

1 **Physical fitness reference standards for preschool children: The PREFIT Project**

2 **Abstract**

3 Objectives: Reference values are necessary for classifying children, for health screening, and for
4 early prevention as many non-communicable diseases aggravate during growth and
5 development. While physical fitness reference standards are available in children aged 6 and
6 older, such information is lacking in preschool children. Therefore, the purposes of this study
7 were 1) to provide sex-and age-specific physical fitness reference standards for Spanish
8 preschool children; and 2) to study sex differences across this age period and to characterise
9 fitness performance throughout the preschool period.

10 Design: Cross-sectional study.

11 Methods: A total of 3179 preschool children (1678 boys) aged 2.8 to 6.4 years old from 10
12 cities geographically distributed across Spain were included in the present study. Physical
13 fitness was measured using the PREFIT battery.

14 Results: Age- and sex-specific percentiles for the main physical fitness components are
15 provided. Boys performed better than girls in the cardiorespiratory fitness, muscular strength,
16 and speed-agility tests over the whole preschool period studied and for the different percentiles.
17 In contrast, girls performed slightly better than boys in the balance test. Older children had
18 better performance in all fitness tests than their younger counterparts.

19 Conclusions: Our study provides age- and sex-specific physical fitness reference standards in
20 preschool children allowing interpretation of fitness assessment. Sexual dimorphism in fitness
21 tests exists already at preschool age, and these differences become larger with age. These
22 findings will help health, sport, and school professionals to identify preschool children with a
23 high/very low fitness level, to examine changes in fitness over time, and to analyse those
24 changes obtained due to intervention effects.

25 *Keywords:* cardiorespiratory fitness, muscular strength, motor skills, reference values.

26 **Introduction**

27 Physical fitness is considered a powerful marker of health in children and adolescents. For
28 instance, low fitness levels have been associated with a higher risk of developing cardiovascular
29 diseases, overweight/obesity, mental disorders, and skeletal problems later in life^{1,2}. In line with
30 this notion, Ortega et al. analysed a sample of over one million Swedish adolescents and
31 observed that those with low muscular strength presented higher risk of mortality later in life³.
32 Likewise, in a recent systematic review, the relationship between fitness and health indicators
33 among children and adolescents was examined (including pre-schoolers aged 5)⁴. Although few
34 studies were found in pre-schoolers (n=5, 3.5%), the results showed significant associations
35 between cardiorespiratory fitness and health indicators, as in studies performed in children and
36 adolescents⁴. Briefly, cross-sectional and longitudinal studies showed that preschool children
37 aged 5 with higher fitness levels presented lower adiposity and better attention⁴. Furthermore,
38 our group recently observed that not only cardiorespiratory fitness but also muscular strength,
39 speed-agility, and balance were associated with total and central body fat in 3-5-year-olds⁵. For
40 this reason, there is a need to include physical fitness testing in health and educational
41 monitoring systems and to examine the associations between fitness and health-related
42 outcomes in 3 to 5-year-olds.

43 Fitness reference data have been reported in children and adolescents (>6 years old) from
44 different countries using standardised measures⁶⁻⁸. However, literature addressing reference data
45 of national or international samples including pre-schoolers (3-5 years old) is rather scarce. In
46 fact, as far as we know, only one study provided reference values for one specific test (standing
47 long jump) in pre-schoolers from one province in the south of Spain (Jaén)⁹. Reference values
48 are necessary for classifying children based on their performance on basic motor abilities, for
49 health screening, and for early prevention of biological risk factors for non-communicable
50 diseases (obesity, diabetes, cardiovascular diseases, etc.). Thus, studies providing reference
51 values in preschool children for all fitness components with harmonised measures are
52 warranted.

53 Therefore, the main objective of this study was to provide sex- and age-specific physical fitness
54 reference standards from a sample of preschool children aged 3 to 5 years old geographically
55 distributed across Spain. This study also addressed sex-related differences across this age period
56 and characterised fitness performance at preschool ages.

57 **Methods**

58 This study was conducted under the PREFIT Project framework (<http://profith.ugr.es/prefit>).
59 The main objective of this project was to assess physical fitness and anthropometric
60 characteristics in preschool children from 10 different cities/towns in Spain (i.e. Almería, Cádiz,
61 Castellón de la Plana, Cuenca, Granada, Las Palmas de Gran Canaria, Madrid, Palma de
62 Mallorca, Vitoria-Gasteiz, and Zaragoza). The data collection took place from January 2014 to
63 November 2015. The study protocol was approved by the local Review Committee for Research
64 Involving Human Subjects (n°845), in accordance with the Declaration of Helsinki 1961
65 (revision of Edinburgh 2013).

66 A total of 4338 pre-schoolers and their parents were invited to participate in the PREFIT
67 Project. The teaching staff from each school delivered an information sheet and an informed
68 consent to parents and/or guardians. These included the purpose of the study and brief
69 explanations concerning the applied tests. Finally, 3198 parents agreed to participate in the
70 study (participation rate: 73.7%). Among them, 19 children were excluded after the assessments
71 (i.e. they presented a motor or cerebral disease that limited the test performance reported by the
72 school teachers, they cried during most tests, they had a cough and mucus, or they did not
73 understand the instructions of the tests correctly). As a result, a total of 3179 preschool children
74 (4.6 ± 0.9 years old, 1678 boys, 52.8%) participated in the PREFIT Project (**Fig. S1**).

75 We measured children attending first, second, and third grades of preschool centres. In Spain,
76 this mainly includes children aged 3, 4, and 5 years old. However, these groups included some
77 children younger than 3 (n=44, 1.4%) and some older than 5.9 (n=112, 3.5%). Tables and
78 Figures report reference values from 3.0 (few participants aged 2 years old) to 6.25, which

79 corresponds to the first trimester of the 6th year of age. In all the analyses, we merged the data
80 obtained from the youngest and oldest pre-schoolers (i.e., participants aged <3 and ≥ 6) with the
81 closest groups for a higher statistical power. Throughout this article, we generally refer to the 3
82 to 5-year-old sample, since there are roughly 1000 children who are 3, 4, and 5 years old and the
83 proportionality out of this range is little ($n= 116$, 4.6%).

84 Weight (kg) and height (cm) were assessed without shoes and wearing light clothes using a
85 balance scale (SECA 213, Hamburg, Germany) and a stadiometer (SECA 213, Hamburg,
86 Germany), respectively. Thereafter, we calculated body mass index (BMI) (body mass /body
87 height² [kg/m²]).

88 Physical fitness (i.e. cardiorespiratory fitness, muscular strength, speed-agility, and balance)
89 was assessed with the PREFIT battery¹⁰. Feasibility, reliability, maximality, and practical
90 recommendations of these tests have been published elsewhere^{11, 12}. Just before the
91 measurements, we told a motivating fairy tale based on *Cofito and his adventures on the Lipid*
92 *Island* with the aim to encourage children and make the tests more appealing. More information
93 about this strategy has been published elsewhere¹¹. Cardiorespiratory fitness was assessed with
94 the PREFIT 20m shuttle run test that was performed as the last test. Briefly, the test consisted in
95 running back and forth between two lines (20m apart) following an audio signal. From the
96 original version proposed by Leger et al.¹³ two modifications were introduced for preschool
97 children¹²: 1) the test started at 6.5 km/h with an increment of 0.5 km/h every minute, and 2) one
98 evaluator ran in front of the pre-schoolers and another behind them (e.g. 4-8 pre-schoolers of the
99 same age) in order to help them to maintain the pace. The test finished for each child when they
100 could not reach the line with the audio signal on two consecutive occasions or when they
101 stopped due to fatigue. One of the evaluators was the person responsible for taking out the
102 children of the test when they finished. The test was carried out only once and the resulted laps
103 were registered.

104 Upper-limb muscular strength was assessed with the handgrip strength test. This test consisted
105 in squeezing as much as possible for 2-3 seconds. The analogue version of TKK dynamometer
106 (TKK 5001, Grip-A, Takei, Tokyo) was used and the grip span was fixed at 4.0 cm^{14, 15}. The
107 elbow had to be extended without being in contact with anything except for the hand touching
108 the dynamometer. Pre-schoolers performed two non-consecutive attempts with each hand. We
109 chose the best result of each hand and registered the average of both hands in kg.

110 Lower-limb muscular strength was assessed with the standing long jump test. This test consisted
111 in jumping forward as far as possible, with the feet separated at the shoulders' width, and
112 landing upright. We drew footprints on the floor to guide the pre-schoolers towards the starting
113 line to jump. We recorded the distance between the starting line and the location of the foot
114 closest to the starting line. The children performed the test three times and had time to rest
115 between the attempts. We registered the best of three attempts in cm.

116 Speed-agility was assessed with the PREFIT 4x10m shuttle run test. In this test, the children had
117 to run four times between two lines (10m apart) as fast as possible. Two evaluators stood at each
118 line and the pre-schoolers had to touch the evaluator's hand and return to the starting line as fast
119 as possible. The best of two attempts was manually registered by an experienced evaluator
120 (lowest duration in seconds).

121 Static balance was assessed with the one-leg stance test. The test consisted in standing on one-
122 leg still and bending the other leg at approximately 90°. The beginning of the test starts when
123 one of the legs is no longer in contact with the floor. The children had to maintain the balance
124 position for as long as they could. In accordance with the original protocol, there were no upper-
125 limb movement restrictions. The test finished when the child could not continue in the required
126 position. The children had one attempt with each leg, and the average time was registered in
127 seconds.

128 Familiarisation trials and explanations providing examples of how to perform the tests were
129 very important to ensure that the children had understood the process correctly. More

130 information about practical recommendations and how we approached several situations during
131 the assessments can be found in Table S1. The manual of operations, audio of the PREFIT 20m
132 shuttle run test, and videos showing how to perform and score the fitness tests are freely
133 available in Spanish and English at: <http://profith.ugr.es/recursos-prefit>. All the tests were
134 performed by trained evaluators and the protocol was standardised and homogenised across all
135 of the centres involved.

136 Anthropometric and physical fitness characteristics of the study sample are presented as mean
137 and standard deviation (SD) for the whole sample and stratified by sex and age. We tested
138 differences by sex and age group (3, 4, and 5 years old) with a two-way analysis of variance
139 (ANOVA). The statistical tests were all conducted with a significance level of $\alpha=0.05$. We
140 conducted this statistical analysis using SPSS (v.20, IBM Corporation, New York, USA).

141 To obtain percentile curves for preschool children, we applied the Generalized Additive Model
142 for Location, Scale and Shape (GAMLSS)¹⁶. We used the GAMLSS package (version 4.4-0) of
143 the statistical software R (version 3.3.1). GAMLSS is able to model up to four parameters of
144 different distributions: μ accounts for the location, σ for the scale, ν for the skewness, and τ for
145 the kurtosis. The Box-Cox Cole and Green, Box-Cox t , and Box-Cox power exponential
146 distribution were fitted to the observed data. Furthermore, the influence of age on the
147 distribution parameters was modelled constantly, linearly, or as a cubic spline function. We
148 assessed the goodness of fit applying the Bayesian information criterion and worm plots. More
149 information about the procedure has been described elsewhere¹⁷. Percentile curves for the 1st,
150 3rd, 5th, 10th, 15th, 20th, 30th, 40th, 50th, 60th, 70th, 80th, 85th, 90th, 95th, 97th, and 99th percentiles
151 were calculated based on the model that showed the best goodness of fit (Table S2 and
152 Supplementary material 2). We provide reference standards with a precision of 0.25 years of age
153 (every trimester) as the main outcome of this article and also every 0.025 years of age as
154 supplementary material 1 (equivalent to 9 days). To test sex differences in fitness across age
155 groups and percentiles 25th, 50th, and 75th, we performed a one-way ANOVA including the sex
156 differences as dependent variables and age groups (3, 4, and 5 years old) as factor. We used

157 these percentiles to test sex differences in fit pre-schoolers (percentile 75th), in averagely fit pre-
158 schoolers (percentile 50th), and in unfit pre-schoolers (percentile 25th). We had previously
159 calculated and depicted mean differences for every 0.05 years of age (i.e. 9 days) and
160 percentiles.

161 **Results**

162 Anthropometric and physical fitness characteristics of the study sample (whole sample and
163 separated by sex and age) are shown in Table S3.

164 Reference standards for the 1st, 3rd, 5th, 10th, 15th, 20th, 30th, 40th, 50th, 60th, 70th, 80th, 85th, 90th,
165 95th, 97th, and 99th percentiles and for every 0.025 years of age (i.e. 9 days) are provided in
166 supplementary material 1 (as Excel file). A summary of these reference standards (i.e.
167 percentiles: 10th, 20th, 30th, 40th, 50th, 60th, 70th, 80th, 90th, 99th) for each trimester (i.e. 0.25 years
168 of age, 3 months) is provided in **Tables 1 and 2**. **Fig. 1** depicts sex- and age-specific fitness
169 reference data according to the 1st, 5th, 15th, 25th, 50th, 75th, 85th, 95th, and 99th percentiles. We
170 found higher values in boys compared to girls in the entire fitness tests battery except for the
171 one-leg stance test, where girls showed better performance in all analysed percentiles. Also,
172 along the analysed percentiles, the performance improved with age. We found larger differences
173 between P95 and P99 in older preschool children than in their younger counterparts in the
174 PREFIT 20m shuttle run test (for girls), the standing long jump test (for girls), and the one-leg
175 stance test (for boys and girls) (Fig. 1). In the 4x10m shuttle run test, younger children showed
176 larger differences (for boys and girls) not only between P95 and P99 but also for P1 and P5
177 percentiles (Fig. 1). Fig. S2 shows sex differences across the preschool age and percentiles 25th,
178 50th, and 75th. Table S4 shows mean differences between boys and girls in all examined age
179 groups. We found significant differences in most of the fitness components and percentiles
180 studied (all $p \leq 0.001$), except in the standing long jump (for P75 between 3 and 5 years old) and
181 speed-agility (P50 and P75, between 4 and 5 years old and between 3 and 5 years old,
182 respectively) tests, where no differences between boys and girls were observed (all $p > 0.05$).

183 **Discussion**

184 The present study provides, for the first time, reference standards for the main fitness
185 components (i.e. cardiorespiratory fitness, muscular strength, speed-agility, and balance) by sex
186 and age (for every 0.025 year of age increment, i.e. 9 days) in preschool children. Boys
187 performed better than girls in cardiorespiratory fitness, muscular strength, and speed-agility
188 tests, whereas girls performed slightly better in balance tests. Older preschool children
189 performed better in all fitness tests than younger pre-schoolers. Furthermore, we observed sex-
190 related differences in fitness across all preschool ages and percentiles, being greater in older
191 children.

192 In regard to sex differences, from as early as preschool age, boys showed higher levels of
193 physical fitness (i.e. cardiorespiratory fitness, muscular strength, and speed-agility) than girls, as
194 it had previously been reported in older children and adolescents^{7, 18, 19}. However, in relation to
195 balance, girls performed slightly better in the one-leg stance test compared to boys. These
196 differences might be explained by differential sex development and growth. Our results are in
197 accordance with recent studies that analysed motor proficiency at early childhood. Such studies
198 conclude that boys perform better than girls in activities such as catching, throwing, or standing
199 long jump^{20, 21}. Conversely, girls perform better in activities involving balance or flexibility^{21, 22}.
200 These differences have previously been addressed in adolescents, but not in preschool
201 children^{23, 24}. Studies showed that boys have higher levels of cardiorespiratory fitness because
202 they are more physically active²³ and have lower levels of fat mass²⁴⁻²⁶. In the same way, based
203 on the previous literature, fat-free mass is higher in boys²⁷, which allows them to perform better
204 in muscular strength tests²⁴. In regard to speed-agility, other authors showed that the
205 performance seems to be influenced by genetics (neuromuscular components, muscle fibre
206 quality, degree of gene transfer, etc.), and thus, there is no explanation for the sex-related
207 differences^{24, 25}. Likewise, it is possible that height could explain the sex-differences observed in
208 balance, since taller children (in average boys are taller than girls already at these ages) would
209 have a higher postural instability and thus worse balance as a result of a higher location of the

210 centre of body mass²⁸. Our results cannot support these assumptions since we have not
211 measured fat-mass, fat-free mass, or physical activity. However, although these differences have
212 been observed in adolescents, there is no reason to believe that similar physiological differences
213 could also be found already in children aged 3-5 years old.

214 Preschool children are in a period of continuous motor, physiological, and psychological
215 developing changes²⁹. We observed that older children performed better in all fitness tests since
216 their motor development and fitness were higher (i.e. better aerobic capacity, muscular strength,
217 coordination, agility, etc.) compared to their younger counterparts. We observed similar patterns
218 in children and adolescents in most of the study test^{7, 18, 30}, except in those assessing flexibility.
219 Flexibility tests showed that, overall, the performance improved with age in girls^{7, 18, 30} and
220 remained stable or became worse in boys³⁰. Other factors that could explain these differences
221 are motivation, concentration, degree of the motor skills, physical activity, or body composition
222 (fat mass and fat-free mass)²⁹.

223 To the best of our knowledge, our study is the first to provide age- and sex-specific reference
224 standards of a complete set of physical fitness components (i.e., cardiorespiratory fitness,
225 muscular strength, speed-agility, and balance) in preschool children. A strength is that the
226 applied tests were selected based on a systematic review in preschool children¹⁰, together with
227 the existing evidence in older children and adolescents¹. Previous studies^{7, 8, 18} showed reference
228 data for cardiorespiratory fitness, muscular strength, speed-agility, and flexibility in children
229 and adolescents (>6 years old) using evidence-based fitness tests batteries (e.g. ALPHA).
230 Nevertheless, our data are not fully comparable due to the differences in age groups and tests
231 applied.

232 In regard to cardiorespiratory fitness, we observed that the differences of the 50th percentiles
233 (P50) between both sexes increased with age (Fig. 1). Although we conducted different tests
234 (original 20m shuttle run vs. PREFIT 20m shuttle run) and analysed different age groups, other
235 studies in children and adolescents showed a similar trend in P50 (e.g. sex-differences in 18-

236 year-old adolescents: +38 laps for boys)^{7, 19}. Higher age-related differences by sex observed in
237 adolescents compared to children or even preschool children might be explained by more
238 pronounced physiological changes (due to the pubertal development) that occur as age
239 increases³¹.

240 Upper-limb muscular strength, assessed by the handgrip strength test, showed sex-differences in
241 P50 of approximately 1kg as age increased 0.5 years. Despite differences between studies and
242 devices (analogue vs. digital dynamometer), our results are in accordance with the results of De
243 Miguel-Etayo et al.⁷, who observed the same sex-differences in children aged 6-9 years old.

244 Given that the methodology of the test is the same and the inter-instrument reliability is high
245 (mean difference, digital minus analogue dynamometer = -0.35kg)¹⁵, our results are comparable
246 with groups of 6-year-olds. The preschool children from the PREFIT Project were stronger than
247 the IDEFICS children (mean differences: 1.28 and 1.29kg for boys and girls, respectively).

248 Roriz et al.³⁰ also provided reference values for Portuguese children aged 6 to 10 (age-range 1
249 year), showing similar sex-differences (nearly 1kg). They also reported upper-limb muscular
250 strength reference standard in P50, which is slightly lower compared to our results. The
251 differences between studies might be due to the limitations of the digital dynamometer, since
252 the range of measurement is from 5kg to 100kg and the fact that 6-year-olds had several
253 attempts below this range (i.e., 0kg)¹⁵. Another remarkable difference between the IDEFICS and
254 the PREFIT studies is that overweight and obese children were not included in the calculation of
255 reference standards in IDEFICS. Indeed, children and adolescents with higher BMI performed
256 better in tests assessing absolute strength (also called non-weight bearing test)²⁴ compared to
257 those with lower BMI.

258 Regarding lower-limb muscular strength, we observed that the differences in P50 between both
259 sexes ranged from 6 to 8 cm as age increased 0.5 years. La Torre et al.⁹ provided reference data
260 in standing long jump in a group of 3 to 6-year-olds from Jaén (a region from the south of
261 Spain). It can be observed that P50 of the PREFIT reference data of this study was slightly
262 higher in boys and girls and for all age groups than in the aforementioned study (La Torre et al.,

263 differences ranging from 3 to 6 cm). Likewise, P50 depicts higher differences in the group of 3
264 to 4-year-olds (from +11 to +18 cm for boys and girls) and lower age differences in older
265 groups (i.e. in 5 to 6-year-olds: from 4 to 8 cm). Other studies^{7,30} provided reference values for
266 children from 6 to 10 years old, showing lower variances between 0.5 and 1 year of difference.
267 Moreover, P50 of 6-year-olds from the European IDEFICS study⁷ was higher than in both
268 Spanish pre-schoolers participating in the PREFIT and in Portuguese children³⁰. Once again,
269 this result could be due to the exclusion of overweight and obese children from the data analyses
270 in the IDEFICS study⁷. In contrast, the Portuguese study and the PREFIT study provide
271 reference values for the whole sample, including all weight status categories³⁰. The standing
272 long jump test is a weight-bearing test where children have to move and lift their body mass. As
273 a result, heavier children usually perform worse than their counterparts with lower body mass.
274 In line with this, Henriksson et al.³² confirmed that a better performance on weight-bearing tests
275 in preschool children was associated with a lower fat mass index. The researchers concluded
276 that the more favourable body composition you have, the fitter you are.

277 Given the differences in the applied methodology, it is not possible to compare our results on
278 motor fitness with any previous study in children and adolescents. In speed-agility (i.e., 4x10m
279 shuttle run test), P50 showed the same trend for boys and girls, improving their performance
280 with age, and the range of difference between ages being practically systematic (from 0.2 to
281 nearly 1.8 seconds). The reason for the better performance in older children could be explained
282 by the development produced in motor coordination during the preschool period and childhood.
283 As an example, this improvement was also demonstrated in European children from 6 to 9 years
284 old (P50) who reduced the performance time by one second in 40m sprint⁷. Concerning
285 balance, little is known about the reference standards of this fitness component in pre-schoolers.
286 To the best of our knowledge, only one study³³ provided static balance norms in children from 4
287 to 15 years old, yet the data provided for pre-schoolers (4-5 years old) were extracted from a
288 small sample size (n=25) and provided for boys and girls together. In comparison with our data,
289 P50 from the study of Condon et al.³³ was between 2-5 seconds higher than P50 observed in

290 PREFIT. In our study particularly, P50 of boys and girls followed a similar pattern. Differences
291 between younger and older pre-schoolers were greater in P50 both for boys and girls
292 (approximately 18 seconds of difference). It is important to note that, although this test showed
293 low reliability¹¹, we decided to provide its reference standard in order to help professionals to
294 detect low levels of this fitness component. Nevertheless, researchers should be cautious when
295 comparing pre-post values on two different occasions or after an intervention programme, due
296 to the low reliability found.

297 The sex differences observed showed that already from preschool ages differences between
298 boys and girls increased with age. Similar findings were reported by Castro-Piñero et al.³⁴, who
299 observed sex differences during the stage from childhood to adolescence. Growth and,
300 particularly, the early maturational status of girls play an important role. Our results are novel
301 because they add the existence of sexual dimorphism in preschool children to the literature and
302 characterise the age-specific pattern of the different development course in both sexes.
303 Nevertheless, further studies are needed in order to corroborate or contrast these findings.

304 Overall, the present physical fitness reference standards allow other researchers or professionals
305 to classify preschool children in sex- and age-percentiles. Preschool children can also be
306 classified into fitness categories such as very low ($X < P10$), low ($P10 \leq X < P25$), medium
307 ($P25 \leq X < P75$), high ($P75 \leq X < P95$), and very high ($X \geq P95$) and also scaling them from 0 to
308 10. In line with this definition, we uploaded an excel-based calculator to the website. With this
309 tool, the researcher or practitioner can copy and paste age, sex, and the result of the fitness test,
310 and the calculator will inform at which percentile that fitness value belongs to. The calculator
311 functions entering either the data of one child, or copying and pasting columns from a data set,
312 for instance of 3000 participants (freely available at <http://profith.ugr.es/recursos-prefit>).

313 Thereby, professionals (sports practitioners, teachers, health care, trainers, etc.) can identify and
314 help young children classified into the lower categories, implementing strategies to promote
315 physical fitness and physical activity to prevent or reduce future health-related problems. This
316 paper provides valid national specific reference standards for preschool children and thus, our

317 results are valid to compare with Spanish preschool children. However, since no data are
318 available from other countries concerning this population, these reference standards could help
319 and guide professionals in other countries in fitness classification until their own and/or
320 international reference standards are available.

321 The cross-sectional design of this study does not allow to examine inter- and intra-individual
322 differences, resulting in the need for studies with repeated measurements. The lack of validation
323 studies of the physical fitness applied in preschool children due to the logistic problems inherent
324 to the age of the children is another limitation, yet it must be highlighted that the tests are
325 reliable in pre-schoolers (except for balance) ^{10,11}. In addition, the difficulty to differentiate
326 between motivation and performance limitations is another study limitation to acknowledge. We
327 also consider the lack of sensitivity analyses for obese children a limitation, since it does not
328 allow to provide specific reference standards for them. Although the analysed sample is not
329 representative for the Spanish population, it is a large sample size that covers cities from north
330 to south and from east to west in Spain. The harmonisation and standardisation of the physical
331 fitness, as well as the use of the GAMLSS as a strong tool to obtain smooth age-dependent
332 reference curves are notable strengths of the study.

333 **Conclusion**

334 Our study provides age- and sex-specific physical fitness reference standards in Spanish
335 preschool children. This study in children from 3 to 5 years old extends to pre-schoolers the
336 already existing reference standards in older children^{6,7} and adolescents^{6,8,35}. Older children
337 perform better than their younger counterparts. Likewise, sexual dimorphism is detectable
338 already at the age of 3 and increases with age. Overall, boys show better performance than girls
339 in the majority of the applied fitness tests, except for the balance component where girls
340 perform slightly better. These findings will help health, sport, and school professionals to
341 identify preschool children with a high/very low fitness level and to examine changes over time,

342 including those obtained due to intervention effects. Further studies should examine and provide
343 reference standards at international level.

344 **Practical implications**

- 345 • Reference values are necessary for classifying children, for health screening, and for
346 early prevention as many non-communicable diseases aggravate during growth and
347 development. Therefore, as far as we know, this is the first study providing reference
348 values in preschool children for all fitness components with harmonised measures.
- 349 • This study provides sex- and age-specific physical fitness reference standards from a
350 sample of preschool children aged 3 to 5 years old geographically distributed across 10
351 cities in Spain (n=3179).
- 352 • Boys performed better than girls in the cardiorespiratory fitness, muscular strength, and
353 speed-agility tests, whereas girls performed slightly better in balance tests. Older
354 preschool children performed better in all fitness tests than younger pre-schoolers.
355 Furthermore, sex-related differences in fitness across all preschool ages and percentiles
356 were observed, being greater in older children.
- 357 • Tables, Figures, and Supplementary materials help health, sport, and school
358 professionals to identify preschool children with high/very low fitness levels and to
359 examine changes over time, including those obtained due to intervention effects.

360 **Conflict of interest**

361 The authors declare no conflict of interest.

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Figures legend

Fig. 1. Percentile curves of the PREFIT 20m shuttle run (Fig.1a), handgrip strength (Fig.1b), standing long jump (Fig.1c), 4x10m shuttle run (Fig.1d), and one-leg stance (Fig.1e) tests in preschool children from 3 to 6.25 years old.

*In the 4x10m shuttle run test, lower scores (less seconds in running the fixed distance) indicate a better performance (children are faster and more agile).