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Obesity Status and Physical Activity Level in Children and Adults with Autism Spectrum Disorders: A Pilot Study

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Abstract

The purpose of the present study was to compare body composition and physical activity level between children and adults with Autism Spectrum Disorders (ASD). A sample of 78 children, adolescents and adults participated in the study. Anthropometrics and physical activity, using GT1M accelerometer, were assessed. Overweight and obesity prevalence was higher in men vs. male children (p < 0.001) and in men vs. women (p = 0.035). Children recorded more moderate to vigorous physical activity (p = 0.040) than adults. Normal-weight children and adolescents combined as one age group, accomplished more moderate to vigorous physical activity, steps and less sedentary time compared to their overweight and obese counterparts during the weekend. Obesity status may negatively affect physical activity level in ASD individuals.

Keywords Physical activity · Sedentary behavior · Obesity · Autism Spectrum Disorders

Introduction

The prevalence of obesity in children and adults has increased greatly during the past few decades, and is considered a serious medical disorder (Berghofer et al. 2008). The consequences of overweight and obesity do not manifest immediately, but could lead to type 2 diabetes, hypertension, cardiovascular disease, gallbladder disease, postmenopausal breast cancer, colon and other cancers, osteoarthritis, back pain, and physical and mental disabilities (Guo et al. 2002; He and Baker 2004; Yan et al. 2004). Regular physical activity helps to improve health and psychosocial outcomes, because it offers many other health benefits in addition to body weight regulation (Cavill et al. 2006; Warburton et al. 2006).

Autism Spectrum Disorders (ASD) are characterized by impairments in social and communication abilities associated with symptoms varying from difficulty in communication and social interaction, to restricted and repetitive behaviors (American Psychiatric Association 2013). People with ASD may be overly dependent on routines and are highly

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sensitive to changes in their environment (American Psychiatric Association 2013). Inactivity is a concern for individual with ASD. One of the reasons is because they have fewer opportunities to participate in exercise and physical activity programs, which exposes them to the risk of developing other health problems (Pan et al. 2011).

Individuals with disabilities are more likely to be physically inactive and experience more barriers to participation in sports activities compared to people without disabilities (Kinne et al. 2004; Rimmer et al. 2007). It has been reported that youths with ASD were less active than peers without disabilities (Pan and Frey 2006), with a substantial reduction in activity levels across the adolescent years (Macdonald et al. 2011; Memari et al. 2013) and especially in girls (Ruiz-Vicente et al. 2015). Parents and clinicians report, that social, cognitive, behavioral and physical limitations experienced by people with ASD tend to prevent them from participating in physical activities (Bandini et al. 2013). The existing literature on physical activity levels in people with ASD is sparse; most studies are limited by the lack of a comparison group (Memari et al. 2013; Pan and Frey 2006), a sample age restricted to children and adolescents (Bandini et al. 2013; Sandt and Frey 2005), or because they focus on a particular part of the day only (Pan 2008; Sandt and Frey 2005).

Despite the emergence of a growing body of literature on obesity and physical activity habits in typically developing



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children and adults during the last few decades, little has been done to investigate this aspect in children and adults with development disabilities, and in particular with ASD (Curtin et al. 2010). Only a few studies have reported data on overweight and obesity status in children with ASD, and the results from these studies suggest that the prevalence of obesity in such children is at least as high, if not higher, than that seen in typically developing children (Broder-Fingert et al. 2014; Curtin et al. 2010, 2014). Nevertheless, the prevalence of obesity in ASD adults remained unexplored.

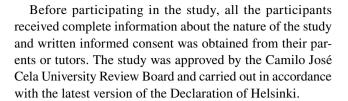
Physical activity habits and a healthy body composition during child and adulthood is a major public health concern for people with or without ASD, because a sedentary lifestyle and related consequences can predispose them to greater health problems during adulthood (Rimmer et al. 2007). Therefore, the purpose of the present pilot study was to compare body composition and physical activity level in children and adolescents compared to adults with ASD, to further examine physical activity levels related to anthropometric factors. We hypothesized that physical activity levels and body composition would be healthier in children compared to adolescents and adults data, particularly in normalweight compared to overweight and obese participants. To the knowledge of the authors, this is one of the first studies to investigate the physical activity status and anthropometric characteristics among children and adults with ASD.

Materials and Methods

Sample

The evaluation was performed on 78 children, adolescents and adults (56 boys/men and 22 girls/women), aged 7–48 years, recruited from special schools and centers for children and adults with ASD in Madrid (Spain). All the centers were geographically distributed throughout the different districts of the Madrid region. All the participants had received a clinical diagnosis of ASD from an experienced and recognized psychiatrist or neurologist in public hospitals according to the Diagnostic and Statistical Manual of Mental Disorders criteria (fifth edition) (American Psychiatric Association 2013). The individuals met clinical cut-off scores for ASD using either the Autism Diagnostic Observation Schedule-2 (ADOS-2) (Lord et al. 2012) or the Autism Diagnostic Interview—Revised (ADI-R) (Lord et al. 1994).

The total sample was distributed into three groups: children (n=29, 22 boys and 7 girls), with ages between 7 and 12 years (mean age= 9.41 ± 1.55 years); adolescents (n=15, 11 boys and 4 girls), with ages between 13 and 18 years (mean age= 15.20 ± 1.78 years); and adults (n=34, 23 men and 11 women) aged 19 and over (mean age= 31.09 ± 6.85 years).



Measurements

The study was carried out at four different centers. One team comprising a physical education teacher and a field research assistant conducted all the measurements, which were taken at around the same time of the day for consistency.

Anthropometric Characteristics

Anthropometric measures were assessed for each participant. Height was measured in the upright position using a stadiometer (SECA 206, Seca Corp, Hanover, Maryland USA) to the nearest 0.1 cm. Body fat composition was calculated using bioimpedance (BC-418, Tanita, Japan), following appropriate standards for this measurement. Height, sex and age were entered manually; weight was recorded with a 0.5 Kg adjustment for the weight of their clothes. The Tanita software uses equations to estimate fat mass and fat free mass. Prevalence of overweight and obesity in the adults was assessed with reference to the body mass index, according to the standard definitions (25 and 30 kg/m², respectively), and for the children and adolescents based on age-specific BMI percentile reference guidelines (Serra et al. 2003); values higher than those corresponding to the 85th percentile were considered as overweight, and higher than those corresponding to the 95th were considered as obese.

Physical Activity and Sedentary Habits

Physical activity was measured using the GTIM accelerometer (Actigraph LLC, Pensacola, FL, USA) during 6 consecutive days. The device used a motion sensor that integrated the degree and intensity of movement and produced a voltage output signal the magnitude of which was recorded as activity counts. Counts were recorded over periods of 60 s. All the children wore the accelerometer in an elastic band fastened on the right hip during the daytime, excepting during aquatic activities or while bathing. Parents and children were provided with verbal and written instructions for care and placement of the monitor. Validation studies suggest that this accelerometer is a valid and reliable measurement of children's and adults' physical activity with highly significant correlations (children, r=0.86; adults, r=0.92) with energy expenditure determined by multiplying the average VO₂ by the caloric equivalent of the mean respiratory exchange ratio (Brage et al. 2003; Trost et al. 1998). We



established two criteria to consider data valid: a minimum of four consecutive days including 1 weekend day and a minimum of 10 h of data registered per day (Ekelund et al. 2004). In order to calculate the time spent doing moderate or vigorous intensity physical activity, we used an application of count thresholds corresponding to moderate or vigorous intensity activity. These cut off points are obtained from calibration studies that relate the amount of accelerometer counts to physical activity energy expenditure, recommending the use of different cut off points for children and adults (Troiano 2007). Count ranges for activity intensities in children and adolescents were: 0-100 for sedentary, 101-2295 for light, 2296-4011 for moderate, 4012 and above for vigorous and very vigorous (Evenson et al. 2008). Count ranges for activity intensities in adults were: 0–99 for sedentary, 100-1951 for light, 1952-5724 for moderate, 5725-9498 for vigorous, 9599 and above for very vigorous (Freedson et al. 1998). We considered periods of 20 continuous minutes of activity with intensity counts of 0 as non-wearing time, and these were excluded from the analysis.

Seasonal influences are relatively strong in physical activity assessment (Riddoch et al. 2007), and so to avoid this potential confounder, all the participants were the accelerometer during the same seasonal period: March and April.

All the participants attended two one-hour physical education classes a week. These classes included 10 min to change clothes, 20 min in moderate intensity cardiorespiratory fitness activities, 10 min in moderate intensity muscular fitness activities, 10 min for agility and coordination activities and 10 min for flexibility, cool down and relaxation activities. The program was different from children to adults in the ludic aspect of the activities, but not in the amount or intensity of the exercise. All the physical activity program information (e.g. frequency, intensity, duration, contents and type of exercise offered as educational facilities) was provided by director center, and physical education teachers.

Statistics

The SPSS for Windows (version 19.0) statistical package was used for this study. The Kolgomorov–Smirnov test was used to verify the normality of the variables. Means and standard deviations were used to describe the physical characteristics and all physical activity variables.

A two-way ANOVA was used to examine differences in physical activity level and anthropometric factors among groups of age, gender and obesity status (overweight/obesity vs. normal-weight) with Bonferroni adjustment when needed, and the Chi square test for nominal variables. The level of statistical significance for the study was established at p < 0.05. The effect size (ES) $\pm 90\%$ confidence intervals was calculated according to the formula proposed by Cohen (1992) in all pairwise comparisons in which the differences

reached statistical significance. The magnitude of the ES was interpreted as follows: an effect size lower than 0.2 was considered as small, an effect size around 0.5 was considered as medium and an effect size over 0.8 was considered as large.

Results

Anthropometric characteristics of the study sample are shown in Table 1. Overweight/obesity affected more than half of the adult sample (61.80%), especially the men (73.9%). Overweight/obesity prevalence was significantly higher in adults than in children for the overall sample $(\chi^2 = 15.04; p = 0.001)$ and for males $(\chi^2 = 11.90; p < 0.001)$. Though overweight/obesity prevalence was higher in female adults compared to female children, the difference was not statistically significant (p = 0.132). Overall the adult's and adolescent's sample showed significantly higher values than children's in height (F = 57.5; df = 2; adults: p < 0.001; $ES = 2.4 \pm 0.54$; adolescents: p < 0.001; $ES = 2.36 \pm 0.67$), weight (F = 41.8; df = 2; adults: p < 0.001; ES = 2.28 ± 0.53 ; adolescents: p < 0.001; ES = 1.99 ± 0.63), and BMI $(F = 22.2; df = 2; adults: p < 0.001; ES = 1.71 \pm 0.49; ado$ lescents: p = 0.005; ES = 1.14 \pm 0.56). Similar results were found when comparing men vs. male children and male adolescents, and women vs. female children and female adolescents in height and weight. Nevertheless, male children showed significantly lower values (F=22.5; df=2) vs. male adolescents (p = 0.009; ES = 4.52 ± 2.20) and male adults $(p < 0.001; ES = 9.32 \pm 3.38)$ of BMI, while there were not statistical differences in females. Body fat percentage was significantly higher in females: women (F = 7.5; df = 1;p = 0.0008; ES = 0.99 ± 0.64), adolescent (F = 11.0; df = 1; p = 0.001; ES = 1.68 ± 1.08) and children (F = 4.2; df = 1; p = 0.043; ES = 0.90 ± 0.74).

Children with ASD recorded significantly more moderate to vigorous physical activity (F=3.1; df=2) and steps (F=8.2; df=2) than adults with ASD during the whole week (moderate to vigorous physical activity, p=0.040; ES = 0.59 ± 0.43 ; steps, p=0.001; ES = 1.01 ± 0.44), and steps during weekdays (F=5.5; df=2; p<0.001; ES = 0.95 ± 0.44) and weekend days (F=7.7; df=2; p=0.005; ES = 0.89 ± 0.43). Adolescents showed significantly more total steps than adults (p=0.041; ES = 0.68 ± 0.46) and steps on weekdays (p=0.036; ES = 0.69 ± 0.46). Consequently, adults recorded significantly more sedentary time for the whole week (F=10.2; df=2; p<0.001; ES = 1.10 ± 0.45), weekdays (F=8.5; df=2; p<0.001; ES = 0.99 ± 0.44) and weekend days (F=9.1; df=2; p<0.001; ES = 0.99 ± 0.44) and weekend days

Figure 2 presents physical activity and sedentary behavior for the study sample classified according to the BMI standard definition. Normal-weight and overweight/obesity



Table 1 Anthropometric and descriptive characteristics of study sample according to age and sex

•	•		•)					
	Overall sample (n=78)	= 78)		Male (n = 56)			Female $(n=22)$		
	Children $(n = 29)$ Adolescents $(n = 15)$	Adolescents $(n=15)$	Adults $(n=34)$	Children $(n = 22)$ Adolescents $(n = 11)$	Adolescents (n=11)	Adults $(n=23)$	Children $(n=7)$ Adolescents $(n=4)$	Adolescents $(n=4)$	Adults (n=11)
Age (years)	9.41 ± 1.55	15.20 ± 1.78 *	$31.09\pm6.85^{*\dagger}$	9.41 ± 1.47	15.73 ± 1.74 *	32.39±7.27*†	9.43 ± 1.90	$13.75\pm0.96*$	$28.36\pm5.14^{*}$
Height (m)	139.1 ± 13.0	168.7 ± 10.8 *	$166.8\pm9.8*$	139.9 ± 12.5	$170.5\pm9.9*$	$170.7 \pm 8.7 *$	136.4 ± 15.4	$163.8 \pm 13.2 *$	$158.6\pm6.4^{*\ddagger}$
Weight (kg)	36.26 ± 13.70	$67.92 \pm 18.98*$	$73.07 \pm 17.60*$	36.15 ± 14.28	$68.07 \pm 17.85*$	79.45 ± 15.84 *	36.66 ± 12.73	$67.50\pm24.85*$	$59.73 \pm 13.43 *$
Body fat (%)	21.15 ± 7.62	22.42 ± 10.16	24.54 ± 8.06	19.55 ± 8.03	18.59 ± 6.57	22.12 ± 7.32	$26.18 \pm 2.54 \ddagger$	$32.93 \pm 11.69^{\ddagger}$	$29.59 \pm 7.41^{\ddagger}$
$BMI (kg/m^2)$	18.23 ± 4.25	23.62 ± 5.31 *	26.04 ± 4.73 *	17.96 ± 4.70	$23.20\pm5.11*$	$27.18\pm4.43*$	19.09 ± 2.42	24.78 ± 6.48	$23.65 \pm 4.61^{\ddagger}$
Overweight + obe- 13.80 sity (%)	. 13.80	40.00	61.80*	13.60	27.30	73.90*	14.30	75.0	36.40‡
Obesity (%)	06.90	13.30	14.70	9.10	9.10	17.40	0.00	25.00	9.10
Mean±SD									

*Significant differences vs. children

†Significant differences vs. adolescents

significant differences male vs. female among age groups; p < 0.05

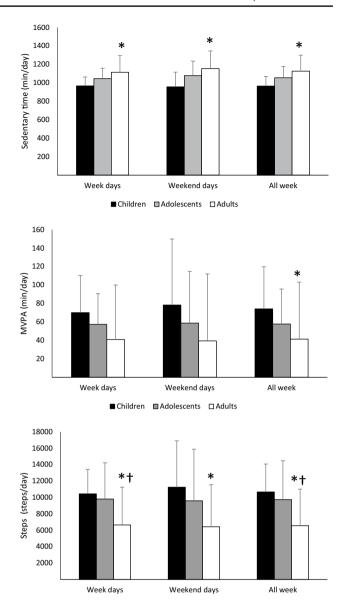
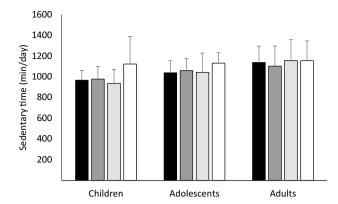


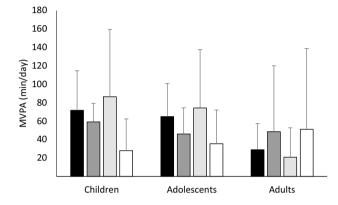
Fig. 1 Sedentary time, moderate to vigorous physical activity (MVPA) and steps per day for children, adolescents and adults for all week, weekdays and weekend days. *Significant differences vs. children, † Significant differences vs. adolescents; p < 0.05

■ Children ■ Adolescents □ Adults

participants reached similar levels of physical activity on weekdays and weekend days (p > 0.05). Nevertheless, we should highlight that if we combine children and adolescents groups, during the weekend, overweight/obese children and adolescents recorded significantly lower levels of moderate to vigorous physical activity (F = 4.4; df = 1; p = 0.034, ES = 0.81 ± 1.28) and steps (F = 7.7; df = 1; p = 0.010, ES = 1.01 ± 1.29) and higher levels of sedentary time (F = 6.2; df = 1; p = 0.008, ES = 1.03 ± 1.30), compared to normal-weight children and adolescents.







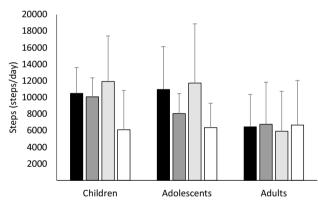


Fig. 2 Sedentary time, moderate to vigorous physical activity (MVPA) and steps per day for study sample classified in normal-weight weekdays (filled black square), overweight/obese weekdays (filled dark gray square), normal-weight weekend days (filled light gray square) and overweight/obese weekend days (open square) for children, adolescents and adults

Normal-weight children were significantly more active than normal-weight adults in light physical activity (all week, F = 5.5; df = 2; p = 0.001, ES = 0.52 ± 1.13 ; week-days, F = 4.4; df = 2; p = 0.003, ES = 0.51 ± 1.13 ; weekend days, F = 4.3; df = 2; p = 0.004, ES = 0.87 ± 0.62), moderate to vigorous physical activity (all week, F = 3.8; df = 2; p = 0.024, ES = 1.11 ± 1.13 ; weekedays, F = 3.4; df = 2; p = 0.036, ES = 0.54 ± 1.13 ; weekend days, F = 4.0; df = 2;

p=0.019, ES = 0.96 ± 0.62) and steps (all week, F=6.0; df=2; p=0.005, ES = 1.04 ± 0.56 ; weekdays, F=5.0; df=2; P=0.014, ES = 1.02 ± 0.57 ; weekend days, F=4.6; df=2; p=0.014, ES = 0.87 ± 0.62). No significant differences were revealed between overweight/obese children' and adults' physical activity behavior (p>0.05).

Discussion

The aim of the investigation was to compare body composition and physical activity levels among children, adolescents and adults with ASD and physical activity and sedentary levels related to anthropometric factors. The main outcomes were: (1) overweight/obesity prevalence was higher in adults compared to adolescents and children, (2) children recorded a higher physical activity level and less sedentary time than adults, (3) during the weekend, normal-weight children and adolescents tend to record more steps, moderate to vigorous physical activity and lower sedentary times, compared to overweight/obese children and adolescents.

Body composition data were significantly higher for adults vs. children, especially in men compared with male children. Due to the greater prevalence of ASD in males than females, females with ASD tend to be understudied (Lai et al. 2012). Unfortunately, that was also the case here, as this study was underpowered to make age comparisons of prevalence of obesity in the female sub-population. Past research suggests ASD confers an equal or greater risk of obesity on children, however, our research found a lower incidence than in typically developing children. The prevalence of obesity in subjects participating in the study was 6.9% in the children, 13.3% in the adolescents and 14.7% for the adults, lower than the prevalence previously reviewed in American ASD children (30.4%) (Curtin et al. 2014).and ASD adults (34.9%) (Tyler et al. 2011). The prevalence of obesity in our sample was also lower than the prevalence in typically developing Spanish children (13.9%) and adults (21.6%) (Aranceta-Bartrina et al. 2016; Serra et al. 2003). In contrast, the overweight/obesity data was considerably higher in our sample for adolescents: 40% and adults: 61.8% compared to the non ASD Spanish population (adolescents: 21.8%; adults: 39.3%) (Aranceta-Bartrina et al. 2016; Serra et al. 2003). Children with ASD are at risk for obesity at the same or a higher rate than children generally. However, this population is more susceptible to typical risk factors associated with insufficient physical activity (Curtin et al. 2005) and atypical eating patterns, showing more food selectivity with preferred energy dense foods (Schreck and Williams 2006). These results may be due to the fact that the participants in the study were institutionalized in specific educational facilities. All the participants followed a controlled physical exercise program with a 1-h session, twice a week,



including activities for improving flexibility, cardiorespiratory and muscular fitness, focusing on the development of basic motor skills. This issue can be related to an increased level of motor skills, affecting their ability to participate in sports or physical activity programs successfully, and maybe explaining the reduction in these data in our sample. Nevertheless, this can be helpful for obesity avoidance but is not sufficiently effective for overweight prevention. Another limitation of this study affecting generalizability is that secondary conditions and medications of the participants, which could affect the activity and anthropometric factors were not reported to us. One group previously found that psychotropic medications like stabilizers, antipsychotics, antiepileptic drugs, and selective serotonin reuptake inhibitors were associated with obesity in ASD (Corvey et al. 2016), while another group found that controlling for medication use removes any associations of ASD with weight and physical activity (Shedlock et al. 2016). Therefore, it is possible that some of the differences attributed to age that we report, or in our population vs. other populations, could reflect differences in medications.

Children recorded more physical activity levels and less sedentary time than adults did. The decline in physical activity with age has been reported in people without disabilities (Laguna et al. 2013), although this phenomenon is well documented, it is not known whether the mechanism of the decline is environmental or biological (Sallis 2000); nevertheless, to date little is known about the age-related decline in ASD physical activity habits. This fact establishes the need for longitudinal studies to understand the effects of environment, ASD severity, and age on physical activity levels in ASD.

Physical activity levels are considerable lower than those reported for the Spanish population. Our data in moderate to vigorous physical activity showed 460.05 ± 267.71 min/ week against 616.6 ± 578.08 min/week for typically developing children and 287.15 ± 413.09 min/week in our sample to 564.4 ± 509.3 min/week for Spanish adults (Mielgo-Ayuso et al. 2016). Limited physical activity levels in ASD individuals may be attributed to their impairments in motor, social communication, sensory and behavioral domains (Srinivasan et al. 2014), as they prefer to enroll in more solitary physical activities such as cycling and swimming (Potvin et al. 2013). In addition, social communication impairments could limit their abilities to engage in group sports, similarly, behavioral problems or preferences such as restricted interests, inflexible schedules and preference for predictable, structured activities may limit physical activity choices (Borremans et al. 2010). Moreover, as children get older, games get more complex and children with ASD may have trouble following the rules, and the tactics or strategy in sports. Due to the lack of positive experiences, they are more frequently prone to failure that can lead to low self-esteem and competency in physical activity (Memari et al. 2013). Nevertheless, there is considerable evidence that exercise interventions are effective in improving motor, social and behavioral skills in individuals with ASD (Sowa and Meulenbroek 2012). These findings reinforce the importance of developing targeted intervention in physical activity promotion.

Higher levels of physical activity in non-overweight than overweight/obese individuals have been demonstrated in typically developing children (Laguna et al. 2013) and adults (Maher et al. 2013). Our data reflect similar results in ASD individuals, with higher values of weekend days steps for normal-weight children and adolescents compared to overweight/obese children and adolescents. There is a paucity of research on physical activity levels in ASD according to obesity status, future studies are needed to clarify this relationship in ASD individuals. This prevents us from contrasting the results with a similar population. In this sample we found higher differences during the weekend, indicating a tendency in overweight/obese children to be less active than non-obese children at times when activity was more likely to be determined by free choice, reflecting similar results to those found in children without ASD (Page et al. 2005). These findings suggest that the home environment is closely associated with reduced levels of physical activity, supporting the use of family-based intervention to increase physical activity in overweight/obese ASD children and adults (Hinckson et al. 2013).

A particular strength of our study lies in the objective measurement of physical activity using an accelerometer, as there is a paucity of data regarding physical activity habits of ASD individuals, especially in the adult population. However, several limitations should be taken into consideration. The observations are limited by the lack of a control group with non-ASD children and adults, and the cross-sectional design nature, so the direction of causality cannot be determined. Every attempt was made to obtain a homogeneous sample of ASD children and adults; even so differences in symptoms severity, verbal abilities, social skills, behaviors, cognitive abilities, and medications were not evaluated and might have influenced findings. The aforementioned factors and the fact that they were institutionalized subjects engaged in a physical activity program could potentially confound the observed associations, since ASD individuals show variability that may interfere with anthropometric characteristics or physical activity level. Further research is needed to understand the impact of this kind of variables on physical activity behavior in ASD individuals. We should also emphasize that the sample size is relatively small, especially for women and girls, studies with larger samples of females are necessary to examine gender effects on body composition and obesity in ASD. In the future, we should also evaluate secondary conditions and medication of the participants, include a typically developing control group or a not institutionalized



group, in order to find an adjusted impact in weight and related activities.

To summarize, our study suggested that in our sample obesity status in ASD increases with age, especially in men. Children and adolescents that participated in the study tend to be more active and less sedentary than their adult counterparts. During the weekend, our sample of normal-weight children and adolescents tend to record more steps, moderate to vigorous physical activity and lower sedentary times, compared to overweight/obese children and adolescents. Our investigation also showed the need to focus these efforts in improving and enhancing physical activity opportunities in clinical environments and outside school, especially during the weekend in both, ASD adults and overweight/obese children. Longitudinal and/or clinical intervention studies are needed to contrast and confirm these results.

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Author Contributions DR head of the research project. She obtained funding and contacted the director centers to obtain authorization. Coordination of the data acquisition and data interpretation. Reviewing and contributing in the drafting of the manuscript. CIT psychologist specialized in ASD disorders. She assisted the team in the design of the project and collaborated in data acquisition. Reviewing and contributing in the drafting of the manuscript. JJS specialized in statistics. He assisted the team in the design of the project and in data interpretation and statistics. Reviewing and contributing in the drafting of the manuscript. TG specialized in obesity and physical activity assessment. She assisted the team in the design of the project, physical activity and anthropometrics data acquisition and interpretation. First author and creator of the first version of the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed were in accordance with the ethical standards of the Camilo Jose Cela University Review Board and with the 1964 Helsinki declaration and its later amendments.

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