1 **Title:**

2 Effect of prior injury on changes to biceps femoris architecture across an AFL season.

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- 18 **Running title:**
- 19 Hamstring architecture changes in-season
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22 ABSTRACT

Purpose: To assess in-season alterations of biceps femoris long head (BFlh) fascicle length in
elite Australian footballers with and without a history of HSI.

25 Methods: Thirty elite Australian football players were recruited. Twelve had a history of

26 unilateral HSI. Eighteen had no HSI history. All had their BFlh architecture assessed at

27 approximately monthly intervals, six times across a competitive season.

28 Results: The previously injured limb's BFlh fascicles increased from the start of the season and 29 peaked at week 5. Fascicle length gradually decreased until the end of the season, where they 30 were shortest. The contralateral uninjured limb's fascicles were the longest when assessed at 31 week 5 and showed a reduction in-season where weeks 17 and 23 were shorter than the first. Control group fascicles were longest at week 5 and reduced in-season. The previously injured 32 33 limb's BFlh fascicles were shorter than the control group at all weeks and the contralateral 34 uninjured limb at week 5. Compared to the control group, the contralateral uninjured limb had 35 shorter fascicles from weeks 9 to 23.

36 Conclusion: Athletes with a history of HSI end the season with shorter fascicles than they start.
37 Limbs without a history of HSI display similar BFlh fascicle lengths at the end of the season as
38 they begin with. All athletes increase fascicle length at the beginning of the season however the
39 extent of these differed based on history of HSI. These findings show that a HSI history may
40 influence structural adaptation of the BFlh in-season.

42 INTRODUCTION

43 For more than 20 years hamstring strain injuries (HSIs) have been the leading cause of lost 44 playing and training time in elite Australian football (26). Furthermore, HSIs commonly re-occur 45 and typically result in a reduced level of performance following a return to competitive match 46 play.(35) These injuries represent a significant financial burden for the athlete and/or their 47 organisation(14). Given that a history of HSI has been consistently shown to increase the risk of 48 future a HSI (11, 25), investigations involving previously injured individuals have attempted to 49 determine if retrospective deficits in structure and/or function of the hamstrings contribute to the 50 elevated risk of re-injury (7, 21-23, 27, 30).

51

Recently, variations in biceps femoris long head (BFlh) architectural characteristics and their role in the aetiology of HSI have been brought to the attention of researchers and practitioners (30-33). Elite soccer players with shorter BFlh fascicles were reported to have a 4.1 fold increased risk of future HSI and this was amplified in those athletes with a history of HSI (32). These data, coupled with the finding that a previously injured BFlh consistently displays shorter fascicles than the uninjured contralateral limb (30), suggests that architectural characteristics of those with a history of HSI likely contribute to the elevated rate of re-injury.

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Providing interventions for athletes that present with shorter fascicles following ultrasonic examination would appear to be relatively straight forward. This is due to the increasing evidence that resistance exercise, particularly eccentric training targeting the hamstrings, can increase BFlh fascicle length (6, 33, 34). However those with a prior HSI might exhibit a reduced scope for positive adaptation as a result of a diminished capacity to activate the 65 previously injured muscle, per the inhibition hypothesis (7, 10, 22). This reduced ability to activate the previously injured muscle may also limit the extent of strain within the contractile 66 67 tissue, which in turn may dampen the stimulus needed to increase fascicle length and eccentric 68 strength (4, 13, 18). One study has examined the impact of a prior HSI on the adaptation of the 69 hamstrings, reporting that elite Australian footballers with a HSI in the prior 12 months increased 70 eccentric knee flexor strength to a lesser extent across a pre-season training period than 71 individuals without a HSI(24). A restricted capacity to improve eccentric knee flexor strength is 72 at least one mechanism through which prior HSI could increase the risk of future injury (20, 32).

73

74 Despite the aforementioned findings, it remains unclear as to whether a history of HSI impacts 75 upon the adaptive capacity of other risk factors, such as BFlh fascicle length, particularly during 76 the in-season period. Recovery time and competition travel schedules can also limit when physical training can be implemented during a season. It is well established that physical 77 78 performance variables tend to decline across the in-season period in elite Australian footballers 79 (8). However, it remains to be seen if a specific pathological history might influence these 80 changes. An improved understanding of the in-season changes in BFlh fascicle length, in 81 previously injured and uninjured limbs, may inform on whether those with a history of HSI 82 respond differently to the demands of a competitive season. Such data may have implications for 83 the provision of risk mitigating interventions that are tailored to individuals based on their injury history. Therefore, the purpose of this study was to observe the in-season time course of changes 84 85 to BFlh architecture in elite Australian footballers, with and without a history of HSI.

87 METHODS

88 **Participants**

89 **Participants**

90 Paragraph

91 In total, 30 elite male Australian footballers participated in this study. All participants provided 92 written informed consent prior to collection of any data. For all athletes, team medical staff 93 completed a retrospective injury questionnaire that detailed their history of hamstring, 94 quadriceps, groin and calf strain injuries and chronic groin pain in the past 12 months, as well as the history of anterior cruciate ligament (ACL) injury at any stage throughout their career. This 95 96 information was sourced from club medical records via the team doctor or physiotherapist. Of 97 the 30 participants, 18 had no history of HSI or any other significant lower limb injury (including 98 ACL) and formed the control group. Twelve athletes had suffered a unilateral BFlh strain injury 99 in the prior 12 months and formed the previously injured group. Ethical approval for the study 100 was granted by the Australian Catholic University Human Research Ethics Committee (approval 101 number 2016-145E).

102 Study design

103 Paragraph

This observational, retrospective cohort study was completed during the 2016 Australian Football League season which consists of 23 weeks of competitive matches (March 2016 to August 2016). All participants had their BFlh architecture assessed via two-dimensional ultrasound (Figure 1) approximately once every month on six separate occasions throughout the in-season period, at a consistent tie of day. These assessments occurred at weeks 1, 5, 9, 13, 17 and 23 (final week of competitive games) of the in-season period. . .

110 **BFlh architecture assessment**

111 Paragraph

112 The protocol for the collection of BFlh muscle architecture has been described previously (29-113 33). Muscle thickness, pennation angle and fascicle length of the BFlh was determined from 114 ultrasound images taken along the longitudinal axis of the muscle belly utilising a two 115 dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm) (GE 116 Healthcare Vivid-i, Wauwatosa, U.S.A). The scanning site was determined as the halfway point 117 between the ischial tuberosity and the knee joint fold, along the line of the BFlh. All architectural 118 assessments were performed with participants in a prone position, with the hip in neutral and the 119 knee fully extended, following at least 5 minutes of inactivity. To gather ultrasound images, the 120 linear array ultrasound probe, with a layer of conductive gel, was placed on the skin over the 121 scanning site and aligned longitudinally and perpendicular to the posterior thigh. Care was taken 122 to ensure minimal pressure was placed on the skin by the probe. Finally, the orientation of the 123 probe was manipulated slightly by the assessor (RGT) if the superficial and intermediate 124 aponeuroses were not parallel. Reliability of the assessor (RGT) has been previously reported for 125 the assessment of BFlh architectural characteristics (intraclass correlations range from 0.93 to 126 0.98 and typical error as a % coefficient of variation range from 2.1 to 3.4)(30). The assessor 127 (RGT) has experience in the assessment of muscle architecture utilising two-dimensional 128 ultrasound, specifically when assessing the BFlh (6, 30-33).

129 Paragraph

Once the images were collected, analysis was undertaken off-line (MicroDicom, Version 0.7.8,
Bulgaria). For each image (Figure 1), fascicle length estimation was performed as described by
Blazevich and colleagues(5). Muscle thickness was defined as the distance between the

superficial and intermediate aponeuroses of the BFlh. A fascicle of interest was outlined and marked on the image and the angle at which it inserted onto the intermediate aponeurosis was determined as the pennation angle. The superficial and intermediate aponeurosis angles were determined as the angle between the line marked as the aponeurosis and an intersecting horizontal reference line across the captured image(5, 16). As the entire fascicle was not visible in probe's field of view, it was estimated via the following equation from Blazevich and colleagues(5, 16):

140 $FL = sin (AA + 90^{\circ}) x MT/sin(180^{\circ} - (AA + 180^{\circ} - PA)).$

Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation angle. Fascicle length was reported in absolute terms (cm) from a single image and fascicle. The same assessor (RGT) collected and analysed all scans and was blinded to participant identifiers (name, limb and group) during the collection and analysis of the images.

145 **Statistical analyses**

146 Paragraph

147 All data (including age, height and weight) were analysed using a custom spreadsheet which 148 assessed the magnitude of difference across the season within groups as well as the extent of any 149 between group differences in muscle architecture, at each time point (15). As there were no 150 differences between limbs in the control group at all weeks, the two-limb averages were used for 151 all comparisons. In order to reduce bias associated with non-uniformity of error, all data were 152 log-transformed and effect sizes (Cohen's d) with \pm 90% confidence interval (CI) were 153 calculated. Effect sizes of ≥ 0.2 , ≥ 0.5 and ≥ 0.8 were defined as small, moderate and large, 154 respectively, with effect sizes of <0.2 deemed as trivial. Finally, any effects where the 90% CI

simultaneously overlapped the positive (≥ 0.2) and negative (≤ -0.2) thresholds of a small effect, were defined as being unclear(2).

157

158 **RESULTS**

159 **Power calculations**

Power analysis was undertaken *a priori* using G-Power(9). The analysis was based on anticipated differences in BFlh fascicle length between the injured and contralateral uninjured limbs, using a split plot ANOVA model. Effect size estimates were based on previous research(30) which reported an effect size of 1.34 when comparing BFlh fascicle length between injured and uninjured limbs. Therefore an effect size of 1.2 was deemed as a reasonable and conservative starting point for determining sample size. A calculated sample size of 10 per group was determined utilising the below parameters:

• Power
$$(1-\beta \text{ err probability}) = 0.80$$

168 • $\alpha = 0.05$

• Effect size = 1.2

170 Participant details

There were no clear differences between the two groups with respect to age (unclear effect; $d = 0.11 \pm 0.60$), height (unclear effect; $d = 0.06 \pm 0.59$) and body mass (unclear effect; $d = 0.26 \pm 0.59$) (previously injured group age = 22.9 ± 2.6 yrs, height = 1.87 ± 0.06 m, body mass = 86.0 ± 6.3 kg; control group age = 23.5 ± 3.9 yrs, height = 1.88 ± 0.10 m, body mass = 88.7 ± 10.4 kg). Percentage of total time on ground throughout the entire competitive season did not differ between the previously injured ($80.6 \pm 3.7\%$) and the control group ($79.8 \pm 5.4\%$; unclear effect; 177 $d = 0.17 \pm 0.58$). There were also no within group differences, across the season, in the 178 percentage of total time on ground for either the previously injured (trivial effects; *d* range: 0.15 179 to 0.17) or control groups (trivial effects: *d* range: 0.13 to 0.17).

Throughout the study, three participants suffered a HSI. Two of these were from the control group with one being from the previously injured group. The injuries for the control group participants occurred between weeks 13 and 17. As a result, these two participants were excluded from analysis at weeks 17 and 23. The previously injured participant's incident occurred after week 23 and was not removed from any analysis due to the injury occurring after the final assessment was completed.

186 **BF**th architectural characteristics

187 Fascicle length

- 188 Temporal changes across the in-season period
- 189 *Previously injured limbs*

Fascicle length in the previously injured limbs increased from week 1 to week 5 (small effect; $d = 0.20 \pm 0.32$) and fascicles were longer at all time points when compared to week 23 (small to moderate effects; d range: 0.22 to 0.75; Table 1 and 2, Figure 2). Furthermore, fascicles were longer at weeks 5 and 9 compared to weeks 13 and 17 (small effect; d range = 0.22 to 0.31; Table 1 and 2, Figure 2)

- 195 Contralateral uninjured limbs
- 196 Fascicle length was longest at week 5 compared to all other weeks (small to large effects; d
- 197 range = 0.40 to 0.89; Table 1 and 2, Figure 2). Furthermore, fascicle lengths were longer at
- 198 weeks 1 and 9 compared to weeks 17 and 23 (small to moderate effects; d range = 0.35 to 0.50;

Table 1 and 2, Figure 2). Week 9 also displayed longer fascicles compared to week 13 (small effect; $d = 0.21 \pm 0.19$; Table 1 and 2, Figure 2), whilst at week 13 fascicles were longer compared to week 23 (small effect; $d = 0.22 \pm 0.17$; Table 1 and 2, Figure 2).

202 *Control group*

Longer fascicles were observed in the control group at weeks 5, 9 and 13 when compared to weeks 1, 17 and 23 (small to large effects; *d* range: 0.34 to 1.01; Table 1 and 2, Figure 2). Furthermore, fascicles were longer at week 5 compared to week 13 (small effect; $d = 0.33 \pm$ 0.23; Table 1 and 2, Figure 2) and longer at week 17 compared to week 23 (small effect; $d = 0.42 \pm 0.26$; Table 1 and 2, Figure 2).

208 Between group comparisons

- 209 Previously injured limbs compared to contralateral uninjured limb
- 210 The previously injured limb displayed shorter fascicle lengths compared to the contralateral
- uninjured limb only at week 5 (moderate effect; $d = -0.76 \pm 0.68$; Table 3).
- 212 Previously injured limbs compared to control group
- 213 Fascicle length of the previously injured limb was shorter than the control group at all time
- 214 points (moderate to large effects; *d* range: -1.15 to -0.77; Table 3).
- 215 Contralateral uninjured limb compared to control group
- 216 The contralateral uninjured limb displayed shorter fascicles compared to the control group
- average at weeks 9, 13, 17, 23 (moderate to large effect; d range = -0.87 to -0.54; Table 3)

218 **Pennation angle**

219 Temporal changes across the in-season period

- 220 Previously injured limbs
- 221 Pennation angle in the previously injured limb was smaller at all weeks compared to week 23
- (moderate to large effects; d = -1.13 to -0.60, Table 1). Pennation angle was also lesser at week 5
- 223 compared to week 17 (small effect; $d = 0.26 \pm 0.44$, Table 1).
- 224 Contralateral uninjured limb
- 225 Pennation angle was less at week 5 compared to all other weeks (moderate to large effect; d
- range = -1.61 to -0.71, Table 1). In contrast, pennation angle was larger at week 23 compared to
- all other time points (small to large effects; d range = 1.61 to 0.35, Table 1). Pennation angle was
- also lesser at week 1 compared to week 13 (small effect; $d = 0.36 \pm 0.50$, Table 1).
- 229 *Control group*
- 230 Pennation angle was greatest at weeks 1 and 23 when compared to all other weeks (small to large
- effects; d range = 0.21 to 0.94, Table 1). Further, pennation angle was greater at weeks 13 and 17
- when compared to weeks 5 and 9 (small effects; d range = 0.23 to 0.33, Table 1).
- 233 Between group comparisons

234 Paragraph

- 235 Previously injured limbs compared to contralateral uninjured limb
- 236 Pennation angle in the previously injured limb was larger compared to the contralateral uninjured
- limbs at weeks 5 and 23 (moderate to large effects; d range = 0.61 to 1.04; Table 3).
- 238 Previously injured limbs compared to control group
- 239 When compared to the control group, previously injured limbs had greater pennation angles at
- 240 weeks 5, 9, 13 and 23 (moderate to large effects; d range = 0.50 to 1.01; Table 3).

- 241 Contralateral uninjured limb compared to control group
- 242 The contralateral uninjured limb's pennation angle was greater than the control group average at
- 243 week 9 ($d = 0.61 \pm 0.60$) and 13 ($d = 0.46 \pm 0.62$).
- 244
- 245 Muscle thickness
- 246 Temporal changes across the in-season period
- 247 Previously injured limbs
- 248 Muscle thickness was greater at week 23 compared to week 1 (small effect; $d = 0.26 \pm 0.45$,
- 249 Table 1).
- 250 Contralateral uninjured limb
- 251 No small, moderate or large effects were detected for muscle thickness across all time points.
- 252 *Control group*
- 253 Muscle thickness was greater at week 5 ($d = 0.29 \pm 0.19$, Table 1) and week 13 ($d = 0.20 \pm 0.13$,
- Table 1) compared to week 17.
- 255

256 Between group comparisons

- 257 Previously injured limbs compared to contralateral uninjured limb
- 258 No small, moderate or large effects were detected for muscle thickness between the previously
- 259 injured and uninjured contralateral limbs.
- 260 *Previously injured limbs compared to control group*
- 261 Compared to the control group the previously injured limbs had decreased muscle thickness at
- weeks 1, 5 and 13 (moderate effect; d range -0.56 to -0.48; Table 3)
- 263 Contralateral uninjured limb compared to control group

264 No small, moderate or large effects were detected.

265 **DISCUSSION**

The main findings of this study were 1) those with a history of unilateral HSI end the in-season period with shorter BFlh fascicles compared to the start of the in-season period in both their previously injured and contralateral uninjured limb; 2) uninjured limbs display similar BFlh fascicle lengths at the start of the in-season period compared to the end of the in-season period; 3) increases in BFlh fascicle length were observed early in-season across all athletes, however the magnitude of this increase differed based on history of HSI.

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BFlh fascicle length has been identified as a modifiable risk factor for HSI (32), however, it was 273 274 previously unclear as to how or if this parameter changed across a season in elite Australian 275 footballers. In the current study all groups increased BFlh fascicle length during the early part of 276 the in-season period, which then progressively shortened until the end of the competitive season. 277 Of note, the increase was largest in the control group (moderate effect, $d = 0.67 \pm 0.33$), followed 278 by the contralateral uninjured limbs (small effect, $d = 0.47 \pm 0.27$) and finally the previously 279 injured limbs (small effect, $d = 0.20 \pm 0.32$). This divergence in early in-season responses across 280 groups appears to be a factor that ultimately results in both limbs from the previously injured 281 athlete possessing shorter fascicles at the conclusion of the season compared to the start of the 282 season. From weeks 5 to 23, the control group displays the largest decline in fascicle length 283 (large effect, $d = -1.01 \pm 0.31$), followed by the contralateral uninjured limbs (large effect, $d = -1.01 \pm 0.31$) 284 0.89 ± 0.35) and then the previously injured limbs (moderate effect, $d = -0.75 \pm 0.37$). These 285 findings differ to work which has examined in-season alterations in vastus lateralis fascicle 286 length, in softball and track and field (3, 19). In these studies, an initial decline in the first half of the competitive season was counteracted by an increase at the end of the season(3, 19). However as the vastus lateralis acts in an anti-gravity nature, it is likely that the differing roles of the knee extensors and flexors contribute to these divergent findings, as would the differing demands between the sports examined.

291

292 The current data suggest that the early in-season period (i.e. within the first one to two months of 293 the commencement of the season) may be an important time to continue to implement 294 interventions to increase BFlh fascicle length, particularly in Australian footballers with a history 295 of HSI. Simplistically, there is the possibility that this could be achieved with high-intensity, 296 eccentric loading strategies that can elicit favourable adaptations within 2 weeks (33). However, 297 there are likely a number of practical considerations that may limit or preclude such a strategy in 298 elite sporting environments compared to those observed from lab-based studies in recreational 299 athletes. These may include coach/athlete apprehension towards eccentrically induced muscle 300 damage often reported in response to unaccustomed training(1) (which can be accentuated by the 301 extent of the muscle strain undertaken during lengthening contractions (17)). Also a greater 302 emphasis placed on recovery between matches at the expense of loading exposures (12, 28), as 303 well as the presence or accumulation of other lower limb injuries that might not result in on-field 304 time loss but do require modifications to resistance exercise prescription. Prior evidence has 305 suggested that the de-training effect for BFlh fascicles following eccentric training interventions 306 can occur in as little as four weeks(33), which would justify the need for constant application of 307 an eccentric strength training stimulus, yet implementation appears to be challenging in practice 308 (1).

310 It should be acknowledged that the current paper is limited as no architecture data was captured 311 during the pre-season period, which spans November to February. It is certainly possible that the 312 previously injured athletes increased fascicle length substantially during this period and future 313 work should seek to explore this possibility. Nevertheless, across the entire in-season period, the 314 previously injured hamstrings possessed shorter fascicles than the control group at all weeks 315 (moderate to large effects throughout). These findings are likely to at least partly explain the high 316 rates of HSI recurrence seen in Australian footballers (26). Therefore consideration should be 317 given to what previously injured Australian footballers are capable of doing during their off-318 season program as a means of minimising any deficits at the commencement of the season. As 319 exposure to high speed running can be minimised in the off-season, this may allow for the 320 application of high-intensity strength training interventions targeted at increasing or at least 321 minimising reductions in BFlh fascicle length, leading into the next pre- and in-season periods.

322

323 The current study indirectly infers the possibility that previously injured athletes/limbs are less 324 capable of adapting positively to the rigours of in-season demands compared to those without a 325 history of injury. Similar observational research has found that previously injured Australian 326 footballers display less improvement in eccentric knee flexor strength across the pre-season 327 compared to their uninjured counterparts(24). Such limited adaptation in previously injured 328 athletes could be partly attributed to prolonged neuromuscular inhibition(10), which has been 329 noted in previously injured athletes even after returning to pre-injured levels of competition(7, 330 22, 23, 30). For example, a previously injured BFlh has been shown to be significantly less 331 active than uninjured contralateral muscles during performance of the Nordic hamstring curl(7), 332 which is an exercise commonly used in HSI rehabilitation(1). It is possible that this limited

activation may result in a reduced amount of strain within the tissue and limit the stimulus required to increase fascicle length (4, 13). However, from a mechanistic perspective, this phenomenon requires further investigation. No study has investigated whether individuals with and without a prior history of HSI respond differently to controlled interventions aimed at increasing eccentric strength and fascicle length. Should differences exist, further exploration as to whether inhibition manifests at the spinal or supraspinal level would be necessary to guide interventions targeted at restoring voluntary activation capacity after injury.

340

341 The authors acknowledge there are limitations in the current study. First, there are 342 methodological limitations with the use of two-dimensional ultrasound to estimate BFlh fascicle 343 length. As the fascicles which were measured are longer than the field of view which was 344 utilised, the entire fascicle was not captured. Therefore, estimation was required to determine 345 BFlh fascicle length. The estimation process used has been previously validated against 346 cadaveric samples (5, 16). However, it must be recognised that there is still error associated with 347 the determination of BFlh fascicle length (in this assessment typical error is approximately 348 0.30cm). Secondly, there was no concurrent collection of match and training exposure, internal 349 and external training load and resistance training programming variables. As several factors are 350 likely modulators of fascicle length, examining the interaction between previous injury status 351 and the aforementioned variables needs to be the focus of the next series of studies in this area.

353 CONCLUSION

354 Paragraph

355 Elite Australian footballers with a history of HSI display shorter BFlh fascicles at the completion 356 of the season compared to the start, in both their injured and uninjured limbs. In contrast, athletes 357 without a history of HSI finish the season with similar fascicle lengths to what they started with. 358 Yet they do experience lengthening shortly after the commencement of the season which is then 359 succeeded by a sustained period of shortening for the rest of the season. The impact of injury 360 history on the structural and functional adaptations of the hamstrings requires further 361 examination, as practitioners and clinicians search for novel strategies to mitigate the risk of 362 recurrent HSI in their athletes.

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366 CONFLICT OF INTEREST

The authors wish to disclose that there were no conflicts of interest associated with professional relationships, that the study does not constitute endorsement by ACSM and that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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472 Figure 1

A two-dimensional ultrasound image of the biceps femoris long head. The image was along the longitudinal axis of the posterior thigh. From these images, it is possible to determine the superficial and intermediate aponeuroses, muscle thickness and angle of the fascicle in relation to the aponeurosis. Estimates of fascicle length can then be made via trigonometry using an equation validated against cadaveric tissue (5).

478 Figure 2

479 Fascicle length changes of the biceps femoris long head in previously hamstring strain injured 480 limbs, the contralateral uninjured limb and two-limb average of the control group without a 481 history of hamstring strain injury from elite Australian footballers. The weeks are each separated 482 by ~28 days and all data were collected during the in-season period. Error bars represent 483 standard deviations.

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