children ($p < 0.001$).

## Discussion

The principal finding of this study is that children diagnosed with dyslipidemia do not present elevated CRP in their plasma when compared with a matched cohort of normal children. Notably the affected cohort was clearly dyslipidemic with diminished HDL-C and increased total cholesterol levels presenting the classic pattern of elevated total cholesterol, and decreased HDL-C, differences that were statistically significant ($p < 0.001$). There was no particular trend in the anthropometric data, with the exception of weight that was somewhat greater for controls ($p < 0.05$).

The result that the concentration of CRP in children diagnosed as dyslipidemic was not statistically significantly different from control is unexpected and required further scrutiny of the population characteristics. As an example a recent study conducted in dyslipidemic children and adolescents with different combinations of low and high HDL-C and triglycerides and average body mass index (BMI) ranging from 21.3 to 24.8 kg/m$^2$ (control 20.1 kg/m$^2$) had significantly elevated levels of CRP. Notably the prevalent level of exercise was not evaluated in these populations.[10] Analysis of the clinical questionnaires administered to the population sampled in our study revealed that both groups (diagnosed and controls) routinely carried out a regular regime of daily, one hour or more exercise. Therefore this population may be markedly different from the more sedentary individuals that manifest early signs of overweight or obesity, who encompass a greater number of factors linked to the metabolic syndrome.

A significant factor of cardiovascular risk (particularly in adults) is the presence of pro-inflammatory conditions evidenced by elevated CRP. The significant decrease of this marker in the population studied confirms that the inherent risk factors are reduced in individuals that perform regular exercise,[11] an effect noted in previous studies primarily in adults and older people. The present finding shows that exercise is necessary and beneficial at the earliest age. The incidence of increasingly sedentary life styles evidenced also in children should be a factor on the increased incidence of diabetes in younger individuals, and the lowering of the threshold at which these diseases appear in the population.

It has been recently recognized that exercising skeletal muscle is the source of antiinflammatory cytokines (IL-6) which significantly lower the incidence of inflammatory markers, including CRP.[12] This effects has been observed in young and elder individuals[13] who do consistent daily exercise. This mechanism is now considered to be the basis for the noted beneficial effects resulting from the performance of exercise,[14–16] as well as the generally improved health condition of exercising individuals compared to sedentary persons. It should be noted however that although reducing CRP concentrations through lifestyle changes or pharmacological interventions is widely held to be of clinical benefit[17,18] there remains a critical need for long-term studies to determine whether reductions in CRP concentrations translate into a decreased cardiovascular risk factor.[19]

Regular exercise has the additional benefit of increasing blood flow in the circulation, with the effect of increasing shears stress and the production of nitric oxide (NO) by the endothelium, increasing NO bioavailability. This effect is generally considered to produce an antiinflammatory environment.[20,21]

The results and interpretations of the present study are directly linked to physical activity as a determining factor, which is a challenge to evaluate since there is no gold standard to obtain this information. Continuous recording of single axis body acceleration by a transducer designed to measure and record acceleration as a function of time is an option for obtaining reliable first order evaluation of this parameter.[22] A doubly labeled water method[23] is also considered to be a valid method for measuring energy expenditure. However, both methods are complex and expensive to implement, and not suitable for comparatively large studies in children populations.

In view of these considerations we adopted the "self questionnaire" technique developed by Aadahl and Jørgensen[9] minimally modified to account for the youth of our population and environmental differences, since their approach was developed for adults in the population of Denmark. The significant correlation found by these authors between results of their questionnaire and the combined data from actual measurement and a diary of daily activity suggested that their approach could be effectively used in this study, a perception in part supported by the low within group variability, and the significant difference between active and the low level exercising normal group found in our study.

A tentative conclusion of this study is that children that report similar high levels of physical exercise have low levels of CRP whether they are normal or are diagnosed dyslipidemic. Notably, similar studies in children where exercise was not quantified report a significant difference in CRP. The data on CRP and exercise presents some dispersion; however this was not sufficient for determining a statically significant association between CRP and levels of exercise. The possibility exists that the recorded levels of exercise are sufficient to overcome the inflammatory component manifested by the increase of concentration of CRP, a process that, however, may not be complete since there is a statistically not significant remnant increase in CRP in the dyslipidemic children. Verification of this conjecture rests on the possibility of testing a significant number of nonobese dyslipedemic children that engage in a low level of physical activity, which in the population tested is a small sub-group.

Although exercise is a significant factor in reducing inflammatory and pro-inflammatory conditions, it cannot be excluded that in the absence of obesity, the expression of pro-inflammatory markers such as CRP may be absent. Therefore in a population of active and thin adolescents dyslipedemia may not be necessarily be liked to inflammatory conditions, and may be due to genetic and familial antecedents or unusual dietary habits.[24,25]

In conclusion, there is a population of children with a median age of 10 years that presents diminished plasma concentration of HDL-C and increased cholesterol, a condition classified as dyslipidemia, that is prognostic and precursor to the metabolic syndrome, cardiovascular dysfunction and diabetes. However, an elevated level of CRP associated with these syndromes is not present in this population of nonobese children that perform regular exercise. Exercise is regarded as a critical component of the maintenance of "healthy" conditions. This study shows that the effects of exercise may be important in very young individuals. The effects of inflammatory conditions, some of them derived from dietary habits, are most likely cumulative, thus there should be health benefits in engaging in moderate but not inconsequential routine exercise, at an early age.

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# Appendix

## Exercise self-report questionnaire

### *PERSONAL INFORMATION*

| | | | |
|---|---|---|---|
| Name: | Last name: | | |
| Age: | | O Female | O Male |

### Home address

| | |
|---|---|
| Street: | |
| Number: | |
| Zip code: | |
| Phone number: | |

### Occupation or work

| | |
|---|---|
| Street: | |
| Number: | |
| Zip code: | |
| Phone number | |

| | | | |
|---|---|---|---|
| Weight | | Height | |
| Waist | | Hip | |
| Blood pressure | | Heart rate | |
| Body mass index | | | |
| Product ≥ 4kg | | Product < 2 kg | |

### FISICAL ACTIVITY

Of the following activities, please mark these that you have done in your free time in the last 12 months and select the circle that better indicates the frequency whereupon the activity was performed:

| **Activity** | **¿Qué día las realizó?** | | | | | | | **5–14** | **15–30** | **31–60** | **1–2 hr** | **3–4 hr** | **5–6 hr** | **>6 hr** | **Cada actividad la realiza en forma:** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **L** | **M** | **M** | **J** | **V** | **S** | **D** | **min sem** | **min sem** | **min sem** | **sem** | **sem** | **sem** | **hr sem** | **Light** | **Moderate** | **Intense** |
| None O | | | | | | | | | | | | | | | | | |
| To walk | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| To run | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| Cycling | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| Baseball | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| Soccer | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| Volleyball | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| Aerobics | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| To swim | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| Bowling | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| To dance | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| Other _____ _____ | | | | | | | | | | | | | | | | | |

**Notes: Light**: like the activity that is resembled to walk in calm form. **Moderate**: activity that realizes with effort, causing in you perspiration. **Intense**: activity that realizes like training or preparing themselves for a sport competition

| **Daily activities** | **1 Monday**<br>**2 Tuesday**<br>**3 Wednesday**<br>**3 Wednesday**<br>**4 Thursday**<br>**5 Friday**<br>**6 Saturday**<br>**7 Sunday** | | | | | | | **5 min** | **10 min** | **30 min** | **1 hr** | **2–3 hr** | **4–5 hr** | **6–7 hr sem** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | | | | | | | |
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| | | | | | | | | O | O | O | O | O | O | O |
| | | | | | | | | O | O | O | O | O | O | O |
| | | | | | | | | O | O | O | O | O | O | O |

Additional notes by the interviewer

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_____

| **Some familiar has suffered or suffers** | **Grandparents to father** | **Maternal grandparents** | **Mother** | **Father** | **brothers** |
|---|---|---|---|---|---|
| ¿Hypertension? | O | O | O | O | O |
| NO Yes (¿Who?) | O | O | O | O | O |
| ¿Diabetes mellitus? | O | O | O | O | O |
| NO Yes (¿Who?) | O | O | O | O | O |
| ¿myocardial infarction? | O | O | O | O | O |
| NO Yes (¿Who?) | | | | | |
| Renal disease?<br>NO Yes (¿Who?) | | | | | |
| ¿Obesity?<br>NO Yes (¿Who?) | | | | | |
| some other disease in its family | | | | | |

What medications do you take in the present time?

| Medicine | 1–4 times per day | 1–4 times per week | 1–4 times per month | 1–6 times per year |
|---|---|---|---|---|
| None | | | | |
| Aspirin | | | | |
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| Please check if you ever had done one of the procedures mentioned below or if any doctor has told that you had or have any of the next diseases and the year that you were told for the first time | | | | | |
|---|---|---|---|---|---|
| just check what you have had | | | | | |
| Diabetes | | | | | |
| Blood pressure | | | | | |
| Cholesterol | | | | | |
| Triglycerides | | | | | |
| Renal disease | | | | | |
| | | | | | |
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