Clinical Nutrition 43 (2024) 936–942

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Assessment of muscle endocrine function and inflammatory signalling in male school children following a physical activity programme

Javier Diaz-Castro [a, b, c, 1], Daniela Reyes-Olavarría [a, b, d, e], Juan M. Toledano [a, b, d, **], María Puche-Juarez [a, b, d, ***], Jose Eulogio Garcia-Vega [a, b, d], Julio J. Ochoa [a, b, c, 1], Jorge Moreno-Fernandez [a, b, c, *, 1]

[a] Department of Physiology, Faculty of Pharmacy, Campus Universitario de Cartuja, University of Granada, 18071 Granada, Spain
[b] Institute of Nutrition and Food Technology "José Mataix Verdú", University of Granada, 18071 Granada, Spain
[c] Instituto de Investigación Biosanitaria (IBS), 18016 Granada, Spain
[d] Nutrition and Food Sciences Ph.D. Program, University of Granada, 18071 Granada, Spain
[e] Department of Physical Education, Sport, and Recreation, Universidad de La Frontera, Temuco 4780000, Chile

ARTICLE INFO

Article history:
Received 29 September 2023
Accepted 22 February 2024

Keywords:
Children
Physical activity
Muscle tissue
Myokines
Cytokines

SUMMARY

Background & aims: Regular and planned physical activity can diminish the risk of numerous illnesses. However, school children and teenagers often exercise intermittently and for brief periods, restricting potential benefits. Furthermore, previous studies mainly focused on body composition, without providing molecular mechanisms elucidating the role of physical activity in muscle tissue and inflammatory signalling. The objective of this study was to determine the effect of a vigorous physical activity intervention on endocrine muscle function and cytokine output in children.

Methods: 103 boys were divided into two groups: control (n = 51, did not perform additional physical activity) and exercise (n = 52, performed vigorous physical activity). Body composition measurements, endocrine muscle function and inflammatory signalling biomarkers were assessed at enrolment and after 6 months of intervention.

Results: No statistical significance was found for fractalkine, oncostatin, EGF, TNF-α and eotaxin. However, LIF, FBAP3, IL-6, FGF21 and IL-15 increased in the exercise group at the end of the protocol, though myostatin got decreased. In contrast, IFN-γ was increased in the exercise group at the beginning and end of the exercise protocol, IL-10 was also increased in this group, IL-1α decreased in the exercise group before and after the exercise protocol, and IP-10 and MCP-1 also decreased in the exercise group.

Conclusion: It can be affirmed that a physical activity programme for boys was shown to produce changes in body composition (decreased fat mass, increased lean mass) and in markers of endocrine muscle function and cytokine release. It is possible that these changes, if sustained, could reduce the risk of chronic disease.

1. Introduction

Exercise is possibly the best approach to forestall physiological maladjustments, especially those related to cardiovascular health. Regular and planned physical activity diminishes the risk of numerous illnesses, including hypertension, diabetes, overweight and obesity [1]. According to this, physical inactivity in children and adolescents, together with other factors causes overweight, which leads to a poor level of cardiovascular health and increases the possibility of developing hypertension and raised cholesterol in this group of population. These conditions may additionally prompt the appearance of persistent illnesses such as hypertension and diabetes, during adulthood [2]. Owing to the connection between physical activity and well-being in children and teenagers that also contributes to overall health, physical activity should be regularly performed so as to maintain clinical benefits, vigorous exercise providing greater benefit [3]. In this sense, the World Health Organisation (WHO) [4] suggests moderate-to-vigorous physical

* Corresponding author. Department of Physiology, University of Granada, Campus Universitario de Cartuja, University of Granada, 18071 Granada, Spain.
** Corresponding author. Department of Physiology, University of Granada, Campus Universitario de Cartuja, University of Granada, 18071 Granada, Spain.
*** Corresponding author. Department of Physiology, University of Granada, Campus Universitario de Cartuja, University of Granada, 18071 Granada, Spain.
E-mail addresses: jmtoledano@ugr.es (J.M. Toledano), mpuchej@ugr.es (M. Puche-Juarez), jorgemf@ugr.es (J. Moreno-Fernandez).
[1] These authors have contributed equally to this work and share senior authorship.

https://doi.org/10.1016/j.clnu.2024.02.024

activity for children and teenagers (around 60 min each day). Nevertheless, physical activity levels have begun to diminish along with the appearance of new devices and leisure alternatives, including cell phones, PCs, tablets, computer games and web-based entertainment [1].

Physical inactivity is one of the main elements involved in the appearance and worsening of risk factors associated with obesity [5,6]. Interventions orientated to increased physical activity are effective and low-cost methods that help improve weight control, as well as with other clinical conditions such as bone and muscle reinforcement, rest and sleep, emotional well-being, also leading to a decrease in the risk of cardiovascular diseases [3,7]. In a longitudinal study performed on 6000 seven years old children performing planned physical activity, it was reported that physical activity level was related to muscle/fat ratio measured four years later (when children were 11 years old) [8]. Additionally, studies conducted on youngsters between 4 and 18 years old showed that regular physical activity induces clinical improvements, including the amelioration of bone turnover, blood pressure and cardiorespiratory capacity [3,9]. It is important to remark that, during physical activity performance, skeletal muscle releases several endocrine factors called myokines into the bloodstream [10–12] some of which have beneficial effects on metabolism and body composition by promoting an increase in insulin sensitivity, glucose tolerance and thermogenesis.

Guidelines by sports medicine authorities recommend physical activity protocols in children and adults. However, despite its benefits, school children and teenagers often exercise intermittently and for brief periods, restricting potential benefits and, surprisingly, poorly documented [13]. In addition, results of previous studies were mainly focused on body composition, without providing the molecular mechanisms that help elucidate the role of physical activity in muscle tissue and inflammatory signalling. Taking all the mentioned above into consideration, the objective of this study was to determine the effect of a vigorous physical activity intervention on endocrine muscle function and cytokine output in children.

2. Materials and methods

2.1. Subjects

One hundred and twenty-two boys were invited to take part in the research. Of those, 14 students declined to join, primarily because they were already involved in extracurricular sporting activities several days a week after school hours, and one of them had a chronic illness (diabetes). Furthermore, five adolescents who consented to participate in the study eventually dropped out because their parents failed to sign the informed consent form. All other parents signed it. With regards to Sample Size Calculation, a minimum sample size of 40 children per intervention is required, per prior findings [14], to detect differences in physical activity across groups with a power of 80% and $\alpha = 0.05$. Thus, 80 participants in total are needed, 40 in each group. A total of 103 students were enlisted in order to account for a 25% possibility of follow-up loss. 103 children (boys), were randomly separated into two groups based on their baseline weight. The control group ($n = 51$) did not engage in any additional physical activity, while the exercise group ($n = 52$) engaged in a vigorous physical activity. Boys were enrolled at a Primary and Secondary Education Center (Málaga, Spain) during the second semester of the academic course. The mean age in the control group was 11.21 ± 0.17 years, while in the exercise group, the mean age was 11.16 ± 0.18 years. The study was accepted by the Ethics committee (ref. 29/01/2018/2/2018). After receiving the boys' acceptance to participate in the study, all parents provided signed approval to participate in this study after receiving informed consent. To eliminate a significant confounder, we conducted a three-day diet questionnaire, including one weekend day, to examine the nutritional conditions of the participants. Nutritional software was used to analyse the data collected in this survey. Blood samples were taken from all participants, and they completed a physical activity questionnaire.

2.2. Physical exercise performance protocol

The detailed exercise protocol and sessions, along with specific exercises, can be found elsewhere [14].

Schoolchildren in the exercise group participated in a concurrent-training program during six months consisting of a combination of aerobic-resistance exercises of moderate-to-vigorous intensity. The exercise routine was created in collaboration with an expert group of sports and physical activity scientists. At the start of the study, all of the participants (control and exercise groups) completed three 1-h training sessions per week, each consisting of three sections (A + B + C): A) Light-intensity warm-up (10 min): children start with light-intensity movements (e.g., wrist rotations; leg swings). B) Technique exercises (15 min): passes, catches, feints, dribbles, shots on goal, control exercises, skill circuits. Tactic drills (15 min): rounds, defence drills, attack drills, counterattacks, set plays, superior attack, ball possession drills, pressures, field positions, lines, set pieces. Real game situation “match” (15 min). C) Stretching to cool down (5 min). The intervention period took place during the second semester of the academic course (from January to June). For the six months of the trial, control subjects followed the same routine. However, the exercise group participated in a six-month physical activity program led by a physical education teacher, that included an increase in time and days of activity per week, as well as in activities, in a progressive manner, so during the last two months of the study, the exercise protocol consisted of 100 min a day, 5 days. A complete and more detailed protocol can be found in the Supporting Information.

2.3. Blood sampling

Two blood samples were collected from the subjects in each group: when enrolled in the study (T1, basal value), and after 6 months (T2). An aliquot od the blood was used for the measurement of hematological parameters and the rest of the blood was centrifuged at 1750×*g* for 10 min at 4 °C in a Beckman GS-6R refrigerated centrifuge (Beckman, Fullerton, CA, USA) using heparin as anticoagulant to separate plasma from cells to assess biochemical parameters and the endocrine function of the muscle tissue biomarkers (see Supporting Information for a detailed description).

2.4. Body composition measurements

The assessment of body composition described [14] was carried out by electrical bioimpedance (EIB) and is shown in more detail in Supplementary Digital Content. A multifrequency TANITA MC-980MA equipment (Biológica Tecnología Médica S.L., Barcelona, Spain) with software Suite Biológica 7.1 (Version 368) was used, following the protocol previously described. Age and gender-specific standardised child body mass index (BMI) (kg/m^2) z-score was also recorded (see Table S1 in Supporting Information for seeing the Anthropometric characteristics).

2.5. Physical activity measurement

The IPAQ-C questionnaire [15] was used to calculate the number of metabolic equivalents of task (MET) and the physical activity level during the last 7 days as previously described [14], which is shown in a Table S1 in Supporting Information.

2.6. Nutritional assessment

A three-day diet questionnaire was carried out, which is a retrospective method to calculate the nutrient intake. Data from the food consumption recall survey were processed through the Nutriber computer program (Nutriber, v1.1.1.5.r5, FUNIBER, Barcelona, Spain, 2005) as previously described [14]. For further details of how the questionnaire was carried out, please see Supporting Information. The results of the nutritional intake analyses and diet composition are shown in Table S2 in Supporting Information.

2.7. Endocrine function of the muscle tissue

Fractalkine, cytokine leukaemia inhibitory factor (LIF), Interleukin (IL)-15, Myostatin, Fatty acid binding protein 3 (FABP3), Oncostatin, IL-6 and Fibroblast growth factor 21 (FGF21) were determined using the HMYOMAG-56K MILLIPLEX MAP Human Myokine Magnetic Bead Panel assay (Millipore Corporation, Missouri, USA), based on immunoassays on the surface of fluorescent-coded beads (microspheres), following the specifications of the manufacturer (50 events per bead, 50 μl sample, gate settings: 8000–15000, time out 60 s). Plates were read on LABScan 100 analyzer (Luminex Corporation, Texas, USA) with xPONENT software for data acquisition. Average values for each set of duplicate samples or standards were within 15% of the mean. All the analytes in plasma samples were determined by comparing the mean of duplicate samples with the standard curve for each assay.

2.8. Inflammatory parameters

Epidermal growth factor (EGF), Eotaxin, Interferon gamma (IFN-γ, Interleukin (IL)-10, IL-1α, Interferon-γ-Inducible Protein 10 (IP-10), Monocyte chemoattractant protein-1 (MCP-1), Tumour necrosis factor alpha (TNF-α) were determined using the HCYTOMAG-60K Millipore MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Corporation, Missouri, USA), as previously described.

2.9. Statistical analysis

Results are reported as mean values with their standard errors. All variables were tested for the criteria of normality and homogeneity, making use of the Kolmogorov-Smirnoff's and Levine's tests, respectively. To compare general attributes of the subjects in both groups, unpaired Student's t test was utilised. In order to compare the differences between periods, data were analysed with the t-test for paired samples. Finally, statistical analyses were carried out through the SPSS computer program (version 26.0, 2021, SPSS Inc., Chicago, IL).

3. Results

No statistically significant differences were found for age, height and bone mass between both groups. Weight, BMI and z-score BMI were lower in the exercise group in comparison with the control group ($P < 0.05$) at the end of the intervention. Fat mass was much lower in the exercise group compared to the control group ($P < 0.001$). Accordingly, lean mass and total water was increased in the exercise group in comparison with the control group at the end of the study ($P < 0.01$). Physical activity noticeably increased in the exercise group after the exercise protocol compared to the baseline ($P < 0.001$) (Table S1). Regarding energy intake and macronutrients, no statistically significant differences between both groups were found (see Table S2).

Endocrine function parameters of muscle tissue are shown in Fig. 1. No statistical significance was found for fractalkine and oncostatin. However, LIF, FBAP3 and IL-6 increased in the exercise group at the end of the protocol ($P < 0.01$ for LIF and $P < 0.001$ for FABP3 and IL-6). FGF21 decreased in the control group after the 6 months follow-up ($P < 0.001$), though it increased in the exercise group after the exercise protocol ($P < 0.01$). IL-15 increased in the exercise group compared to the control group after the 6 months follow-up ($P < 0.01$) and in the exercise group after the physical activity protocol ($P < 0.01$). Regarding myostatin, it decreased in the exercise group after the protocol ($P < 0.001$).

Inflammatory signalling is summarised in Fig. 2. No statistical significance was found for EGF, TNF-α and eotaxin. In contrast, IFN-γ increased in the exercise group at the beginning and end of the exercise protocol ($P < 0.001$). IL-10 increased in the exercise group in comparison with the control group after the 6 months follow-up ($P < 0.01$), and after the 6 months protocol ($P < 0.001$). IL-1α decreased in the exercise group before and after the exercise protocol ($P < 0.05$). IP-10 decreased in the exercise group compared with the control group after the 6 months protocol, also decreasing after the protocol in the exercise group compared to the baseline ($P < 0.01$). Finally, MCP-1 decreased in the exercise group when compared with the control group after the 6 months follow-up ($P < 0.01$).

4. Discussion

Although previous interventions have been carried out in order to promote physical activity in children [16–19] no previous study has examined the influence of exercise on the endocrine muscle function and inflammatory signalling as the current study does. As previously reported [14], this protocol intended to boost the advantages of physical activity and limit the possibility of injury, with a minimal expense and a simple implementation, making use of scant equipment and facilities.

In the current study, LIF concentration increased in the exercise group at the end of the physical activity protocol. This is a contraction-induced myokine whose production gets augmented in human skeletal muscle after vigorous physical activity, stimulating satellite cell proliferation for muscle regeneration and hypertrophy, and increasing skeletal muscle strength and mass [20]. This would explain the increased lean mass recorded in the exercise group at the end of the exercise protocol [14]. The cytokine is induced by acute exercise (aerobic and resistance) and improves bone turnover [21], which is noteworthy during the rapid growth in the peri adolescence period. However, no differences were recorded in bone mass between groups [14], probably due to the short period of intervention (6 months follow-up).

FABP3 decreased in the control group and increased in the exercise group after the 6 months tracking. This myokine improves the transport of intracellular fatty acids from the cell membrane to mitochondria for oxidation. Results agree with previous reports, revealing that physical activity increases the expression of this molecule [22]. On the other hand, FABP3 shows a positive correlation with maximal oxygen uptake and skeletal muscle type I fibres ratio, revealing a key role for this myokine in muscle aerobic metabolism [23]. It has also been correlated with body weight and glucose uptake via activation of AMP-activated protein kinase,

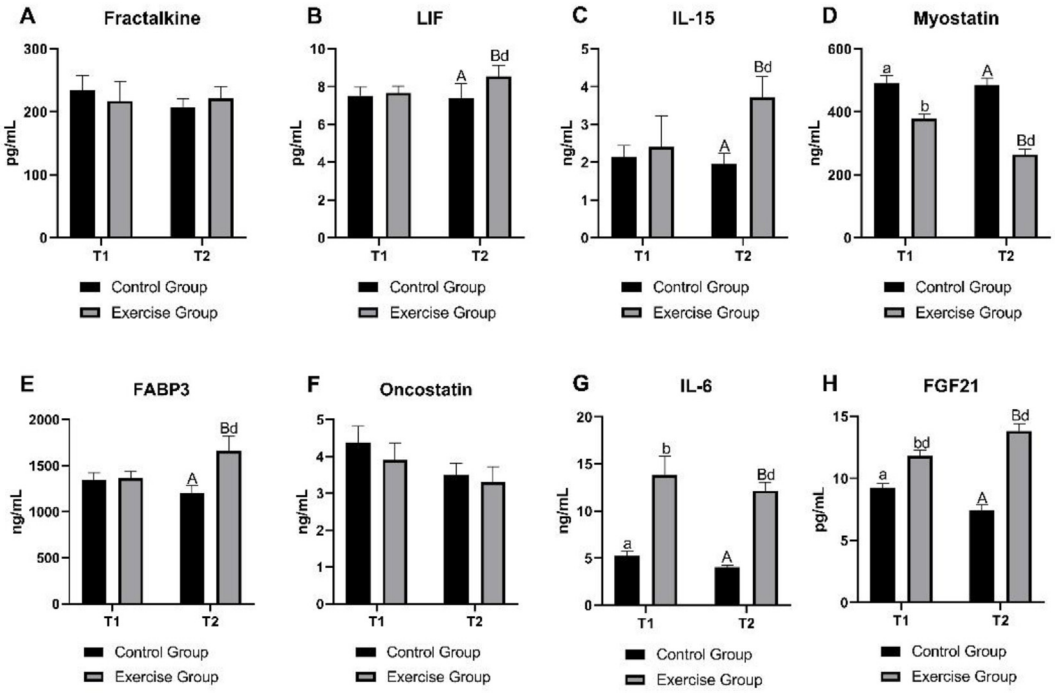

Fig. 1. Endocrine function parameters of the muscle tissue from the control and exercise groups. Mean values among groups with different letters differ ($P < 0.05$) (a,b for T1 and A,B for T2 by Student's t test between different groups; d for T1 vs. T2 in the same group by t-test for paired samples). (A) Fractalkine, (B) LIF, (C) IL-15, (D) Myostatin, (E) FABP3, (F) Oncostatin, (G) IL-6, (H) FGF21.

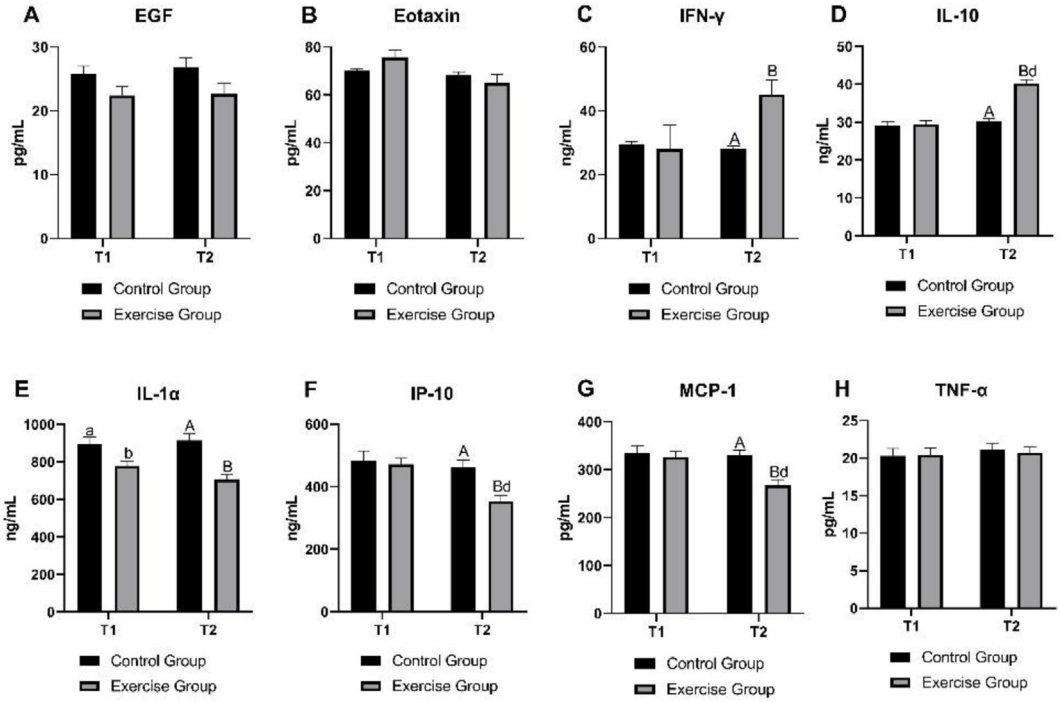

Fig. 2. Cytokines from the control and exercise groups. Mean values among groups with different letters differ ($P < 0.05$) (a,b for T1 and A,B for T2 by Student's t test between different groups; d for T1 vs. T2 in the same group by t-test for paired samples). (A) EGF, (B) Eotaxin, (C) IFN-γ, (D) IL-10, (E) IL-1α, (F) IP-10, (G) MCP-1, (H) TNF-α.

providing a first link between physical activity and reduced insulin resistance [24].

An increase in IL-6 concentration within the exercise group was also recorded. This cytokine has been previously linked to pro-inflammatory signalling and insulin resistance. However, when released after exercise periods, it releases without any sign of muscle damage, exerting a beneficial anti-inflammatory and insulin-sensitising effect, which suggests that this cytokine has a role in metabolism in spite of its role in inflammation [25,26]. In addition, IL-6 has a key role in the loss of visceral adipose tissue

induced by physical activity, increasing glucagon-like peptide 1 [27]. IL-6 also stimulates sympathetic nervous system, increasing white adipose tissue lipolysis and brown adipose tissue thermogenesis [28]. By doing this, it also delivers a metabolic response to exercise in skeletal muscle [29] which improves glucose disposal and muscle utilisation of free fatty acids derived from visceral adipose tissue lipolysis. This would explain the reduction of fat mass previously recorded in children performing physical activity [14]. In addition, an increase of IL-10 in the exercise group compared with the control group after the 6 months follow-up, and in the exercise group after the 6 months protocol has been recorded in the current study. It has been documented that well trained individuals have higher IL-10 production, which is linked to higher circulating T regulatory cell levels, revealing an anti-inflammatory response to challenge when facing training [30]. This can be correlated with the higher concentration of IL-6 recorded in the current study, results that are in agreement with previous studies [31].

FGF21 concentration decreased in the control group after the 6 months follow-up, although they increased in the exercise group after the exercise protocol. It is a myokine which gets increased by exercise in lean subjects [32], being involved in the regulation of energy homeostasis, lipid metabolism and insulin sensitivity, also acting as a stress-responsive endocrine factor which improves the stress adaptation by promoting gluconeogenesis, ketogenesis and adaptive thermogenesis [33,34]. In the adipose tissue, FGF21 promotes insulin-independent glucose uptake, regulates lipolysis and increases mitochondrial biogenesis [33,35].

IL-15 showed to be increased in the exercise group after the protocol. This cytokine stimulates protein accretion and myosin heavy chain accumulation in differentiated myocytes [36] and myotubes [37], while reducing protein degradation [38]. The increase of IL-15 features an anabolic mechanism in muscle growth, linked to decreased proteolysis [39] and apoptosis [40]. In addition, IL-15 plays a key role in muscle-adipose tissue interaction [41], reducing adipose mass [42,43]. These findings reveal that IL-15 functions in a muscle-to-fat endocrine axis that modulates body composition and insulin sensitivity [44], explaining once more, the benefits in BMI recorded in the exercise group [14].

Myostatin decreased in the exercise group after the 6 months exercise intervention, which coincides with previous reports revealing that it is the only myokine downregulated by acute and chronic physical activity [45]. Its expression is elevated in overweight subjects, and it is strongly linked to insulin resistance because it downregulates glucose transporter type 4 (GLUT4) expression and decreases insulin receptor substrate 1 (IRS1) phosphorylation [46]. On the other hand, inhibition of myostatin upregulates oxidative metabolism in skeletal muscle [45]. The decrease of myostatin during exercise results in increased gene expression of enzymes and transcription factors involved in lipolysis, mitochondrial fatty acid oxidation, and white adipose tissue browning, as well as decreased visceral adipose tissue mass [47]. This can be correlated with the improvement of body mass index, increase in lean mass and reduction of fat mass previously recorded in children performing physical activity [14].

IFN-γ was increased in the exercise group at the end of the protocol. It is a cytokine with multiple physiological roles, including antiviral gene stimulation and contribution to barrier against microbial attack [48]. IFN-γ also stimulates macrophages, which play an essential role in muscle regeneration, also having a direct impact on skeletal muscle cells [49]. In addition, IFN-γ increases immune and inflammatory responses, favouring myogenesis [48], which can be correlated with the higher lean mass measured in the exercise group [14].

IL-1α concentration decreased in the exercise group after the protocol. It is a proinflammatory cytokine [50] released after cell damage [51]. This molecule also regulates autocrine and paracrine cytokines within active skeletal muscles, favouring their performance and its glucose homeostasis [52]. In the present study, the

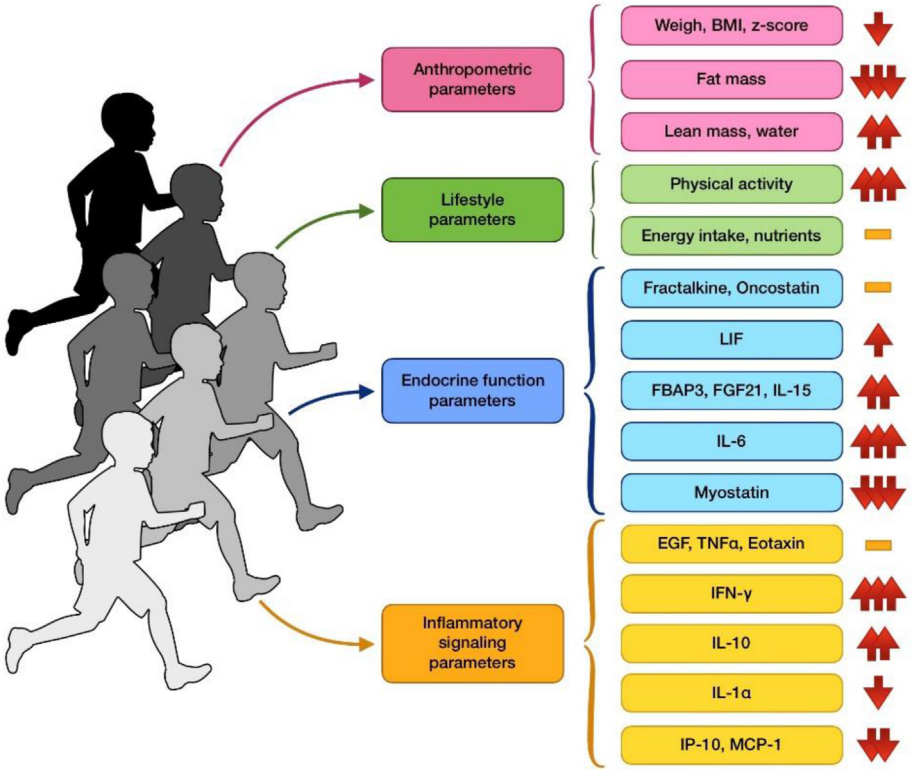

Fig. 3. Main attained results of muscle endocrine function and inflammatory signalling in school children after the implementation of a validated physical activity programme.

reduction of IL-1α concentration indicate that glucose homeostasis is functionally sufficient for glucose disposal and utilisation during physical activity [52], and also that, although the exercise protocol is vigorous, no skeletal muscle cells damage has taken place.

IP-10 decreased in the exercise group before and after the exercise protocol. This molecule induces apoptosis in several cell types through activation of caspase-3 [53], and it is an angiostatic factor which suppresses angiogenesis, as it antagonises the activities of other fibroblast growth factors and vascular endothelial growth factor [54,55]. It has been previously reported that excessive glucose and saturated fatty acids induce oxidative stress and IP-10 expression [56], and as mentioned above, FABP3 increased in the exercise group, fact that favours the transport of intracellular fatty acids from cell membranes to mitochondria for oxidation [22], which explains the reduction of the cytokine observed in the current study.

Finally, MCP-1 remarkably decreased in the exercise group in comparison with the control group after the 6 months follow-up. This cytokine is a monocyte chemoattractant, mainly produced by monocytes, macrophages and dendritic cells, inducing adhesion molecules expression, inflammation and insulin resistance [57]. Within the physiological range, MCP-1 is the only cytokine secreted by the adipose tissue capable of impairing insulin signalling and glucose metabolism in skeletal muscle [58]. In this sense, a decrease of this cytokine during physical activity improves insulin sensitivity [59].

5. Conclusions

Considering the previously mentioned results (Fig. 3) and the fact that the muscular system is found throughout the body, its role as an endocrine organ may be an important contributing factor for children's growth and development during this critical period. The physical programme developed in male schoolchildren modifies the myokine and cytokine profiles studied; in particular, this protocol of physical activity increased LIF, IL-6 and FABP3, which could induce a better development of lean mass and an ergogenic advantage, due to the improvement of the transport of intracellular fatty acids for oxidation and decreased the concentration of IP-10, IL-1α and MCP-1, revealing a better insulin sensitivity and glucose homeostasis and improving angiogenesis compared to control group. Taking all these findings together, a physical activity programme for boys was shown to produce changes in body composition (decreased fat mass, increased lean mass) and in markers of endocrine muscle function and cytokine release. It is possible that these changes, if sustained, could reduce the risk of chronic disease.

Funding

Funding for open access charge: Universidad de Granada/CBUA.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

Daniela Reyes-Olavarría, Juan M. Toledano and María Puche-Juarez are grateful to the Excellence Program “Nutrición y Ciencias de los Alimentos” from the University of Granada. This publication is part of the doctoral thesis of Daniela Reyes-Olavarría. Juan M. Toledano was supported by a FPU contract with grant reference FPU21/04865 funded by Ministry of Education of Spain. Authors would like to thank the students for their participation in the current study. The authors also thank to Susan Stevenson for her efficient support in the revision of the manuscript writing. Fig. 3 has been created with BioRender.com tool.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2024.02.024.

References

[1] Chaddha A, Jackson EA, Richardson CR, Franklin BA. Technology to help promote physical activity. Am J Cardiol 2017;119(1):149–52.
[2] Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA 2006;295(13):1549–55.
[3] Janssen I, Leblanc AG. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. Int J Behav Nutr Phys Activ 2010;7:40.
[4] Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. Br J Sports Med 2020;54:1451–62.
[5] Kosti RI, Tsiampalis T, Kouvari M, Gketsios I, Kanellopoulou A, Notara V, et al. Clustering of activity-related behaviors in relation to self-reported causes of stress among pre-adolescents: results from a national epidemiological study. Life 2023;13(3).
[6] Jongenelis MI, Scully M, Morley B, Pratt IS, Slevin T. Physical activity and screen-based recreation: prevalences and trends over time among adolescents and barriers to recommended engagement. Prev Med 2018;106:66–72.
[7] Sasayama K, Imura T, Adachi M, Aoki T, Li M. Positive relationships of character strengths with fitness and physical activity in primary school children. Heal Psych Behav Med 2023;11(1):2278290.
[8] Griffiths LJ, Sera F, Cortina-Borja M, Law C, Ness A, Dezateux C. Objectively measured physical activity and sedentary time: cross-sectional and prospective associations with adiposity in the Millennium Cohort Study. BMJ Open 2016;6(4):e010366.
[9] Ekelund U, Luan J, Sherar LB, Esliger DW, Griew P, Cooper A. Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. JAMA 2012;307(7):704–12.
[10] Duran AT, Gertz E, Judelson DA, Haqq AM, Clark SJ, Tsang KW, et al. Cytokine responses to acute intermittent aerobic exercise in children with Prader-Willi Syndrome and Nonsyndromic obesity. Pediatr Exerc Sci 2015;27(4):525–34.
[11] Giudice J, Taylor JM. Muscle as a paracrine and endocrine organ. Curr Opin Pharmacol 2017;34:49–55.
[12] Nishii K, Aizu N, Yamada K. Review of the health-promoting effects of exercise and the involvement of myokines. Fujita Med J 2023;9(3):171–8.
[13] Martínez-Vizcaíno V, Sánchez-López M. [Relationship between physical activity and physical fitness in children and adolescents]. Rev Esp Cardiol 2008;61(2):108–11.
[14] Diaz-Castro J, Garcia-Vega JE, Ochoa JJ, Puche-Juarez M, Toledano JM, Moreno-Fernandez J. Implementation of a physical activity program protocol in schoolchildren: effects on the endocrine adipose tissue and cognitive functions. Front Nutr 2021;8:761213.
[15] Crocker PR, Bailey DA, Faulkner RA, Kowalski KC, McGrath R. Measuring general levels of physical activity: preliminary evidence for the Physical Activity Questionnaire for Older Children. Med Sci Sports Exerc 1997;29(10):1344–9.
[16] Parrish AM, Okely AD, Stanley RM, Ridgers ND. The effect of school recess interventions on physical activity : a systematic review. Sports Med 2013;43(4):287–99.
[17] Ducheyne F, De Bourdeaudhuij I, Lenoir M, Cardon G. Effects of a cycle training course on children's cycling skills and levels of cycling to school. Accid Anal Prev 2014;67:49–60.
[18] Russ LB, Webster CA, Beets MW, Phillips DS. Systematic review and meta-analysis of multi-component interventions through schools to increase physical activity. J Phys Activ Health 2015;12(10):1436–46.
[19] Tercedor P, Villa-González E, Ávila-García M, Díaz-Piedra C, Martínez-Baena A, Soriano-Maldonado A, et al. A school-based physical activity promotion intervention in children: rationale and study protocol for the PREVIENE Project. BMC Publ Health 2017;17(1):748.
[20] Ahsan M, Garneau L, Aguer C. The bidirectional relationship between AMPK pathway activation and myokine secretion in skeletal muscle: how it affects energy metabolism. Front Physiol 2022;13:1040809.
[21] Broholm C, Laye MJ, Brandt C, Vadalasetty R, Pilegaard H, Pedersen BK, et al. LIF is a contraction-induced myokine stimulating human myocyte proliferation. 1985 J Appl Physiol 2011;111(1):251–9.
[22] Lammers G, Poelkens F, van Duijnhoven NT, Pardoel EM, Hoenderop JG, Thijssen DH, et al. Expression of genes involved in fatty acid transport and insulin signaling is altered by physical inactivity and exercise training in

human skeletal muscle. Am J Physiol Endocrinol Metab 2012;303(10):E1245–51.
[23] Parikh H, Nilsson E, Ling C, Poulsen P, Almgren P, Nittby H, et al. Molecular correlates for maximal oxygen uptake and type 1 fibers. Am J Physiol Endocrinol Metab 2008;294(6):E1152–9.
[24] Primo D, Izaola O, Gomez JJL, Luis D. Anthropometric, muscle and serum myokine levels effects of physical exercise with an online platform in female patients with obesity. Endocrinologia, diabetes y nutricion 2023;70(7):484–91.
[25] Pedersen BK. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. Eur J Clin Invest 2017;47(8):600–11.
[26] Pal M, Febbraio MA, Whitham M. From cytokine to myokine: the emerging role of interleukin-6 in metabolic regulation. Immunol Cell Biol 2014;92(4):331–9.
[27] Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nat Med 2011;17(11):1481–9.
[28] Larabee CM, Neely OC, Domingos AI. Obesity: a neuro immunometabolic perspective. Nat Rev Endocrinol 2020;16(1):30–43.
[29] van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, et al. Interleukin-6 stimulates lipolysis and fat oxidation in humans. J Clin Endocrinol Metab 2003;88(7):3005–10.
[30] Handzlik MK, Shaw AJ, Dungey M, Bishop NC, Gleeson M. The influence of exercise training status on antigen-stimulated IL-10 production in whole blood culture and numbers of circulating regulatory T cells. Eur J Appl Physiol 2013;113(7):1839–48.
[31] Macpherson RE, Huber JS, Frendo-Cumbo S, Simpson JA, Wright DC. Adipose tissue insulin action and IL-6 signaling after exercise in obese mice. Med Sci Sports Exerc 2015;47(10):2034–42.
[32] Ji M, Cho C, Lee S. Acute effect of exercise intensity on circulating FGF-21, FSTL-1, cathepsin B, and BDNF in young men. J Exerc Sci Fitness 2024;22(1):51–8.
[33] Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, et al. FGF21 regulates PGC-1α and browning of white adipose tissues in adaptive thermogenesis. Genes Dev 2012;26(3):271–81.
[34] Liang Q, Zhong L, Zhang J, Wang Y, Bornstein SR, Triggle CR, et al. FGF21 maintains glucose homeostasis by mediating the cross talk between liver and brain during prolonged fasting. Diabetes 2014;63(12):4064–75.
[35] Park JG, Xu X, Cho S, Hur KY, Lee MS, Kersten S, et al. CREBH-FGF21 axis improves hepatic steatosis by suppressing adipose tissue lipolysis. Sci Rep 2016;6:27938.
[36] Quinn LS, Haugk KL, Grabstein KH. Interleukin-15: a novel anabolic cytokine for skeletal muscle. Endocrinology 1995;136(8):3669–72.
[37] Furmanczyk PS, Quinn LS. Interleukin-15 increases myosin accretion in human skeletal myogenic cultures. Cell Biol Int 2003;27(10):845–51.
[38] Quinn LS, Anderson BG, Drivdahl RH, Alvarez B, Argilés JM. Overexpression of interleukin-15 induces skeletal muscle hypertrophy in vitro: implications for treatment of muscle wasting disorders. Exp Cell Res 2002;280(1):55–63.
[39] Busquets S, Figueras MT, Meijsing S, Carbó N, Quinn LS, Almendro V, et al. Interleukin-15 decreases proteolysis in skeletal muscle: a direct effect. Int J Mol Med 2005;16(3):471–6.
[40] Figueras M, Busquets S, Carbó N, Barreiro E, Almendro V, Argilés JM, et al. Interleukin-15 is able to suppress the increased DNA fragmentation associated with muscle wasting in tumour-bearing rats. FEBS Lett 2004;569(1–3):201–6.
[41] Argilés JM, López-Soriano J, Almendro V, Busquets S, López-Soriano FJ. Cross-talk between skeletal muscle and adipose tissue: a link with obesity? Med Res Rev 2005;25(1):49–65.
[42] Docherty S, Harley R, McAuley JJ, Crowe LAN, Pedret C, Kirwan PD, et al. The effect of exercise on cytokines: implications for musculoskeletal health: a narrative review. BMC Sports Sci Med Rehab 2022;14(1):5.
[43] Alvarez B, Carbó N, López-Soriano J, Drivdahl RH, Busquets S, López-Soriano FJ, et al. Effects of interleukin-15 (IL-15) on adipose tissue mass in rodent obesity models: evidence for direct IL-15 action on adipose tissue. Biochim Biophys Acta 2002;1570(1):33–7.
[44] Pérez-López A, McKendry J, Martin-Rincon M, Morales-Alamo D, Pérez-Köhler B, Valadés D, et al. Skeletal muscle IL-15/IL-15Rα and myofibrillar protein synthesis after resistance exercise. Scand J Med Sci Sports 2018;28(1):116–25.
[45] Baig MH, Ahmad K, Moon JS, Park SY, Ho Lim J, Chun HJ, et al. Myostatin and its regulation: a comprehensive review of myostatin inhibiting strategies. Front Physiol 2022;13:876078.
[46] Das DK, Graham ZA, Cardozo CP. Myokines in skeletal muscle physiology and metabolism: recent advances and future perspectives. Acta Physiol 2020;228(2):e13367.
[47] Zhang C, McFarlane C, Lokireddy S, Masuda S, Ge X, Gluckman PD, et al. Inhibition of myostatin protects against diet-induced obesity by enhancing fatty acid oxidation and promoting a brown adipose phenotype in mice. Diabetologia 2012;55(1):183–93.
[48] Guttridge DC, Mayo MW, Madrid LV, Wang CY, Baldwin Jr AS. NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. Science 2000;289(5488):2363–6.
[49] Panduro M, Benoist C, Mathis D. T(reg) cells limit IFN-γ production to control macrophage accrual and phenotype during skeletal muscle regeneration. Proc Natl Acad Sci U S A 2018;115(11):E2585–93.
[50] Oguri S, Motegi K, Iwakura Y, Endo Y. Primary role of interleukin-1 alpha and interleukin-1 beta in lipopolysaccharide-induced hypoglycemia in mice. Clin Diagn Lab Immunol 2002;9(6):1307–12.
[51] Galozzi P, Bindoli S, Doria A, Sfriso P. The revisited role of interleukin-1 alpha and beta in autoimmune and inflammatory disorders and in comorbidities. Autoimmun Rev 2021;20(4):102785.
[52] Chiba K, Tsuchiya M, Koide M, Hagiwara Y, Sasaki K, Hattori Y, et al. Involvement of IL-1 in the maintenance of masseter muscle activity and glucose homeostasis. PLoS One 2015;10(11):e0143635.
[53] van Marle G, Henry S, Todoruk T, Sullivan A, Silva C, Rourke SB, et al. Human immunodeficiency virus type 1 Nef protein mediates neural cell death: a neurotoxic role for IP-10. Virology 2004;329(2):302–18.
[54] Moore BB, Arenberg DA, Stoy K, Morgan T, Addison CL, Morris SB, et al. Distinct CXC chemokines mediate tumorigenicity of prostate cancer cells. Am J Pathol 1999;154(5):1503–12.
[55] Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, et al. CXC chemokines in angiogenesis. J Leukoc Biol 2000;68(1):1–8.
[56] Ishiuchi-Sato Y, Hiraiwa E, Shinozaki A, Nedachi T. The effects of glucose and fatty acids on CXCL10 expression in skeletal muscle cells. Biosci Biotechnol Biochem 2020;84(12):2448–57.
[57] Panee J. Monocyte chemoattractant protein 1 (MCP-1) in obesity and diabetes. Cytokine 2012;60(1):1–12.
[58] Sell H, Dietze-Schroeder D, Kaiser U, Eckel J. Monocyte chemotactic protein-1 is a potential player in the negative cross-talk between adipose tissue and skeletal muscle. Endocrinology 2006;147(5):2458–67.
[59] Khalafi M, Symonds ME, Sakhaei MH, Ghasemi F. The effects of exercise training on circulating adhesion molecules in adults: a systematic review and meta-analysis. PLoS One 2023;18(10):e0292734.