PPARα and PPARβ/δ Expression is Associated with Proinflammatory Markers in an Obese Pediatric Population

Karina Vargas-Sánchez  
Universidad de los Andes

Laura Vargas  
Universidad Antonio Narino

Yenny Urrutia  
Universidad Antonio Nariño

Iván Beltrán  
Universidad Antonio Nariño

Ana Beatriz Rossi  
Universidad Antonio Narino

Hemán Yupanqui Lozano  
Dexa diab

Jorge Guarín  
Universidad Antonio Nariño

Monica Losada-Barragán  
monica.losada@uan.edu.co  
Universidad Antonio Nariño  
https://orcid.org/0000-0003-1141-1828

Research

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Abstract

Background

Obesity configures a pathophysiological profile that predisposes the development of metabolic and cardiovascular diseases, critically impacting public health. The chronic dysregulation of immunometabolic components triggered by obesity directly affects the metabolism of various tissues. Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptors and transcription factors essential for energy homeostasis of different tissues. Despite its wide-ranging expression and function, the functional mismatch of the PPAR subtypes establish biological mechanisms responsible for metabolic diseases from childhood. In addition, the glucagon-like peptide-1 receptor (GLP-1R) activation can influence insulin secretion, but also the cytokine profile possibly mediated through PPARγ activation. However, to date, the role of PPARs and GLP-1R in leukocytes from obese pediatric patients has been unclear. Therefore, we examined the expression of PPARs isotypes and GLP-1R, and its correlation with metabolic, hormonal, inflammatory, and anthropometric markers in an obese pediatric population.

Results

Obese children and adolescents presented a significant increase in anthropometric and body composition parameters, TG, VLDL, TG/HDL, A/G index, and HOMA score when compared to the control group. Obese participants exhibited a pro-inflammatory profile with an augment of IL-8 (p = 0.0081), IL-6 (p = 0.0005), TNF-α (p = 0.0004), IFN-γ (p = 0.0110), MCP-1 (p = 0.0452), and adipsin (p = 0.0397), whereas displayed a reduction of adiponectin (p = 0.0452). The expression of PPARα and GLP-1R was lower in the leukocytes from obese participants than in lean subjects. Furthermore, PPARα correlates negatively with TNF-α (p = 0.0383), while GLP-1R did not show correlation with any inflammatory variable. However, both receptors correlate negatively with the abdominal skinfold. Although PPARβ/δ expression was similar between groups, it was negatively associated with IL-8 levels (p = 0.0085).

Conclusions

PPARα and PPARβ/δ expression are negatively associated with the proinflammatory markers TNF-α and IL-8, respectively, suggesting participation in the regulation of inflammation observed in pediatric obesity. Furthermore, PPARα and GLP-1R are downregulated in leukocytes from obese children and adolescents, and both receptors are associated with the abdominal skinfold suggesting a role in fat distribution that could indirectly affect the function of different immune cells and the secreted cytokines. Thereby, these findings may impact the understanding and implementation of PPARα or GLP-1R agonists in the clinic.

Background
Obesity is a chronic disease with a high impact on public health due to its increased prevalence, and it constitutes a risk factor for associated comorbidities. Obese subjects have a metabolic phenotype that determines their predisposition to the development of cardiometabolic diseases, metabolic syndrome (MS), and type 2 diabetes (T2D) [1].

Childhood and adolescence are stages of greater vulnerability to adopt unhealthy lifestyles, e.g., low physical activity and increased consumption of foods loaded with sugar or salt. However, those habits can be modified with early interventions [2]. The development of obesity at the early stages has become a topic of great interest in the formulation of preventive and protective public health policies to reach healthy adulthood life [3].

According to global obesity trends, between 1975 and 2016, there has been an increase in the global prevalence of obesity from 0.7–5.6% and 0.9–7.8% in girls and boys, respectively [4]. Likewise, Colombia presented between 2010 and 2015, an augment of excess weight of 5.6% in children and 2.4% in adolescents, without any difference in gender [5]. In 2007, the International Diabetes Federation (IDF) established abdominal obesity as one of the main risk factors for cardiovascular disease (CVD), MS, and insulin resistance (IR), based on expert agreements, which considered only studies of children between 10 and 15 years [6, 7].

The metabolic phenotype in obese subjects is determined by alterations in metabolic regulation of triglycerides and glucose, inflammatory balance, regulation of oxidative stress, among others [8]. Notably, the lipid profile displayed an association with exacerbated alterations in obesity that could lead to the development of a chronic inflammatory status [8, 9].

A chronic inflammatory profile is characterized by increased circulation of proinflammatory cytokines, affecting tissues with a high metabolic rate. Inflammation is a determining factor in the complications of patients with obesity, which highlights the relationship between nutrition, tissues responsible for high metabolic expenditure, and the immune system [10, 11].

Peroxisome proliferator-activated receptors (PPARs) are recognized transcription factors, comprising a group of three isotypes: PPARα, PPARβ/δ, and PPARγ. They play an essential role in regulating the expression of genes responsible for lipid and carbohydrate metabolism, as well as maintenance mitochondrial metabolic rate [12]. Its function aimed at maintaining the proper balance between the formation of adipose tissue, caloric expenditure, and a proven anti-inflammatory role [11, 13].

PPARs participate in the regulation of the expression of the cytokine profile from adipose tissue but also in immune cells [12, 14]. In particular, PPARα plays a vital role in the modulation of target genes that control lipid metabolism, absorption, and transport [15]. Furthermore, PPARα overlaps its expression with PPARβ/δ, and PPARγ in tissues such as the skin, gut, skeletal, heart muscles, adipose tissue, brain, vascular cells, and immune cells, being linked in processes of cell repair, differentiation, and proliferation [11]. Nevertheless, studies of PPARs are scarce, particularly on leukocytes from obese children and adolescents.
An additional factor that can contribute to modulate the inflammation in obesity is the activation of the glucagon-like peptide-1 (GLP-1)/GLP-1R axis. GLP-1 is a hormonal factor that increases the pancreatic release of insulin [16, 17]. Its cytoprotective role has been reported mainly in pancreatic β-cells, delaying the establishment of obese T2D [16, 18]. Indeed, the activation of GLP-1R affects the profile of cytokines, since GLP-1R signaling on iNKT cells can enhance the expression of anti-inflammatory cytokines such as IL-10 [16]. Besides, GLP-1R also downregulates proinflammatory cytokines expression mediated through PPARγ activation, followed by NF-kB inhibition [19].

Therefore, this study aimed to evaluate the expression of key metabolic genes, such as PPARs, and GLP1R in leukocytes and its association with metabolic, hormonal, and inflammatory markers, body composition, and anthropometric factors in a Colombian's obese pediatric population.

**Results**

Obese children and adolescents have an impaired metabolic and anthropometric profile

The anthropometric, body composition, and biochemical variables of the studied individuals stratified by BMI percentile status are shown in Table 1. Age and gender of the participants were not significantly different among the control and obese group, the median age was 11 years old, and 48% were girls. All mean values of skinfold thickness, waist circumference (WC), arm circumference (AC), waist-to-height ratio (WHR), arm muscle circumference, and neck circumference were significantly increased in participants with obesity in comparison with healthy weight children and adolescents (Table 1). Regarding body composition, the percentage of arm, leg, trunk, android, gynoid, and body fat were significantly elevated in obese patients compared to control subjects (Table 1). In obese children, fat distribution was similar between different body areas, and there were no significant differences in mean values of lean mass between groups.
Table 1
Anthropometric, body composition and clinical cardiometabolic risk factors in children and adolescents†

|                           | Control     | Obesity     | p value    |
|---------------------------|-------------|-------------|------------|
|                           | n = 19      | n = 18      |            |
| Gender                    |             |             |            |
| Female                    | 8           | 10          |            |
| Male                      | 11          | 8           |            |
| Age (years)               | 10,47 ± 2,87| 11,33 ± 2,93| 0,3515     |
| Tanner                    | 2,88 ± 1,72 | 2,72 ± 1,32 | 0,8826     |
| BMI                       | 49,92 ± 28,03| 96,37 ± 8,50| <0,0001****|
| Arm circumference (cm)    | 20,41 ± 3,23| 27,45 ± 3,50| <0,0001****|
| Arm muscle circumference (cm)| 16,77 ± 2,83| 20,64 ± 3,19| 0,0008***  |
| Neck circumference (cm)   | 28,29 ± 3,03| 32,47 ± 2,82| 0,0003***  |
| Triceps skinfold (cm)     | 11,68 ± 4,61| 21,64 ± 4,94| <0,0001****|
| Subscapular skinfold (cm) | 6,85 ± 2,27 | 21,17 ± 5,40| <0,0001****|
| Suprailiac skinfold (cm)  | 10,91 ± 4,78| 26,61 ± 5,79| <0,0001****|
| Abdominal skinfold (cm)   | 10,53 ± 5,01| 27,64 ± 6,17| <0,0001****|
| Waist circumference (WC) (cm)| 63,75 ± 7,53| 83,75 ± 6,94| <0,0001****|
| Waist/Height ratio (WHR)  | 0,45 ± 0,03 | 0,58 ± 0,04 | <0,0001****|
| Arm fat (%)               | 33,07 ± 9,44| 45,91 ± 7,73| <0,0001****|
| Leg fat (%)               | 32,82 ± 7,74| 44,41 ± 5,82| <0,0001****|
| Trunk fat (%)             | 21,06 ± 8,95| 42,9 ± 6,79 | <0,0001****|
| Android fat (%)           | 18,58 ± 9,93| 44,33 ± 7,53| <0,0001****|
| Gynoid fat (%)            | 28,88 ± 8,21| 44,68 ± 5,96| <0,0001****|
| Body fat (%)              | 26,28 ± 7,35| 39,9 ± 5,89 | <0,0001****|
| Lean mass (kg)            | 32,83 ± 10,31| 22,84 ± 11,25| 0,0073**  |

†BMI: body mass index. HDL: high-density lipoprotein. VLDL: very low-density lipoprotein. LDL: low-density lipoprotein. HOMA-IR: homeostatic model of assessment for insulin resistance. GLP-1: glucagon-like peptide 1. GIP: gastric inhibitory polypeptide. Values are presented as percentage or mean ± standard deviation (SD). Differences between groups were evaluated using Wilcoxon signed-rank tests. * p < 0.05, ** p < 0.005, *** p < 0.0001.
Control | Obesity | p value
--- | --- | ---
Fasting blood glucose (mg/dL) | 87.61 ± 5.55 | 87.59 ± 6.25 | 0.936
2 hours glucose (mg/dL) | 91.74 ± 13 | 94.23 ± 13.58 | 0.7617
Insulin (mcU/mL) | 7.77 ± 4.78 | 19.77 ± 8.02 | <0.0001****
Total cholesterol (mg/dL) | 150.15 ± 21.83 | 158.29 ± 24.91 | 0.3758
Triglycerides (mg/dL) | 76.48 ± 18.47 | 117.27 ± 31.64 | 0.0001***
HDL (mg/dL) | 50.34 ± 8.22 | 41.39 ± 9.51 | 0.0077**
LDL (mg/dL) | 84.55 ± 18.04 | 94.70 ± 20.67 | 0.2547
VLDL (mg/dL) | 15.29 ± 3.69 | 23.45 ± 6.33 | 0.0001***
HOMA-IR (score) | 1.50 ± 1.19 | 4.28 ± 1.72 | <0.0001****
TG/HDL ratio | 1.60 ± 0.61 | 3.02 ± 1.24 | <0.0001****
Android-gynoid % fat ratio | 0.61 ± 0.17 | 0.99 ± 0.08 | <0.0001****
GLP-1 (ng/mL) | 15.98 ± 0.69 | 17.70 ± 0.66 | 0.0725
GIP (pg/mL) | 2637 ± 486.89 | 3137 ± 512.44 | 0.6299

†BMI: body mass index. HDL: high-density lipoprotein. VLDL: very low-density lipoprotein. LDL: low-density lipoprotein. HOMA-IR: homeostatic model of assessment for insulin resistance. GLP-1: glucagon-like peptide 1. GIP: gastric inhibitory polypeptide. Values are presented as percentage or mean ± standard deviation (SD). Differences between groups were evaluated using Wilcoxon signed-rank tests. * p < 0.05, ** p < 0.005, *** p < 0.0001.

The mean of fasting glucose and 2-hours glucose fell within the normal ranges, as recommended by the American Academy of Pediatrics (Table 1) [20]. However, insulin was significantly higher in children with obesity than in the standard weight group. Accordingly, the HOMA score was increased in obese children; this score overcomes the 3.4 cut-off indicating IR (Table 1) [21]. TG and VLDL levels were higher in obese children than those of lean subjects, whereas HDL-c levels were significantly reduced in obese subjects in contrast with the control group (Table 1). The levels of total cholesterol were similar among groups (Table 1).

TG/HDL and A/G ratios are frequently used as indicators of cardiometabolic risk, and here, those ratios were significantly elevated in the obese group in comparison with the lean patients. Based on this data, obese children and adolescents have an increased risk of developing cardiovascular disease and T2D. Incretins gastric inhibitory polypeptide (GIP) and GLP-1 did not show significant differences between the study groups (Table 1).

Triceps skinfold displayed a positive correlation with body composition parameters
Relationships between fat body distribution and anthropometric data are shown in Table 2. *Triceps skinfold* displayed a positive correlation with all body composition parameters, the stronger correlation was observed with *total body fat percentage* \((r = 0.6554, p = 0.0071)\), and a weaker association with *Android fat percentage* \((r = 0.4974, p = 0.0516)\) (Table 2). Furthermore, *WHT ratio* showed a positive association with *arm* \((r = 0.691, p = 0.0038)\), *leg* \((r = 0.5245, p = 0.0388)\), *android* \((r = 0.5528, p = 0.0282)\), and *total body fat percentages* \((r = 0.5379, p = 0.0334)\) (Table 2).

|                      | Arm fat (%) | Leg fat (%) | Trunk fat (%) | Android fat (%) | Gynoid fat (%) | Body fat (%) |
|----------------------|-------------|-------------|---------------|-----------------|---------------|-------------|
| BMI                  | 0.060       | 0.131       | -0.013        | -0.063          | -0.028        | 0.081       |
| Arm circumference    | -0.118      | 0.014       | -0.167        | -0.217          | -0.179        | -0.046      |
| Arm muscle circumference | -0.340     | -0.202      | -0.437        | -0.419          | -0.425        | -0.299      |
| Neck circumference   | -0.449      | -0.278      | -0.411        | -0.496          | -0.391        | -0.355      |
| Triceps skinfold     | 0.590\(a\) | 0.627\(a\) | 0.604\(a\)   | 0.497\(a\)      | 0.579\(a\)    | 0.655\(a\)  |
| Subscapular skinfold | 0.148       | 0.022       | 0.294         | 0.186           | 0.179         | 0.171       |
| Suprailiac skinfold  | -0.041      | -0.186      | 0.099         | 0.140           | 0.031         | -0.027      |
| Abdominal skinfold   | 0.189       | -0.029      | 0.117         | 0.289           | -0.004        | 0.119       |
| Waist circumference (WC) | -0.105    | -0.110      | -0.255        | -0.217          | -0.234        | -0.149      |
| Waist/Height ratio (WHR) | 0.691\(b\) | 0.525\(a\) | 0.442         | 0.553\(a\)      | 0.482         | 0.538\(a\)  |

† BMI: body mass index. Spearman’s rank correlation test, \(a p < 0.05\) and \(b p < 0.005\).

*Trunk fat percentage* presented a significant association with *A/G ratio* \((r = 0.558, p = 0.027)\). Likewise, *Android fat percentage* also displayed a positive correlation with *A/G ratio* \((r = 0.558, p = 0.006)\). *Arm circumference* parameter correlated negatively with LDL \((r=-0.572, p = 0.013)\) and no-HDL \((r=-0.502, p = 0.034)\) whereas *arm muscle circumference* exhibited a weak negative correlation with LDL \((r=-0.486, p = 0.041)\) ([Supplementary table 2](#)).

The correlation of different variables with the *suprailiac skinfold* indicates a positive association with *Total Cholesterol* \((r = 0.658, p = 0.003)\), and *no-HDL* \((r = 0.724, p = 0.001)\) while showed a weaker association with *LDL* \((r = 0.550, p = 0.018)\) ([Supplementary Table 2](#)). There was a positive correlation between *Abdominal skinfold* with *HOMA-IR* \((r = 0.459, p = 0.055)\).

*PPARα* and *GLP-1R* gene expression is reduced in leukocytes from obese subjects.
To determine the expression of PPAR isotypes in the leukocytes, the expression of each isotype was analyzed by qPCR. **PPAR-α** expression showed a significant reduction in the leukocytes from obese patients in comparison with the control group, with a 50% reduction in the expression of this gene ($p = 0.0484$) (Fig. 1a). **PPAR-β** did not show differential expression between the study groups (Fig. 1b). In contrast, **PPAR-γ** expression was undetectable in leukocytes from all the samples. To corroborate this finding, we used adipose tissue cDNA as a positive control, since this isotype is mainly present in adipose cells. This sample presented a positive amplification and a single peak in the dissociation curve, indicating that the reaction was specific (data not shown). Likely, **PPAR-γ** transcripts were not expressed on leukocytes or are expressed at low levels but just in some cell lineages of these samples.

Besides, analysis from mRNA expression levels of incretin receptors was also evaluated in the studied individuals. **GLP-1R** expression was significantly reduced in the obese group as compared to the healthy weight participants ($p = 0.1358$) (Fig. 2a), whereas no difference was observed in **GIPR** expression between the two groups (Fig. 2b).

Obese children and adolescents showed a proinflammatory profile of adipokines and cytokines

To identify the inflammatory serum profile in obese children and adolescents, the levels of several adipokines and cytokines were measured by flow cytometry using a multiplex assay. Obese subjects showed a significant increase in the levels of IL-8 ($p = 0.0081$), IL-6 ($p = 0.0005$), TNF-α ($p = 0.0004$) IFN-γ ($p = 0.0110$), and MCP-1 levels ($p = 0.0452$) in comparison with the normal weight group (Fig. 3a and b). Serum levels of IL-10 and IP-10 were detected but did not differ significantly between the groups.

The serum adiponectin concentration presented a significant reduction in the obese group ($p = 0.0452$) compared to the control group (Fig. 4). Conversely, adipisin displayed a concentration significantly higher ($p = 0.0397$) in the obese group in comparison with the control group, which had a concentration of 7,688 ± 6,116 pg/mL. (Fig. 4). Resistin showed similar levels between the study groups, with concentrations of 13,102 ± 8,342 pg/mL and 16,792 ± 8,302 pg/mL for the obese and control participants, respectively (Fig. 4). Leptin was also analyzed; however, it presented considerably high levels in the group with obesity, which exceeded the concentrations determined in the calibration curve, which did not allow to quantify it using the test used. Finally, RBP4 did not present a representative concentration in any of the study groups, indicating that it is not secreted in serum at detectable levels under the evaluated conditions.

**PPARα and PPARβ/δ correlate negatively with proinflammatory markers**

Analysis of the relationships between **PPAR-α**, **PPAR-β**, and **GLP-1R** expression and the levels of cytokines, chemokines, adipokines and anthropometric parameters of the obese subjects showed that **PPAR-α** transcript levels had a significant negative correlation with TNF-α levels ($r = -0.583$, $p = 0.03883$) (Table 3), as well as with abdominal skinfold ($r = -0.712$, $p = 0.0016$). Besides **PPAR-β** showed a significant negative correlation with IL-8 levels ($r = -0.667$, $p = 0.0085$) and arm fat percentage ($r = -0.651$, $p = 0.0132$) (Tables 3 and 4).
Table 3
Correlation between hormones and cytokines with PPAR-α, PPAR-β and GLP-1R expression in children and adolescents†

|                | PPAR-α | PPAR-β | GLP-1R |
|----------------|--------|--------|--------|
| Adiponectin    | 0.496  | -0.330 | -0.084 |
| Adipsin        | 0.067  | -0.667 | 0.357  |
| MCP-1          | 0.324  | 0.011  | 0.235  |
| IP-10          | 0.370  | -0.300 | 0.033  |
| IL-10          | -0.125 | 0.000  | -0.136 |
| IL-8           | 0.233  | -0.664\(^b\) | -0.007 |
| IL-6           | -0.343 | -0.200 | 0.317  |
| IFN-γ          | 0.130  | -0.411 | -0.130 |
| Resistin       | 0.172  | -0.311 | 0.323  |
| TNF-α          | -0.583\(^a\) | -0.059 | 0.304  |

† Spearman's rank correlation test. \(^a\) \(p = 0.0383\), \(^b\) \(p = 0.0085\)
Table 4
Correlation between anthropometric and biochemical parameters with gene expression of metabolic markers in children and adolescents†

|                        | PPAR-α | p value | PPAR-β | p value | GLP-1R | p value |
|------------------------|--------|---------|--------|---------|--------|---------|
| BMI                    | 0,049  | 0,854   | -0,257 | 0,354   | -0,086 | 0,773   |
| Arm fat (%)            | 0,052  | 0,850   | -0,651 | 0,013   | -0,364 | 0,246   |
| Leg fat (%)            | 0,208  | 0,438   | -0,515 | 0,061   | -0,266 | 0,404   |
| Trunk fat (%)          | 0,096  | 0,723   | -0,436 | 0,118   | -0,322 | 0,309   |
| Android fat (%)        | -0,028 | 0,915   | -0,339 | 0,231   | -0,420 | 0,177   |
| Gynoid fat (%)         | 0,108  | 0,690   | -0,513 | 0,062   | -0,189 | 0,546   |
| Body fat (%)           | 0,096  | 0,723   | -0,511 | 0,063   | -0,343 | 0,276   |
| Arm circumference      | -0,094 | 0,713   | 0,306  | 0,266   | -0,147 | 0,608   |
| Arm muscle circumference| -0,173 | 0,501   | 0,411  | 0,128   | 0,042  | 0,892   |
| Waist circumference (WC)| -0,308 | 0,226   | 0,050  | 0,860   | 0,059  | 0,844   |
| Neck circumference      | -0,048 | 0,852   | 0,190  | 0,496   | 0,090  | 0,758   |
| Waist/Height ratio (WHR)| -0,262 | 0,291   | -0,448 | 0,090   | -0,326 | 0,241   |
| Triceps skinfold       | 0,210  | 0,415   | -0,390 | 0,146   | -0,277 | 0,330   |
| Subscapular skinfold   | 0,142  | 0,586   | -0,225 | 0,413   | 0,183  | 0,527   |
| Suprailliac skinfold   | -0,429 | 0,084   | 0,285  | 0,301   | -0,236 | 0,405   |
| Abdominal skinfold     | -0,712 | 0,002   | 0,379  | 0,163   | -0,678 | 0,009   |
| Fasting blood glucose  | -0,364 | 0,149   | 0,340  | 0,214   | -0,279 | 0,333   |
| 2 hours glucose        | 0,042  | 0,876   | -0,412 | 0,125   | -0,051 | 0,868   |
| Insulin                | -0,185 | 0,471   | 0,293  | 0,287   | -0,288 | 0,318   |
| Total cholesterol      | -0,021 | 0,934   | 0,075  | 0,790   | 0,209  | 0,473   |
| Triglycerides          | -0,247 | 0,335   | -0,064 | 0,815   | -0,125 | 0,671   |
| HDL                    | 0,416  | 0,098   | -0,054 | 0,845   | 0,354  | 0,215   |
| VLDL                   | -0,248 | 0,333   | -0,064 | 0,815   | -0,117 | 0,685   |
| LDL                    | 0,033  | 0,900   | -0,086 | 0,756   | 0,077  | 0,797   |

† BMI: body mass index. HDL: high-density lipoprotein. VLDL: very low-density lipoprotein. LDL: low-density lipoprotein. HOMA-IR: homeostatic model of assessment for insulin resistance. Spearman’s rank correlation test.
In contrast, GLP-1R expression did not correlate with any inflammatory parameters (Table 3). However, GLP-1R showed a negative correlation with Abdominal skinfold (r = -0.678, p = 0.009) (Table 4).

**Discussion**

Childhood and adolescence are critical periods where fundamental metabolic and hormonal changes start and have an impact on adulthood health. Excess weight at these life stages is a critical factor for the onset of cardiometabolic disease and T2D. Thus, those stages are vital periods for timely intervention. We evaluated the expression of essential metabolic genes, such as PPARs and GLP-1R, in leukocytes and its correlation with metabolic, immune, and anthropometric factors in a Colombian's obese pediatric population.

PPARs participate in the control of the inflammatory process produced by obesity, modulating the expression of proinflammatory cytokines in adipose cells [12]. However, other tissues can contribute to the inflammatory process. Studies of PPARs, particularly of PPARβ/δ and PPARα, on leukocytes from obese children and adolescents, are scarce. However, the assessment of its expression and relation with variables associated with obesity represents an accessible tool for developing therapeutic strategies and understanding the pathophysiology of obesity [22].

In this work, we identify the expression of PPARα and PPARβ/δ, but not PPARγ in leukocytes from obese children and adolescents. The activation of PPARβ/δ has been mainly studied in "endurance-exercise mimetics" particularly focused on the liver, skeletal muscle, and adipose tissue, to reduce inflammation as an obesity treatment [23, 24]. Moreover, PPARβ/δ is involved in lipid metabolism, regulation of fatty acid oxidation (FAO), inflammation, and modulation of glucose and cholesterol levels [25]. Direct activation of this isotype improves insulin sensitivity and the disorders of metabolic syndrome in humans [26]. The use of the agonist GW0742 for PPARβ/δ in obese mice influences the improvement of metabolic parameters positively, increases Foxp3+CD4+ T cells, induced FAO gene expression in lymph node and increases FAO in CD4+ T cells [27, 28]. FAO can also influence not exclusively the metabolism, but also the T cells lineages and functions [29]. Those effects are not observed only with exercise and suggest that this transcription factor can present additional effects that need to be explored.

In this study, PPARβ/δ did not show significant differences between the standard weight and obese groups. However, this gene displayed a significant negative correlation with IL-8 levels and arm fat.
IL-8 (CXCL8) is a proinflammatory chemokine that mediates the crosstalk between obesity and cardiovascular disease [30, 31]. IL-8 is mainly secreted by adipose tissue, fibroblasts, endothelial cells, monocytes, and macrophages in conditions such as exposure to IL-1β, TNF-α, or LPS [32]. Indeed, we detected a significant increase in TNF-α levels from the serum of obese subjects, which could contribute to the IL-8 secretion in those individuals.

Although the direct link of PPARβ/δ to IL-8 is suggestive, some studies have already related the activation of PPARβ/δ with the modulation of expression of IL-8 in endothelial cells and endometrium tissue participating in inflammation [33–35]. In line with this evidence, our results suggest that a decrease in the PPARβ/δ expression in leukocytes might consequently augment the IL-8 levels and, thereby, the inflammatory response during obesity. Indeed, experiments in mice have associated the deletion in the expression of PPARβ/δ, with the reduction in the number of hepatic macrophages of the M2 type, which results in alterations in lipid metabolism, IR, and decreased expression of anti-inflammatory cytokines [36]. PPARβ/δ senses the effects that chylomicron-derived FAs have on macrophages inducing overexpression of angiopoietin-related protein 4 (ANGTPL4), inhibiting postprandial uptake of saturated FAs into mesenteric lymph nodes macrophages and preventing macrophage activation in an inflammatory response derived from fat accumulation [37]. Those findings show the protective role of PPARβ/δ in the control of lipid metabolism and the development of inflammatory processes.

On the other hand, PPARα acts as a lipid sensor actively expressed in tissues with high metabolic rates, such as the liver, heart, muscle, kidney, intestinal mucosa, and brown adipose tissues [38, 39]. The use of natural ligands or agonists for this isotype has revealed its participation in glucose homeostasis, IR development, inflammation, atherogenesis, and in the expression of genes related to lipid metabolism [40, 41]. In leukocytes, PPARα expression has barely been studied. However, the expression of PPARα has been reported in immune cells such as monocytes/macrophages, peripheral blood mononuclear cells (PBMCs), and CD4+ T cells [12, 42, 43]. Also, subjects with MS significantly decreased PPARα expression in eosinophils by approximately 21% [44].

Our pediatric obese population showed reduced expression of this isotype in leukocytes when compared to the lean group. Likewise, studies on mice with induced obesity exhibited reduced expression of PPARα and carnitine palmitoyltransferase I (CPT-1) in the liver [45]. PPARγ expression is also reduced in PBMC from obese children and adolescents [46]. Indeed, obese mice treated with Bezafibrate (BZ), an agonist that preferentially activates PPARα and PPARγ, improves biochemical parameters and reduce the WAT proinflammatory state in obese mice [45]. These findings suggest that obesity in the early stages of life influences the expression of PPARα in leukocytes, and consequently, its reduction can be involved in the observed altered lipid profile and reduced insulin sensitivity.

We observed a significant increase in TG and a reduction in HDL levels in obese children and adolescents in comparison with the control group. Both parameters overcome the reference values for pediatric individuals. Insulin sensitivity was also affected in this group. We used the HOMA index to evaluate the IR status, which is the most used method for its diagnosis in the pediatric population. We observed that
obese subjects displayed a mean value of 4.28, significantly higher than lean subjects, which showed a 1.50 score, predicting IR for the obese group.

As a complementary measure to confirm the prevalence of IR, we calculated the \( TG/HDL \) ratio, which closely correlated with this parameter in adults at a value \( \geq 3 \) [47]. This ratio is also considered as a predictor of cardiometabolic risk and reflects an atherogenic lipid profile [48]. In the pediatric population, a high \( TG/HDL \) ratio is associated with lower insulin sensitivity and cardiovascular risk [49]. We found a significantly increased \( TG/HDL \) ratio for the obese group \( (3.02 \ p < 0.0001) \) in comparison with the lean group \( (1.60) \). These results confirm a prevalent condition of IR and cardiovascular risk in obese participants that could be associated with their downregulation of PPARα. Regarding this, PPARα has been reported to improve insulin sensitivity and give a protective property against the development of IR [44, 50].

Besides, PPARα is recognized by its anti-inflammatory role. In this study, we observed a proinflammatory profile in obese pediatric subjects with an increase in the serum levels of adipin, MCP-1, IL-8, IL-6, IFN-γ, and TNF-α, and a decreased levels of the anti-inflammatory adipokine adiponectin. Hence, PPARα downregulation in leukocytes from obese children suggests an association with their proinflammatory profile. Indeed, PPARα displayed a significant negative correlation with TNF-α levels and the abdominal skinfold. TNF-α promotes IR, decreases the uptake of fatty acids by adipocytes, increasing them in the circulation while stimulating lipolysis [51, 52].

Furthermore, TNF-α increases the production of inflammatory cytokines such as IL-6 and suppresses the production of adiponectin [52]. TNF-α altogether with IFN-γ and IL-6 leads to an infiltration of immune cells in adipose tissue and dysfunction of inflammatory immune cells [53]. In this work, it was found that the concentration of TNF-α was significantly higher in obese patients than in the control group. These findings are consistent with the high HOMA index, IL-8 and IL-6 levels, and the decreased adiponectin concentration in obese children and adolescents.

The consequences of obesity on the development of IR and activation of a chronic inflammatory response has allowed establishing a relationship between GLP-1 and PPARs. GLP-1 is a hormonal factor that improves insulin synthesis and secretion on the pancreatic β-cells. GLP-1 signaling through GLP-1R is recognized for its cytoprotective role in those cells [16, 18]. Increased GLP-1R expression and improved GLP-1 sensitivity into pancreatic islets may be favored by the activation of PPARα [54]. Interestingly, GLP-1R is expressed in some lineages of immune cells that have an immunomodulatory role in the migration and differentiation of T and B lymphocytes [16, 55, 56]. GLP-1R also displays anti-atherogenic effects during obesity by reducing monocyte/macrophage migration and inflammatory cytokine production [57, 58]. In this study, we observed a reduced expression of GLP-1R in obese children and adolescents. Although its expression did not show a significant association with inflammatory parameters, \( GLP-1R \) and \( PPARα \) expression exhibited a negative correlation with abdominal skinfold. These findings suggest a role of GLP-1R and PPARα in the lipid distribution that could indirectly affect the role of different immune cells and the secreted cytokines. Previous studies show that GLP-1R induce downregulation of
proinflammatory cytokines mediated through PPARγ activation and NF-kB inhibition [19], indicating that it is possible that both receptors present crosstalk in leukocytes from obese pediatric population. This association is highly needed to be explored in the future.

In summary, adipose tissue accumulation in obesity is associated with disturbances in the homeostasis of several tissues, disruption of the mechanisms controlling lipid and glucose metabolism, and dysregulation at the secretion profile for proinflammatory cytokines and adipokines [53]. Accordingly, it was possible to establish a relationship between anthropometric alterations and body fat distribution observed in obesity with associated comorbidities, such as IR, chronic inflammatory profile, and risk for the development of cardiovascular diseases. Thus, PPARα and GLP-1R are potential gene targets to explore its role in fat distribution and inflammatory profile as essential aspects in pediatric obesity.

**Conclusion**

We have shown that PPARα and PPARβ/δ expression are negatively associated with the proinflammatory markers TNF-α and IL-8, respectively, suggesting participation in the regulation of inflammation observed in pediatric obesity. Furthermore, PPARα and GLP-1R are downregulated in leukocytes from obese children and adolescents, and both receptors are associated with the abdominal skinfold suggesting a role in fat distribution that could indirectly affect the function of different immune cells and the secreted cytokines. Thereby, these findings may impact the understanding and implementation of PPARα or GLP-1R agonists in the clinic.

**Materials And Methods**

**Participants**

This is a cross-sectional study with primary data collection performed in children and adolescents from February of 2017 to June of 2018 in Bogota-Colombia. Children and adolescents aged 7 to 17 years old from seven public schools were invited to participate voluntarily with parental authorization. The study population included participants from low- to middle-income, which represent most of the city population. Finally, 37 participants (female, n = 18, and male, n = 19), which fulfilled the inclusion criteria, agreed to participate in this study. The inclusion criteria included female and male participants aged 7–17, with a healthy weight (lean control group) and obesity (defined as BMI, ≥ 85th, and ≥ 95th percentile) [2]. Exclusion criteria included: children and adolescents with diabetes mellitus (both type I and II), secondary causes of obesity, psychiatric illness, mental disorders, with weight equal to or greater than 100 kilograms, as well as those who were receiving medications which promoted weight loss or gain or altered in a way the metabolic profile including glycemia.

The Institutional Review Board of the Antonio Nariño University and the Medical Ethics Committee at DEXA DIAB (CE-CC-00721) approved the study protocol. Written informed parental consent and child assent from participants were obtained before any research procedures. This study was carried out
following the ethics committee code of the American Medical Association endorsed by the National Institutes of Health (NIH) [59].

**Anthropometric assessment**

Anthropometric measures were obtained using standard methods [60]. Weight was measured to the nearest 0.1 kg on a calibrated digital scale without shoes and wearing light clothing. Height was measured to the nearest 0.1 cm on a stadiometer. To evaluate the nutritional status of children and adolescents, the BMI Z-score was used, according to the criteria proposed by the World Health Organization [2]. Waist circumference (WC) was measured via flexible measuring tape with an accuracy of 1 mm. The measurement was made with the subject standing, taken at the midpoint between the right lower costal ridge and the iliac crest, without skin compression from the measuring tape, and at the end of a normal expiration. BMI and waist-height were calculated as ratios. Skinfold thicknesses were assessed using a Harpenden Skinfold Calliper, according to a previous protocol [60]. The measurements were performed in triplicate at the non-dominant side on biceps, triceps, subscapular, and suprailiac to the nearest 0.1 mm, and the mean values were calculated [60].

The pubertal stage was assessed according to Tanner criteria [61]. Briefly, the participants were classified as pre-pubertal when they reached a pubertal state between 2–4 and post-pubertal in state 5.

**Measurement of body composition by Dual X-ray Absorptiometry**

Measurements of total and regional body composition were acquired using a Dual-Energy X-ray Absorptiometry (DEXA) scan (GE Lunar Prodigy advance, GE Healthcare) by a trained technician. Before each acquisition, the scanner was calibrated according to the manufacturer's instructions. The total effective radiation dose during each examination was < 0.05 micro Sievert for a three-minute scan. For analysis, reference data for standard deviation scores were provided from GE Lunar Body composition software (enCORE 2010; version 11.3; GE Healthcare, Madison, WI, USA)

**Biochemical analyses**

Blood samples for laboratory analysis were collected by venipuncture from all participants after an overnight fast (10–12 hours). Participants underwent a 2-hour oral glucose tolerance test (OGTT) (1.75 g/kg, maximum 75 g) according to the American Diabetes Association criteria [62]. Fasting blood samples were obtained for fasting plasma glucose (FPG) insulin levels, total cholesterol, High-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c) VLDL-cholesterol (VLDL-c) and triglycerides (TG). IR was scored using the HOMA-IR index by calculating the product of fasting plasma insulin (U/mL) and fasting plasma glucose (mmol/L) divided by 22.5. TG/HDL ratio was calculated as TG (mg/dL)/HDL (mg/dL) and android-gynoid percent fat ratio was calculated as android fat (%) / gynoid fat (%) (A/G %).

**Gene expression analysis**
Leukocyte total RNA was extracted using Trizol reagent (Life Technologies, Inc) according to the manufacturer’s instructions. RNA was quantified with a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies), and cDNA was synthesized from 1 µg ARN with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Cat. # 4368814). Real-time PCR was performed in duplicate using CFX96 equipment (BioRad) and SYBR® Green PCR Master Mix, according to the manufacturer’s protocol. PPAR-α, PPAR-β, PPAR-γ, incretins receptor genes GLP-1R and GIPR, and reference genes PGK1 and YWHAZ were quantitated using the gene-specific primers (Supplementary Table 1). cDNAs were amplified for 40 cycles consisting of 10 s of denaturation at 95 °C, 15 s of annealing temperature for each primer (Supplementary Table 1), and 10 s of extension at 72 °C. Standard curves for all genes were generated using serial dilutions of pooled cDNAs from all samples. Relative mRNA expression was calculated with the method ΔΔCt. Data are shown as normalized ratios between target gene expression and geometric media of the two reference genes [63]. All expression assays were performed following the MIQE guidelines [64].

Hormone and cytokine levels

Preprandial GIP and GLP1 levels were measured in serum using a specific enzyme-linked immunosorbent assay (Elabscience, Cat. #E-EL-H2061 and E-EL-H6025) according to the procedures provided by the manufacturer. Levels were expressed in ng/mL and pg/mL. The presence of adiponectin, adipsin, RBP4, MCP-1, IL-1β, IP-10, IL-10, IL-8, leptin, IL-6, IFN-γ, resistin, and TNF-α in serum samples was analyzed using a multiplex immunoassay based on fluorescence-encoded beads according to the manufacturer’s instructions (No. 40196, BioLegend). The acquisition was performed in an Accuri C6 flow cytometer. Off-line analysis was performed with LEGENDplex™ data analysis software (version 8.0), and the data were expressed as the mean reporter fluorescence intensity PE (MFI) as a function of concentration (pg/mL). Each assay was performed with two technical replicates.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6. Descriptive statistics were expressed as mean ± standard deviation (SD) whenever applicable, and variables were tested for normality by Kolmogorov-Smirnov test. Non-parametric tests were used whenever data were not normally distributed or Student t-test when data were normally distributed. We used Wilcoxon signed-rank tests to assess the significance of inter-group differences. The correlation of target genes and evaluated parameters were determined using the Spearman rank test. For all analyses, statistical significance was considered as p < 0.05.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of the Antonio Nariño University and the Medical Ethics Committee at DEXA DIAB (CE-CC-00721). Written informed parental consent, and child
assent from participants were obtained before any research procedures were conducted. This study was carried out in accordance with the ethics committee code of the American Medical Association endorsed by the National Institutes of Health (NIH).

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests

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**Authors' contributions**

KVS, JG, and MLB. conceived and designed the research; MLB, KVS, ABR, LV, YU, IB, HYL, and JG performed experiments; KVS, LV, YU, IB, and MLB, prepared figures and wrote the manuscript; MLB, and KVS analyzed data; MLB, and KVS edited, and all authors revised and approved the final manuscript version.

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Figures

![a. PPAR-α](image1)

![b. PPAR-β](image2)

**Figure 1**

Leukocytes from obese children and adolescents displayed a reduction of PPAR-α gene expression. (a) PPAR-α and (b) PPAR-β mRNA expression levels were measured by qPCR in leukocytes from each group. The values are expressed as a normalized ratio between the target gene expression and the geometric median of the PGK and YWHAZ endogenous genes. Statistical differences were analyzed by Student’s t test (* p < 0.05).
Figure 2

Leukocytes from obese children and adolescents displayed a reduction of GLP-1R gene expression. GLP1R and GIPR mRNA expression levels were measured by qPCR in leukocytes from each group. The values are expressed as a normalized ratio between the target gene expression and the geometric median of the PGK and YWHAZ endogenous genes. Statistical differences were analyzed by Student’s t test (* p < 0.05).
Figure 3

Serum proinflammatory cytokines and chemokines are increased in children and adolescents with obesity. IL-8, IL-6, TNF-α, IFN-γ, IL-10, MCP-1 and IP-10 serum levels were measured by flow cytometry using a Legendplex multiplex assay. Statistical differences were analyzed by Mann Whitney test (* p < 0.05, ** p < 0.005, *** p < 0.0005).

Figure 4

Serum proinflammatory adipokines are increased in children and adolescents with obesity. Adiponectin, adipisin and resistin serum levels were measured by flow cytometry using a Legendplex multiplex assay. Statistical differences were analyzed by Mann Whitney test (* p < 0.05, ** p < 0.005, *** p < 0.0005).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable2.xlsx
- Supplementarytable1.docx