



**Inflammation and fatness in adolescents with and without
Down Syndrome. UP&DOWN Study**

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Abstract

Background: The main objective of this study was to describe the inflammatory status of adolescents with Down Syndrome (DS) and their relationship with fatness. **Methods:** 95 DS adolescents (44.2% girls) and a control group of 113 adolescents (47.8% girls), 11-18 years, from the UP&DOWN Study participated. Serum C-reactive protein (CRP), C3 and C4 complement factors, total proteins, interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), insulin, cortisol, leptin, adiponectin, galactin-3 and visfatin were analyzed; HOMA-index was calculated. Body fat indicators: weight, height, waist circumference and skinfold thicknesses were measured. Birth weight was obtained by questionnaire. BMI, waist-to-height-ratio (WHtR) and body fat percentage (BF%) were calculated. **Results:** DS group showed higher levels of BMI, WHtR, waist circumference, BF%, and lower birth weight than controls ($p < 0.001$). In the general lineal model in the total sample, WHtR was positively associated with C3 and C4 ($p < 0.001$) as well as with leptin levels ($p = 0.015$). BF% was positively associated with total proteins ($p = 0.093$) and leptin levels ($p < 0.001$). DS was positively associated with total proteins ($p < 0.001$), C3 ($p = 0.047$) and C4 ($p = 0.019$). Despite the higher levels of fatness found in DS group, no direct association was found between BF% and leptin levels, opposite to the control group. **Conclusions:** these findings suggest that abdominal obesity should be controlled in adolescents due to its relationship with acute phase-inflammatory biomarkers, but especially on DS adolescents who may show a peculiar metabolic status according to their relationship between fatness and inflammatory biomarkers.

Key words: inflammatory biomarkers; fatness; adolescents; Down Syndrome.

Background

Down Syndrome (DS) is a human genetic disorder due to the triplication of chromosome 21 that is associated with several chronic pathologies, such as cardiovascular diseases, obesity, diabetes mellitus or Alzheimer (Roizen & Patterson 2003), together with a variety of dysmorphic physical characteristics, and immunodeficiency (Kusters et al. 2009). Autoimmune diseases occur more frequently in individuals with DS than in those without DS. However, the etiology of this immunological disorder has not been fully described (Santos-Silva CR, Biselli-Périco JM et al. 2016).

Inflammation is a natural defense mechanism of body tissues from the immune system. Nevertheless, chronic inflammation is associated with the development of cardiovascular and metabolic diseases, such as atherosclerosis and type 2 diabetes. Fat tissue excess is related to a low-grade inflammation status that produces immune-related mediators, like adipokines that play an important role in sugar and fat metabolism, immunity and cardiovascular function (Smekal & Vaclavik 2017).

Inflammatory conditions seem to have a crucial role in DS. However, this status of low-grade inflammation in DS has to be confirmed in humans (Fructuoso et al. 2018). Therefore, the main aim was to describe the inflammatory status of adolescents with Down Syndrome (DS) and their relationship with fatness.

Methods

Sample and study design

The UP & DOWN Study is a two-year follow-up study designed to assess the impact over time of physical activity and sedentary behaviors on health indicators, such as physical fitness, metabolic and cardiovascular disease risk factors, inflammatory biomarkers and mental health,

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3 as well as to identify the psycho-environmental and genetic determinants of physical activity in
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5 a Spanish sample of adolescents with and without DS (Castro-Piñero et al. 2014). Parents and
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7 school supervisors were informed by letter about the nature and purpose of the study, and
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9 written informed consent was obtained.
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12 For the current study, we included initial data collected from September 2011 to June 2012 in
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14 Madrid (Spain), all the participants aged between 11 and 18 years with available blood samples
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16 were included. The sample included a total of 95 DS adolescents (44.2% girls) (DS group) and
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18 113 adolescents (47.8% girls) (control group). The study protocols were approved by the Ethics
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20 Committee of Puerta de Hierro Hospital (Madrid, Spain) and the Bioethics Committee of the
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22 Spanish National Research Council (Madrid, Spain). The study conforms to recognized
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24 Declaration of Helsinki standards.
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31 **Inflammatory biomarkers**

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34 Fasting blood samples were collected early in the morning. In all cases, blood was extracted
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36 from the cubital vein according to the established protocol (Castro-Piñero et al. 2014). Twelve
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38 key biomarkers involved in the inflammatory process were analyzed for this study: C-reactive
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40 protein (CRP, mg/L), C3 (C3, mg/dL) and C4 component factors (C4, mg/dL) by turbidimetry
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42 (AU2700 Olympus Analyzer; Olympus UK Ltd, Watford, UK); total proteins (g/L) by
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44 colorimetric assay (AU2700 Olympus analyser); tumor necrosis factor- α (TNF- α pg/mL),
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46 interleukin-6 (IL-6, pg/mL), adiponectin ($\times 10^6$ pg/mL), insulin (pg/mL) and leptin (pg/mL) by
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48 Immunoassay (xMAP Technology) using a kit (5 + 1) plex: 171B5006M Bio-Plex Human IL-
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50 6 set; 171B5026M Bio-Plex Human TNF- α set; 171D50001 Bio-Plex Human Cytokine Stds;
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52 171-A7003M Bio-plex Pro Human Diabetes Adiponectin Assay; YB0000002Y Bio-Plex
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54 Human Diabetes 3-Plex Assay; visfatin (ng/mL) by Enzyme-linked ImmunoSorbent Assay
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56 (Human visfatin Elisa kit; Cusabio Biotech); galactin-3 (pg/mL) by enzyme-Linked
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3 ImmunoSorbent Assay (Omnikine TM Human Galectin-3 Elisa Kit, Assay biotech) and cortisol
4 (pg/mL) by enzyme-Linked ImmunoSorbent Assay (ARBOR ASSAYS kit). Also, homeostatic
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6 model assessment for insulin resistance (HOMA-IR) was calculated by the following equation:
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8 Fasting glucose (mmol/L)×fasting insulin (mIU/L)/22.5.
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14 **Fatness measurements**

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17 Body fat indicators were assessed following standardized procedures (Ruiz et al. 2011). Weight
18 was measured using an electronic scale (model SECA 701, Hamburg, Germany) and height by
19 using a telescopic height-measuring instrument (model SECA 220, Hamburg, Germany). Body
20 mass index was expressed as kg/m². As indices of abdominal obesity, both waist circumference
21 [indicator of abdominal body fat, was measured with a non-elastic tape (SECA 200; SECA,
22 Hamburg, Germany) at the level of the narrowest part of the torso] and waist-to-height ratio
23 (WHtR) (indicator of abdominal body fat, was calculated as waist circumference/height) were
24 used for this purpose. Body fat percentage (BF%) was calculated from triceps and subscapular
25 skinfold thicknesses, that were measured with a Holtain caliper, using the Slaughter's equations
26 (Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD 1988).
27 These equations accurately predict body fat by these skinfolds in both populations (González-
28 Agüero et al. 2011; Rodríguez et al. 2005). Birth weight was obtained by a questionnaire. The
29 whole protocol of the study had been previously described (Castro-Piñero et al. 2014).
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50 **Statistical analysis**

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52 Differences between groups (DS vs control) in age, birth weight, BMI, waist circumference,
53 WHtR, BF%, C3 and C4 complements, CRP, total proteins, IL-6, TNF- α , insulin, HOMA-IR,
54 cortisol, leptin, adiponectin, galactin-3 and cortisol were calculated using Student's t-test for
55 independent samples (continuous variables). Kolmogorov-Smirnov test was performed to
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3 confirm the normal distribution of the variables. To normalize galactin-3 variable, natural
4 logarithm was calculated. IL-6 and TNF- α did not present a normal distribution either with the
5 calculation of box transformations.
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10 Chi square test was analyzed to determine the differences between groups of visfatin detectable
11 levels. In order to minimize the effect of multicollinearity for each biomarker, the most
12 explicative covariates (age, gender, control or DS group, WHtR, BMI group, BF% and birth
13 weight) were previously identified by a Stepwise Regression Analysis. Afterwards, a General
14 Linear Model (GLM) was implemented to each biomarker from these fixed factors: DS, gender
15 and BMI groups; and the following covariates: BF%, WHtR, birth weight and age.
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20 The analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 24.0
21 for WINDOWS; SPSS INC, Chicago) and the level of significance was set at $p < 0.05$.
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33 **Results**

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36 Characteristics of the total sample are shown in Table 1. DS group showed higher levels of
37 BMI, waist circumference, WHtR, and BF% while lower birth weight in comparison with the
38 control group ($p < 0.001$). Regarding inflammatory biomarkers, DS group showed higher levels
39 of CRP, C3 and C4 complement factors, total proteins and visfatin while lower levels of
40 adiponectin, insulin and galactin-3 than the control group ($p < 0.05$).
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48 Visfatin levels were below the level of detection in 64.5% of the total sample, these levels were
49 only detected in 44 (38.9%) adolescents from the control group and 23 (24.2%) DS adolescents
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3 In the GLM, relationships between some body fat indicators and inflammatory biomarkers were
4 found. WHtR was positively associated with C3 and C4 complement factors and leptin levels.
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6 The BF% was positively associated with leptin levels (Table 2).
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10 The feature to show this genetic disorder due to the triplication of chromosome 21 (DS group)
11 seems to be related to higher levels of C3 and C4 complement factors and total proteins (Table
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13 2). In DS subjects no direct association was found between BF% and leptin levels, opposite to
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15 the results found in the control group (Figures 1 and 2).
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23 Discussion

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26 This study provides original and useful information about the relationship between body fat
27 indicators and metabolic and inflammatory status in healthy adolescents with DS through the
28 evaluation of fatness and inflammatory biomarkers in this population.
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34 According to our knowledge, the scientific literature is scarce regarding the immune system in
35 the population with DS. Some studies have assessed some immune biomarkers in a relatively
36 low number of subjects (<43) with DS (Santos-Silva CR, Biselli-Périco JM et al. 2016; Corsi
37 et al. 2009; SN et al. 2008) or in other studies showing conjunctly other diseases (Rohrer TR,
38 Hennes P & Dost A, Grabert M, Rami B, Wiegand S 2010; Magni et al. 2004). On the other
39 hand, the number of biomarkers analyzed in this study is much wider than in other studies.
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42 Nowadays, DS has been demonstrated to reveal an anthropometric dimorphism associated with
43 their trisomy 21 (Real de Asua et al. 2014), what is clearly found in our results since practically
44 all the body fat indicators determined, such as BMI, waist circumference, WHtR and BF%,
45 showed higher values than the control group. These findings coincide with the results reported
46 by other authors in both children and adolescents (Basil et al. 2016; Bertapelli et al. 2016), as
47 well as in adults (Leti et al. 2015; Nespoli L, Burgio GR, Ugazio AG 1993). This outcome is in
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3 agreement with the characteristics of DS subjects who suffer from overweight and obesity
4 (Bertapelli et al. 2016), and also from abdominal obesity (Real de Asua et al. 2014).
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7 Despite the evaluation of the immune system in DS population acquired certain interest few
8 years ago (Nespoli L, Burgio GR, Ugazio AG 1993), in our opinion these studies lacked enough
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10 depth, especially when DS subjects are healthy and young. Therefore, our study aimed to
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12 evaluate the inflammatory status of this population with a battery of inflammatory biomarkers
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14 of acute phase, such as CRP, C3, C4 and total proteins. All these markers showed higher levels
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16 in the DS group than in the control group. Other studies have found similar results related to
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18 C3 and C4 complement factors, but in adult population with DS and with other pathologies
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20 (Nanjo et al. 2014). However, to our knowledge there are not studies on healthy youth with DS.
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22 All these molecules analyzed in our study are considered early cardiovascular risk markers,
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24 whose high values are indicative of low-grade inflammation, especially in adolescents with
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26 overweight and obesity. We have found a positive relationship between abdominal obesity
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28 (assessed by WHtR) and C3 and C4 complement factors (Table 2) in the total adolescents, both
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30 with and without DS, that means both biomarkers are related to body fat distribution, in
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32 agreement with previous results evaluating the excess of abdominal fat in children and
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34 adolescents without DS (Warnberg et al. 2018). On the other hand, the DS group showed higher
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36 levels of total proteins than controls (Tables 1,2). Total protein values include serum albumin
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38 and globulins that were also assessed since DS subjects are more prone to suffer from infectious
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40 diseases (Santos-Silva CR, Biselli-Périco JM et al. 2016). According to Mahan et al. (Mahan
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42 LK, Escott-Stump, S 2013) this result could be related to an increase of globulins revealing an
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44 immune response triggered by an infectious agent. On the other hand, an association between
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46 total proteins and lean body mass has also been observed in adults with renal treatment (Gallar-
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48 Ruiz et al. 2012). Nevertheless, we must highlight that no infections were detected in the DS
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3 group assessed in the current study, therefore, the evaluation of all markers, as a whole, is
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5 necessary to understand the real inflammation status of this population.
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9 Due to the higher levels of body fat showed by adolescents with DS, we included the assessment
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11 of some molecules such as insulin and HOMA-IR, cortisol, leptin, adiponectin, galactin-3 and
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13 visfatin in order to evaluate their impact at the metabolic level. In addition, it is important to
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15 highlight that DS subjects show an increased risk of both type 1 (Rohrer TR, Hennes P & Dost
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17 A, Grabert M, Rami B, Wiegand S 2010) and type 2 diabetes (Helguera et al. 2013).
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21 Adolescents with DS showed lower insulin concentration and a trend towards lower HOMA-
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23 IR values in comparison with controls (Table 1), according to other studies performed in
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25 children and adolescents (Bertapelli et al. 2016) or adults (Leti et al. 2015) with DS and also in
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27 validated DS mice models (Peiris et al. 2016). According to Rohrer et al. (Rohrer TR, Hennes
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29 P & Dost A, Grabert M, Rami B, Wiegand S 2010), youth with DS use less insulin since they
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31 seem to have a better metabolism control. On the other hand, HOMA-IR remained unmodified
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33 between both groups (Table 1), in agreement with other authors (SN et al. 2008). However, a
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35 relationship between obesity and hyperinsulinemia has been found in children with DS (Basil
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37 et al. 2016), although there is not a consistent association.
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42 Cortisol is well-known to be a steroid hormone that is produced in the hypothalamic-pituitary-
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44 adrenal axis and has been positively associated with adipose tissue and low-grade inflammation,
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46 especially in obese subjects (Smekal & Vaclavik 2017). However, in our sample cortisol levels
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48 remained similar in both groups (DS and control) even though DS adolescents showed higher
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50 BMI and BF% than the control group. This result is not in accordance with that found in another
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52 study (Bricout et al. 2008) reporting lower levels of cortisol in young adults (22.5 years old)
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54 with DS in comparison with a control group. In addition, since cortisol levels raise with age in
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3 the adult general population (Pal & Singh 2014), we could elucidate that this result could be
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5 due to the different age range in both studies.
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8 Leptin is another adipokine assessed in the current study as a hormone involved in food and
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10 appetite regulations. Leptin levels remained unmodified between both groups in the current
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12 study (Table 1), opposite result to that found in youth (Corsi et al. 2009; SN et al. 2008) and
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14 adults (Leti et al. 2015) with DS, despite leptin has been associated with high BMI in prepuberal
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16 youth with DS (Magni et al. 2004). Indeed, this result coincides with our findings in the general
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18 lineal model, where trisomy 21 did not present the same direct relationship between body
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20 composition and WHtR and leptin levels in comparison with the control group (Table 2, Figures
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22 1 y 2). May be, this is the reason why other studies have accepted a lack in the knowledge of
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24 the relationship between adiposity and inflammation in DS subjects (Fructuoso et al. 2018).
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29 Adiponectin is a hormone involved in glucose regulation and fatty acid catabolism, with
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31 cardioprotective characteristics and probably with an anti-inflammatory effect (Shetty et al.
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33 2009). In the current study, DS adolescents showed lower adiponectin levels than the control
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35 group (Table 1). These results are in agreement with Martínez-Gómez et al. (Martinez-Gomez
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37 et al. 2012) and Turer et al. (Turer et al. 2011) studies in healthy adolescents and adults,
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39 respectively, and in a smaller sample of children with DS (Tenneti et al. 2017). However, such
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41 inflammatory risk seems to be diminished with aging, decreasing also the cardiovascular risk
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43 (Corsi et al. 2009). Maybe this fact could contribute to a lower prevalence of cardiovascular
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45 mortality in the DS population (Uppal et al. 2015).
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51 The high percentage of congenital coronary alterations found in the DS population (Diogenes
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53 et al. 2017) acquires a great interest, despite not being the main cause of mortality (Uppal et al.
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55 2015). Therefore, we included the assessment of galactin-3 as a marker of cardiovascular
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57 pathologies (de Boer et al. 2012; De Boer et al. 2014; Vassalle et al. 2017). In the current study,
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3 adolescents with DS showed lower levels of galactin-3 than the control group (Table 1).
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5 However, opposite results in Ts65Dn have been found in adult mice, a validated trisomy 21
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7 model (Fructuoso et al. 2018). These controversial results could be due to the different age-
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9 range in these studies since galactin-3 increases with age in general population (de Boer et al.
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11 2012).

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15 Visfatin is secreted by visceral fat (Smekal & Vaclavik 2017), being usually increased in
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17 cardiovascular diseases (Turer et al. 2012), and related to insulin resistance and type 2 diabetes
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19 (Chen et al. 2006) in general population. DS subjects showed higher visfatin values than the
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21 control group (Table 1). In the same way, several authors have pointed out a positive association
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23 between visfatin and BMI as well as WHtR index in DS adolescents (Blüher et al. 2017); and
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25 also with BF% in children (García-Hermoso et al. 2017) and adults (Turer et al. 2012) without
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27 DS. It is highlighted that despite most DS subjects had undetectable levels of visfatin, when
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29 these levels were detectable, they were higher in DS subjects than in the control group (Table
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36 Finally, our findings demonstrate that adolescents with DS show higher body fat indicators,
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38 which lead to a higher risk of obesity. Furthermore, abdominal obesity, through WHtR
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40 assessment, is positively related to inflammatory and cardiovascular risk biomarkers such as
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42 C3 and C4 complement factors and leptin. Therefore, in agreement with other authors, this
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44 anthropometric index should be taken into account in children and adolescents, being especially
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46 important in DS population where exist other idiosyncratic factors such as trisomy 21 that may
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48 get worse an inflammatory status. However, in view of these results we could interpret that this
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50 chromosomal alteration may be a metabolic modulator effect on variables such as leptin. Thus,
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52 it seems that adolescents with DS may show a peculiar inflammatory and metabolic behavior,
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54 different from control subjects at the same age.
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3 The principal limitation of the current study is the cross-sectional design, which does not allow
4 us to read any conclusion about the causal direction of associations. Likewise, the sample could
5 not be adjusted by pubertal development due to complications found in the self-reported data
6 collection in the DS group. However, as strength, the relative large sample of adolescents with
7 DS and the large number of biomarkers analyzed should be highlighted, in the context of few
8 previous studies assessing the interactions between inflammatory biomarkers and body fat
9 indicators in adolescents with DS.
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20 Indeed, the knowledge of the relationship between body fat indicators and inflammatory
21 biomarkers in DS could be a big help in the control of associated pathologies to the DS
22 chromosomal alteration. In future investigations, the assessment of molecules such as C3 and
23 C4 complement factors, total proteins, visfatin and leptin could be helpful to understand their
24 different behavior in youth with DS. In addition, interventions in subjects with DS addressed
25 to enhance their body composition must be performed in order to avoid any inflammation status
26 risk, especially nowadays when their life expectancy is increasing.
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For Peer Review

Table 1. Description of main variables in both groups, Down Syndrome and control groups

| | Control | | Down Syndrome | | p-value |
|---------------------------------------|---------|------------------------|---------------|------------------|------------------|
| | n | Mean (SD) ^a | n | Mean (SD) | |
| Age (years) | 113 | 14.64 (2.16) | 95 | 14.22 (2.00) | 0.172 |
| Birth weight (kg) | 108 | 3.16 (0.64) | 67 | 2.77 (0.81) | <0.001 |
| BMI ^b (kg/m ²) | 112 | 18.50 (2.77) | 95 | 23.98 (4.24) | <0.001 |
| Waist circumference (cm) | 113 | 62.21 (7.88) | 95 | 74.03 (9.72) | <0.001 |
| WHtR ^c (cm/cm) | 112 | 0.41 (0.03) | 95 | 0.49 (0.06) | <0.001 |
| BF ^d % | 113 | 18.91 (6.12) | 91 | 29.26 (5.44) | <0.001 |
| CRP ^e (mg/L) | 57 | 0.90 (2.68) | 87 | 4.49 (9.31) | 0.001 |
| C3 (mg/dL) | 105 | 91.90 (24.65) | 90 | 119.75 (22.52) | <0.001 |
| C4 (mg/dL) | 105 | 20.11 (9.30) | 90 | 30.06 (9.09) | <0.001 |
| Total proteins (g/L) | 77 | 58.74 (15.68) | 90 | 72.21 (5.37) | <0.001 |
| IL-6 ^f (pg/mL) | 112 | 28.18 (25.07) | 87 | 37.75 (46.31) | 0.086 |
| TNF- α ^g (pg/mL) | 100 | 78.04 (76.40) | 68 | 71.75 (112.4) | 0.666 |
| Insulin (pg/mL) | 81 | 413.5 (368.4) | 56 | 243.9 (347.6) | 0.008 |
| HOMA-IR ^h (mmol/L*mIU/L) | 78 | 2.34 (2.24) | 53 | 1.57 (2.28) | 0.061 |
| Cortisol (pg/mL) | 102 | 197972 (102877) | 87 | 173669 (86454) | 0.083 |
| Leptin (pg/mL) | 103 | 7187.7 (6563.6) | 77 | 9169.9 (10800.7) | 0.157 |
| Adiponectin (x10 ⁶ pg/mL) | 105 | 14.78 (9.18) | 90 | 11.49 (5.21) | 0.002 |
| Galactin-3 (pg/mL) | 101 | 3598.9 (3010.6) | 87 | 2453.7 (1583.5) | 0.001 |
| Visfatin (ng/mL) | 44 | 0.93 (0.78) | 23 | 1.40 (0.75) | 0.019 |

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3 ^a Standard deviation; ^b Body mass index; ^c Waist to height ratio; ^d Body fat; ^e C-reactive protein;
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5 ^f Interleukin-6; ^g Tumor necrosis factor- α ; ^h Homeostatic model assessment for insulin
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7 resistance (HOMA-IR).
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10 *Statistical significance was determined by Student's t-test.
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Table 2. Analysis of inflammatory biomarkers in adolescents with and without Down Syndrome. General Lineal Model

| Outcome variable | Independent variables | B | Standard error | p-value |
|--|-----------------------|----------------|----------------|------------------|
| C3 Adjusted R Squared=0.347 | Control | -9.480 | 4.749 | 0.047 |
| | DS ^b | 0 ^a | | |
| | WHtR ^c | 195.094 | 36.940 | <0.001 |
| C4 Adjusted R Squared=0.280 | Control | -4.056 | 1.899 | 0.019 |
| | DS ^b | 0 ^a | | |
| | WHtR ^c | 58.070 | 14.773 | <0.001 |
| Total proteins Adjusted R Squared=0.264 | Control | -10.660 | 0.5 | <0.001 |
| | DS ^b | 0 ^a | | |
| | BF ^d % | 0.261 | 0.155 | 0.093 |
| Leptin Adjusted R Squared=0.213 | Control | 7286.864 | 1872.830 | <0.001 |
| | DS ^b | 0 ^a | | |
| | BF ^d % | 566.268 | 109.627 | <0.001 |
| | WHtR ^c | 35660.685 | 14543.828 | 0.015 |

^a Down Syndrome; ^b This parameter is set to zero because it is redundant; ^c Waist to height ratio; ^d Body fat.

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3 **Figure legends**
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6 Figure 1
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8
9 Title: Relationship between leptin levels and body fat percentage in youth with and without
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11 Down Syndrome
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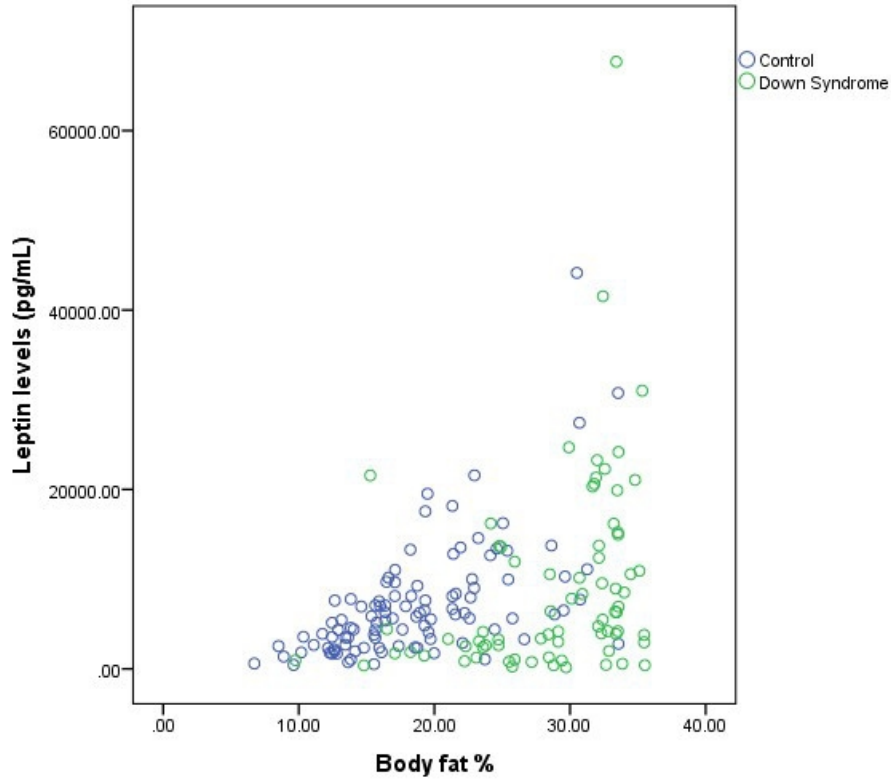
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20 Title: Relationship between leptin levels and WHtR in youth with and without Down Syndrome
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23 Foot of figure: WHtR, Waist to height ratio
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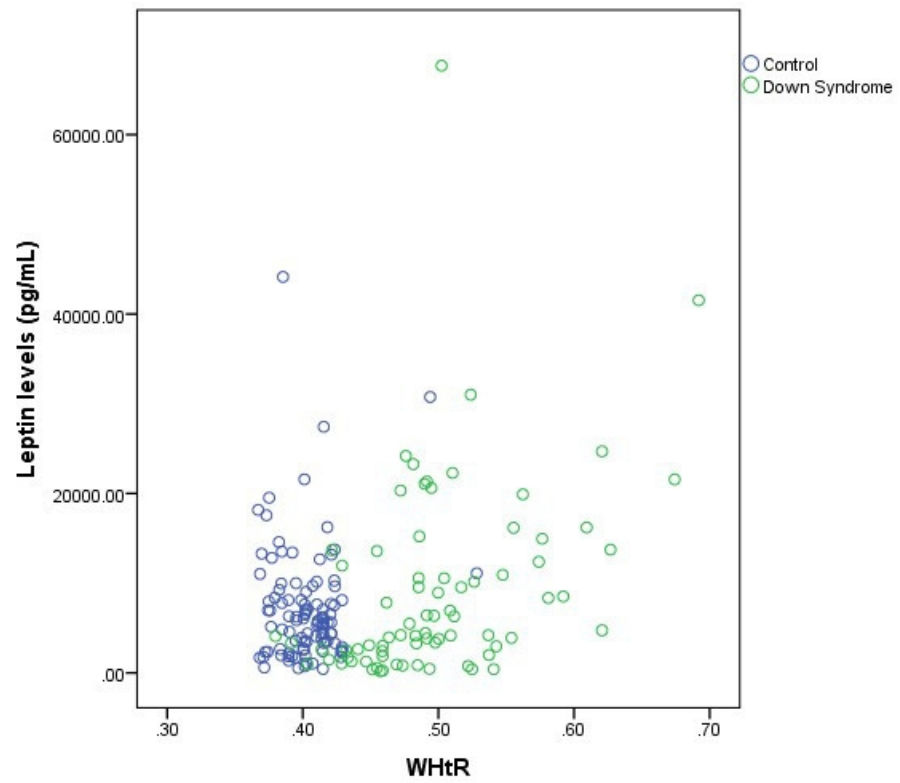
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