Title:

Sedentarism, physical activity, steps, and neurotrophic factors in obese children

Authors' names:

Jose Mora-Gonzalez[1*], Jairo H. Migueles[1], Irene Esteban-Cornejo[2,1], Cristina Cadenas-Sanchez[1], Belén Pastor-Villaescusa[3,4], Pablo Molina-García,[1] Maria Rodríguez-Ayllon[1], María C. Rico[4], Angel Gil[4,5,6], Concepción M. Aguilera[4,5,6], María Victoria Escolano-Margarit,[7] Anne Kaer Gejl[8], Lars Bo Andersen[9], Andrés Catena[10], Francisco B. Ortega[1].

Authors' affiliations:

[1]PROFITH "PROmoting FITness and Health through physical activity" Research Group, Department of Physical and Sports Education, Faculty of Sports Science, University of Granada, Spain. [2]Department of Psychology, Northeastern University, Boston, Massachusetts, USA. [3]LMU - Ludwig-Maximilians-Universität München, Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, Univ. of Munich Medical Center, Munich, Germany. [4]Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix", Center of Biomedical Research, University of Granada, Granada, Spain. [5]Instituto de Investigación Biosanitaria ibs. Granada, Granada, Spain. [6]CIBER Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Madrid, Spain. [7]Hospital Universitario San Cecilio, Granada, Spain. [8]Department of Physiotherapy and Occupational Therapy, University College Copenhagen, Copenhagen, Denmark. [9]Western Norway University of Applied Sciences, Campus Sogndal, Faculty of Education, Arts and Sport, Norway. [10]Department of Experimental Psychology, Mind, Brain and Behaviour Research Centre (CIMCYC), University of Granada, Granada, Spain.

*Corresponding author: Jose Mora-Gonzalez, Department of Physical and Sports Education, Faculty of Sports Science, University of Granada; Carretera de Alfacar, 21. Granada 18071, Spain; +(34) 958 24 66 51, fax: +(34) 958 24 94 28; email: jmorag@ugr.es

ABSTRACT

Purpose: This study aimed to examine the associations of sedentary time, physical activity (PA) and step-related behaviors with neurotrophic growth factors. **Methods:** A total of 97 children with overweight/obesity aged 8-11 years participated in this study. Sedentary time, PA, and steps were measured by GT3X+ accelerometers in hip and non-dominant wrist. Estimates of light, moderate, vigorous, and moderate-to-vigorous PA (MVPA) were obtained. Steps/daytime, peak 60-, 30-, and 1-min cadence were computed. The time accumulated (min/day) in different cadence bands of steps was also computed from hip accelerometer. Plasma levels of brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and insulin growth factor-1 (IGF-1) were determined by the XMap technology (Luminex IS 100/200 system, Luminex Corporation, Austin, TX). **Results:** Light PA, moderate PA, MVPA and the peak 60-min cadence were positively related with BDNF concentrations (all $P<0.05$), and only light PA to VEGF ($P=0.048$). No association was observed for IGF-1 ($P>0.05$). The associations of light PA with BDNF and VEGF disappeared (all $P>0.05$) after performing analyses with non-dominant wrist-placement data. However, moderate PA and MVPA remained significantly associated with BDNF (both $P<0.05$). The time accumulated in cadence bands of 40-59 steps/min and 60-79 steps/min (i.e., walking at slow pace) was positively associated with plasma BDNF (all $P<0.05$). **Conclusion:** In conclusion, PA is positively related to plasma BDNF, whereas no relationship was observed for VEGF or IGF-1. Higher amounts of time spent in slow walking cadence bands could increment BDNF levels. Exercise-based randomized controlled trials in children with overweight/obesity should be carried out to better understand the influence of PA behaviors on the neurotrophic factors.

Key words: accelerometry; BDNF; cognition; obesity; walking; youth

INTRODUCTION

Childhood obesity has shown to be negatively related to cognitive functions and detectable structural abnormalities in the brain (2,1). Likewise, obesity may also influence stored and circulating neurotrophic factors such as brain-derived neurotrophic factor (BDNF) in humans (3), although literature to this respect is inconsistent (4). It has been observed that BDNF missense mutations in its receptor, TrkB, have been associated with weight gain both in humans (4). Further, evidence have shown a significant reduction of circulating BDNF levels in children with obesity compared to normal-weight peers (5,6). Importantly, BDNF plays a key role in synaptic plasticity, neuronal transmission, and cell growth and survival throughout the cortex (7). This factor is produced in the brain and in selected peripheral tissues such as platelets (8). Platelets are the major non-neural source of BDNF from which it reaches plasma and is able to pass the blood-brain barrier, only when it is not bound to platelets (10,9). Interestingly, the positive correlation between BDNF in the brain and circulating BDNF suggests that circulating BDNF levels may reflect the levels in the central nervous system (11). Furthermore, BDNF may be released from the brain to the periphery during the practice of physical activity (PA) (12). Apart from BDNF, other neurotrophic factors such as vascular endothelial growth factor (VEGF) or insulin growth factor-1 (IGF-1) are important for neural growth and neuron survival (13). Hence, it seems of relevance to examine how protective environmental factors, such as lifestyle behaviors (e.g., sedentarism or PA), may influence neurotrophic factors in a particularly vulnerable population such as children with overweight/obesity.

Emerging evidence suggest that PA has a beneficial effect on the brain and cognitive processes in children (14). Neurotrophic factors have been suggested as potential mechanisms underlying this relationship (15). From all these factors, BDNF

may be the most important one that has been suggested to be upregulated by PA (13). Indeed, BDNF may play a crucial role in the PA's influence on brain structure and as an underlying factor of the PA-induced cognitive improvement. However, in humans, there is inconsistent evidence on the role of PA on neurotrophic factors (19,18,17,16). PA may increment serum BDNF concentrations in adolescents (19,16) and adults (18), although there are other studies showing a negative association between PA and BDNF (17). In children, to the best of our knowledge, there are only two observational studies and they did not find significant associations (20,21). However, no previous cross-sectional studies have focused on obese children nor have analyzed the role of step-related behaviors on neurotrophic factors. In addition, the BDNF plays a key role in the energy homeostasis and the appetite regulation (22), which highlights even more the importance of examining the potential relationship of sedentary time and PA with brain in the context of obesity during childhood. Particularly, walking (hereinafter step-related behaviors) is the most popular PA behavior, as well as the ideal PA intervention to improve health across sedentary populations, such as the obese ones (23,24). Thus, the aim of the present study was to analyze the association of sedentary time, PA and step-related behaviors with BDNF and other neurotrophic factors (i.e., VEGF and IGF-1) in children with overweight/obesity.

METHODS

Participants. The present cross-sectional study was developed under the framework of the ActiveBrains project (http://profith.ugr.es/activebrains) (25). A total of 110 children with overweight/obesity aged 8–11 years were recruited from Granada (Spain) after meeting the defined inclusion criteria, which have been described elsewhere (25). The study was conducted in three waves. The present cross-sectional analyses used baseline data from 97 children with overweight/obesity (10.0 ± 1.2 years old; 58% boys)

with complete baseline data on sedentary time, time-based PA, steps-related behaviors, and neurotrophic factors. For VEGF analyses, a sample of 88 participants was used after excluding those children with lower VEGF levels than the kit could detect. The baseline data collection took part from November 2014 to February 2016.

A description of the purpose and characteristics of the study was given to the parents or legal guardian and written informed consent was provided by them allowing the child to participate. The ActiveBrains project was approved by the Ethics Committee on Human Research of the University of Granada, and was registered in ClinicalTrials.gov (identifier: NCT02295072).

Sedentary time, physical activity, and steps metrics. Sedentary time, PA, and step-related behaviors were assessed by accelerometer (GT3X+, ActiGraph, Pensacola, FL, USA) taking into account the latest advances in data processing (26). Children wore simultaneously two accelerometers located on the right hip and non-dominant wrist for 7 consecutive days (24h/day). They were instructed to remove them only for water activities (i.e., bathing or swimming) and to record waking-up and sleep onset times during the 7 days on a diary. Raw data were collected at a sampling frequency of 100 Hz were loaded in ActiLife (ActiGraph, Pensacola, FL, USA) and processed then in R (v. 3.1.2, https:// www.cran.r-project.org/) using the GGIR package (v. 1.6-0, https://cran.r-project.org/web/packages/GGIR/) (27). We calculated the Euclidean Norm Minus One *G* metric (ENMO, 1 *G* ~ 9.8 m/s2) after auto-calibrating the acceleration signal (27,28). The mean of ENMO with negative values rounded to zero was calculated over 5 s epochs. Simultaneously, we derived the number of steps/minute (step cadence) from the hip-worn accelerometer using the ActiLife software. Then, we imported steps information to R for further analyses in the GGIR package.

Accelerometric information processing in GGIR consisted in: a) Non-wear time detection by the Van Hees et al. approach (29). b) Detection of abnormally and sustained high acceleration values (i.e., clipped time). c) Replacement of the non-wear and clipped time by the mean acceleration recorded within the same time frame for the rest of the measurement (29). A replacement by 0 for all metrics was performed if no data were collected for a specific time frame for the rest of the days. d) Identification of waking and sleeping hours based on an automatized algorithm guided by the diaries completed by the participants (30). The inclusion criterion for a valid day was wearing the accelerometer ≥16 h/day. A minimum of 4 valid days (3 weekdays and 1 weekend day) per week was required to be included in the analyses. The compliance wearing the accelerometer was high, with 98% of the sample wearing the accelerometers for ≥6 days.

Sedentary time and PA were classified into different intensities following Hildebrand et al. hip- and wrist-based cut-off points for the ENMO metric (31,32). Since ActiGraph's step detection algorithm is adapted to the hip location, the main analyses of the present study were performed using hip data, although analyses for sedentary time and PA were replicated using estimates from the non-dominant wrist-worn accelerometer and presented as supplementary material.

The PA variables included in this study were total minutes per day at light, moderate, vigorous, and MVPA for hip and wrist. With regards to steps, the volume of steps/day and the peak 60-, 30-, and 1-min cadences were computed following previously published procedures (33). We also derived time spent in the following cadence bands intensities (i.e., steps/min): 0 (Non-movement), 1–19 (Incidental movement), 20–39 (Sporadic movement), 40–59 (Purposeful movement), 60–79 (Slow walking), 80–99 (Medium walking), 100–119 (Brisk walking), and 120+ steps/min (Faster locomotor movements, e.g., running) (33).

Neurotrophic factors. Blood samples were obtained for biochemical and hematological screening tests between 08.30 AM and 10.30 AM after a minimum of 8 hours overnight fasting condition at the San Cecilio University Hospital and the Virgen de las Nieves Maternity Hospital (Granada, Spain). All participants had up to 11 ml of blood drawn from the antecubital vein. The blood for plasma samples was drawn into tubes containing ethylenediaminetetraacetic acid (EDTA) and kept on ice for around 60 min. After collection and transportation of the samples, they were centrifuged (10 min at 4°C, 1000xg), aliquoted under cold conditions by ice, and immediately stored at -80°C in the Center of Biomedical Research (Granada, Spain) until analysis.

The analysis of mature BDNF, VEGF, and IGF-1 levels in plasma was performed using the Luminex IS 100/200 system (Luminex Corporation, Austin, TX, USA) with the XMap technology and using human monoclonal anti-bodies (Milliplex Map Kit, Millipore, Billerica, MA, USA). For mature BDNF, we used the Human Neurodegenerative Disease Magnetic Bead Panel 3 (Catalog #HNDG3MAG-36K; EMD Millipore Corporation, Billerica, MA, USA); for VEGF, we used the Human Angiogenesis/Growth Factor Magnetic Bead Panel (Catalog #HAGP1MAG-12K; EMD Millipore), and for IGF-1, we used the Human IGF-1, II Magnetic Bead Panel (Catalog #HIGFMAG-52K; EMD Millipore). In the Luminex IS 100/200 system, assay sensitivities or minimum detectable concentrations for BDNF, VEGF-A, and IGF-1 assays were 0.23 ng/ml, 8.1 pg/ml, and 15 ng/ml, respectively. Those samples not reaching the minimum detectable were excluded from the analyses. The intra-assay % coefficient of variation for BDNF, VEGF-A, and IGF-1 was estimated to be <5.4, 3.5 and 10, respectively, and inter-assay at <5.3, 10, 15, respectively.

Potential confounders. After testing with correlation analyses which of the variables could be a potential confounder, sex, peak height velocity (PHV), fat mass

177 index, wave of participation, and the parental educational level were used as potential
178 confounders in the analyses. PHV is an indicator of maturity offset during childhood and
179 adolescence (34). We used age and anthropometric variables (height –girls- and seated
180 height -boys-) to calculate PHV following Moore's equations (34). The difference in
181 years between PHV and chronological age was defined as a value of maturity offset. Fat
182 mass index (kg/m^2) was assessed by Dual-energy X-ray absorptiometry (DXA, Discovery
183 densitometer from Hologic). Wave of participation was a categorical variable according
184 to the first moment of participation of each child in the study (wave 1, 2, or 3). Parental
185 educational level was assessed by a self-reported questionnaire completed by parents, and
186 we combined responses of both of them as: neither had a university degree; one had a
187 university degree; or both had a university degree (35).

188 **Statistical analysis.** The characteristics of the study sample are presented as
189 means and standard deviations (SD) or percentages. Non-normally distributed outcomes
190 are presented as median and interquartile range (IQR). Prior to all analyses, the extreme
191 values were winsorized to limit their influence; this was done by replacing raw scores
192 with less than the 1^{st} percentile of the cohort-wide distribution with the value of the 1^{st}
193 percentile and replacing scores greater than the 99^{th} percentile with the 99^{th} percentile
194 value (36). Furthermore, all outcomes were checked for normal distribution and BDNF,
195 VEGF, and IGF-1 were normalized since they showed skewed distributions. Interaction
196 analyses were performed between sex and sedentary time, PA and steps-related behaviors
197 on the neurotrophic factors. No significant interactions with sex were found ($P \geq 0.10$);
198 therefore, analyses were performed for all the participants together.

199 Linear regression analyses were performed to examine the association of
200 estimations from hip-worn accelerometers of sedentary time, PA, and step-related
201 behavior with neurotrophic factors (i.e., BDNF, VEGF and IGF-1) adjusting by potential

confounders. Sedentary time and PA analyses were replicated for the non-dominant wrist-placement data. We also performed linear regression analyses to examine the association between time accumulated (min/day) in different cadence bands of 0, 1–19, 20–39, 40–59, 60–79, 80–99, 100–119, and 120+ steps/min and the BDNF, adjusting by potential confounders. We performed collinearity diagnosis between physical activity intensities and between step cadences. No multi-collinearity was observed among any of the independent variables (variance inflation factor, VIF < 10). A significance level of $P<0.05$ was used. All the statistical procedures were performed using the SPSS software for Mac (version 22.0, IBM Corporation).

RESULTS

Descriptive characteristics of the sample are shown in **Table 1**. Times accumulated at different cadence bands are shown in **Table 2**. A significant association was found between light PA, moderate PA, MVPA, and peak 60-min steps cadence with BDNF (β ranging from 0.195 to 0.242, all $P<0.037$) (**Table 3**). An association was also found between light PA and VEGF ($\beta=0.207$, $P=0.048$). No significant associations were found for the relationship of sedentary time with any of the neurotrophic factors nor for the relationship between PA, step-related behaviors, and IGF-1 ($P>0.05$). When performing analyses with non-dominant wrist-placement data (**Table S1**), the associations of light PA with BDNF and VEGF disappeared (all $P>0.05$). However, moderate PA and MVPA remained significantly associated with BDNF ($\beta=0.220$, $P=0.041$ and $\beta=0.246$, $P=0.027$, respectively). An association was also observed between vigorous PA and BDNF ($\beta=0.244$, $P=0.032$).

Figure 1 shows the relationship between time accumulated at different steps cadence bands and BDNF, adjusting for potential confounders. Among all the cadence

bands, a significant association was found for the time spent in the 40-59 steps/min cadence band (i.e., Purposeful movement) and the time spent in 60-79 steps/min cadence band (i.e., Slow walking) with BDNF (β=0.198, P=0.044, and β=0.205, P=0.040, respectively).

DISCUSSION

The main finding of the present study was that objectively-measured PA and step-related behaviors, but not sedentary time, were positively associated with BDNF in children. Particularly, light PA, moderate PA, MVPA, and peak 60-min steps cadency were related to BDNF, being the associations of moderate PA and MVPA consistent from either hip or wrist accelerometer data. No significant associations were found between PA and steps with VEGF and IGF-1, apart from the borderline association observed between light PA and VEGF. No association was found between sedentary time and the neurotrophic factors. In addition, the time spent in purposeful movements (i.e., 40–59 steps/min) and slow walking (i.e., 60–79 steps/min) was associated with BDNF. Our findings suggest that different intensities and types of PA, mainly moderate and MVPA and walking at slow-medium cadences may increase plasma BDNF levels in children with overweight/obesity. However, these findings must be interpreted with caution due to the methodological limitations when measuring neurotropic factors (37), as well as to the complexity of PA analyses and the emerging variety of methods to analyze it (26).

To the best of our knowledge, this is the first study that analyzes the association between objectively-measured sedentary time, PA and step-related behaviors with neurotrophic factors (i.e., BDNF, VEGF and IGF-1) in a sample of children with overweight/obesity. Only two observational studies in healthy normal-weight children have previously analyzed this relationship. In line with our results, Gabel et al. did not

250 find any association between sedentary time and plasma BDNF levels in 7–10-year-old
251 children (21). In contrast to our cross-sectional results, a recent 2-year longitudinal study
252 did not find a relationship between objectively-measured PA and serum BDNF in children
253 aged 8–11 years (20). When analyzing steps, our positive results between the peak 60-
254 min cadence and BDNF are in contrast to the negative associations found by another
255 study in adults (38). The inconsistency and contradictory findings regarding the
256 relationship between PA, steps and BDNF might be due to differences between studies
257 with respect to the sample's characteristics (i.e., overweight/obese versus normal weight
258 peers); the age group analyzed (i.e., children versus adolescents or adults); the study
259 design (i.e., cross-sectional versus longitudinal); and the methodology followed for
260 assessing and processing PA (i.e., objective versus subjective methods) or for analyzing
261 the neurotrophic factors levels (i.e., differences regarding kits used, pre-storage
262 treatments of blood samples –clotting/icing time, centrifugation strategy– or the way
263 BDNF is measured in peripheral blood –plasma BDNF versus serum BDNF). With
264 respect to the differences in BDNF measurements, much higher concentrations of BDNF
265 has been observed in serum in comparison to plasma (8,39). On one hand, the clotting
266 time methodology chosen can be critical for serum BDNF levels (8,40). On the other
267 hand, plasma is obtained from blood samples drawn into tubes containing anti-coagulants,
268 preventing coagulation and thereby activation of platelets and BDNF release. Due to the
269 smaller amount of platelet-associated BDNF in plasma, BDNF measured in plasma may,
270 to a higher extent than serum BDNF, reflect the concentration of free BDNF. However,
271 there is still a need to better understand how much it reflects brain levels and how it relates
272 to PA.

273 Despite findings from most observational studies suggest an inverse relationship
274 between PA and peripheral BDNF levels (18), the positive associations found in our study

275 are supported by previous literature focusing on the effects of physical exercise on BDNF
276 in humans (18,41). Particularly, two studies analyzed the changes in children's BDNF
277 level after a lifestyle intervention which included an exercise component. Corripio et al.
278 (42) observed that BDNF in plasma was increased in prepubertal obese children after a
279 2-year lifestyle intervention which included 30 to 45 min of moderate exercise 3 times
280 per week. On the contrary, another study did not find any significant change in serum
281 BDNF in children of different weight loss after one-year exercise therapy (i.e., physical
282 games) once per week (6). In adults, a recent meta-analysis showed that both acute and
283 regular programmed exercise had a significant impact on BDNF concentrations,
284 reflecting a moderate and small effect size (Hedges'*g*=0.46, P<0.001; and
285 Hedges'*g*=0.28, P=0.005, respectively for acute and regular exercise intervention studies)
286 (41). Another study found that the impact on adult's BDNF levels might be exercise
287 intensity-dependent (19). In fact, we observed a significant association between vigorous
288 PA and BDNF when the wrist-location data was used. No information is yet available
289 regarding which accelerometer-location is more valid and reliable in children (26), what
290 highlight the need of reporting both hip and wrist data whenever this is feasible. In our
291 study, moderate PA and MVPA intensities were consistently associated with BDNF when
292 using either hip or wrist PA data. This fact suggest that a moderate intensity of PA could
293 be a higher stimulus for children with overweight/obesity to increase BDNF levels.
294 However, further investigations are needed in order to clarify the effects of different PA
295 intensities accelerometer-locations on neurotrophic factors.

296 Another interesting finding of this study was the consistently (with both hip and
297 wrist data) no significant associations between sedentary time, PA and steps with VEGF
298 and IGF-1 (only a borderline association was found between light PA and VEGF).
299 Although BDNF, VEGF and IGF-1 are all considered neurotrophic factors and have

several characteristics in common, each of them has a different functionality. Whereas BDNF is an important nerve growth factor that facilitates the growth and survival of various neurons and regulates synaptic plasticity (7), both VEGF and IGF-1 contribute to the stimulation of angiogenesis and hippocampal neurogenesis (13). Thus, the influence of PA may be different depending on the factor, what could explain the significant associations found for BDNF and the non-associations for VEGF and IGF-1.

When analyzing which of the steps cadence bands were associated to BDNF, we observed a significant association of the time accumulated in purposeful movement (i.e., 40–59 steps/min) and in slow walking (60–79 steps/min) with BDNF. In this regard, walking is the most popular PA behavior, as well as the ideal PA intervention to be recommended to improve health across sedentary populations, such as the one of the present study (23,24). Additionally, the fact that our sample only accumulate an average of 7.8 min/day in bands over 100 steps/min limits the possibility to detect any significant relation between these high cadences and BDNF. To the best of our knowledge, no previous studies have analyzed the relation between time in different cadence bands and neurotrophic factors. The cadence bands appearing significantly associated to BDNF in children with overweight/obesity could be considered as bands of light PA. This, together with the fact that we also found an association between light PA and BDNF, may suggest that light activities such as walking may be enough to increase levels of the BDNF in children with overweight/obesity. In fact, children with overweight/obesity have shown a higher metabolic cost when walking at same speeds in comparison with normal-weight peers (43). This fact may indicate that children with overweight/obesity could be more sensible to neurophysiological changes at lower absolute intensities, yet the relative intensity (e.g., % of maximal heart rate) might be similar to higher cadences conducted by leaner children. Additionally, obese children do not achieve cadences that are as high

as those reached by either overweight or normal-weight children, and therefore it may be difficult to investigate whether high cadences are associated with neurotrophic factors (33,43). Taking into account the difficulties to perform physical activities of higher intensity for this type of population, walking may be of help to increase total PA levels and health (24), and therefore have neurotrophic benefits (44).

Several explanations have been suggested in order to physiologically explain our associations between PA and BDNF.(18) First, BDNF can pass through the blood-brain barrier in both directions (10), and it may be speculated whether peripheral BDNF circulating in blood is more efficiently uptaken or released by the brain or platelets in physically active individuals (12). However, this must be interpreted with caution since platelets cannot pass the blood-brain barrier, and at least 80% of the BDNF in plasma comes from platelets (8). Second, exercise may have beneficial effects on platelet function, being platelets a main storage for peripheral BDNF (45). Third, aerobic exercise increases hippocampal levels of BDNF in animals (13). Animal models have also shown that BDNF can pass the blood-brain barrier from the brain to the plasma (10), and it is likely that exercise cause a production of BDNF in human brain. All these neurobiological mechanisms may explain the association of PA and steps with BDNF in the present study. However, further studies are needed to elucidate the underlying mechanisms on the association between PA and BDNF.

Caution must be applied when interpreting our findings due to several limitations. Firstly, the cross-sectional design does not allow inferences about causality to any of the associated outcomes. Secondly, plasma BDNF bound to platelets cannot cross the blood-brain barrier (46) and therefore the BDNF level in the brain may be rather reflected by the amount of free BDNF in plasma (not bounded to platelets) (39). Further, normal plasma still contains a large number of platelets after centrifugation, and since BDNF is

released from platelets due to activation (e.g. when a blood vessel is punctured), this fact may highly affect the level of BDNF in plasma measured in vitro (8). Thirdly, in our study we used a statistical approach to analyze PA that has been previously used in the literature focusing on neurotrophic factors and that allows us to make direct comparisons with previous studies. However, nowadays it is complex to choose a way to analyze PA, what is reflected in the wide variety of statistical approaches to analyze PA in the literature. Many of these ways to analyze PA should be performed when a large sample size is available, as they require all predictors (i.e., sedentary time, light physical activity, moderate physical activity and vigorous physical activity) coexisting in the same model, therefore decreasing the degrees of freedom and, also, the statistical power. Our relatively small sample (N = 97) discourage any attempt of applying statistical models requiring larger sample sizes to answer these questions. Thus, in order to find a consensus and clarify which is the best method to analyze PA, future studies using larger sample sizes should address different type of PA analysis when analyzing its association with neurotrophic factors. On the other hand, the main strength of this study was its novelty, being the first study to investigate the relationship between sedentary time, PA and steps with neurotrophic factors in a sample of children with overweight/obesity. Additional strengths include the objective measurements of sedentary time, PA and steps using raw accelerations in two different locations (hip and non-dominant wrist) and the use of the most advanced technology to analyze neurotrophic factors (i.e., Luminex 200).

CONCLUSION

The results of the present study suggest that light to moderate PA intensity and step-related behaviors, but not sedentary time, are positively associated to BDNF in children with overweight/obesity. Moderate PA and MVPA seem to be consistent in the association with BDNF regardless of the accelerometer location. Particularly, the time

spent in walking at slow cadences may be stimulus enough to influence the levels of BDNF in children with overweight/obesity. No associations were found between PA, sedentary time, and VEGF and IGF. Importantly, we revealed for the first time that light PA, moderate PA, MVPA and time spent in walking at slow cadences, but not sedentary time, were associated with BDNF in children with overweight/obesity. These findings shed light on that children in an overweight/obesity status may have more room for BDNF increments induced by physical activity. Further, walking at slow cadences may be stimulus enough for this population to influence levels of BDNF. Result from the present study must be interpreted with caution taking into account the limitations and variety of methods used to measure neurotrophic factors and to analyze PA. Thus, further studies using other methods must confirm or contrast our results.

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CONFLICT OF INTEREST

The results of the present study do not constitute endorsement by ACSM. We declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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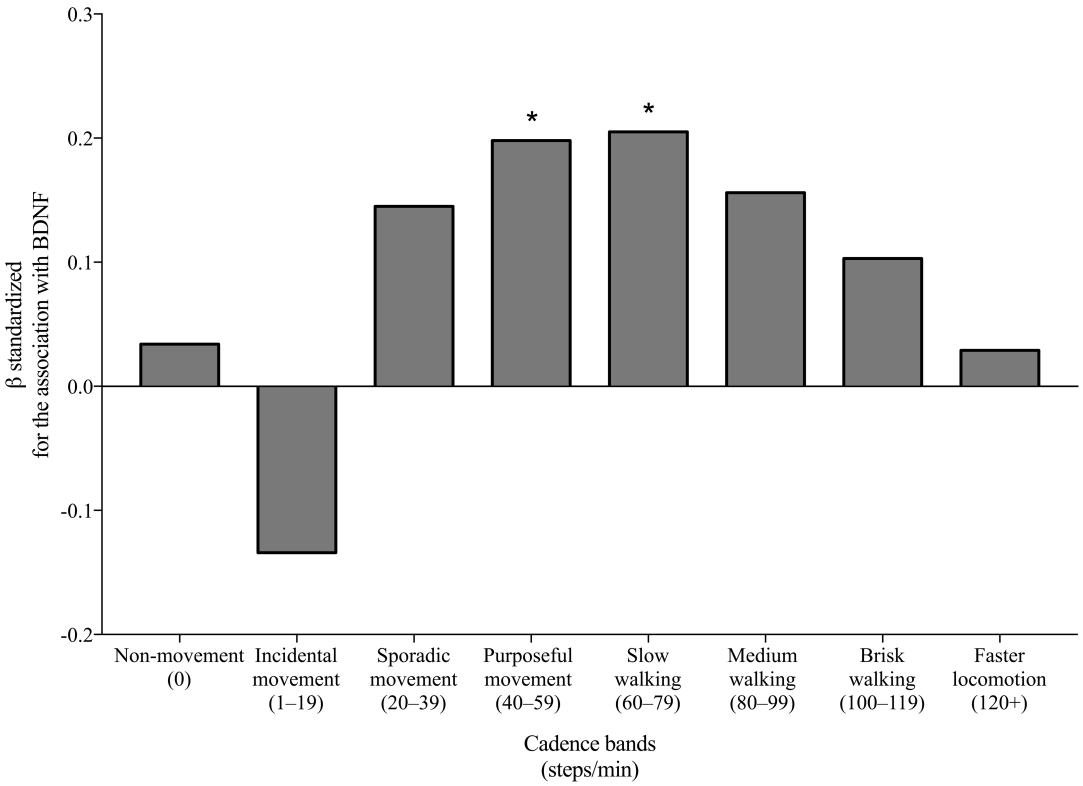

Figure 1. Relationship between time (min/day) accumulated at different cadence bands and levels of brain-derived neurotrophic factor (BDNF).

These analyses were adjusted for the following covariates: sex, peak height velocity, fat mass index, wave of participation and parental educational level. The asterisk (*) is used to highlight significance level at $P<0.05$.

Table 1. Descriptive characteristics of the study sample.

	All (n=97)	Boys (n=56)	Girls (n=41)
Physical characteristics			
Age (years)	10.1 ± 1.2	10.2 ± 1.2	9.9 ± 1.1
Peak height velocity offset (years)	-2.3 ± 1.0	-2.7 ± 0.8	-1.7 ± 1.0
Weight (kg)	56.0 ± 10.7	56.6 ± 10.3	55.2 ± 11.2
Height (cm)	144.1 ± 8.4	144.6 ± 7.9	143.5 ± 9.1
BMI (kg/m^2)	26.8 ± 3.5	26.9 ± 3.6	26.6 ± 3.4
BMI categories (%)*			
Overweight	24	23	24
Obesity grade I	46	50	42
Obesity grade II	21	16	27
Obesity grade III	9	11	7
Fat mass index (kg/m^2)	11.8 ± 2.8	11.5 ± 2.8	12.1 ± 2.8
Wave of participation (%)			
First	16.5	10.7	24.4
Second	42.3	50.0	31.7
Third	41.2	39.3	43.9
Parental university level (%)			
None of them	64.9	71.4	56.1
One of them	17.5	14.3	22.0
Both of them	17.5	14,3	22.0
Sedentary time (min/day)	818.3 ± 45.6	812.2 ± 44.1	826.7 ± 46.9
Physical activity (min/day)			

Light PA	65.3 ± 15.5	67.6 ± 15.1	62.1 ± 15.7
Moderate PA	32.4 ± 13.6	36.8 ± 14.5	26.4 ± 9.6
Vigorous PA	3.0 ± 2.1	3.7 ± 2.2	2.1 ± 1.4
MVPA	35.4 ± 14.1	40.5 ± 16.0	28.5 ± 10.6
Steps			
Volume (steps/day)	8588.6 ± 2176.7	9163.9 ± 2416.0	7802.9 ± 1499.5
Peak 60-min cadence (steps/min)	63.2 ± 13.1	66.1 ± 14.3	59.2 ± 10.2
Peak 30-min cadence (steps/min)	77.5 ± 14.0	79.5 ± 15.0	74.7 ± 12.1
Peak 1-min cadence (steps/min)	111.0 ± 13.0	111.2 ± 13.5	110.9 ± 12.5
Neurotrophic factors (median (IQR))			
BDNF (ng/ml)	3.0 (4.4)	3.0 (4.1)	2.9 (6.1)
VEGF (pg/ml)†	35.4 (35.9)	37.7 (34.8)	34.5 (51.1)
IGF-1 (ng/ml)	86.6 (38.8)	80.2 (39.4)	90.8 (52.7)

Values are means ± standard deviations, unless otherwise indicated. PA=Physical activity; MVPA=Moderate-to-Vigorous Physical Activity; BDNF=Brain-derived neurotrophic factor; VEGF=Vascular endothelial growth factor A; IGF-1=Insulin-like growth factor-1; BMI=body mass index. Sedentary time, physical activity and step-related behaviors were obtained from the Euclidian norm minus one metric in hip. *BMI categories were defined (i.e. overweight, obesity grade I, II, III) according to Cole and Lobstein (2012). †Sample for VEGF was n=88 (n=55 boys; n=33 girls).

Table 2. Time (min/day) accumulated at different cadence band of steps.

	All (n=97)	Boys (n=56)	Girls (n=41)
Cadence bands (steps/min)			
Non-movement (0)	346.4 ± 79.7	342.5 ± 82.7	351.9 ± 76.2
Incidental movement (1-19)	440.3 ± 64.6	437.0 ± 63.9	444.8 ± 65.9
Sporadic movement (20-39)	71.1 ± 17.8	71.8 ± 18.8	70.1 ± 16.6
Purposeful movement (40-59)	27.0 ± 8.6	29.4 ± 9.2	23.7 ± 6.6
Slow walking (60-79)	15.7 ± 7.5	18.2 ± 8.1	12.2 ± 4.7
Medium walking (80-99)	10.4 ± 6.2	12.0 ± 7.1	8.1 ± 3.8
Brisk walking (100-119)	6.4 ± 5.4	7.4 ± 6.5	5.1 ± 3.2
Faster locomotion (120+)	1.4 ± 1.9	1.6 ± 2.1	1.1 ± 1.6

Values are means ± standard deviations.

Table 3. Associations of sedentary time, physical activity and steps (measured with Euclidian norm minus one metric in hip) with neurotrophic factors (n=97).

	BDNF (ng/ml)		VEGF (pg/ml)†		IGF-1 (ng/ml)	
	β	P	*β*	P	*β*	P
Sedentary time (min/day)	-0.147	0.107	-0.078	0.479	0.130	0.192
Physical activity (min/day)						
Light PA	0.242	**0.006**	0.207	**0.048**	-0.187	0.055
Moderate PA	0.237	**0.016**	0.148	0.203	-0.095	0.385
Vigorous PA	0.098	0.343	0.156	0.195	-0.018	0.875
MVPA	0.234	**0.019**	0.159	0.180	-0.091	0.413
Steps						
Total number of steps/day	0.182	0.055	0.110	0.331	-0.105	0.314
Peak 60-min cadence (steps/min)	0.195	**0.037**	0.055	0.621	-0.061	0.556
Peak 30-min cadence (steps/min)	0.171	0.064	0.039	0.724	-0.036	0.724
Peak 1-min cadence (steps/min)	0.034	0.720	-0.041	0.712	-0.046	0.658

β values are standardized. These analyses were adjusted for the following covariates: sex, peak height velocity, fat mass index, wave of participation and parental educational level. The bold font is used to highlight significance level at P<0.05. PA=Physical activity; MVPA=Moderate-to-vigorous physical activity; BDNF=Brain-derived neurotrophic factor; VEGF=Vascular endothelial growth factor A; IGF-1=Insulin-like growth factor-

1. †Sample for VEGF was n=88 (n=53 boys; n=35 girls). Normalized values were used in the analyses.

Supplemental digital content 1. Data processing of sedentary time and physical activity measurements.

Raw data collected at a sampling frequency of 100 Hz were loaded in ActiLife (ActiGraph, Pensacola, FL, USA) and processed then in R (v. 3.1.2, https:// www.cran.r-project.org/) using the GGIR package (v. 1.5-12, https://cran.r-project.org/web/packages/GGIR/) (1). We calculated the Euclidean Norm Minus One G metric (ENMO, 1 G ~ 9.8 m/s2) after auto-calibrating the acceleration signal (1,2). The mean of ENMO with negative values rounded to zero was calculated over 5s epochs. Simultaneously, we derived the number of steps/minute (step cadence) from the hip-worn accelerometer from the ActiLife software. Additionally, waking up, and sleeping times were recorded during the 7 days on a diary by the participant.

Accelerometric information processing in GGIR consisted in: 1) non-wear time detection by the Van Hees et al. approach (3); 2) detection of abnormally and sustained high acceleration values (i.e., clipped time); 3) replacement of the non-wear and clipped time by the mean acceleration recorded within the same time frame for the rest of the measurement (3). A replacement by 0 for all metrics was performed if no data were collected for a specific time frame for the rest of the days; 4) identification of waking and sleeping hours based on an automatized algorithm guided by the diaries completed by the participants (4). The inclusion criterion for a valid day was wearing the accelerometer with ≥600 min/day of waking hours and ≥240 min/day of sleeping hours. A minimum of 4 valid days (3 weekdays and 1 weekend day) per week was required to be included in the analyses. The compliance wearing the accelerometer was high, with 98% of the sample wearing the accelerometers for ≥6 days.

Sedentary time and PA were classified into different intensities following Hildebrand et al. hip- and wrist-based cut-off points for the Euclidean Norm Minus One (ENMO) metric (5,6).

All accelerometer data were processed in R by using the GGIR package v.1.5-24 (https://cran.r-project.org/web/packages/GGIR/). Steps estimations were derived from ActiLife and then imported to R for further analyses in the GGIR package.

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Table S1. Associations between sedentary time, physical activity, measured with Euclidian norm minus one metric in non-dominant wrist, and neurotrophic factors (n=97).

	BDNF (ng/ml)		VEGF (pg/ml)†		IGF-1(ng/ml)	
	β	P	*β*	P	*β*	P
Sedentary time (min/day)	-0.097	0.288	-0.055	0.609	0.074	0.460
Physical activity (min/day)						
Light PA	0.022	0.801	0.073	0.478	-0.028	0.775
Moderate PA	0.220	**0.041**	0.071	0.577	-0.078	0.516
Vigorous PA	0.244	**0.032**	0.229	0.088	-0.054	0.666
MVPA	0.246	**0.027**	0.110	0.406	-0.080	0.516

β values are standardized. These analyses were adjusted for the following covariates: sex, peak height velocity, fat mass index, wave of participation and parental educational level. The bold font is used to highlight significance level at P<0.05. PA=Physical activity; MVPA=Moderate-to-vigorous physical activity; BDNF=Brain-derived neurotrophic factor; VEGF=Vascular endothelial growth factor A; IGF-1=Insulin-like growth factor-1. †Sample for VEGF was n=88 (n=53 boys; n=35 girls). Normalized values were used in the analyses.