



Association of physical inactivity with blood pressure and cardiovascular risk factors in Amerindian schoolchildren

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Abstract

Objectives: There is a wealth of published data on blood pressure (BP) and physical activity in adults of European descent, but less information is available for Amerindian schoolchildren. The aim of this study was to investigate whether BP and other cardiometabolic risk factors differ by physical activity levels in schoolchildren from different ethnic backgrounds.

Methods: This was a cross-sectional study of 540 schoolchildren (6–13 years) from two ethnic backgrounds ($n = 119$ Mapuche Amerindians and $n = 421$ of European descent). Physical activity was measured using international physical activity recommendations and a standard questionnaire, and ethnicity was classified according to previous methods using surnames. The population was divided into the following four groups: (a) physically active Mapuches ($n = 45$) and of European descent ($n = 101$); and (b) physically inactive Mapuches ($n = 74$) and of European descent ($n = 320$).

Results: In physically inactive schoolchildren, significant differences were found between schoolchildren of Mapuche and European descent in systolic (134 ± 3 mmHg vs 130 ± 5 mmHg, respectively, $P = .034$) and diastolic BP (85 ± 13 mmHg vs 81 ± 3 mmHg, $P = .029$). For other cardiometabolic risk factors, schoolchildren with Mapuche surnames were significantly less likely to be classified with normal weight (5 [6.8%] vs 85 [26.6%]), and more likely to be obese (30 [40.5%] vs 76 [26.6%]) at $P < .0001$ than European peers. Additionally, significant differences were found between Mapuche schoolchildren vs European peers in the outcomes body mass (51.2 ± 18 kg vs 47.0 ± 15), z score of body mass index (2.16 vs 1.70), fat-to-muscle mass ratio (1.68 vs 0.96), resting heart rate (81.7 ± 10 vs 86.8 ± 16), waist circumference (89.3 ± 5 cm vs 76.3 ± 2 cm), muscle mass (18.2 ± 3 kg vs 16.2 ± 2 kg), and handgrip strength normalized by body mass (0.68 vs 0.57 kg).

Conclusion: Under conditions of similar physical inactivity, Mapuche schoolchildren have a more adverse cardiovascular and adiposity profile than their counterparts of European descent.

Abbreviations: BP, blood pressure; BMI, body mass index; DBP, diastolic blood pressure; HGS, handgrip strength; SBP, systolic blood pressure; WC, waist circumference.



1 | INTRODUCTION

Hypertension and high blood pressure (BP) in childhood has increased during the last four decades (Din-Dzietham, Liu, Bielo, & Shamsa, 2007). While genetic and environmental factors are known to play important roles in the etiology of hypertension (Carretero & Oparil, 2000), physical inactivity, sedentary-related behaviors, and poor quality of diet are also main factors contributing to childhood high BP and the development of hypertension (Whincup et al., 2002). Furthermore, there is strong evidence to suggest that the health burden of hypertension differs by ethnicity (Harding et al., 2010).

Chile has a diverse ethnic population including European and Amerindians, with the Mapuche—*people of the land*—comprising the largest Amerindian group. Data from adult populations indicate that the Mapuche have a higher cardiovascular risk than those of European ancestry; however, little is known about cardiovascular risk factors in children and adolescents among this ethnic group. A recent study reported that Mapuche children have a higher prevalence of hypertension than European schoolchildren (28.9% vs 18.6%) (Álvarez et al., 2016). This situation is essentially mirrored in other ethnic groups around the world, such as South Asians. Indeed, children of school age from a South Asian background have a more adverse cardiometabolic profile than white European children, which has been linked to a higher risk of developing cardiovascular diseases during adulthood (Harding et al., 2010).

With the increase in the prevalence of physical inactivity, obesity, and other cardiometabolic risk factors, including high BP, in Chilean children, more information is needed to determinate whether the association between physical activity/inactivity and health outcomes differ by ethnicity. This knowledge could inform relevant stakeholders and increase the motivation for future action to tackle physical inactivity, obesity, and an adverse metabolic profile during early life for preventing hypertension development at adulthood. The aim of this study was, therefore, to investigate whether BP and other cardiometabolic risk factors differ by physical activity levels in schoolchildren of different ethnic backgrounds.

2 | MATERIALS AND METHODS

2.1 | Design

This cross-sectional study included girls and boys from first to seventh grade of a primary public school in the South of Chile. Parents and guardians were informed about the study and provided signed written consent for participation. Additionally, all children gave their written assent on the day of the assessment.

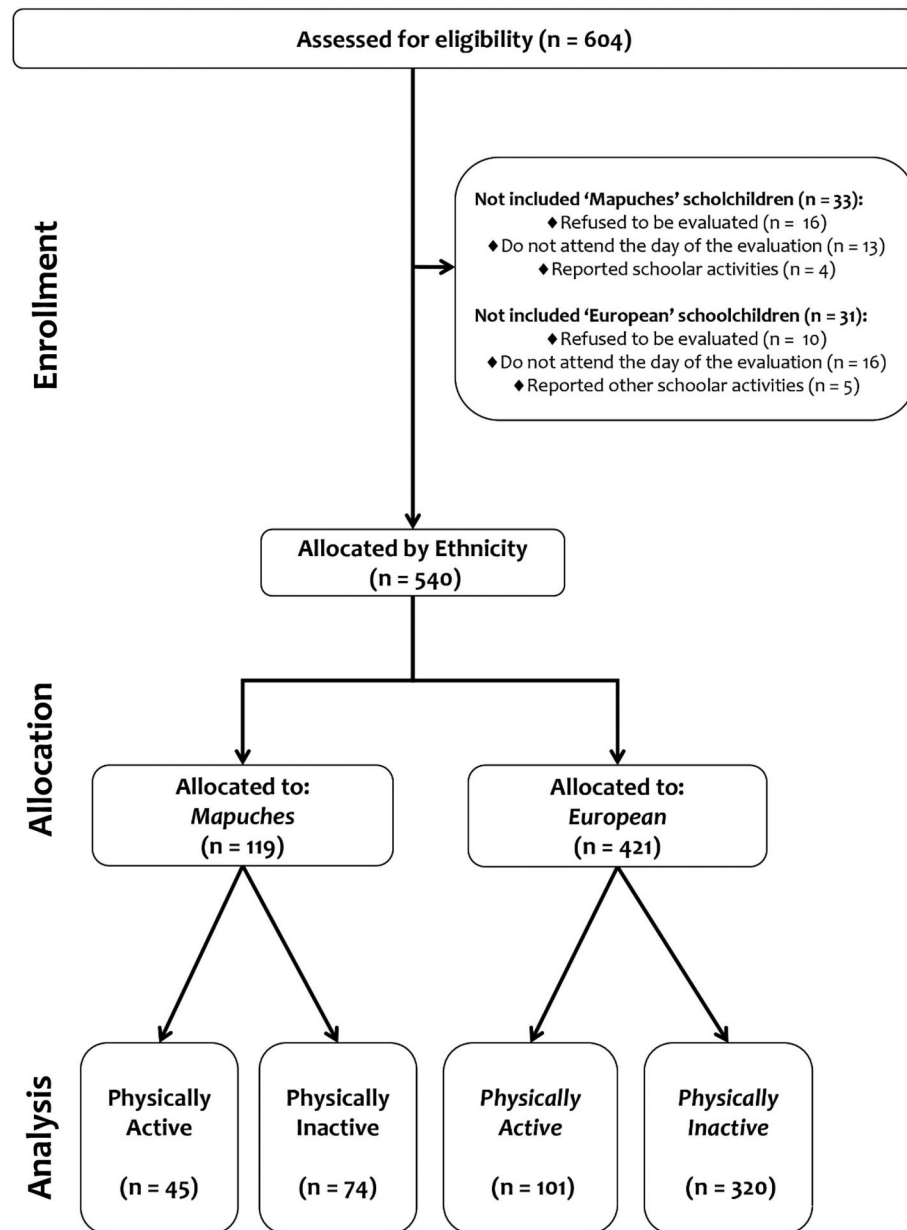
The inclusion criteria were the following: (a) aged 6 to 13 years, (b) student attendance rate at school > 70%, (c) parents or guardians signed consent, and (d) to have both mother and father of either Mapuche or European ancestry. Exclusion criteria were: (a) participants who refused to be measured, (b) who did not attend the day of the evaluation, or (c) who reported schedule conflicts with scholarly activities. Overall, $n = 604$ children took part in the enrollment stage; however, $n = 64$ were excluded because they did not meet inclusion criteria ($n = 33$ Mapuche and $n = 31$ European). Schoolchildren who reported scholarly activities included, among Mapuches, (a) an application test $n = 2$ and extra-scholarly activities $n = 2$, and, among European, (b) an application test $n = 1$, extra-scholarly activities $n = 3$, and left the school before class time was finished officially in the day of the measurement $n = 1$. The final sample included $n = 119$ Mapuches ($n = 55$ boys and $n = 64$ girls), and $n = 421$ European ($n = 199$ boys and $n = 222$ girls). The study was developed according to the Declaration of Helsinki, and was approved by the Ethical Committee of the Family Healthcare Center Tomas Rojas, Los Lagos and by the Physical activity And Sports Sciences Department Committee Review Board. A description of the study enrollment and final sample is shown in Figure 1.

2.2 | Classification of ethnicity

To classify the ancestry of the Mapuche and European schoolchildren, we examined the two family surnames of the participants relating to the first degree of Mapuche or European or a second familial degree of Mapuche or European ancestry, similar to previous Chilean (Álvarez et al., 2016) and international studies (Morgan, Wei, & Virnig, 2004).

2.3 | Classification of physical activity and inactivity

Children who did not adhere to the international physical activity recommendation by the World Health Organization (WHO, 2010) and international expert panels (O'Donovan et al., 2010), accumulating 60 minutes of physical activity per day, were classified as “physically inactive”. Accordingly, all participants reporting more than this amount of physical activity time per day were classified as “physically active”. Physical activity was determined using a questionnaire that counted children's total number of minutes of physical activity per day, as previously applied (Álvarez et al., 2016).

FIGURE 1 Study design

2.4 | Body composition assessment

Trained research staff including nurses and technicians performed all the assessments using a standardized procedures. Height (Ht) was assessed with a standard stadiometer (Health o Meter Professional, Sunbeam Products, Inc., Chicago, Illinois, USA) with an accuracy of 0.1 cm. Waist circumference (WC) was measured with an inextensible tape with an accuracy of 0.1 cm (Hoechstmass, Sulzbach, Germany). Body mass, fat mass (percentage) and muscle mass (kg) were measured by electrical bioimpedance analysis (BIA) using an InBody120 tetrapolar 8-point tactile electrode system (model BPM040S12F07, Biospace, Inc., Seoul, Korea) with 0.1 kg precision, as described (Ramirez-Campillo et al., 2018). The classification of BMI was performed according to the cut-offs points for age and sex proposed by the World Health

Organization (2007) (de Onis et al., 2007) and the International Obesity Task Force (2012) (Cole & Lobstein, 2012). Waist-to-height ratio was calculated as the ratio of WC (in cm) to Ht (in cm). A ratio of fat mass to muscle mass, (both in kilograms) was calculated as described previously (Xu et al., 2018)

2.5 | Blood pressure, oxygen saturation and heart rate

Systolic (SBP) and diastolic (DBP) BP was assessed by a standardized protocol using a digital monitor (Omron HEM 7114TM, Omron Healthcare, Inc., Japan); BP was measured three times with 2 minute intervals, and the average of the three readings was used (Álvarez et al., 2016). The oxygen saturation (SpO₂) was assessed by a pulse saturometer OMRON with

TABLE 1 Characteristics of the sample groups

	Mapuches		European		Physically inactive mean (95%CI)	Physically active Mapuches vs European†&	Physically inactive Mapuches vs European†&
	Physically active mean (95%CI)	Physically inactive mean (95%CI)	Physically active mean (95%CI)	Physically inactive mean (95%CI)			
Age (y)	9.8 (8.6, 11.1)	10.5 (8.5, 12.4)	9.1 (8.4, 9.8)	10.4 (9.5, 11.3)	0.692	0.633	
<i>Body composition</i>							
Body mass (kg)	37.8 (29.1, 46.6)	51.2 (38.2, 64.2)	37.6 (33.2, 43.1)	47.0 (40.8, 53.1)	<i>P</i> = .355	<i>P</i> = .036	
Height (cm)	139.6 (130.8, 148.4)	144.2 (131.0, 157.4)	136.3 (131.7, 140.8)	144.0 (137.7, 150.2)	<i>P</i> = .191	<i>P</i> = .411	
Body fat (kg)	9.5 (6.0, 13.4)	16.8 (4.7, 29.5)	10.2 (8.6, 12.9)	15.6 (11.1, 20.1)	<i>P</i> = .322	<i>P</i> = .277	
Waist-to-height ratio	0.49 (0.44, 0.55)	0.61 (0.51, 0.73)	0.52 (0.49, 0.55)	0.52 (0.50, 0.56)	<i>P</i> = .452	<i>P</i> = .211	
Fat-to-muscle Ratio	0.65 (0.43, 0.91)	1.68 (0.51, 1.38)	0.56 (0.64, 0.88)	0.96 (0.79, 1.15)	<i>P</i> = .089	<i>P</i> < .0001	
Nutritional state by BMI WHO¶							
Very-underweight (<i>n</i> , %)	0 (0.0%)	0 (0.0%)	1 (0.9%)	1 (0.3%)	<i>P</i> = .633	<i>P</i> = .611	
Underweight (<i>n</i> , %)	1 (2.1%)	0 (0.0%)	2 (1.8%)	1 (0.3%)	<i>P</i> = .599	<i>P</i> = 5.892	
Normal (<i>n</i> , %)	46 (95.8%)	5 (6.8%)	104 (94.5%)	85 (26.6%)	<i>P</i> = .461	<i>P</i> < .0001	
Overweight (<i>n</i> , %)	0 (0.0%)	39 (52.7%)	0 (0.0%)	156 (48.8%)	<i>P</i> = .773	<i>P</i> = .163	
Obesity (<i>n</i> , %)	1 (2.1%)	30 (40.5%)	2 (1.8%)	76 (26.6%)	<i>P</i> = .512	<i>P</i> < .0001	
<i>Cardiorespiratory</i>							
HRR (b/min)	95.1 (86.5, 103.6)	81.7 (68.9, 94.5)	89.8 (85.5, 94.3)	86.8 (80.8, 92.9)	<i>P</i> < .0001	<i>P</i> < .0001	
SpO ₂	98.1 (95.8, 99.9)	93.5 (90.0, 96.9)	96.9 (95.7, 98.1)	97.1 (95.4, 98.7)	<i>P</i> = .211	<i>P</i> = .121	
<i>Strength muscle</i>							
HGSd (kg)	22.3 (17.5, 27.1)	17.2 (14.0, 20.4)	17.3 (15.1, 19.6)	16.6 (15.0, 18.3)	<i>P</i> < .0001	<i>P</i> = .244	
HGSnd (kg)	21.2 (16.9, 25.4)	17.0 (14.2, 19.8)	16.4 (14.4, 18.4)	15.3 (13.9, 16.8)	<i>P</i> < .0001	<i>P</i> = .311	
Mean HGS (kg)	21.7 (17.2, 26.2)	17.1 (14.1, 20.1)	16.8 (14.7, 19.0)	15.9 (14.4, 17.5)	<i>P</i> < .022	<i>P</i> = .131	
Normalized HGS (kg/body mass (kg)	0.71 (0.53, 0.90)	0.68 (0.40, 0.96)	0.67 (0.61, 0.73)	0.57 (0.51, 0.62)	<i>P</i> = .351	<i>P</i> = .011	

Data are presented as mean with 95% confidence interval (CI). (BMI) body mass index, (¶) nutritional state classified according with the World Health Organization guide, (HRR) heart rate at rest, (SpO₂) oxygen saturation, (HGSd) handgrip strength of dominant arm, (HGSnd) handgrip strength of nondominant arm. (†) Analyzed by General Linear Model. (&) Analyzed by X² test to categorical variables). Bold values denote significant differences at each respective *P*-value (*P* < .05 or less) between both “physically active” and “physically inactive” groups.

70 to 99% range of measurement, 1% resolution, and $\pm 2\%$ precision. With the same device was assessed the heart rate with a 30 to 240 b/min range, ± 2 b/min of precision.

2.6 | Handgrip strength

Handgrip strength in the dominant (HGSd), and nondominant (HGSnd) arm was assessed as determined elsewhere (Ramírez-

Vélez, Tordecilla-Sanders, Correa-Bautista, Peterson, & Garcia-Hermoso, 2016). HGS was assessed by the handgrip test using a hand dynamometer with an adjustable grip (Baseline, model 12-0286, China). Participants were instructed to gradually and continuously tighten their grip for at least 5 seconds of maximal effort, while receiving standardized verbal motivation, performing the test twice (alternately with both hands). The HGS score (kg) was calculated as the average of the HGSnd and

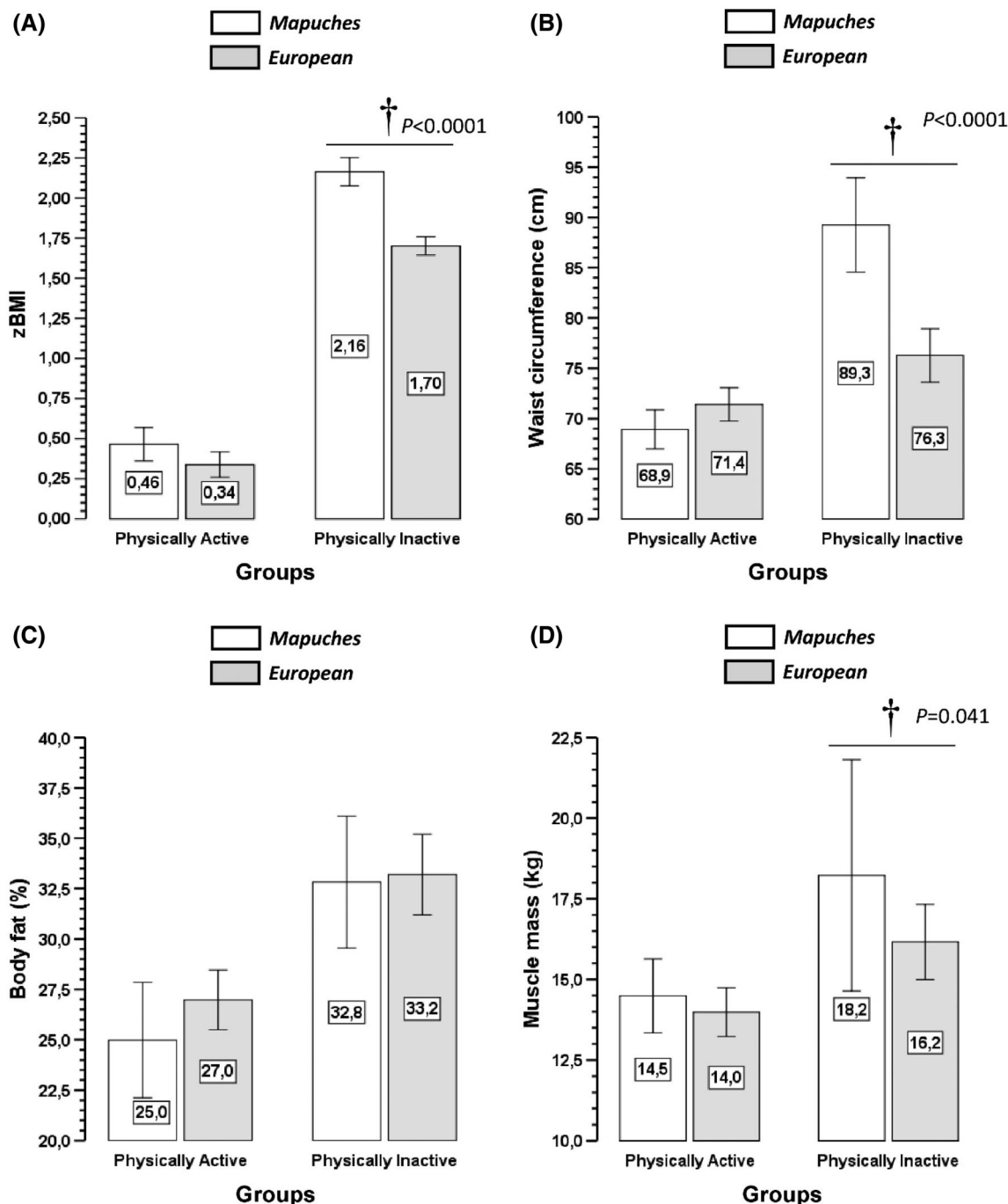


FIGURE 2 Body composition differences between schoolchildren ascendants of Mapuche and European parents. A, z score of body mass index; B, denotes waist circumference; C, body fat; and D, muscle mass. (†) Denotes significant differences between Mapuches vs European at $P < .05$

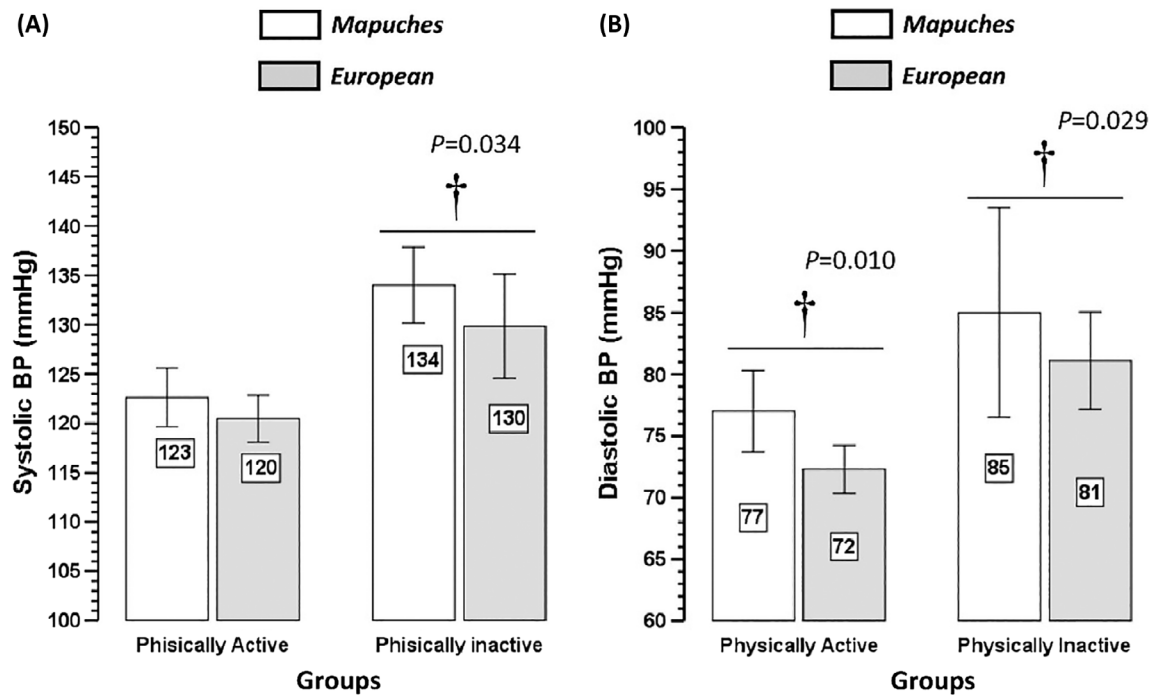


FIGURE 3 Systolic (A) and (B) diastolic blood pressure in schoolchildren ascendants from Mapuches and European. A, Systolic blood pressure; and B, diastolic blood pressure. (†) Denotes significant differences at $P < .05$ between Mapuche vs European schoolchildren

HGSd and then expressed per kilogram. Lastly, HGS was normalized as handgrip strength per body mass, that is, handgrip strength in kg/body mass in kg.

2.7 | Statistical analyses

Data are presented as the mean and 95% confidence interval (95% CI) for continuous variables, and as a percentage for categorical variables in Table 1. In Figures 2 and 3, data are presented as mean and SE. The General Linear Model was used to compare ethnicity for identifying differences in continuous variables between schoolchildren of Mapuche and European ethnicity using the “physically active” and “inactive” groups. We adjusted by age, gender, adiposity, and muscle mass the analyses. For categorical variables, the Chi-square test X^2 was used to identify differences between groups. Data were analyzed using a $P < .05$ alpha level.

3 | RESULTS

Table 1 shows groups compared by physically active and inactive group. In the physically active group, there were significant differences between schoolchildren of Mapuche and European ancestry for fat-to-muscle mass ratio 1.68 (95% CI; $0.51, 1.38$) vs 0.96 (95% CI; $0.79, 1.15$) at $P < .0001$, resting heart rate 95.1 beats/min, (95% CI; $86.5, 103.6$) vs 89.8 beats/min, (95% CI; $85.5, 94.3$) at $P < .0001$, in the handgrip strength of the dominant arm [HGSd] 22.3 kg (95% CI; $17.5,$

27.1) vs 17.3 kg (95% CI; $15.1, 19.6$) at $P < .0001$, handgrip strength of the nondominant arm [HGSnd] 21.2 kg (16.9, 25.4) vs 16.4 kg (14.4, 18.4) at $P < .0001$, and in the mean HGS 21.7 kg (95% CI; $17.2, 26.2$) vs 16.8 kg (95% CI; $14.7, 19.0$). In schoolchildren categorized as physically inactive, there were significant differences between schoolchildren of Mapuche and European ancestry in body mass 51.2 kg (95% CI; $38.2, 64.2$) vs 47.0 kg (95% CI; $40.8, 53.1$) at $P = .036$, in the percentage of “normal weight” 5 (6.8%) vs 85 (26.6%), and “obese” BMI at $P < .0001$, in resting heart rate 81.7 (95% CI; $68.9, 94.5$) vs 86.8 (95% CI; $80.8, 92.9$) at $P < .0001$, and in normalized HGS/body mass 0.68 (0.40, 0.96) vs 0.57 (0.51, 0.62) at $P = .011$ (Table 1).

No differences were found between groups for BMI and body fat percentage, irrespective of whether they were physically active or not (Figure 2, panel A, and Figure 2, panel C). However, in children classified as physically inactive, there were significant differences in WC between Mapuche (89.3 ± 5 cm) vs European (76.3 ± 2 cm) schoolchildren at $P < .0001$ (Figure 2, panel B). Similarly, higher levels of muscle mass were found in physically inactive Mapuche (18.2 ± 3 kg) vs European (16.2 ± 2 kg) schoolchildren at $P = .041$ (Figure 2, panel D).

In physically inactive schoolchildren, SBP was significantly higher in Mapuches (134 ± 3 mmHg) than in European counterparts (130 ± 5 mmHg, $P = .034$) (Figure 3, panel A). A similar finding was found for DBP (85 ± 13 mmHg vs 81 ± 3 mmHg, $P = .029$) (Figure 3, panel B).

By contrast, in physically active schoolchildren, SBP was unchanged between the two groups, whereas DBP was significantly higher in Mapuche (77 ± 3 mmHg) than in European schoolchildren (72 ± 2 mmHg, $P = .010$) (Figure 3, panel B).

4 | DISCUSSION

We sought to compare BP levels and other cardiometabolic risk factors for hypertension development between schoolchildren of different ethnicities—that is Amerindian Mapuche and European. The main findings of the study were: (a) “physically inactive” Mapuche schoolchildren show higher levels of systolic and diastolic BP than their European counterparts (Figure 3), (b) “physically active” European schoolchildren have lower DBP than equivalent Mapuche pupils. These results were reported with, additionally, physically inactive Mapuche schoolchildren having higher muscle mass, and WC than their European counterparts (Figure 2). Other body composition differences included high fat-to-muscle ratio in Mapuches compared to European, as well as a higher mean HGS among Mapuches compared to European in the “physically inactive” group (Table 1).

In children and adolescents who are physically inactive and have high BP, the condition is usually maintained in adulthood if no lifestyle interventions are implemented to decrease BP (Sorof, Lai, Turner, Poffenbarger, & Portman, 2004). Accordingly, BP screening programs should be offered by health care providers, especially when schoolchildren are physically inactive (Álvarez et al., 2016). In a cross-sectional study of BP patterns in West African schoolchildren (8–16 years), rural boys had lower age-adjusted systolic and diastolic BP than equivalent semi-urban boys and lower SBP than urban boys. A similar pattern was seen in girls between rural and semi-urban areas (Agyemang, Redekop, Owusu-Dabo, & Bruijnzeels, 2005). The authors concluded that there is an explicit effect of the urban environment for BP. Forty years of follow-up by the United States National Survey in a population of American African, European, and Mexican American ancestry of (8–17 years of age at baseline) reported that the mean increase in age-adjusted BP between 1988 and 2002 was greater for diastolic (~ 8 mmHg) than for systolic BP (~ 1 mmHg), and that the diastolic BP increase during this period was greater for lean (~ 9 mmHg) than for those at risk of overweight (~ 7 mmHg) and overweight (~ 5 mmHg) schoolchildren, suggesting some familial heritability in these values (Din-Dzietham et al., 2007). Thus, the prevalence of hypertension is increasing in urban areas in younger age groups, implicating environmental factors related to their sedentary lifestyle, altered eating habits, increased fat content in the diet and lack of physical activity (Mohan et al., 2004).

In a large cohort of 5102 schoolchildren with a mean age of 13 years, BP was compared between different ethnic groups, with a distribution of 44% white, 25% Hispanic, 22% African American, and 7% Asian children (Sorof et al., 2004). The authors reported in this study that after three different screenings, the prevalence of high BP decreased progressively from the first to the second and third measurement by 19.4%, 9.5%, and 4.5%, respectively, showing that it might be relevant to include more than one measurement in the BP screening of epidemiological studies as we have applied (Sorof et al., 2004). The major finding was that the frequency of elevated BP was highest among Hispanics (25%) and lowest among Asians (14%). It is also relevant to mention that overweight prevalence was, by ethnicity, 31% for Hispanics, 20% for African Americans, 15% for those with European ancestry, and 11% for Asians, with BMI highly related to high BP. Thus, the prevalence of hypertension increased as the BMI percentile rose from 2% in the ≤ 5 th percentile to 11% in the ≥ 95 th percentile (Sorof et al., 2004). Other studies have highlighted differences in the prevalence of hypertension among schoolchildren of different ethnic origins living in central Europe. For example, Turkish ethnicity was more highly associated with hypertension development than European ethnicity (Martin et al., 2015).

In the present study, we found that WC (+13 cm) and BP levels (SBP +4 mmHg, and DBP +4 mmHg) were higher in physically inactive Mapuche schoolchildren than in their European peers (Figure 3). These data are consistent with international studies in children and adolescents, in which BP was highly associated with several body composition markers including BMI and WC (Ramos-Arellano et al., 2011), as well as with more altered metabolic markers such as fasting insulin (Harding et al., 2010). Exceptionally, a New Zealand study of children and adolescents with obesity, showed that the BP differences between indigenous Māori and nonindigenous peers were minimal (Anderson et al., 2017). Additionally, we found here that resting heart rate was higher in European than in Mapuche schoolchildren, and also that, whereas the height was similar, body mass was different between the groups (Table 1). We speculate that an increase in muscle mass (Figure 2B) might stimulate capillary growth and blood flow (Baron, Brechtel-Hook, Johnson, & Hardin, 1993).

On the other hand, anthropometric indicators typically regard only the effect of fat on metabolism, and discount the effect of skeletal muscle, another pertinent tissue. Indeed, skeletal muscle is regarded as the body's principal nonfat component and can supply up to 40% of the adult human body weight and can also account for 30% of energy expenditure (Rasmussen & Phillips, 2003). In this study, we observed that physically inactive children of both ethnicities had comparable handgrip strength in the HGSd and HGSnd arm

(Table 1), whereas physically active Mapuche schoolchildren performed better than European peers in both arms, and in the mean HGS, which is consistent with the slightly greater muscle mass involved in this response (Figure 2B). Along this line, Kurinami et al. (2016) found that the muscle-to-fat ratio also has clinical utility in assessing the presence of insulin resistance. Furthermore, Park and Kim (2016) reported that the muscle-to-fat ratio indicator was practical in the early prevention and management of the metabolic syndrome among $n = 6256$ Koreans subjects. Nevertheless, differences in ethnicity, age range, and lifestyle of these study populations investigated might mean that the results are not directly comparable.

Contrary to our expectations (ie, we speculated that more muscle mass would be related to lower levels of BP, or could be a protective factor in Mapuche schoolchildren), even though skeletal muscle mass was greater in physically inactive Mapuche schoolchildren than in European peers after statistical analyses (to see Figure 2D), SBP and DBP was also higher in Mapuche schoolchildren. We presume that lifestyle and possibly genetic factors (not included in this study), and other more novel cardiometabolic risk factors nonincluded (ie, artificial light exposure, chronodisruption in tissues, or other from lifestyle) might contribute to a detrimental effect in physically inactive Mapuche schoolchildren. But, rarely, this was in spite the high muscle mass observed in the Mapuches, suggesting that potential genetic factors can be playing a major role. We did not find oxygen saturation differences between groups; however, cardiorespiratory tests were not included in this study. The above-mentioned results support the importance of promoting physical training to prevent fat mass accumulation and maximize muscle mass to provide metabolic health benefits among schoolchildren.

There are some limitations to this study. Although we used standardized, previously used physical activity questionnaires, we did not use accelerometer devices for more precise and objectively physical activity quantification. We adjusted analyses for age and gender of the participants, showed BMI by z score, and have adjusted the body composition analysis by body fat (ie, adiposity) and muscle mass (according to biological maturation). In addition, in intergroup (ie, Mapuche vs European schoolchildren) comparisons, we did not detect significant differences with age or adiposity. Similarly, the HGS was adjusted by body mass for each participant. We recognize some differences in the number of each ethnic group. However, these are common in the design of ethnic studies in populations poorly explored, such as Amerindians. Some strengths of this study are that we examined an underexplored Mapuche Amerindian population ascendants, and we included not only BP analysis but also respiratory and performance data of these schoolchildren participants.

In conclusion, our data suggest that under conditions of similar physical inactivity, Mapuche schoolchildren have higher systolic and diastolic BP, as well as a greater body weight and resting heart rate than their European peers. These results suggest that more robust and complex research is needed to establish more clearly the risk for early hypertension development in schoolchildren of different ethnicity.

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AUTHOR CONTRIBUTIONS

C.A. conceived and designed the project and conducted data extraction. R.R.C., R.S.L., and A.A.M. reviewed the literature. P.D.F., C.M.S., and C.C.M. reviewed the literature and approved the final version. R.R.V. conducted the statistical analyses and reviewed the manuscript. M.I. and all authors approved the final version.

CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

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