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EXHIBIT 10

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WILENCHIK & BARTNESS

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's domination of a female soccer league are campaigning for the competition to ban transgender women from female soccer teams.

Angry parents fed-up with a trans woman

The trans woman, who Daily Mail Australia has chosen not to identify, has led Football NSW's League One Women's 1st Grade goal kickers table, with seven goals.

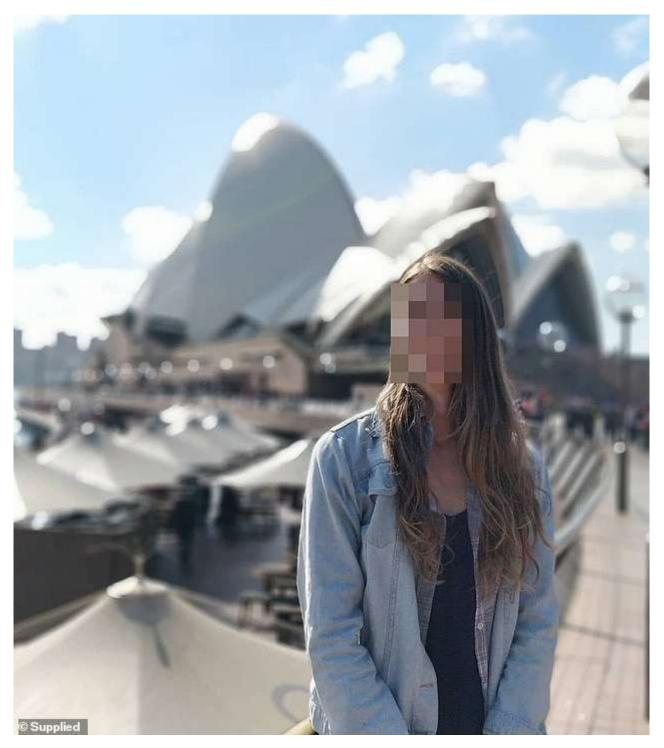
But it is allegations the trans athletes injured females from an opposing side in a match last weekend that has caused the ire of some fed-up parents and players.



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Angry parents fed-up with a trans woman's domination of a female soccer league are campaigning for the competition to ban transgender women from female soccer teams



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The trans woman is an active member of her community and came to Australia from the United States

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Daily Mail Australia has been told Football NSW, which governs the sport, refuses to address concerns surrounding the inclusion of trans women on female sides.

Kirralie Smith, a spokeswoman for Binary Australia, said she had spent months trying to speak with officials at the sporting body about its policy on the subject.

It is understood at least five trans women actively participate in the league across New South Wales.

Ms Smith claimed on Friday that her supporters sent thousands of emails to Football NSW, and 'no one has had any reasonable response despite all of the emails, warnings of injuries and how unfair it is.'

'Football NSW has failed to answer the simple questions, 'What is a woman?' and 'Why have a woman's division if men can play in it?'.

'They have failed in safeguarding fairness and safety for girls and women.'

A Facebook page dedicated to discussing the NPL league blew-up about the issue this week and has since received more than 260 comments.

Most of them take aim directly at Football NSW.



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The trans woman is a top goal scorer in the competition

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One person posted images of the American trans woman on field next to a much smaller female competitor.

'Totally unfair for all the females in the competition,' the woman wrote.

'So many players have gotten seriously injured because of this ridiculous situation'.

A man who claimed to have coached men and boys' soccer teams for 20 years said the league needed to 'take a good hard look at itself'.

'After what happened on the weekend at a couple of games, family and friends are looking at pulling their kids out of their teams,' he wrote.

'This needs to be addressed by the powers that be before anyone else gets hurt or players refuse to take the field or players quit the game.'

Daily Mail Australia contacted both Football Australia and Football NSW for comment.

Both clubs involved in last weekend's controversial match were also contacted as was the trans player at the centre of controversy. None were willing to comment on the issue.

'Football NSW is the administrator of their respective leagues and competitions, you'll need to contact them,' a Football Australia spokeswoman said.

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Parents and players are concerned the trans woman has a greater advantage

Ms Smith claimed images of trans women had recently been removed from official websites promoting the competition.

'Why? Why are transgender players' feelings being protected while female and their parents who raise concerns are being ignored?' she said.

Ms Smith said women and girls deserved single-sex divisions based upon the sex they were born as, adding: 'Transgender players do not need to be excluded.

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She noted that 'males retain an unfair, and sometimes unsafe, advantage due to the physical advantages of male development including bone density and structure, heart and lung capacity, blood volume, fast twitch muscle fibres, height, reach, strength, speed and stamina.'

Last year, world soccer's governing body FIFA and World Athletics said they were reviewing their transgender eligibility policies after swimming passed new rules that restricted transgender participation in women's events.

However reports from earlier this year suggested transgender footballers would compete at the FIFA Women's World Cup in Australia and New Zealand this July and August.

FIFA women's program director Sarai Bareman told The Australian in January three transgender players had contacted her about the review process and that she believed there were others.

'I think it's very sensitive, and we need to be very careful about how we deal with it. That's something we're taking very, very seriously.

'And we certainly don't want to rush it (the decision on new rules), given the impact that it will have for many generations to come,' she said.

Ms Bareman said FIFA had been consulting various groups including human rights groups, nongovernment organisations, athletes and other sports as well as the International Olympic Committee.

'We have to be very careful as you know, we have 211 member associations and essentially what we do seem to blueprint for those member associations, which is why the consultation process is very extensive and we will take our time to ensure that we get it right," she said.

The Australian Human Rights Commission shared guidelines about the the inclusion of transgender people in sport in 2019, noting that 'transgender and gender diverse people are sometimes excluded from sport, or may experience discrimination and sexual harassment when they do participate.

'While some reported positive experiences of inclusion, others described how they had been excluded from the sports they loved because of their sex or gender identity.

'Some spoke of disengaging from sport during their transition journey because of their concern about how their team mates would treat them.'

The Australian Professional Association for Trans Health did not respond to Daily Mail Australia's inquiry.

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EXHIBIT 11

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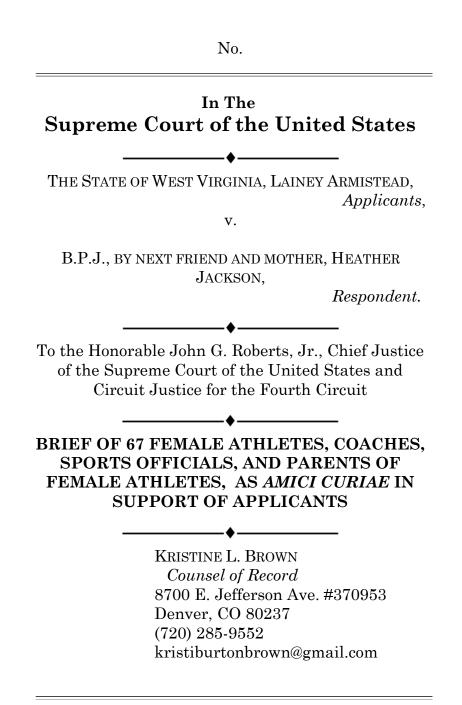


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INTEREST OF AMICI CURIAE¹

Since the founding of this nation, equality has been a constant struggle for various people groups. When the struggle has reached this Court, this Court has consistently been willing to consider how to uphold equal protection, equality of opportunity, and equal rights for all persons in these United States.

In the emergency application sought by West Virginia, the need to protect the equality of women is at issue. Sports is the new playing field, but the ultimate victory sought is for equality. *Amici* are 67 female athletes, coaches, teammates, parents, and relatives of these athletes². These athletes come from many levels of playing – from elementary school to collegiate; from professional to Olympic. No matter their level of accomplishment, their years in their chosen sport, or their age – some minors and some adults – all have been forced to compete against males or to suffer the psychological impact of helplessly watching the forced competition of men against women. Each of the *amici* who have signed on to this brief have a unique story to tell that all weave

¹ This *amicus* brief is filed in connection with an application under Rule 22. Under the time-sensitive provisions laid out in Rule 37.4, electronic transmission of the brief has been provided to both parties in accordance with Rule 29.3. In accordance with Rule 37.6, counsel affirms that no counsel for any party authored this brief in whole or in part and that no person or entity other than *amici* or their counsel made a monetary contribution intended to fund the preparation and submission of this brief.

² Athletes, coaches, and family members are identified in the Appendix.

a common thread essential to this Court's consideration of West Virginia's application.

Among the 67 athletes, coaches, and family members are: (i) Martina Navratilova, 59x Grand Slam Champion; (ii) Jennifer Sees, former NCAA track and field athlete, high school track coach, and parent of an NCAA soccer player; (iii) Summer Sanders, Olympic Gold Medalist; (iv) Courtney DeSoto, mother of a high school female athlete; (v) Jill Sterkel, an Olympic swimmer, former world record holder, and former University of Texas head swim coach; (vi) Pam Etem, an Olympian in rowing; (vii) Madisan Debos, current NCAA track athlete; (viii) Laura Wilkinson, an Olympian and World Champion in diving and parent to a daughter; (ix) Donna de Varona, an Olympic Gold Medalist and world record holder; and (x) Evie Edwards, a cyclist and the mother of an elementary-age female cyclist.

Amici demonstrate, through painfully lived experiences, that West Virginia's situation is not unique. Female athletes across the country, at all levels of sports, stand on the precipice of permanently losing their access to equal opportunity and safety in sports. Based on their biological sex, they are at risk of being pushed aside in law and in life in a permanently damaging and irreversible way.

By bringing their names, their voices, and their personal stories to this Court's attention, *amici* hope to highlight the plea of women and girls across the nation: that this Court affirm their continued right to equal opportunity and to set in granite that females

may not be put at a clear and targeted disadvantage based on their biological sex.

SUMMARY OF ARGUMENT

Amici offer that an argument uniquely supplements West Virginia's bold defense of females' equal rights. By vacating the injunction against West Virginia's law, this Court can reaffirm that women should not lose their equal opportunity to compete in sports on a level playing field. By affirming West Virginia's right to stand with women and girls, this Court can ensure that females' basic right to be treated equally is still the legal norm in the United States. In order for women and girls to be able to talk frankly about their biology and the impact of their sex in sport and in life, the words "female," "girl," and "woman" are an essential recognition of scientific reality throughout this brief. Accurate language that clearly describes the biological existence of female humans must have priority over language of preferred personal identification. This is the only way to protect the rights, equality, and safety of female athletes.

A growing number of women and girls have been facing the humiliating and damaging experience of being forced to compete against males who identify as transgender in the women's sports category. Lawmakers in West Virginia passed a law to put a stop to this abusive and discriminatory practice, but this law has been enjoined. *Amici* ask this Court to allow for the protection of women and girls while the lower courts work through further arguments. One male competing against women and girls negatively

affects every girl he competes with as well as every girl who loses a playing opportunity and every girl who must witness a female athlete being asked to step aside for the feelings of a male despite the knowledge that it is unfair to ask her to do so.

Amici's experiences as athletes, coaches, parents, and relatives of female athletes prove that females are uniquely and adversely affected when they are forced to compete against males in sports. Their personal stories demonstrate that females and males cannot experience or compete in physical sports in the same way; that the psychological, tangible, and longterm harm suffered by females forced to compete against males is irreversible; and that females across the nation at all levels of sports are suffering real harm that threatens their right to basic equality, safety, and equal opportunity under the law.

ARGUMENT

I. Females are uniquely and adversely affected when they are forced to compete against males in sports.

It is hard to express the pain, humiliation, frustration, and shame women experience when they are forced to compete against males in sport. It is public shaming and suffering, an exclusion from women's own category. The message to women and girls, 50% of our population, is shared by the parents, teammates, and spectators who watch it unfold. The shame does not disappear after competition is over. It stays forever as a memory of sanctioned public ridicule and a reminder of how women should expect

to be treated and set aside for the needs and desires of males.

At every age and every level, a female athlete deserves to know she is worthy of respect and fair competition against other females. She should not have to reach elite status to finally be deemed good enough to play without facing sex discrimination. College women's teams do not play against college men's teams; the high school girls' basketball team does not play against the boys' basketball team. The individual men's and women's state champion in tennis do not play against each other to determine who is the actual champion. The women's Olympic sprint champion does not race the men's champion.

This kind of competition is not allowed because we understand the result would almost always serve to humiliate women. It is not real or fair competition. We know the outcome because the numbers, science, and physical realities predict it with concrete assurance. A far less talented and skilled male will soundly beat a female. With this knowledge, we know the contests would merely be a predetermined public display of the physical differences between males and females. Such competition robs women and girls of a place to be held up in equal value to boys and men. In fact, it solidifies and reinforces that they are not worthy of equal opportunity and recognition.

Each stage of a girl's sports development path provides the opportunity to play – from granting her the last spot on the team to the first-place podium. These experiences – which start at a young age – create inspiration, self-belief, resilience, and

confidence – things every girl should be able to seek fairly and with equal opportunity to her male counterparts. We know the names of women like Martina Navratilova because these pathways and opportunities have been protected for females from a young age to the most competitive levels.

A. Females and males cannot experience or compete in physical sports in the same way.

There is enormous emotional trauma that accompanies women and girls when they are subject to competing against males in their sports opportunities. This trauma is grounded in real science and an understanding of our physical world as women; it is not a figment of our imagination. As athletes, coaches, and spectators of sport, we know there is a clear and obvious physical difference between boys and girls. We know the physical development of boys – beginning in utero – results in a performance difference between boys and girls. We see this play out in sports and physical activity at every age and every level.

Physical fitness tests and records for youth sports showcase a measurable performance disparity between males and females at every age. The genetic gene expressions that differ between males and females number over 6,000 and are not limited to: height, body mass, skeletal structure, strength, muscle quality, center of gravity, limb length ratios, cardiovascular performance, and, of course, reproductive influence. The effects of any amount of

male puberty and androgenization make those early performance differences explode even further.

As athletes, coaches, and parents of female athletes, we know this because we live it. We see and understand that the average age at which male athletes will beat the world records of women is 14-15 years of age. The use, weight, and design of sports equipment such as bikes, balls, bats, javelins, discs, and suits, as well as playing fields and net heights reflect the biological differences between boys and men and girls and women and are designed to optimize the competition. At every level, we are aware that less skilled, less determined males beat higher level female athletes because of innate physical difference in the sexes. Physical and developmental differences should not mean that girls and women are less worthy of participating, competing, and winning in sport. Females are half of the world's population and deserve equal opportunities as much as males.

B. The psychological, tangible, and longterm harm suffered by females forced to compete against males is irreversible.

When women and girls are asked to compete against male athletes, they are asked to ignore biological reality, the reality that defines female physical bodies. They are asked to pretend there is no hardship or difference in competing against male development that began in utero and resulted in differences in muscle structure, bone structure, response and reaction times, bone density, and finally, reproductive influences, such as monthly

cycles and possible pregnancy. They are asked to ignore almost all their lived experiences. This expected disconnection from reality has a very real psychological impact. It tells female athletes, their coaches, and their family members that female bodies don't matter enough to be recognized. This message is received when girls and women are told that rules in their sports don't need to be fair and that female bodies don't have equal representation on a playing field. Women's and girls' positions can be taken by a male if he requests to play with females, despite his physical advantages. Women and girls must stand by as boys and men now have a claim to female spaces, in addition to their own full male teams.

The girl who loses her place or her chance to compete must watch a male take a place that was set aside for her. She not only has to deal with a loss; but she must also deal with the psychological trauma that comes from knowing that the loss was not just, fair, or equal; it was an 'extra' spot given to a male – one she had no equal opportunity to compete for. The girls around her must watch a male supplant a place that was once set aside for women. This is mental torture for them. Women and girls know this means their fair treatment and their equal opportunity are no longer recognized as important. They are not protected or safe in their own sports. The girls competing receive the message that their competition is not important enough to demand integrity and fairness, while they witness firsthand that the males' competition and demands are always enough. The damage this causes is irreversible.

As athletes, coaches, and parents of female athletes, we are left with questions. How are we not seen working and training and striving – only to be beaten by a male who has less objective talent and skill but is able to rely on innate male advantage? How have we come to a place where we no longer have fair and equal opportunities in sports and where females are excluded from our own podiums, our own teams, and our own championships because we are expected to affirm males who wish to have our place? How are we expected to compare ourselves to males who everyone knows are physically stronger and biologically different? Why are women not allowed to have a female champion when there is already a male champion - whose place we could never take? Why does a male get to take a female's place on a team or in a race because it will help him feel better? If the measurement is feelings, why do our feelings not count? Will this Court agree that females no longer have the right to equal opportunities in real competitions? Females are suffering irreversible psychological damage that compounds every day this unequal treatment continues.

C. Females are suffering real harm that threatens their right to basic equality and equal opportunity.

Women and girls had begun to believe that the measured and known performance gap between males and females did not have to be viewed as a stamp of male superiority, but rather as the understood physiological and biological divergence

between equally respected members of society. The sex of female bodies, organized around and along divergent developmental paths, affects every cell and system. from reproduction and muscular development to skeletal and cardiovascular systems. Females had come to appreciate that these innate differences did not strip them of an expected place of equality and fair treatment. Sport and physical competition is the one public place where males and females have been guaranteed the right to celebrate their entirely independent and incomparable physical limits under laws like Title IX. Now, these same rules are being interpreted to remove women's access to equal protection and equal opportunities.

Women's sports were created and set aside to be a place where 50% of the population could finally be included and seen as worthy of the title, "champion"; where they could be held up as valuable members of schools, teams, and society.

Now, the nation is being told that fair sports for women and girls was a lie. As athletes, coaches, and parents of female athletes, we know that asking women to compare their bodies to male bodies is not a just request. The athletes know the competition is unfair. The coaches, officials, and sports scientists know the competition is unfair. The parents and spectators know the competition is unfair. And we all know exactly why. We even know that many of the male athletes taking our positions and titles were not exceptional male athletes in comparison to other males; they now serve as a reminder that a physically unexceptional male is entitled to showcase physical prowess against women and demand women

relinquish their opportunity to compete, their place in the event, their hard-earned title, and even their records. The girls and women must comply; the officials must congratulate; the parents must cheer; and the records must be etched for all to appreciate the reinforced reality that males are entitled to replace and show dominance over females in what was once an equal society.

These policies and actions are violating the spirit of the formation of women's sports and laws like Title IX. People in positions of power are looking for the right words to justify this deprivation of equal opportunity. As women, we can only think that it is because we are "just women;" and that even our biological reality is a debatable concept.

The realization that the laws and rights written to protect women are being used against females and the knowledge that people in power cannot or will not see - even in a publicly visible contest - that females are not being treated with the same respect and honor as men is a message that cuts deep into the psyche of women. The women that have experienced this feel the weight of unequal treatment, the stripping of rights, the loss of rewards, and the erasure of fair and equal representation. Women feel the weight of the message that female physical bodies are only good enough if they are able to compete with a physical development of biology that does not match their own. Women see and hear the rule makers and lawmakers argue the purpose of women's sports and, indeed, that the purpose of female athletes is to make a male athlete feel welcome and honored above the female athletes. Girls and women hear the message that it is

a female's job to consider our kindness above our demands for fair and equal treatment. Female athletes are told that "there are only a few" male athletes who want to compete against women, and so females must step aside and make room for them. The awards and record boards, originally meant to help girls and women share new possibilities for those of us born female, are rewritten with male names. Female existence and accomplishment in sports is being erased, name by name. When women compete in races against males and compete on teams with males, females know they are supporting the premise of male dominance; they are supporting the idea that females can only respond with meek compliance when treated as less than men; women and girls are forced to support the erasure of something females were proud of and once esteemed for.

As athletes, coaches, and parents of female athletes, what is our choice? To cede to participate? To give up entirely? Do we not then also give away our rights and our dignity? There is no solution for women and girls without the protection of laws that recognize equal opportunity for females.

The forced competition against males is humiliating. It cannot be fair or equal and yet, girls and women must either walk away from sports opportunities or accept the humiliation. Frustratingly, the rules and laws written for the expressed purpose of equality have not been enough; states now need to write new laws on top of the old ones to prevent the redefinition of the physical reality of being female.

A question being asked is if there is a way to make competition fair for women while still including some of the males who wish to participate with women. This is also an insulting proposition. Competition is how human beings find their physical limits. It is an invitation to bring one's absolute personal best and match it with the personal best of others in a fair and clean contest. Bringing a body forward to compete that is intentionally and artificially hindered is not in the spirit of that ethos. The question the lawmakers and governing bodies of sport are asking as they try to make guidelines to include males is just how much rules must impair male performance and development to be equated to that of women. This argument – this experiment – is not empowering for women; it is damaging to an entire generation of females. It is deeply misogynistic and demeaning. Girls and women are not encumbered male bodies. Girls and women are uniquely and innately female, and females should not have to fight for representation and see only biology that does not compare to their own rewarded

As athletes, coaches, and parents of female athletes, we are hurt and shamed that people in power do not find female athletes important enough to speak up for. We are left to cry and sink into depression on our own as we embrace our new understanding of girls' and women's place in the world. We are left with the shame of not being able to compete physically with a male who wants our place, the shame that laws have not been enough, the shame of losing while others cheered in a competition with no integrity, the shame of seeing males so easily take over that which was established for us, the

shame of having to speak out for something so plainly obvious, and the shame of having been silent, even if just for a moment, while we suffered or watched other women suffer. Not one more girl should go through this. Not one more parent should have to watch their daughter sidelined for a male who is deemed more important than her fair and equal chance.

The rules and record boards have not been fixed. We are not just haunted by our memories and experiences. We are forced to reckon with a public record that condones and historically celebrates our abuse and marginalization. This cannot be the legacy we leave for women and girls; for millions of human beings who are born female.

1. Reka Gyorgy, 2016 Hungarian Olympian, 2x ACC Champion from Virginia Tech

I was a senior competing in my last swim meet at the NCAA Women's Swimming & Diving Championships on March 17th, 2022. I swam the 500 freestyle in preliminaries where I got 17th, which means I did not make it back to the finals and was first alternate. I watched Lia Thomas [a biological malel from the pool deck win a women's national title in a finals that I deserved to be in because the rules in place did not support biological women. I couldn't help but cry and feel frustrated, angry, and sad. It hurt me, my team, and other women in the pool. A year later, there is still no response to my letter to the NCAA. This is an ongoing, painful reminder of how little all the women at that swim meet matter to the people running our schools and sports competitions.



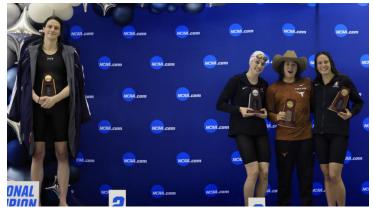


Photo 1: The podium at the 2022 NCAA Women's Swimming and Diving Championships where a male took the place of Reka Gyorgy.

2. Macy Petty, current NCAA volleyball player

While in high school, I competed in club volleyball tournaments across the country with hopes of being recruited to a college volleyball team. At one of these tournaments, with several college recruiters watching, I had to play against a boy in a girls' volleyball tournament. While trying to evaluate our skills, the recruiters instead watched this athlete repeatedly slam the ball in our faces. Because the girls' volleyball net heights are different from boys', this athlete was competing on a net 7.5 inches shorter than he should have as a male. As an athlete, this was humiliating; as a woman, I was horrified to see a boy so easily steal the right to play in brackets that were designed specifically to make volleyball safe and competitive for female bodies. I thought this was a

mistake everyone could see, and it would never happen again.

3. Riley Gaines, 12x All-American, SEC Record Holder

I have been a devout swimmer from the age of seven. It's hard to explain the amount of time, hard work, and sacrifice I have made in an effort to be successful at my sport. Every opportunity and every small victory along the way made the next step possible.

Last year, a 6'4" male (Lia Thomas) and I raced at the National Championships, which ultimately resulted in a tie. Upon tying, I was told Thomas (the male athlete) would get the trophy instead of myself as it was necessary for photo purposes. Everything I worked my entire life for was reduced to a photo-op to validate a male's feelings and identity of himself. All the women in the race faced unfair competition and the silencing of our voices through intimidation, emotional blackmail and gas-lighting by these large organizations and institutions.

I still struggle with knowing the people who were supposed to shield us from harm and make sure our sport is ethical were the same people who were silent and allowed us to be discriminated against. This goes against everything federal civil rights laws and Title IX were intended to protect. Women's sports were created to recognize and celebrate the unique physical accomplishments of female athletes. I feel neither recognized nor celebrated. I feel betrayed,

belittled, and traumatized. Please don't make any other women go through this.



Photo 2: Riley Gaines is forced to share the podium with a male.

4. Catrina Allen, World Champion in Disc Golf

I have been playing sports since I was five, and although I've lost many times over the course of my career, I've never felt as defeated as the day I had to compete against a male opponent in the disc golf female professional division. As tears ran down my face, during an elite series tournament, I realized that even though I have a strict practice regiment,

workout plan and am known as a fighter, there is no outworking the physical advantages that a male has. I have since faced four different males in the female category in 26 different tournaments. The worst part is if the women speak out and share their feelings of defeat and frustration, they fear loss of sponsorships and the very public wrath of those defending the male athletes. The women feel helpless, scared, voiceless and isolated.



Photo 3: A male becomes the women's champion in disc golf in competition against Catrina Allen and other females.

5. Hannah Arensman, professional women's cycling, Cyclocross National Champion

I was born into a family of athletes. Encouraged by my parents and siblings, I competed in sports from a young age, and I followed in my sister's footsteps, climbing the ranks to become an elite cyclocross racer. Over the past few years, I have had to race directly with male cyclists in women's events. As this has become more of a reality, it has become increasingly discouraging to train as hard as I do only to have to lose to a man with the unfair advantage of an androgenized body that intrinsically gives him an obvious advantage over me, no matter how hard I train.

I have decided to end my cycling career. At my last race at the recent UCI Cyclocross National Championships in the elite women's category in December 2022, I came in 4th place, flanked on either side by male riders awarded 3rd and 5th places. My sister and family sobbed as they watched a man finish in front of me, having witnessed several physical interactions with him throughout the race.

Additionally, it is difficult for me to think about the very real possibility I was overlooked for an international selection on the US team at Cyclocross Worlds in February 2023 because of a male competitor.

Moving forward, I feel for young girls learning to compete and who are growing up in a day when they no longer have a fair chance at being the new record

holders and champions in cycling because men want to compete in our division. I have felt deeply angered, disappointed, overlooked, and humiliated that the rule makers of women's sports do not feel it is necessary to protect women's sports to ensure fair competition for women anymore.



Photo 4: Hannah Arensmen misses the podium while a male takes her place.

6. Courtney DeSoto, parent of current high school track athlete

I am the parent of a minor daughter who runs varsity track in a public school in California. A male freshman joined the women's team this year and is running varsity track and winning every race while the girls watch in bewilderment. This same

individual is using the girl's locker room to change and shower. The girls are so uncomfortable that some are not using the locker room themselves anymore. The head coach is about to quit over the injustice of it all. Complaints and concerns for the girls are made to school and district administration, but no one is willing to say anything because our state laws and legislators will not protect our daughters. I have a younger daughter who is also interested in sports, but I am concerned for the future of all our girls.

CONCLUSION

Every day that girls' and women's equal opportunity in sports is denied, is a day that females suffer irreversible harm and psychological trauma. By vacating the injunction against West Virginia's law, this Court can reaffirm that females have not lost their equal opportunity to compete in sports on a level playing field. By affirming West Virginia's right to stand with girls and women, this Court can ensure that the basic right to be treated equally as a person born female is still the legal norm in the United States.

Respectfully submitted,

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March 9, 2023

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APPENDIX

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APPENDIX

LIST OF 67 AMICI CURIAE FEMALE ATHLETES, COACHES, SPORTS OFFICIALS, AND FEMALE ATHLETES' FAMILY MEMBERS, MANY OF WHOM HAVE BEEN FORCED TO COMPETE AGAINST BIOLOGICAL MALES AND ALL OF WHOM HAVE SUFFERED THE PSYCHOLOGICAL DAMAGE FROM WITNESSING FORCED AND UNEQUAL MALE DOMINANCE OVER FEMALES IN SPORTS¹

Brianna Alexander* Cyclist

Catrina Allen*

World Champion – professional disc golf

Hannah Arensman* Professional cyclist Sarah Powers Barnhard Professional volleyball player, current coach

Lauren Bondly

Masters National Champion – Triathlon

Carol Brown Olympian – rowing

¹ *Amici* submit this brief solely in their capacities as private citizens. To the extent an *Amicus*'s employer, institution, or association is named, it is solely for descriptive purposes and does not constitute endorsement by the employer, institution, or association of the brief or any portion of its content.

^{*} An asterisk by a name indicates that the athlete has personally faced a male in sports competition or the parent has a daughter who has personally faced this.

Abigail Buckley*

Mother of two female athletes who have been forced to compete against males

Mariah Burton Nelson

Former professional women's basketball player

Monika Burzynska* Current NCAA swimmer

Kathy Smith Connor* Former NCAA athlete and mother of daughter who competed in the 2022 NCAA Swim Championship

Madisan Debos* Current NCAA track athlete

Courtney DeSoto* Mother of a female high school track athlete iii

Donna de Varona *Olympic Gold Medalist, World Record holder*

Evie Edwards* Cyclist, mother of

elementary age female cyclist

Stephanie Elkins

Olympian – swimming

Pat Etem Olympian – rowing

Dianna Fussner* *Pro Masters disc golf*

Riley Gaines*

All-American swimming, tied Lia Thomas in the 200 free at the NCAA Swim Championships

Shawna Glazier*

Cyclist, Triathlete

Annie Grevers

U.S. National Team – swimming

Case 4:23-cv-00185-JGZ Document 92-15 Filed 06/29/23 Page 29 of 32

iv

Reka Gyorgy*

Olympian – swimming, missed finals by one placement at NCAA Swim Championships in the 500 free where Lia Thomas won first place

Rena Hedeman*

Mother of female rowing athlete

Nancy Hogshead-Makar Olympic Gold Medalist

Sarah Hokom* World Champion – professional disc golf

Lacey John Olympic Silver Medalist, NCAA Woman of the Year

Kim Jones*

All American – tennis, mother of a female swimmer Raime Jones* Current NCAA athlete – swimming, lost a finals spot in Ivy League Championships to Lia Thomas

Margot Kackzorowski* Current NCAA swimmer

Ronda Key* Disc golf athlete

Samantha Keddington* Former professional disc golf athlete, missed payout qualification by one placement won by a male, current coach

Danielle Keen* Professional disc golf,

Marshi Kokmeyer NCAA Champion – swimming v

Jess Kruchoski*

Fiancé of female athlete who competed against a male

Holly LaVasser* Cyclist

Donna Lopiano 6x National Champion, Former AD University of Texas

Valerie McClain Olympian – rowing

Riona C. McCormick *Current rowing athlete*

Cynthia Monteleone*

Masters track athlete, mother of female track athlete, both of whom competed against male athletes

Martina Navratilova 59x Grand Slam Tennis Champion

Sarita Nori* Mother of female

rowing athlete

Mary O'Connor Olympian – rowing

Keri Olson NCAA Champion – tennis, mother of female athlete

Jan Palchikoff Olympian – rowing

Macy Petty* Current NCAA athlete – volleyball

Mary T. Plant Olympian – swimming

Lori Post*

Mother of NCAA female swimmer who competed against Lia Thomas

Genoa Rossi

Current NCAA water polo athlete, U.S. Jr. National Team

Jennifer Sees

NCAA pole vaulter, current high school track coach, mother of NCAA soccer player vi

Jennifer Sey

U.S. National Champion – gymnastics

Jeri Shanteau National Champion, U.S. National Team member – swimming

Sandy Shasby* Family member of a female athlete

DeNee Shepherd* Disc golf athlete

Taylor Silverman*Skateboard athlete

Anne Simpson Rowing athlete

Summer Sanders Olympic Gold Medalist

Lori Stenstrom

National Champion, former American Record holder, mother of female athletes Jill Sterkel Olympian – swimming, former University of Texas head swim coach

Tracy Sundlan 5x Olympic coach, manager, and administrator – track and field

Maya Tait* NCAA rowing athlete

Leanne Venema* Mother of female NCAA swimmer

Eric Venema* Father of female NCAA swimmer

Michelle Venema* Aunt of female NCAA swimmer

Sue Walsh

Olympian – swimming, coach, sports official

Claudia Westholder *Female athlete, mother of female athlete* vii

Max Wettstein

Father of U.S. Olympic skateboard team member

Val Whiting

National Champion, WNBA

Laura Wilkinson

Olympian and World Champion – diving, mother of female athlete Case 4:23-cv-00185-JGZ Document 92-16 Filed 06/29/23 Page 1 of 7

EXHIBIT 12

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WILENCHIK & BARTNESS

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Fifty-fourth Legislature Second Regular Session

COMMITTEE ON HEALTH & HUMAN SERVICES HOUSE OF REPRESENTATIVES AMENDMENTS TO H.B. 2706 (Reference to printed bill)

6/8/23, 8:16 AM Case 4:23-cv-00185-JGZ HD2706IFA92Haber & Filesh & Filesh 660/264/268 pt Rage 3 of 7

Page 1, line 16, strike "ALL OF"

Page 2, between lines 20 and 21, insert:

"Sec. 2. Legislative findings and purpose

The legislature finds that:

1. There are "'[i]nherent differences' between men and women," and that these differences "remain cause for celebration, but not for denigration of the members of either sex or for artificial constraints on an individual's opportunity." <u>United States v. Virginia</u>, 518 U.S. 515, 533 (1996).

2. These "inherent differences" range from chromosomal and hormonal differences to physiological differences.

3. Men generally have "denser, stronger bones, tendons, and ligaments" and "larger hearts, greater lung volume per body mass, a higher red blood cell count, and higher hemoglobin." Neel Burton, <u>The Battle of the Sexes</u>, PSYCHOL. TODAY, July 2, 2012, https://www.psychologytoday.com/us/blog/hide-and-seek/201207/the-battle-the-sexes?amp.

4. Men also have higher natural levels of testosterone, which affects traits such as hemoglobin levels, body fat content, the storage and use of carbohydrates and the development of Type 2 muscle fibers, all of which result in men being able to generate higher speed and power during physical activity. Doriane Lambelet Coleman, <u>Sex in Sport</u>, 80 LAW & CONTEMP. PROBS. 63, 74 (2017) (quoting Gina Kolata, <u>Men, Women and Speed. 2 Words: Got Testosterone?</u>, N.Y. TIMES, Aug. 21, 2008).

5. The biological differences between females and males, especially as they relate to natural levels of testosterone, "explain the male and female secondary sex characteristics which develop during puberty and have lifelong effects, including those most important for success in sport: categorically different strength, speed, and endurance." Doriane Lambelet Coleman & Wickliffe Shreve, <u>Comparing Athletic Performances: The Best Elite Women to Boys and Men</u>, DUKE LAW CTR. FOR SPORTS LAW AND POLICY, https://web.law.duke.edu/sports/sex-sport/comparative-athletic-performance/ (last visited Feb. 10, 2020).

6. While classifications based on sex are generally disfavored, the United States Supreme Court has recognized that "[s]ex classifications may be used to compensate women for particular economic disabilities [they have] suffered, ... to promote equal employment opportunity, ... [and] to advance full development of the talent and capacities of our Nation's people." <u>United States v.</u> <u>Virginia</u>, 518 U.S. 515, 533 (1996) (internal citations and quotation marks omitted).

7. One place where sex classifications allow for the "full development of the talent and capacities of our Nation's people" is in the context of sports and athletics.

8. Courts have recognized that the inherent, physiological differences between males and females result in different athletic capabilities. *See*, *e.g.*, <u>Kleczek v. R.I. Interscholastic League, Inc.</u>,

612 A.2d 734, 738 (R.I. 1992) ("Because of innate physiological differences, boys and girls are not similarly situated as they enter athletic competition."); <u>Petrie v. Ill. High Sch. Ass'n</u>, 394 N.E.2d 855, 861 (Ill. App. Ct. 1979) (noting that "high school boys [generally possess physiological advantages over] their girl counterparts" and that those advantages give them an unfair lead over girls in some sports like "high school track").

9. A recent study of female and male Olympic performances since 1983 found that, although athletes from both sexes improved over the time span, the "gender gap" between female and male performances remained stable. "These suggest that women's performances at the high level will never match those of men." Valerie Thibault, et al., <u>Women and Men in Sport Performance: The Gender Gap has not Evolved since 1983</u>, 9 J. SPORTS SCI. & MED. 214, 219 (2010).

10. As Duke Law professor and all-American track athlete Doriane Coleman, tennis champion Martina Navratilova and Olympic track gold medalist Sanya Richards-Ross recently wrote: "The evidence is unequivocal that starting in puberty, in every sport except sailing, shooting and riding, there will always be significant numbers of boys and men who would beat the best girls and women in head-to-head competition. Claims to the contrary are simply a denial of science." Doriane Coleman, Martina Navratilova, et al., <u>Pass the Equality Act, But Don't Abandon Title IX</u>, WASH. POST, Apr. 29, 2019, https://wapo.st/2VKINN1.

11. The benefits that natural testosterone provides to male athletes are not diminished through the use of puberty blockers and cross-sex hormones. A recent study on the impact of such treatments found that even "after 12 months of hormonal therapy," a man who identifies as a woman and is taking cross-sex hormones "had an absolute advantage" over female athletes and "will still likely have performance benefits" over women. Tommy Lundberg, et al., <u>Muscle strength, size and composition following 12 months of gender-affirming treatment in transgender individuals: retained advantage for the transwomen</u>, Karolinksa Institutet, (Sept. 26, 2019).

12. Having separate sex-specific teams furthers efforts to promote sex equality. Sex-specific teams accomplish this by providing opportunities for female athletes to demonstrate their skill, strength and athletic abilities while also providing them with opportunities to obtain recognition and accolades, college scholarships and the numerous other long-term benefits that flow from success in athletic endeavors."

Renumber to conform Amend title to conform 6/8/23, 8:16 AM Case 4:23-cv-00185-JGZ HD27061F9421Rt-92+42fb & Filesh 869/264/26bpt Rage 6 of 7

6/8/23, 8:16 AM Case 4:23-cv-00185-JGZ HB2706IFB42Rt-92+abth & Hileah 66//28/28 pt Rage 7 of 7

And, as so amended, it do pass

NANCY K. BARTO CHAIRMAN

2706HEALTH HUMAN SERVICES 02/13/2020 11:01 AM H: ra Case 4:23-cv-00185-JGZ Document 92-17 Filed 06/29/23 Page 1 of 6

EXHIBIT 13

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WILENCHIK & BARTNESS

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House Engrossed

interscholastic athletics; biological sex

State of Arizona House of Representatives Fifty-fourth Legislature Second Regular Session 2020

HOUSE BILL 2706

AN ACT

AMENDING TITLE 15, CHAPTER 1, ARTICLE 1, ARIZONA REVISED STATUTES, BY ADDING SECTION 15-120.01; RELATING TO INTERSCHOLASTIC ATHLETICS.

(TEXT OF BILL BEGINS ON NEXT PAGE)

6/8/23, 8:14 AM Case 4:23-cv-00185-JGZ Document+927017542Filedv06/29/23 Page 3 of 6

Be it enacted by the Legislature of the State of Arizona:

Section 1. Title 15, chapter 1, article 1, Arizona Revised Statutes, is amended by adding section 15-120.01, to read:

15-120.01. Designation of athletic teams; educational institutions; cause of action;

<u>definition</u>

A. AN INTERSCHOLASTIC OR INTRAMURAL ATHLETIC TEAM OR SPORT THAT IS SPONSORED BY AN EDUCATIONAL INSTITUTION IN THIS STATE MUST BE EXPRESSLY DESIGNATED AS ONE OF THE FOLLOWING BASED ON BIOLOGICAL SEX:

1. MALES, MEN OR BOYS.

2. FEMALES, WOMEN OR GIRLS.

3. COED OR MIXED SEX.

B. ATHLETIC TEAMS OR SPORTS DESIGNATED FOR FEMALES, WOMEN OR GIRLS MAY NOT BE OPEN TO STUDENTS OF THE MALE SEX.

C. IF DISPUTED, A STUDENT MAY ESTABLISH THE STUDENT'S SEX BY PRESENTING A SIGNED PHYSICIAN'S STATEMENT THAT INDICATES THE STUDENT'S SEX BASED ON AN ANALYSIS OF THE STUDENT'S GENETIC MAKEUP.

D. A GOVERNMENTAL ENTITY, A LICENSING OR ACCREDITING ORGANIZATION OR AN ATHLETIC ASSOCIATION OR ORGANIZATION MAY NOT ENTERTAIN A COMPLAINT, OPEN AN INVESTIGATION OR TAKE ANY OTHER ADVERSE ACTION AGAINST AN EDUCATIONAL INSTITUTION FOR MAINTAINING SEPARATE INTERSCHOLASTIC OR INTRAMURAL ATHLETIC TEAMS OR SPORTS FOR STUDENTS OF THE FEMALE SEX.

E. ANY STUDENT WHO IS DEPRIVED OF AN ATHLETIC OPPORTUNITY OR SUFFERS ANY DIRECT OR INDIRECT HARM AS A RESULT OF A VIOLATION OF THIS SECTION HAS A PRIVATE CAUSE OF ACTION FOR INJUNCTIVE RELIEF, DAMAGES AND ANY OTHER RELIEF AVAILABLE UNDER LAW AGAINST THE EDUCATIONAL INSTITUTION.

F. ANY STUDENT WHO IS SUBJECT TO RETALIATION OR OTHER ADVERSE ACTION BY AN EDUCATIONAL INSTITUTION OR ATHLETIC ASSOCIATION OR ORGANIZATION AS A RESULT OF REPORTING A VIOLATION OF THIS SECTION TO AN EMPLOYEE OR REPRESENTATIVE OF THE EDUCATIONAL INSTITUTION OR ATHLETIC ASSOCIATION OR ORGANIZATION OR TO ANY STATE OR FEDERAL AGENCY WITH OVERSIGHT OF EDUCATIONAL INSTITUTIONS IN THIS STATE HAS A PRIVATE CAUSE OF ACTION FOR INJUNCTIVE RELIEF, DAMAGES AND ANY OTHER RELIEF AVAILABLE UNDER LAW AGAINST THE EDUCATIONAL INSTITUTION OR ATHLETIC ASSOCIATION OR ORGANIZATION.

G. ANY ELIGIBLE INSTITUTION THAT SUFFERS ANY DIRECT OR INDIRECT HARM AS A RESULT OF A VIOLATION OF THIS SECTION HAS A PRIVATE CAUSE OF ACTION FOR INJUNCTIVE RELIEF, DAMAGES AND ANY OTHER RELIEF AVAILABLE UNDER LAW AGAINST THE GOVERNMENTAL ENTITY, LICENSING OR ACCREDITING ORGANIZATION OR ATHLETIC ASSOCIATION OR ORGANIZATION.

H. A CIVIL ACTION UNDER THIS SECTION MUST BE INITIATED WITHIN TWO YEARS AFTER THE HARM OCCURS. A PERSON THAT PREVAILS ON A CLAIM BROUGHT UNDER THIS SECTION IS ENTITLED TO MONETARY DAMAGES, INCLUDING FOR ANY PSYCHOLOGICAL, EMOTIONAL AND PHYSICAL HARM SUFFERED, ANY REASONABLE ATTORNEY FEES AND COSTS AND ANY OTHER APPROPRIATE RELIEF.

I. FOR THE PURPOSES OF THIS SECTION, "EDUCATIONAL INSTITUTION" MEANS ANY OF THE FOLLOWING:

1. A PUBLIC SCHOOL, WHETHER OR NOT THE PUBLIC SCHOOL IS A MEMBER OF AN INTERSCHOLASTIC ATHLETIC ASSOCIATION OR ORGANIZATION.

2. A PRIVATE SCHOOL THAT IS A MEMBER OF AN INTERSCHOLASTIC ATHLETIC ASSOCIATION OR ORGANIZATION.

3. A UNIVERSITY UNDER THE JURISDICTION OF THE ARIZONA BOARD OF REGENTS, WHETHER OR NOT THE UNIVERSITY IS A MEMBER OF ANY ASSOCIATION LISTED IN PARAGRAPH 5 OF THIS SUBSECTION. 4. A COMMUNITY COLLEGE AS DEFINED IN SECTION 15-1401, WHETHER OR NOT THE COMMUNITY COLLEGE IS A MEMBER OF ANY ASSOCIATION LISTED IN PARAGRAPH 5 OF THIS SUBSECTION.

5. ANY OTHER INSTITUTION OF HIGHER EDUCATION THAT IS A MEMBER OF ANY OF THE FOLLOWING:

(a) A NATIONAL COLLEGIATE ATHLETIC ASSOCIATION.

(b) A NATIONAL ASSOCIATION OF INTERCOLLEGIATE ATHLETICS.

(c) A NATIONAL JUNIOR COLLEGE ATHLETIC ASSOCIATION.

Sec. 2. <u>Legislative findings and purpose</u>

The legislature finds that:

1. There are "'[i]nherent differences' between men and women," and that these differences "remain cause for celebration, but not for denigration of the members of either sex or for artificial constraints on an individual's opportunity." <u>United States v. Virginia</u>, 518 U.S. 515, 533 (1996).

2. These "inherent differences" range from chromosomal and hormonal differences to physiological differences.

3. Men generally have "denser, stronger bones, tendons, and ligaments" and "larger hearts, greater lung volume per body mass, a higher red blood cell count, and higher hemoglobin." Neel Burton, <u>The Battle of the Sexes</u>, PSYCHOL. TODAY, July 2, 2012, https://www.psychologytoday.com/us/blog/hide-and-seek/201207/the-battle-the-sexes?amp.

4. Men also have higher natural levels of testosterone, which affects traits such as hemoglobin levels, body fat content, the storage and use of carbohydrates and the development of Type 2 muscle fibers, all of which result in men being able to generate higher speed and power during physical activity. Doriane Lambelet Coleman, <u>Sex in Sport</u>, 80 LAW & CONTEMP. PROBS. 63, 74 (2017) (quoting Gina Kolata, <u>Men, Women and Speed. 2 Words: Got Testosterone?</u>, N.Y. TIMES, Aug. 21, 2008).

5. The biological differences between females and males, especially as they relate to natural levels of testosterone, "explain the male and female secondary sex characteristics which develop during puberty and have lifelong effects, including those most important for success in sport: categorically different strength, speed, and endurance." Doriane Lambelet Coleman & Wickliffe Shreve, <u>Comparing Athletic Performances: The Best Elite Women to Boys and Men</u>, DUKE LAW CTR. FOR SPORTS LAW AND POLICY, https://web.law.duke.edu/sports/sex-sport/comparative-athletic-performance/ (last visited Feb. 10, 2020).

6. While classifications based on sex are generally disfavored, the United States Supreme Court has recognized that "[s]ex classifications may be used to compensate women for particular economic disabilities [they have] suffered, ... to promote equal employment opportunity, ... [and] to advance full development of the talent and capacities of our Nation's people." <u>United States v.</u> <u>Virginia</u>, 518 U.S. 515, 533 (1996) (internal citations and quotation marks omitted).

7. One place where sex classifications allow for the "full development of the talent and capacities of our Nation's people" is in the context of sports and athletics.

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9. A recent study of female and male Olympic performances since 1983 found that, although athletes from both sexes improved over the time span, the "gender gap" between female and male performances remained stable. "These suggest that women's performances at the high level will never match those of men." Valerie Thibault, et al., <u>Women and Men in Sport Performance: The Gender Gap has not Evolved since 1983</u>, 9 J. SPORTS SCI. & MED. 214, 219 (2010).

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and women in head-to-head competition. Claims to the contrary are simply a denial of science." Doriane Coleman, Martina Navratilova, et al., <u>Pass the Equality Act, But Don't Abandon Title IX</u>, WASH. POST, Apr. 29, 2019, https://wapo.st/2VKINN1.

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12. Having separate sex-specific teams furthers efforts to promote sex equality. Sex-specific teams accomplish this by providing opportunities for female athletes to demonstrate their skill, strength and athletic abilities while also providing them with opportunities to obtain recognition and accolades, college scholarships and the numerous other long-term benefits that flow from success in athletic endeavors.

Sec. 3. Severability

If a provision of this act or its application to any person or circumstance is held invalid, the invalidity does not affect other provisions or applications of the act that can be given effect without the invalid provision or application, and to this end the provisions of this act are severable.

Sec. 4. Short title

This act may be cited as the "Save Women's Sports Act".

Case 4:23-cv-00185-JGZ Document 92-18 Filed 06/29/23 Page 1 of 7

EXHIBIT 14

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WILENCHIK & BARTNESS

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Fifty-fourth Legislature Second Regular Session Health & Human Services H.B. 2706

PROPOSED HOUSE OF REPRESENTATIVES AMENDMENTS TO H.B. 2706 (Reference to printed bill)

6/8/23, 8:15 AM Case 4:23-cv-00185-JGZ нВ2000. нэнек 9-20-116 & Filed & filed

Page 1, line 16, strike "ALL OF"

Page 2, between lines 20 and 21, insert:

"Sec. 2. Legislative findings and purpose

The legislature finds that:

1. There are "'[i]nherent differences' between men and women," and that these differences "remain cause for celebration, but not for denigration of the members of either sex or for artificial constraints on an individual's opportunity." <u>United States v. Virginia</u>, 518 U.S. 515, 533 (1996).

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5. The biological differences between females and males, especially as they relate to natural levels of testosterone, "explain the male and female secondary sex characteristics which develop during puberty and have lifelong effects, including those most important for success in sport: categorically different strength, speed, and endurance." Doriane Lambelet Coleman & Wickliffe Shreve, <u>Comparing Athletic Performances: The Best Elite Women to Boys and Men</u>, DUKE LAW CTR. FOR SPORTS LAW AND POLICY, https://web.law.duke.edu/sports/sex-sport/comparative-athletic-performance/ (last visited Feb. 10, 2020).

6. While classifications based on sex are generally disfavored, the United States Supreme Court has recognized that "[s]ex classifications may be used to compensate women for particular economic disabilities [they have] suffered, ... to promote equal employment opportunity, ... [and] to advance full development of the talent and capacities of our Nation's people." <u>United States v.</u> <u>Virginia</u>, 518 U.S. 515, 533 (1996) (internal citations and quotation marks omitted).

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Renumber to conform Amend title to conform 6/8/23, 8:15 AM Case 4:23-cv-00185-JGZ нВ2000. H3742Rt 9-20-all & Filed & Fil

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NANCY K. BARTO

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EXHIBIT 15

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WILENCHIK & BARTNESS

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Case 4:23-cv-00185-JGZ Document 92-19 Filed 06/29/23 Page 2 of 6

Senate Engrossed

interscholastic; intramural athletics; biological sex

State of Arizona Senate Fifty-fifth Legislature Second Regular Session 2022

SENATE BILL 1165

AN ACT

AMENDING TITLE 15, CHAPTER 1, ARTICLE 1, ARIZONA REVISED STATUTES, BY ADDING SECTION 15-120.02; RELATING TO ATHLETICS.

(TEXT OF BILL BEGINS ON NEXT PAGE)

1 Be it enacted by the Legislature of the State of Arizona: Section 1. Title 15, chapter 1, article 1, Arizona Revised 2 3 Statutes, is amended by adding section 15-120.02, to read: 4 15-120.02. Interscholastic and intramural athletics; designation of teams; biological sex; cause of 5 6 action; definition 7 A. EACH INTERSCHOLASTIC OR INTRAMURAL ATHLETIC TEAM OR SPORT THAT IS SPONSORED BY A PUBLIC SCHOOL OR A PRIVATE SCHOOL WHOSE STUDENTS OR 8 TEAMS COMPETE AGAINST A PUBLIC SCHOOL SHALL BE EXPRESSLY DESIGNATED AS ONE 9 OF THE FOLLOWING BASED ON THE BIOLOGICAL SEX OF THE STUDENTS WHO 10 11 PARTICIPATE ON THE TEAM OR IN THE SPORT: "MALES", "MEN" OR "BOYS". 12 1. 13 2. "FEMALES", "WOMEN" OR "GIRLS". 3. "COED" OR "MIXED". 14 B. ATHLETIC TEAMS OR SPORTS DESIGNATED FOR "FEMALES", "WOMEN" OR 15 16 "GIRLS" MAY NOT BE OPEN TO STUDENTS OF THE MALE SEX. 17 C. THIS SECTION DOES NOT RESTRICT THE ELIGIBILITY OF ANY STUDENT TO 18 PARTICIPATE IN ANY INTERSCHOLASTIC OR INTRAMURAL ATHLETIC TEAM OR SPORT DESIGNATED AS BEING FOR "MALES", "MEN" OR "BOYS" OR DESIGNATED AS "COED" 19 20 OR "MIXED". 21 D. A GOVERNMENT ENTITY, ANY LICENSING OR ACCREDITING ORGANIZATION 22 OR ANY ATHLETIC ASSOCIATION OR ORGANIZATION MAY NOT ENTERTAIN A COMPLAINT. 23 OPEN AN INVESTIGATION OR TAKE ANY OTHER ADVERSE ACTION AGAINST A SCHOOL 24 FOR MAINTAINING SEPARATE INTERSCHOLASTIC OR INTRAMURAL ATHLETIC TEAMS OR 25 SPORTS FOR STUDENTS OF THE FEMALE SEX. 26 E. ANY STUDENT WHO IS DEPRIVED OF AN ATHLETIC OPPORTUNITY OR 27 SUFFERS ANY DIRECT OR INDIRECT HARM AS A RESULT OF A SCHOOL KNOWINGLY VIOLATING THIS SECTION HAS A PRIVATE CAUSE OF ACTION FOR INJUNCTIVE 28 29 RELIEF, DAMAGES AND ANY OTHER RELIEF AVAILABLE UNDER LAW AGAINST THE 30 SCHOOL. F. ANY STUDENT WHO IS SUBJECT TO RETALIATION OR ANOTHER ADVERSE 31 32 ACTION BY A SCHOOL OR AN ATHLETIC ASSOCIATION OR ORGANIZATION AS A RESULT 33 OF REPORTING A VIOLATION OF THIS SECTION TO AN EMPLOYEE OR REPRESENTATIVE OF THE SCHOOL OR THE ATHLETIC ASSOCIATION OR ORGANIZATION. OR TO ANY STATE 34 35 OR FEDERAL AGENCY WITH OVERSIGHT OF SCHOOLS IN THIS STATE, HAS A PRIVATE 36 CAUSE OF ACTION FOR INJUNCTIVE RELIEF, DAMAGES AND ANY OTHER RELIEF 37 AVAILABLE UNDER LAW AGAINST THE SCHOOL OR THE ATHLETIC ASSOCIATION OR 38 ORGANIZATION. 39 G. ANY SCHOOL THAT SUFFERS ANY DIRECT OR INDIRECT HARM AS A RESULT 40 OF A VIOLATION OF THIS SECTION HAS A PRIVATE CAUSE OF ACTION FOR 41 INJUNCTIVE RELIEF, DAMAGES AND ANY OTHER RELIEF AVAILABLE UNDER LAW AGAINST THE GOVERNMENT ENTITY. THE LICENSING OR ACCREDITING ORGANIZATION 42 43 OR THE ATHLETIC ASSOCIATION OR ORGANIZATION. 44 H. ALL CIVIL ACTIONS MUST BE INITIATED WITHIN TWO YEARS AFTER THE 45 ALLEGED VIOLATION OF THIS SECTION OCCURRED. A PERSON OR ORGANIZATION THAT

1 PREVAILS ON A CLAIM BROUGHT PURSUANT TO THIS SECTION IS ENTITLED TO 2 MONETARY DAMAGES, INCLUDING DAMAGES FOR ANY PSYCHOLOGICAL, EMOTIONAL OR 3 PHYSICAL HARM SUFFERED, REASONABLE ATTORNEY FEES AND COSTS AND ANY OTHER 4 APPROPRIATE RELIEF. 5 I. FOR THE PURPOSES OF THIS SECTION, "SCHOOL" MEANS EITHER: 6 1. A SCHOOL THAT PROVIDES INSTRUCTION IN ANY COMBINATION OF 7 KINDERGARTEN PROGRAMS OR GRADES ONE THROUGH TWELVE. 2. AN INSTITUTION OF HIGHER EDUCATION. 8 9 Sec. 2. Legislative findings and purpose 10 The legislature finds that: 11 1. "With respect to biological sex, one is either male or female." Arnold De Loof, Only Two Sex Forms but Multiple Gender Variants: 12 13 How to Explain?, 11(1) COMMUNICATIVE & INTEGRATIVE BIOLOGY (2018), https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5824932. 14 15 2. A person's "sex is determined at [fertilization] and revealed 16 birth or, increasingly, in utero." Lucy Griffin et al., Sex, gender at and gender identity: a re-evaluation of the evidence, 45(5) BJPsych 17 18 (2021), https://www.cambridge.org/core/journals/bjpsych-BULLETIN 291 19 bulletin/article/sex-gender-and-gender-identity-a-reevaluation-of-the-20 evidence/76A3DC54F3BD91E8D631B93397698B1A. 21 "[B]iological differences 3. between males and females 22 genetically during embryonic development." determined Stefanie are 23 Eggers & Andrew Sinclair, Mammalian sex determination-insights from 24 20(1) CHROMOSOME humans and mice, Res. 215 (2012), 25 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3279640. 26 4. "Secondary sex characteristics that develop during puberty . . . 27 generate anatomical divergence beyond the reproductive system, leading to adult body types that are measurably different between sexes." 28 Emma N. 29 Hilton & Tommy R. Lundberg, Transgender Women in the Female Category of Sport: Perspectives on Testosterone Suppression and Performance Advantage, 30 51 SPORTS MED. 199 (2021), https://doi.org/10.1007/s40279-020-01389-3. 31 32 5. There are "'[i]nherent differences' between men and women," and 33 that these differences "remain cause for celebration, but not for denigration of the members of either sex or for artificial constraints on 34 35 an individual's opportunity." United States v. Virginia, 518 U.S. 515, 36 533 (1996). 37 6. In studies of large cohorts of children from six years old, 38 "[b]oys typically scored higher than girls on cardiovascular endurance, 39 muscular strength, muscular endurance, and speed/agility, but lower on 40 flexibility." Konstantinos Tambalis et al., Physical fitness normative 41 values for 6-18-year-old Greek boys and girls, using the empirical distribution and the lambda, mu, and sigma statistical method, 16(6) 42 EUR J. SPORT SCI. 736 (2016), https://pubmed.ncbi.nlm.nih.gov/26402318. 43 44 See also, Mark J Catley & Grant R Tomkinson, Normative Health-related fitness values for children: analysis of 85347 test results on 9-17 year 45

1 <u>old Australians since 1985</u>, 47(2) BRIT. J. SPORTS MED. 98 (2013), 2 https://pubmed.ncbi.nlm.nih.gov/22021354.

7. Physiological differences between males and females relevant to sports performance "include a larger body size with more skeletal-muscle mass, a lower percentage of body fat, and greater maximal delivery of anaerobic and aerobic energy." Øyvind Sandbakk et al., <u>Sex Differences in</u> <u>World-Record Performance: The Influence of Sport Discipline and</u> <u>Competition Duration</u>, 13(1) INT'L J. SPORTS PHYSIOLOGY & PERFORMANCE 2 (2018), https://pubmed.ncbi.nlm.nih.gov/28488921.

8. Men also have higher natural levels of testosterone, which affects traits such as hemoglobin levels, body fat content, the storage and use of carbohydrates, and the development of Type 2 muscle fibers, all of which result in men being able to generate higher speed and power during physical activity. Doriane Lambelet Coleman, <u>Sex in Sport</u>, 80 LAW & CONTEMP. PROBS. 63, 74 (2017) (quoting Gina Kolata, <u>Men, Women and Speed.</u> <u>2 Words: Got Testosterone?</u>, N.Y. TIMES (Aug. 21, 2008).

9. There is a sports performance gap between males and females, such that "the physiological advantages conferred by biological sex appear, on assessment of performance data, insurmountable." Hilton, *supra* at 200.

21 While classifications based on sex are generally disfavored, 10. 22 the Supreme Court has recognized that "sex classifications may be used to 23 compensate women for particular economic disabilities [they have] 24 suffered, . . . to promote equal employment opportunity, . . . [and] to 25 advance full development of the talent and capacities of our Nation's 26 United States v. Virginia, 518 U.S. 515, 533 (1996) (internal people." 27 citations and quotation marks omitted).

28 11. One place where sex classifications allow for the "full 29 development of the talent and capacities of our Nation's people" is in the 30 context of sports and athletics.

31 12. Courts have recognized that the inherent, physiological 32 differences between males and females result in different athletic 33 capabilities. See, e.g., Kleczek v. Rhode Island Interscholastic League, 34 Inc., 612 A.2d 734, 738 (R.I. 1992) ("Because of innate physiological 35 differences, boys and girls are not similarly situated as they enter 36 athletic competition."); Petrie v. Ill. High Sch. Ass'n, 394 N.E.2d 855, 37 861 (Ill. App. Ct. 1979) (noting that "high school boys [generally possess 38 physiological advantages over] their girl counterparts" and that those 39 advantages give them an unfair lead over girls in some sports like "high 40 school track").

41 13. The benefits that natural testosterone provides to male 42 athletes is not diminished through the use of testosterone suppression. A 43 recent study on the impact of such treatments found that policies like 44 those of the International Olympic Committee requiring biological males to 45 undergo at least one year of testosterone suppression before competing in

1 women's sports do not create a level playing field. "[T]he reduction in 2 testosterone levels required by [policies like those of the International Olympic Committee] is insufficient to remove or reduce the male advantage, 3 in terms of muscle mass and strength, by any meaningful degree." The 4 study concluded that "[t]he data presented here demonstrate that superior 5 6 anthropometric, muscle mass and strength parameters achieved by males at 7 puberty, and underpinning a considerable portion of the male performance 8 advantage over females, are not removed by the current regimen of testosterone suppression" permitted by the International Olympic Committee 9 and other sports organizations. Rather, the study found that male 10 11 performance advantage over females "remains substantial" and "raises 12 obvious concerns about fair and safe competition." Hilton, supra at 13 207, 209.

14 14. Having separate sex-specific teams furthers efforts to promote 15 sex equality by providing opportunities for female athletes to demonstrate 16 their skill, strength and athletic abilities while also providing them 17 with opportunities to obtain recognition, accolades, college scholarships 18 and the numerous other long-term benefits that flow from success in 19 athletic endeavors.

20

Sec. 3. <u>Severability</u>

If a provision of this act or its application to any person or circumstance is held invalid, the invalidity does not affect other provisions or applications of the act that can be given effect without the invalid provision or application, and to this end the provisions of this act are severable.

26 27

Sec. 4. <u>Short title</u>

This act may be cited as the "Save Women's Sports Act".

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EXHIBIT 16

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Normative health-related fitness values for children: Analysis of 85347 test results on 9-17-year-old Australians since 1985

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Normative health-related fitness values for children: analysis of 85347 test results on 9–17-year-old Australians since 1985

Mark J Catley, Grant R Tomkinson

ABSTRACT

► The appendix to this paper

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Accepted 22 September 2011

Australia, GPO Box 2471,

Received 13 May 2011

Published Online First

21 October 2011

090218).

Australia

Objectives To provide sex- and age-specific normative values for health-related fitness of 9–17-year-old Australians.

Methods A systematic literature search was undertaken to identify peer-reviewed studies reporting health-related fitness data on Australian children since 1985—the year of the last national fitness survey. Only data on reasonably representative s amples of apparently healthy (free from known disease or injury) 9–17-year-old Australians, who were tested using field tests of healthrelated fitness, were included. Both raw and pseudo data (generated using Monte Carlo simulation) were combined with sex- and age-specific normative centile values generated using the Lambda Mu and Sigma (LMS) method. Sex- and age-related differences were expressed as standardised effect sizes.

Results Normative values were displayed as tabulated percentiles and as smoothed centile curves for nine health-related fitness tests based on a dataset comprising 85347 test performances. Boys typically scored higher than girls on cardiovascular endurance, muscular strength, muscular endurance, speed and power tests, but lower on the flexibility test. The magnitude of the age-related changes was generally larger for boys than for girls, especially during the teenage years.

Conclusion This study provides the most up-to-date sex- and age-specific normative centile values for the health-related fitness of Australian children that can be used as benchmark values for health and fitness screening and surveillance systems.

BACKGROUND

Physical fitness is considered to be an important marker of current and future health in children and adults.¹ In children, cardiovascular fitness is a weak-to-strong predictor of total and abdominal adiposity, cardiovascular disease risk factors, cancer and mental health.^{1 2} Certain muscular fitness components (eg, strength and endurance) are moderate predictors of cardiovascular disease risk factors, skeletal health and mental health.¹ Meaningful relationships have also been reported between running speed (another muscular fitness component) and skeletal health.³ In adults, cardiovascular fitness is a strong and independent predictor of all-cause mortality and cardiovascular disease mortality and morbidity,⁴ stroke,⁵ cancer, mental health,⁶ health-related quality of life7 and many other cardiometabolic risk factors and comorbidities.8 9 Moreover, physical fitness tracks moderately well from childhood through to adulthood.10-13 This evidence highlights the need to include health-related fitness testing (ie, the testing of fitness components such as cardiovascular and muscular fitness that have the strongest links with health outcomes) as part of existing health and fitness screening and surveillance systems.

Although the most valid assessments of fitness require sophisticated laboratory equipment and a high level of tester expertise, they unfortunately are not suitable for mass testing. On the other hand, properly conducted field tests offer simple, feasible, and practical alternatives, which typically demon-strate good reliability and validity.² ^{14–17} In Australia, unlike in Europe and North America where standardised test batteries such as the Eurofit¹⁸ or FITNESSGRAM¹⁹ are widely administered, a number of different field-based fitness tests and testing protocols have been used over time. For example, the most popular field test of cardiovascular fitness in Australia in the 1960s and 1970s was the 549-m (600 yd) run; in the 1980s and 1990s, it was the 1600-m run; and over the past decade or so, it has been the 20-m shuttle run.²⁰ Many physical educators and sports coaches in Australia continue to administer tests that are no longer in favour, largely because normative data (which are now several decades old) are available. This makes it difficult to assess the current status of health-related fitness in Australian children. Further compounding the problem is that the last national fitness survey of Australian children was conducted in 1985,²¹ and with convincing evidence of recent temporal changes in several components of fitness,²²⁻²⁴ the usefulness of such data seems to be limited.

Because there has never been a follow-up to the 1985 national survey, this study aimed to locate large and reasonably representative datasets of Australian children to generate normative centile values for health-related fitness. This study also aimed to quantify sex- and age-related differences in health-related fitness. These normative data will facilitate the identification of children with (a) low fitness in order to set appropriate goals and to promote positive health behaviours, and (b) specific fitness characteristics that may be considered important for sporting success.

METHODS

Data sources

A systematic review of the peer-reviewed scientific literature was undertaken to locate studies reporting descriptive summary data on Australian children tested for health-related fitness using field tests. Candidate studies were searched for in

To cite: Catley MJ, Tomkinson GR. *Br J Sports Med* 2013, **47**, 98–109.

		Age (years)	N	Raw data				Tests r	report	ed in inclu	ded studies					
Study	Year				Sampling method	Sample base	Protocol	Push- ups		Standing broad jump	Basketball throw	50 m sprint	Sit- and- reach	Hand- grip	1.6 km run	20 m shuttle run
ACHPER ⁵⁰	1994	9–18	39–104	yes	School-based; stratified, proportional	State (VIC)	ACHPER ⁵⁰		•		•		•		•	•
Barnett <i>et al⁵¹</i>	2007	15–17	21–69	no	School-based; stratified, random	State (NSW)	ACHPER ⁵⁰									•
Birchall ⁵²	1990	5–12	6–184	yes	School-based; convenience	State (VIC)	Pyke ²¹	•	•	•		•	•		•	
Booth <i>et al⁵³</i>	1997	9, 11, 13,15	399–634	no	School-based; stratified, proportional	State (NSW)	ACHPER ⁵⁰		•		•					•
Booth <i>et al⁵⁴</i>	2004	9–15	357–466	no	School-based; stratified, proportional	State (NSW)	ACHPER ⁵⁰									•
Burke <i>et al⁵⁵</i>	2004	10–13	38–117	yes	School-based; stratified, proportional	Capital city (WA)	ACHPER ⁵⁰									•
Cooley and McNaughton ⁵⁶	1998	11–16	339–636	no	School-based; stratified, proportional	State (TAS)	ACHPER ⁵⁰									•
Dollman et al ⁵⁷	1997	10–12	118–450	yes	School-based; stratified, proportional	State (SA)	Pyke ²¹			•		•			•	
Dollman pers. comm.	2002	11–12	19–154	yes	School-based; stratified, random	State (SA)	Pyke ²¹						•	•		
Dollman, pers. comm.	2002	8–12	8–389	yes	School-based; stratified, proportional	State (SA)	ACHPER ⁵⁰ Pyke ²¹			•						•
Hands ⁵⁸	2000	6–12	14–37	yes	School-based; stratified, random	Capital city (WA)	ACHPER ⁵⁰ Pyke ²¹		•	•	•	•	•	•		•
McIntyre, pers. comm.	2009	10–11	23–44	yes	School-based; stratified, random	Capital city (WA)	ACHPER ⁵⁰									•
McNaughton et al ⁵⁹	1995	7–10	30–83	no	School-based; stratified, random	State (TAS)	Pyke ²¹					•			•	
Pyke ²¹	1985	7–15	405–497	yes	School-based; stratified, proportional	National	Pyke ²¹	•	•	•		•	•	•	•	
Vandongen <i>et al⁶⁰</i>	1990	11	485–486	no	School-based; stratified, random	Capital city (WA)	ACHPER ⁵⁰								•	•

Table 1 Summary of the included studies that have been used to assess the health-related fitness of 9–17-year-old Australians since 1985

ACHPER, Australian Council for Health, Physical Education and Recreation; year, year of testing; n, sample size range per sex by age by test group; VIC, TAS, SA, WA, NSW

November 2009 using a computer search of online bibliographic databases (Ausport, CINAHL, Medline, PubMed, Scopus and SPORTDiscus). The search string used for the computer search was: ((((((((((((((((((((((((((())) OR grip strength) OR sprint*) OR jump*) OR push-up*) OR sit-up*) OR strength) OR sprint*) OR jump*) OR push-up*) OR sit-up*) OR grip strength) OR sit and reach) AND (((((((child*) OR paediatric*) OR adolesc*) OR boy*) OR girl*) OR youth*) OR teen*) AND (Australia*). All titles and abstracts (when available) were assessed to identify eligible articles, with full-text articles retrieved if there was doubt in an article's eligibility. A number of Australian researchers were contacted

through email to ask whether they knew of any appropriate studies or unpublished datasets.

Inclusion/exclusion criteria

Studies were included if they explicitly reported descriptive health-related fitness test data for apparently healthy (free from known disease or injury) 9–17-year-old Australians who were tested from 1985 onwards and if they reported data at the sex by age by test level, on children directly measured using fieldbased fitness tests for which explicit testing protocols were available. Studies were excluded if they reported descriptive data that were published in another identified study. The reference

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Original articles

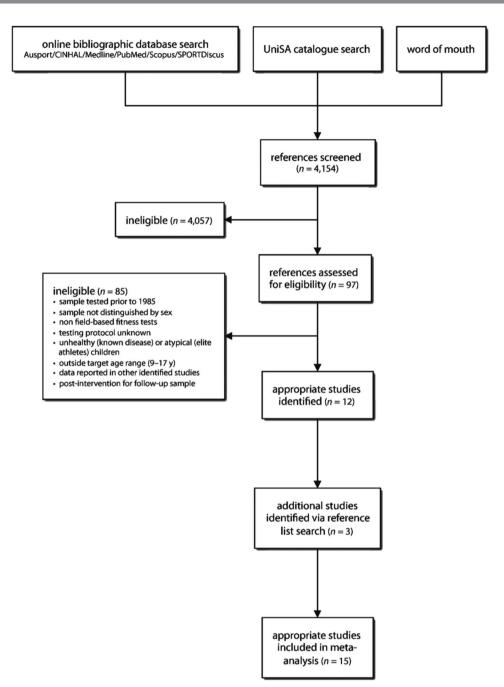


Figure 1 Flowchart outlining the identification of the included studies.

lists of all included studies were examined and cross-referenced to identify additional studies. Attempts were made to contact the corresponding author of each study to request raw data and/ or to clarify study details.

Initial data analysis

The following descriptive data were extracted from each included study: sex, age, year of testing, sample size, mean, SD, fitness test type and test protocol. Only data for commonly used fitness tests that were collected using protocols that were originally described in national or state-based fitness surveys of Australian children were retained for further analysis. Tests were considered to be 'common' if they were used to measure fitness in children across a broad range of ages and in at least two separate studies. Data for each fitness test were expressed in a common metric, and protocol differences were corrected where possible (eg, 20 m shuttle run data were expressed as the number of completed stages using the correction procedures described by Tomkinson *et al*).²⁵ However, if protocol correction was not possible, then only fitness data collected using the most common test protocol were retained. All available raw data were checked for anomalies by running range checks with data ± 3 SDs away from the respective study by sex by age by test mean excluded. Age was expressed in whole years as the age at last birthday.

Statistical analysis

Sex- and age-specific normative centile values were calculated using a dataset comprising raw data and pseudo data that were generated using the method described by Tomkinson.²⁴ Normative centile values were generated using LMSChartmaker Light (v2.3, The Institute of Child Health, London) software,

Age (year)	P ₅	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀	P ₆₀	P ₇₀	P ₈₀	P ₉₀	P ₉₅	L	Μ	S
Boys														
9	750	684	618	578	547	522	499	476	452	423	401	-1.042	521.963	0.183
10	732	666	602	564	535	511	489	469	447	420	400	-1.284	511.053	0.175
11	710	646	585	549	523	500	480	461	441	416	397	-1.466	500.394	0.166
12	682	621	563	530	505	485	467	449	430	408	392	-1.721	484.819	0.157
13	643	587	535	505	483	465	448	432	415	395	380	-1.895	464.529	0.148
14	605	556	509	482	462	446	431	416	401	382	369	-1.987	445.569	0.140
15	575	531	490	465	447	432	418	404	390	373	360	-1.979	431.504	0.133
16	552	514	477	454	437	423	410	397	383	366	354	-1.865	422.693	0.128
17	534	500	467	446	430	417	404	392	379	362	350	-1.707	416.545	0.123
Girls														
9	829	769	706	666	635	609	584	559	533	499	475	-0.779	608.674	0.167
10	820	759	697	657	626	600	576	552	526	494	470	-0.878	600.149	0.166
11	801	741	680	641	611	586	562	539	514	483	460	-0.929	585.820	0.165
12	784	726	666	629	600	575	552	529	505	474	452	-0.921	574.682	0.164
13	771	716	658	621	593	569	546	524	500	469	447	-0.852	568.706	0.163
14	763	711	655	620	592	567	545	523	498	468	445	-0.737	567.486	0.162
15	760	710	656	621	594	570	547	525	500	469	446	-0.591	569.809	0.161
16	757	710	658	624	597	573	550	527	502	471	446	-0.428	572.723	0.160
17	753	708	658	625	598	575	552	529	504	471	446	-0.263	574.536	0.159

L, skew; M, median; P, percentile; S, coefficient of variation.

which analyses data using the LMS method.²⁶ The LMS method fits smooth centile curves to reference data by summarising the changing distribution of three sex- and age-specific curves representing the skewness (L: expressed as a Box-Cox power), the median (M) and the coefficient of variation (S). Using penalised likelihood, the curves can be fitted as cubic splines using nonlinear regression, and the extent of smoothing required can be expressed in terms of smoothing parameters or equivalent degrees of freedom. $^{\rm 27}$

For each fitness test, differences in means between: (a) agematched Australian boys and girls (eg, 10-year-old boys vs 10-year-old girls); (b) sex-matched Australian children of different ages (eg, 10-year-old boys vs 11-year-old boys); and (c) sexand age-matched Australian and international children¹⁸ ²⁸⁻³⁰

Table 3 2	Table 3 20 m shuttle run (completed stages) centile values and LMS summary statistics by sex and age in 9- to 17-year-old Australians													
Age (year)	P ₅	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀	P ₆₀	P ₇₀	P ₈₀	P ₉₀	P ₉₅	L	М	S
Boys														
9	1	1	2	2	2	3	3	3	4	5	6	0.213	2.573	0.568
10	1	2	2	3	3	4	4	5	5	7	8	0.373	3.537	0.543
11	1	2	3	3	4	4	5	5	6	7	8	0.520	4.131	0.517
12	1	2	3	3	4	4	5	6	6	8	9	0.643	4.460	0.486
13	2	2	3	4	4	5	5	6	7	8	9	0.744	4.888	0.453
14	2	3	4	4	5	6	6	7	8	9	10	0.835	5.664	0.418
15	3	3	4	5	6	7	7	8	9	10	11	0.926	6.527	0.380
16	3	4	5	6	7	7	8	8	9	10	11	1.031	7.159	0.343
17	4	5	6	6	7	8	8	9	10	11	11	1.143	7.690	0.306
Girls														
9	1	1	1	1	2	2	2	2	3	4	5	-0.065	1.842	0.535
10	1	1	2	2	2	2	3	3	4	5	6	0.086	2.468	0.557
11	1	1	2	2	2	3	3	4	4	6	7	0.220	2.844	0.573
12	1	1	2	2	3	3	3	4	5	6	7	0.324	3.016	0.577
13	1	1	2	2	3	3	4	4	5	6	7	0.400	3.138	0.569
14	1	1	2	2	3	3	4	4	5	6	7	0.457	3.225	0.554
15	1	1	2	3	3	3	4	4	5	6	7	0.505	3.412	0.536
16	1	2	2	3	3	4	4	5	5	6	7	0.554	3.672	0.518
17	1	2	2	3	4	4	5	5	6	7	8	0.603	4.032	0.499

Percentile data were calculated from 18 075 20 m shuttle run performances collected between 1990 and 2009.

The 20 m shuttle run can be scored in different metrics other than as the number of completed stages, such as the number of completed laps, the speed at the last completed stage and as mass-specific peak oxygen uptake estimates (see Tomkinson *et al*²⁵ for details on how to correct 20 m shuttle run performances to different metrics). L, skew; M, median; P, percentile; S, coefficient of variation.

Original articles

Age (year)	P ₅	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀	P ₆₀	P ₇₀	P ₈₀	P ₉₀	P ₉₅	L	Μ	S
Boys														
9	10.6	10.2	9.8	9.5	9.3	9.1	9.0	8.8	8.6	8.3	8.1	-1.837	9.136	0.078
10	10.5	10.1	9.7	9.4	9.2	9.0	8.8	8.7	8.5	8.2	8.0	-2.185	9.009	0.080
11	10.4	10.0	9.6	9.3	9.1	8.9	8.7	8.5	8.3	8.1	7.9	-2.405	8.877	0.081
12	10.2	9.8	9.3	9.1	8.9	8.7	8.5	8.3	8.1	7.9	7.7	-2.446	8.673	0.081
13	9.8	9.4	9.0	8.8	8.6	8.4	8.2	8.1	7.9	7.7	7.5	-2.489	8.377	0.079
14	9.4	9.0	8.7	8.4	8.2	8.1	7.9	7.8	7.6	7.4	7.2	-2.701	8.063	0.076
15	9.0	8.6	8.3	8.1	7.9	7.7	7.6	7.5	7.3	7.1	7.0	-3.021	7.738	0.073
Girls														
9	11.7	11.3	10.8	10.5	10.3	10.0	9.8	9.6	9.3	9.0	8.8	-0.981	10.033	0.088
10	11.1	10.7	10.3	10.0	9.8	9.5	9.3	9.1	8.9	8.6	8.4	-1.453	9.542	0.084
11	10.7	10.3	9.9	9.6	9.4	9.2	9.0	8.8	8.6	8.3	8.1	-1.803	9.161	0.082
12	10.4	10.0	9.6	9.3	9.1	8.9	8.7	8.6	8.4	8.1	7.9	-1.977	8.919	0.080
13	10.2	9.8	9.4	9.2	9.0	8.8	8.6	8.4	8.3	8.0	7.8	-1.991	8.787	0.078
14	10.0	9.7	9.3	9.1	8.9	8.7	8.5	8.4	8.2	7.9	7.8	-1.884	8.686	0.076
15	9.9	9.6	9.2	9.0	8.8	8.6	8.5	8.3	8.1	7.9	7.7	-1.724	8.638	0.075

Note, percentile data were calculated from 10 104 50 m sprint performances collected between 1985 and 1999.

L, skew; M, median; P, percentile; S, coefficient of variation.

were expressed as standardised effect sizes.³¹ Positive effect sizes indicated that mean fitness test performances for boys (agematched analysis), older children (sex-matched analysis) or Australian children (sex- and age-matched analysis) were higher than those for girls, younger children or international children, respectively. Effect sizes of 0.2, 0.5 and 0.8 were used as thresholds for small, moderate and large.³¹

Table 1 summarises the 15 included studies. Of these, 12 were

identified through bibliographic database searching and word of

mouth, and three were identified through reference list search-

ing. Corresponding authors of all the studies were contacted

through email to clarify study details and/or to request raw data.

All authors satisfactorily clarified study details, and seven of them supplied raw data (figure 1).

The final dataset comprised 85347 individual test results and 142 sex by age by test groups with a median sample size of 537 (range: 54–2612). Data were available for six fitness components and nine fitness tests: cardiovascular endurance (20 m shuttle run, 1.6 km run), muscular strength (hand-grip), muscular endurance (push-ups and sit-ups), muscular power (standing broad jump and basketball throw), muscular speed (50 m sprint) and flexibility (sit-and-reach). Raw data were available for 74% of all data points.

Normative fitness data for 9–17-year-old Australians are presented as tabulated percentiles from 5 to 95 (P_5 , P_{10} , P_{20} , P_{30} , P_{40} , P_{50} , P_{60} , P_{70} , P_{80} , P_{90} , P_{95}) in tables 2–10. The sex- and age-

Age (year)	P ₅	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀	P ₆₀	P ₇₀	P ₈₀	P ₉₀	P ₉₅	L	Μ	S
Boys														
9	2.3	2.5	2.7	2.9	3.1	3.3	3.4	3.6	3.8	4.1	4.4	0.623	3.260	0.198
10	2.5	2.8	3.0	3.3	3.4	3.6	3.8	4.0	4.2	4.5	4.8	0.675	3.608	0.192
11	2.8	3.1	3.4	3.6	3.8	4.0	4.2	4.4	4.7	5.0	5.3	0.733	4.026	0.188
12	3.1	3.4	3.8	4.0	4.3	4.5	4.7	4.9	5.2	5.6	5.9	0.792	4.471	0.188
13	3.5	3.8	4.2	4.5	4.8	5.0	5.3	5.5	5.8	6.2	6.6	0.843	5.012	0.187
14	3.9	4.2	4.7	5.0	5.3	5.5	5.8	6.1	6.4	6.9	7.2	0.898	5.522	0.186
15	4.2	4.6	5.0	5.4	5.7	6.0	6.3	6.6	6.9	7.4	7.8	0.943	5.975	0.185
16	4.4	4.8	5.3	5.6	6.0	6.3	6.5	6.9	7.2	7.7	8.2	0.964	6.254	0.185
17	4.5	4.9	5.5	5.8	6.2	6.5	6.8	7.1	7.5	8.0	8.5	0.966	6.467	0.187
Girls														
9	2.1	2.3	2.5	2.7	2.9	3.0	3.2	3.3	3.5	3.7	3.9	1.116	3.015	0.182
10	2.3	2.6	2.8	3.0	3.2	3.3	3.5	3.7	3.8	4.1	4.3	1.024	3.336	0.181
11	2.6	2.8	3.1	3.3	3.5	3.6	3.8	4.0	4.2	4.5	4.7	0.942	3.646	0.180
12	2.8	3.1	3.4	3.6	3.8	4.0	4.2	4.3	4.6	4.9	5.2	0.873	3.970	0.180
13	3.0	3.3	3.6	3.9	4.1	4.3	4.5	4.7	4.9	5.3	5.6	0.816	4.265	0.179
14	3.2	3.4	3.8	4.0	4.2	4.4	4.6	4.8	5.1	5.4	5.7	0.739	4.410	0.175
15	3.3	3.6	3.9	4.1	4.3	4.5	4.7	4.9	5.1	5.5	5.8	0.606	4.486	0.169
16	3.4	3.7	4.0	4.2	4.4	4.6	4.7	5.0	5.2	5.6	5.9	0.394	4.557	0.162
17	3.6	3.8	4.1	4.3	4.5	4.6	4.8	5.0	5.3	5.6	5.9	0.140	4.634	0.154

Note, percentile data were calculated from 5,541 basketball throw performances collected between 1994 and 1999; L, skew; M, median; P, percentile; S, coefficient of variation.

RESULTS

Age (year)	P ₅	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀	P ₆₀	P ₇₀	P ₈₀	P ₉₀	P ₉₅	L	Μ	S
Boys														
9	105	113	121	127	133	138	142	147	153	161	168	1.244	137.506	0.138
10	109	117	126	133	138	143	148	154	160	168	174	1.490	143.430	0.138
11	112	121	131	138	144	149	154	160	166	174	181	1.654	149.322	0.138
12	117	126	137	144	150	156	161	167	173	182	189	1.704	155.838	0.137
13	126	136	147	154	161	166	172	178	185	194	201	1.629	166.340	0.135
14	137	146	157	165	172	178	184	190	197	206	214	1.526	177.688	0.131
15	148	157	169	177	183	189	196	202	209	219	228	1.446	189.485	0.127
Girls														
9	95	102	110	116	122	126	131	136	142	150	157	1.098	126.379	0.149
10	100	108	117	123	128	133	138	143	149	158	165	1.152	133.177	0.147
11	106	114	123	129	135	140	145	151	157	166	173	1.197	140.142	0.145
12	111	118	128	135	140	145	151	156	163	171	179	1.211	145.432	0.142
13	115	123	132	139	145	150	155	161	167	176	183	1.183	150.080	0.138
14	119	127	136	143	148	154	159	164	171	180	187	1.158	153.551	0.134
15	122	129	139	145	151	156	161	166	173	181	188	1.148	155.661	0.130

Percentile data were calculated from 11 194 standing broad jump performances collected between 1985 and 2002.

L, skew; M, median; P, percentile; S, coefficient of variation.

specific LMS values for all fitness tests are also shown. The LMS values depict the nature of the age-related distributions for boys and girls and can be used to calculate *z*-scores and hence percentile values by looking up a *z*-table, using the following formula:

$$z = \frac{\left(\frac{x}{M}\right)^L - 1}{L \times S}$$

where z is z score, x is performance, L is sex- and age-specific L-value, M is the sex- and age-specific M-value and S is the sex- and age-specific S-value.

Figures 2 and 3 show the smoothed centile curves (P_{10} , P_{50} , P_{90}).

Figure 4 shows the sex-related differences in mean fitness. Boys consistently scored higher than girls on health-related fitness tests, except on the sit-and-reach test, with the magnitude of the differences typically increasing with age and often accelerating from about 12 years of age. Overall, the magnitude of differences between boys and girls was large for the 1.6 km run, 20 m shuttle run, basketball throw and push-ups; moderate for the 50-m sprint, standing broad jump and sit-and-reach; and small for sit-ups and hand-grip strength. Figure 5 shows the age-related changes in mean fitness. The age-related changes were typically larger for boys than for girls, especially during the teenage years, and for muscular fitness tests than for cardiovascular fitness tests. Fitness also tended to peak from about the age of 15 years. Figure 6 shows that the differences in health-related fitness between Australian and international children were generally small, with Australian children scoring slightly higher on hand-grip strength (mean ±95% CI: 0.20±0.03 SDs) and 50 m sprint tests (0.24 ± 0.02 SDs), and slightly lower on sit-and-reach $(-0.36\pm0.02 \text{ SDs})$, standing broad jump $(-0.25\pm0.02 \text{ SDs})$ and 20 m shuttle run tests (-0.49 ± 0.01 SDs).

Table 7 P	Push-ups (no. in 30 s) centile values and LMS summar	y statistics by sex and age in 9-1	o 15-year-old Australians
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Age (year)	P ₅	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀	P ₆₀	P ₇₀	P ₈₀	P ₉₀	P ₉₅	L	М	S
Boys														
9	4	6	8	9	11	12	14	15	17	20	22	0.846	12.310	0.452
10	4	6	8	10	11	13	14	16	18	21	23	0.894	12.943	0.447
11	4	6	8	10	12	13	14	16	18	20	22	0.940	12.942	0.438
12	4	6	9	10	12	13	15	16	18	20	22	0.980	13.200	0.422
13	5	7	9	11	13	14	16	17	19	22	24	1.020	14.255	0.399
14	6	8	11	13	14	16	17	19	21	23	25	1.070	15.954	0.370
15	7	10	13	15	16	18	19	21	23	25	27	1.126	17.697	0.337
Girls														
9	2	3	5	7	8	9	10	12	13	16	18	0.719	8.989	0.550
10	2	3	5	6	7	9	10	11	13	16	18	0.652	8.655	0.583
11	2	3	4	6	7	8	9	11	13	16	18	0.584	8.142	0.624
12	1	2	4	5	6	7	9	10	12	15	18	0.518	7.395	0.672
13	1	2	3	4	6	7	8	10	12	15	18	0.453	6.792	0.720
14	1	2	3	4	5	6	8	9	11	15	18	0.390	6.384	0.765
15	1	2	3	4	5	6	7	9	11	14	18	0.329	5.818	0.812

Percentile data were calculated from 7,342 push-up test performances collected between 1985 and 1991. L, skew; M, median; P, percentile; S, coefficient of variation.

Original articles

Age (year)	P ₅	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀	P ₆₀	P ₇₀	P ₈₀	P ₉₀	P ₉₅	L	М	S
Boys														
9	3	5	8	11	14	17	21	25	30	40	48	0.321	17.046	0.755
10	5	8	13	17	20	24	29	34	40	50	59	0.466	24.459	0.669
11	6	10	16	21	25	29	34	39	45	55	60	0.629	29.422	0.594
12	8	14	21	26	31	36	40	45	51	60	60	0.841	35.561	0.514
13	10	17	25	31	36	40	45	50	55	60	60	1.056	40.288	0.443
14	12	20	29	34	39	43	48	52	57	60	60	1.232	43.454	0.389
15	14	22	31	36	41	45	49	53	58	60	60	1.335	44.942	0.359
16	16	24	32	38	42	46	50	54	58	60	60	1.426	46.209	0.332
17	18	26	34	40	44	47	51	55	59	60	60	1.517	47.466	0.306
Girls														
9	5	8	12	15	18	21	25	29	35	43	51	0.394	21.258	0.642
10	7	10	14	18	22	26	30	34	40	50	58	0.485	25.666	0.605
11	8	11	17	21	25	29	34	39	45	54	60	0.571	29.444	0.569
12	9	13	19	24	28	32	37	42	48	57	60	0.646	32.123	0.534
13	10	15	21	26	30	34	39	44	50	59	60	0.705	34.408	0.504
14	11	15	22	27	31	35	40	45	50	59	60	0.741	35.334	0.482
15	11	16	22	27	31	35	40	44	50	58	60	0.757	35.327	0.464
16	12	17	23	28	32	36	40	44	50	57	60	0.761	35.690	0.447
17	13	18	24	28	32	36	40	45	50	58	60	0.761	36.333	0.431

Percentile data were calculated from 8 837 sit-up test performances collected between 1985 and 1999. L, skew; M, median; P, percentile; S, coefficient of variation.

DISCUSSION

This study provides the most up-to-date sex- and age-specific normative centile values for 9–17-year-old Australians across a range of health-related fitness tests, which can be used as benchmark values for health and fitness screening and surveillance of children. These data complement a growing literature reporting growth percentiles across a range of different health measures, such as body mass index,³² waist girth³³ and blood pressure,²⁸ and a range of other health-related fitness measures.^{29 30} It also quantifies the magnitude and direction of sex- and age-related differences in children's health-related fitness and shows that

boys consistently scored higher than girls on fitness tests (except on the sit-and-reach test of flexibility) and that boys experience larger age-related changes in fitness. The developmental patterns of children's fitness have been well studied and extensively reviewed (eg, for cardiovascular fitness, refer to Armstrong *et al*,³⁴ Krahenbuhl *et al*³⁵ and Rowland³⁶; for muscular fitness, refer to Blimkie and Sale,³⁷ Froberg and Lammert³⁸ and De Ste Croix³⁹). Although the underlying causes of sex- and age-related differences are clear for some fitness test performances, such as those for muscular strength, power and speed, which are largely explained by physical differences (eg, differences in muscle mass

 Table 9
 Hand-grip strength (kg) centile values and LMS summary statistics by sex and age in 9- to 15-year-old Australians (taken as the mean of both hands)

Age (year)	P ₅	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀	P ₆₀	P ₇₀	P ₈₀	P ₉₀	P ₉₅	L	М	S
Boys														
9	11.5	12.5	13.8	14.8	15.6	16.4	17.2	18.1	19.2	20.8	22.1	0.600	16.415	0.197
10	13.1	14.3	15.9	17.0	18.0	19.0	19.9	21.0	22.2	23.9	25.4	0.728	18.967	0.198
11	14.5	15.9	17.7	19.0	20.1	21.2	22.3	23.5	24.9	26.8	28.5	0.764	21.217	0.200
12	15.4	17.0	18.9	20.3	21.5	22.7	23.8	25.1	26.6	28.7	30.5	0.747	22.655	0.203
13	17.5	19.3	21.5	23.1	24.5	25.8	27.2	28.6	30.4	32.8	34.9	0.738	25.819	0.205
14	20.8	22.9	25.5	27.4	29.1	30.7	32.4	34.1	36.2	39.1	41.6	0.742	30.731	0.207
15	24.6	27.1	30.3	32.6	34.6	36.5	38.4	40.5	43.0	46.5	49.5	0.752	36.517	0.207
Girls														
9	9.8	10.8	12.0	12.9	13.7	14.4	15.1	16.0	17.0	18.4	19.5	0.639	14.396	0.205
10	11.4	12.6	14.1	15.2	16.2	17.1	18.0	19.0	20.1	21.8	23.1	0.842	17.072	0.210
11	12.5	13.9	15.5	16.8	17.8	18.8	19.8	20.9	22.1	23.9	25.3	0.932	18.816	0.208
12	14.4	16.0	17.8	19.1	20.3	21.4	22.5	23.6	25.0	26.9	28.5	0.922	21.374	0.200
13	16.4	18.0	19.9	21.3	22.5	23.6	24.8	26.0	27.4	29.5	31.1	0.880	23.641	0.190
14	18.2	19.7	21.6	23.0	24.3	25.4	26.5	27.8	29.2	31.3	33.0	0.828	25.390	0.178
15	19.8	21.3	23.2	24.6	25.8	26.9	28.0	29.2	30.7	32.7	34.4	0.770	26.881	0.165

Percentile data were calculated from the 3 707 hand-grip strength performances collected between 1985 and 1999. L, skew; M, median; P, percentile; S, coefficient of variation.

Table 10	Sit-and	-reach (cn	n) centile	values an	d LMS sur	nmary sta	tistics by	sex and a	ge in 9- t	o 17-year-	old Austra	alians.		
Age (y)	P ₅	P ₁₀	P ₂₀	P ₃₀	P ₄₀	P ₅₀	P ₆₀	P ₇₀	P ₈₀	P ₉₀	P ₉₅	L	М	S
boys														
9	10.4	12.9	15.7	17.7	19.4	20.9	22.4	23.9	25.8	28.2	30.3	1.211	20.877	0.285
10	10.0	12.5	15.3	17.3	19.0	20.5	22.1	23.7	25.5	28.0	30.1	1.190	20.537	0.294
11	9.6	12.1	15.0	17.0	18.7	20.3	21.9	23.5	25.4	28.0	30.1	1.167	20.313	0.305
12	9.3	11.8	14.8	16.9	18.7	20.3	21.9	23.6	25.6	28.3	30.5	1.133	20.292	0.315
13	9.4	12.0	15.1	17.2	19.1	20.8	22.5	24.3	26.4	29.2	31.6	1.091	20.785	0.322
14	9.8	12.5	15.7	18.0	20.0	21.8	23.6	25.5	27.8	30.9	33.4	1.054	21.804	0.328
15	10.4	13.2	16.6	19.1	21.2	23.1	25.1	27.1	29.5	32.9	35.7	1.017	23.112	0.332
16	11.1	14.0	17.6	20.1	22.3	24.4	26.5	28.7	31.3	34.9	37.8	0.984	24.392	0.334
17	11.7	14.8	18.5	21.2	23.5	25.7	27.9	30.2	33.0	36.8	40.0	0.953	25.686	0.335
girls														
9	13.0	15.8	18.9	21.1	22.9	24.6	26.2	28.0	29.9	32.6	34.8	1.285	24.614	0.264
10	13.0	15.7	18.8	20.9	22.7	24.4	26.0	27.7	29.7	32.4	34.6	1.259	24.402	0.265
11	13.2	15.9	19.0	21.2	23.0	24.7	26.4	28.1	30.1	32.8	35.0	1.235	24.705	0.265
12	14.0	16.7	19.9	22.2	24.1	25.8	27.5	29.3	31.3	34.2	36.4	1.230	25.790	0.262
13	15.3	18.2	21.6	24.0	25.9	27.7	29.5	31.4	33.6	36.5	38.9	1.250	27.740	0.256
14	16.5	19.5	23.1	25.5	27.6	29.4	31.3	33.2	35.4	38.4	40.9	1.293	29.440	0.248
15	17.0	20.1	23.7	26.1	28.1	30.0	31.8	33.7	35.9	38.8	41.2	1.350	29.997	0.241
16	17.0	20.0	23.5	25.9	27.9	29.6	31.4	33.2	35.3	38.1	40.3	1.412	29.647	0.235
17	16.8	19.8	23.2	25.5	27.4	29.1	30.7	32.5	34.4	37.1	39.2	1.472	29.074	0.229

Note, percentile data were calculated from 9,124 sit-and-reach performances collected between 1985 and 2000; L = skew; M = median; S = coefficient of variation. Note, a score of "20 cm" corresponds to the participant reaching their toes.

or height), they are less clear for others, such as for cardiovascular endurance, which may be explained by physiological differences (eg, differences in mechanical efficiency and/or fractional utilisation).^{15 36} It is, nonetheless, beyond the scope of this article to discuss the causes that underscore the sex- and age-related changes in fitness test performance.

International comparisons

Although several studies have previously compared the health-related fitness of Australian children with their sex- and age-matched international peers,^{20 40} comparisons have only been made for cardiovascular fitness. Figure 6 compares the 20-m shuttle run, 50 m sprint, standing broad jump, hand-grip strength and sit-and-reach performance of 9-17-year-old Australians with 1 894 971 test results from sex-, age- and testmatched international children from 48 countries who have been measured using the same test protocols as those referenced in table 1 and described in Appendix 1. Figure 6 also shows typically small differences in health-related fitness between Australian and international children. Furthermore, the sex- and age-related differences in fitness of Australian children are strikingly similar to those observed in international children. Given that the differences are generally small, the normative centile data presented in this study could be used as approximate benchmark values for health-related fitness of international children.

Fitness thresholds for cardiometabolic risk

Fitness is widely recognised as a powerful marker of current and future cardiovascular, skeletal and mental health. Unfortunately, there are no universally accepted recommendations for health-related levels of fitness. In recent years however, sex- and age-specific threshold values for cardiovascular fitness (operationalised as mass-specific peak oxygen uptake in ml/kg/min) have been established for European and US children using linked cardiometabolic risk-based values from receiver operator characteristic curve analyses.^{41–44} To estimate the prevalence of Australian children with 'healthy' cardiovascular fitness (ie, those above the thresholds), 'international' sex- and age-specific thresholds for 9–17-year-old children were estimated by determining best-fitting polynomial regression model (quadratic or cubic) relating age (predictor variable) to previously reported threshold values (response variable) in Adegboye *et al*,⁴¹ Lobelo *et al*,⁴² Ruiz *et al*⁴³ and Welk *et al*.⁴⁴ Separate models were generated for boys and girls. Peak oxygen uptake values in Australian children were estimated using 1.6 km run and 20 m shuttle run data and the Cureton *et al*⁴⁵ and Léger *et al*⁴⁶ regression equations, respectively.

Using these thresholds, about 71% of Australian boys (median $\pm 95\%$ CI: 71% $\pm 8\%$) and 77% of Australian girls (median $\pm 95\%$ CI: 77% $\pm 10\%$) apparently have 'healthy' cardiovascular fitness. Although in light of recent secular declines in cardiovascular fitness,²⁰ ²² ²³ ²⁵ and with a median testing year of 1993 in this study's cardiovascular fitness dataset, it is likely that these prevalence rates somewhat overestimate those of today. These prevalence rates are better than (for girls), or similar to (for boys), those observed in European (61% of boys and 57% of girls)²⁹ and US (71% of boys and 69% of girls)⁴² children. Geographical differences in prevalence rates may reflect differences in (a) threshold levels, (b) the year(s) of testing, (c) sampling methodology, (d) test methodology and (e) the way in which peak oxygen uptake was measured or estimated.⁴⁷

Ultimately, it is important to remember that the normative data presented in this study show how well Australian children perform on health-related fitness tests relative to their sex- and age-matched peers. For example, using a percentile classification, children with fitness in the bottom 20% can be classified as having 'very low' fitness; those between the 20th and 40th percentiles as having 'low' fitness; those between the 40th and 60th percentiles as having 'average' fitness; those between the 60th and 80th percentiles as having 'high' fitness; and those

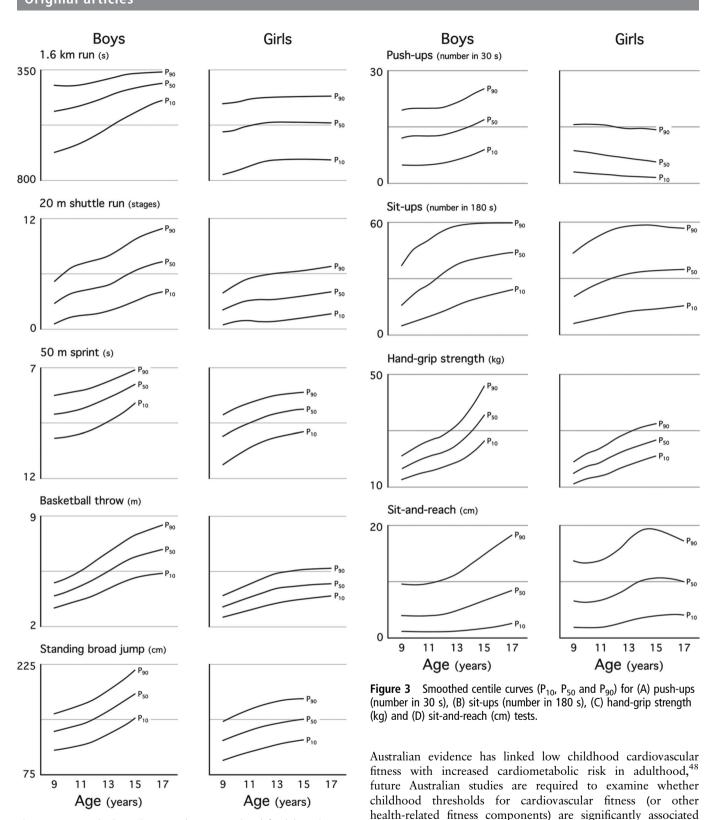


Figure 2 Smoothed centile curves (P_{10} , P_{50} and P_{90}) for (A) 1.6 km run (s), (B) 20 m shuttle run (number of completed stages), (C) 50 m sprint (s), (D) basketball throw (m) and (E) standing broad jump (cm).

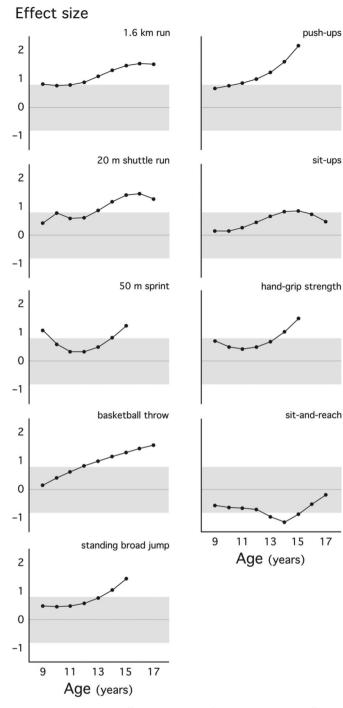
above the 80th percentile as having 'very high' fitness. These data are not criterion-referenced in that they do not indicate whether children with 'very low' or 'low' (or any other classification for that matter) have 'unhealthy' cardiovascular fitness or increased cardiometabolic risk. Despite the fact that previous Despite the fact that the last national fitness survey of Australian children was in 1985, this study provides the most up-to-date

such as mental or skeletal health outcomes).

Strengths and limitations

normative dataset for nine widely administered health-related fitness tests, using cumulated data from 85347 Australian children aged 9–17 years collected between 1985 and 2009. This

with clustered cardiometabolic risk (or other health outcomes,



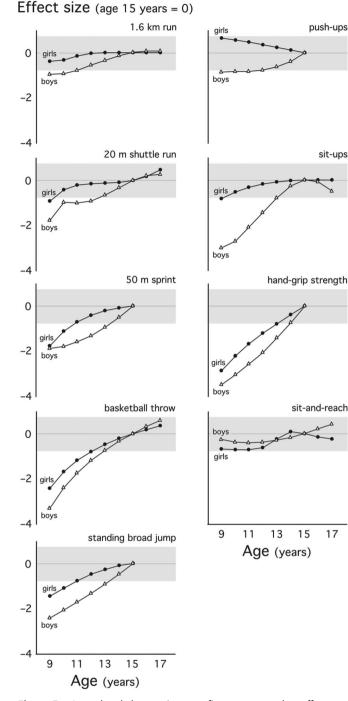


Figure 4 Sex-related differences in mean fitness expressed as effect sizes. Data are shown for 9–17-year-old children tested on the (A) 1.6 km run, (B) 20 m shuttle run, (c) 50 m sprint, (D) basketball throw, (E) standing broad jump, (F) push-ups, (G) sit-ups, (H) hand-grip strength and (I) sit-and-reach tests. The limits of the grey zone represent effects sizes of 0.8 and –0.8, beyond which large differences are observed.

study used a strict set of inclusion and exclusion criteria and rigorous initial data analysis procedures to systematically control for any factors (eg, differences in test methodology) that might have biased the normative values or the estimates of the sexand age-related differences. It used a novel pseudo-data method to allow both descriptive and raw data to be merged before using the LMS method to create sex- and age-specific smoothed percentiles. It also quantified sex- and age-related differences as **Figure 5** Age-related changes in mean fitness expressed as effect sizes standardised to an effect size of age 15 years=0. Data are shown for 9–17-year-old boys (triangles) and girls (circles) separately tested on the (A) 1.6 km run, (B) 20 m shuttle run, (C) 50 m sprint, (D) basketball throw, (E) standing broad jump, (F) push-ups, (G) sit-ups, (H) hand-grip strength and (I) sit-and-reach tests. The limits of the grey zone represent effects sizes of 0.8 and –0.8, beyond which large differences are observed.

standardised effects sizes, allowing for comparison between sexes, among different ages, and with sex, age and test-matched international children.

However, this study is not without limitations. Only one of the 15 included studies was based on a nationally representative sample, which obviously raises the issue of representativeness. Most of the included studies used similar sampling frames

Effect size

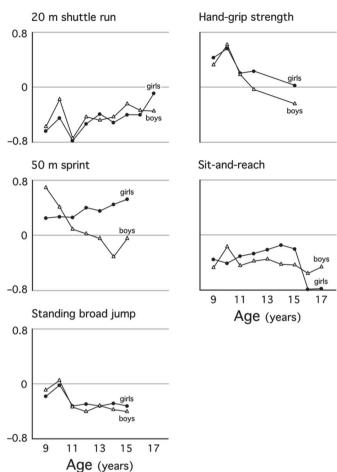


Figure 6 Sex- and age-specific effect sizes for (A) 20 m shuttle run, (B) 50 m sprint, (C) hand-grip strength, (D) sit-and-reach and (E) standing broad jump for 9–17-year-old Australian boys (triangles) and girls (circles) relative to their international peers. Positive effects indicate higher fitness scores for Australian children and negative effects indicate lower fitness scores. Comparative data represent n=284 508 20 m shuttle run performances,^{28–30} n=1 216 452 50 m sprint performances,¹⁸ n=126 361 hand-grip strength performances,²⁹ n=102 664 sit-and-reach performances,²⁹ and n=164 986 standing broad jump performances²⁹ of 9–17-year-old children from 48 international countries.

(table 1). Schools with a greater interest in sport and fitness may have been more willing to participate, and because participation at the individual level was voluntary, it is possible that children with low fitness levels chose not to participate. This might have resulted in fitness test performances unrepresentative of the population, but it should not have affected the sex- and age-related differences. Fitness data were also collected at different times during the 1985-2009 period, and given convincing evidence of recent temporal declines in some (but not all) com-ponents of Australian children's fitness,^{23 49} it is possible that the normative data presented in this study represent a better 'health-related picture' than what would be observed today. A temporal analysis of the data accumulated in this study suggests that these normative data would probably overestimate the fitness of Australian children in 2009 by an average of 0.3 SDs or 13 percentile points, assuming of course that the observed temporal changes remained consistent across the entire 1985-2009 period. Nonetheless, these data represent the best

available and most up-to-date health-related fitness data on Australian children. It must also be remembered that despite being simple, cheap, easy, reliable, reasonably valid and widely used alternatives of laboratory-based criterion measures, field tests are affected by factors other than underlying construct fitness. For example, validity data for field tests of cardiovascular fitness suggest that (at best) only 50-60% of the variance in field test performance is explained by the variance in underlying peak oxygen uptake, indicating that other physiological, physical, biomechanical, psychosocial and environmental factors also play a part.¹⁵ In addition, although criterion-related validity has not been established for all of the included tests, face validity is generally accepted.¹⁷ Most of the included tests are also considered to demonstrate good reliability, although tests requiring a reasonable degree of subjective judgement (eg, the subjective scoring of a properly performed sit-up or push-up) typically demonstrate poorer reliability.14

Conclusion

Physical fitness is considered to be an excellent marker of current and future health. In anticipation of a follow-up national fitness survey, this study provides the most up-to-date and most comprehensive set of sex- and age-specific normative centile values of health-related fitness of Australian children, which can be used as benchmark values for health and fitness screening and surveillance systems. These normative centile values will facilitate the identification of children with low fitness to set appropriate fitness goals, monitor individual changes in fitness and promote positive health behaviours. They will also facilitate the identification of children who possess specific fitness characteristics that may be considered important for sporting success, in the hope of recruiting the high achievers into elite sporting development programs.

Acknowledgements The authors thank the authors of the included studies for generously clarifying details of their studies and/or for providing raw data. The University of South Australia Divisional Development Research Scheme supported this study.

Correction notice This article has been corrected since it was published Online First. The authors have noticed that the normative data in Table 10 are incorrect. The correct table has been inserted.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

- Ortega FB, Ruiz JR, Castillo MJ, et al. Physical fitness in childhood and adolescence: a powerful marker of health. Int J Obes (Lond) 2008;32:1–11.
- 2 Ruiz JR, Castro-Piñero J, Artero EG, et al. Predictive validity of health-related fitness in youth: a systematic review. Br J Sports Med 2009;43:909–23.
- 3 Vicente-Rodriguez G, Ara I, Perez-Gomez J, et al. High femoral bone mineral density accretion in prepubertal soccer players. Med Sci Sports Exerc 2004;36:1789–95.
- 4 Kodama S, Saito K, Tanaka S, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. JAMA 2009;301:2024–35.
- 5 Lee CD, Blair SN. Cardiorespiratory fitness and stroke mortality in men. *Med Sci Sports Exerc* 2002;34:592–5.
- 6 Dishman RK, Washburn RA, Heath GW. *Physical Activity Epidemiology*. Champaign, IL: Human Kinetics 2004:358–9.
- 7 Gillespie LD, Robertson MC, Gillespie WJ, et al. Interventions for preventing falls in older people living in the community. *Cochrane Database Syst Rev* 2009;2: CD007146.
- 8 Katzmarzyk PT, Church TS, Janssen I, et al. Metabolic syndrome, obesity, and mortality: impact of cardiorespiratory fitness. *Diabetes Care* 2005;28:391–7.
- 9 Katzmarzyk PT, Church TS, Blair SN. Cardiorespiratory fitness attenuates the effects of the metabolic syndrome on all-cause and cardiovascular disease mortality in men. Arch Intern Med 2004;164:1092–7.
- 10 Andersen LB, Hasselstrøm H, Grønfeldt V, et al. The relationship between physical fitness and clustered risk, and tracking of clustered risk from adolescence to young

adulthood: eight years follow-up in the Danish Youth and Sport Study. Int J Behav Nutr Phys Act 2004;1:6.

- 11 Cleland VJ, Ball K, Magnussen C, et al. Socioeconomic position and the tracking of physical activity and cardiorespiratory fitness from childhood to adulthood. Am J Epidemiol 2009;170:1069–77.
- 12 Twisk JW, Kemper HC, van Mechelen W. Tracking of activity and fitness and the relationship with cardiovascular disease risk factors. *Med Sci Sports Exerc* 2000;32:1455–61.
- 13 Kristensen PL, Wedderkopp N, Møller NC, et al. Tracking and prevalence of cardiovascular disease risk factors across socio-economic classes: a longitudinal substudy of the European Youth Heart Study. BMC Public Health 2006;6:20.
- 14 Artero EG, España-Romero V, Castro-Piñero J, *et al*. Reliability of field-based fitness tests in youth. *Int J Sports Med* 2011;32:159–69.
- 15 Tomkinson GR, Olds TS. Field tests of fitness. In: Armstrong N, Van Mechelen W, eds. *Pediatric Exercise Science and Medicine*. Oxford: Oxford University Press 2008:109–28.
- 16 Castro-Piñero J, Artero EG, España-Romero V, et al. Criterion-related validity of field-based fitness tests in youth: a systematic review. Br J Sports Med 2010;44:934–43.
- 17 Ruiz JR, Castro-Piñero J, España-Romero V, *et al.* Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. *Br J Sports Med* 2011;45:518–24.
- 18 Council of Europe. *Eurofit: Handbook for the Eurofit Tests Of Physical Fitness*. Rome: Council of Europe 1988.
- 19 The Cooper Institute. *FITNESSGRAM/ACTIVITYGRAM Test Administration Manual*. Fourth edition. Champaign, IL: Human Kinetics 2007.
- 20 Olds TS, Tomkinson GR. The aerobic fitness of 9–15 year old South Australian children: norms, trends and international comparisons. ACHPER Healthy Lifestyles Journal 2003;50:25–30.
- 21 Pyke JE. Australian Health And Fitness Survey 1985: The Fitness, Health And Physical Performance Of Australian School Students Aged 7–15 Years. Parkside, SA: The Australian Council for Health, Physical Education and Recreation 1987.
- 22 Tomkinson GR, Olds TS. Secular changes in pediatric aerobic fitness test performance: the global picture. *Med Sport Sci* 2007;50:46–66.
- 23 Tomkinson GR, Olds TS. Secular changes in aerobic fitness test performance of Australasian children and adolescents. *Med Sport Sci* 2007;50:168–82.
- 24 Tomkinson GR. Global changes in anaerobic fitness test performance of children and adolescents (1958-2003). Scand J Med Sci Sports 2007;17:497–507.
- 25 Tomkinson GR, Léger LA, Olds TS, et al. Secular trends in the performance of children and adolescents (1980-2000): an analysis of 55 studies of the 20m shuttle run test in 11 countries. Sports Med 2003;33:285–300.
- 26 Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med* 1992;11:1305–19.
- 27 Pan H, Cole T. *User's Guide to Lms Chart Maker*. Council MR, ed. 1997–2005 Medical Research Council. UK: Medical Research Council 2005.
- 28 Jackson LV, Thalange NK, Cole TJ. Blood pressure centiles for Great Britain. Arch Dis Child 2007;92:298–303.
- 29 Ortega FB, Artero EG, Ruiz JR, et al. Physical fitness levels among European adolescents: the HELENA study. Br J Sports Med 2011;45:20–9.
- 30 Castro-Piñero J, González-Montesinos JL, Keating XD, et al. Percentile values for running sprint field tests in children ages 6-17 years: influence of weight status. Res Q Exerc Sport 2010;81:143–51.
- 31 Cohen J. Statistical Power Analysis For The Behavioral Sciences. Second edition. New Jersey: Lawrence Erlbaum 1988.
- 32 Cole TJ, Freeman JV, Preece MA. Body mass index reference curves for the UK, 1990. Arch Dis Child 1995;73:25–9.
- 33 Eisenmann JC. Waist circumference percentiles for 7- to 15-year-old Australian children. Acta Paediatr 2005;94:1182–5.
- 34 Armstrong N, McManus AM, Welsman JR. Aerobic fitness. In: Armstrong N, Van Mechelen W, eds. *Paediatric Exercise Science and Medicine*. Oxford: Oxford University Press 2008:269–82.
- 35 Krahenbuhl GS, Skinner JS, Kohrt WM. Developmental aspects of maximal aerobic power in children. *Exerc Sport Sci Rev* 1985;13:503–38.
- 36 Rowland TW. Evolution of maximal oxygen uptake in children. *Med Sport Sci* 2007;50:200–9.

- 37 Blimkie CJR, Sale DG. Strength development and trainability during childhood. In: Van Praagh E, eds. *Pediatric Anaerobic Performance*. Champaign, IL: Human Kinetics 1998:193–224.
- 38 Froberg K, Lammert O. Development of muscle strength during childhood. In: Bar-Or O, ed. The Child and Adolescent Athlete. Oxford: Blackwell Science 1996:42–53.
- 39 De Ste Croix MDA. Muscle strength. In: Armstrong N, Van Mechelen W, eds. Paediatric Exercise Science and Medicine. Second edition. Oxford: Oxford University Press 2008:199–212.
- 40 Olds T, Tomkinson G, Léger L, *et al*. Worldwide variation in the performance of children and adolescents: an analysis of 109 studies of the 20-m shuttle run test in 37 countries. *J Sports Sci* 2006;24:1025–38.
- 41 Adegboye AR, Anderssen SA, Froberg K, et al. Recommended aerobic fitness level for metabolic health in children and adolescents: a study of diagnostic accuracy. Br J Sports Med 2011;45:722–8.
- 42 Lobelo F, Pate RR, Dowda M, *et al.* Validity of cardiorespiratory fitness criterion-referenced standards for adolescents. *Med Sci Sports Exerc* 2009;41:1222–9.
- 43 Ruiz JR, Ortega FB, Rizzo NS, *et al.* High cardiovascular fitness is associated with low metabolic risk score in children: the European Youth Heart Study. *Pediatr Res* 2007;61:350–5.
- 44 Welk GJ, Laurson KR, Eisenmann JC, *et al*. Development of youth aerobic capacity standards using receiver operator characteristic curves. *Am J Prev Med* 2011;41: S111–6.
- 45 Cureton KJ, Sloniger MA, O'Bannon JP, et al. A generalized equation for prediction of VO2peak from 1-mile run/walk performance. *Med Sci Sports Exerc* 1995;27:445–51.
- 46 Léger L, Lambert J, Goulet A, et al. [Aerobic capacity of 6 to 17-year-old Quebecois–20 meter shuttle run test with 1 minute stages]. Can J Appl Sport Sci 1984;9:64–9.
- 47 Tomkinson G. Aerobic fitness thresholds for cardio metabolic health in children and adolescents. *Br J Sports Med* 2011;45:686–7.
- 48 Dwyer T, Magnussen CG, Schmidt MD, *et al*. Decline in physical fitness from childhood to adulthood associated with increased obesity and insulin resistance in adults. *Diabetes Care* 2009;32:683–7.
- 49 Tomkinson GR, Hamlin MJ, Olds TS. Secular changes in anaerobic test performance in Australasian children and adolescents. *Pediatr Exerc Sci* 2006;18:314–28.
- 50 Australian Council for Health, Physical Education and Recreation. Australian Fitness Education Award: User's Manual And Curriculum Ideas. Adelaide, SA: Australian Council for Health, Physical Education and Recreation 1996.
- 51 Barnett LM, Van Beurden E, Morgan PJ, et al. Does childhood motor skill proficiency predict adolescent fitness? *Med Sci Sports Exerc* 2008;40:2137–44.
- 52 Birchall J. Health related fitness testing. In: Went S, ed. A Healthy Start: Holistic Approaches to Health Promotion in School Communities. Second edition. Melbourne: Monash University 1992:251–65.
- 53 Booth M, Macaskill P, McLellan L, et al. NSW Schools Fitness and Physical Activity Survey 1997. Sydney: NSW Department of Education and Training, 1997.
- 54 Booth M, Okely AD, Denney-Wilson E, et al. NSW Schools Physical Activity and Nutrition Survey (SPANS) 2004: Full Report. Sydney: NSW Department of Health, 2006.
- 55 Burke V, Beilin LJ, Durkin K, *et al.* Television, computer use, physical activity, diet and fatness in Australian adolescents. *Int J Pediatr Obes* 2006;1:248–55.
- 56 Cooley D, McNaughton L. Aerobic fitness of Tasmanian secondary school children using the 20-m shuttle run test. *Percept Mot Skills* 1999;88:188–98.
- 57 Dollman J, Olds T, Norton K, *et al*. The evolution of fitness and fatness in 10– 11-year-old Australian schoolchildren: changes in distributional characteristics between 1985 and 1997. *Pediatr Exerc Sci* 1999;11:106–21.
- 58 Hands B. *Fitness and motor skill levels of Western Australian Primary School Children*. Perth, WA: University of Western Australia 2000.
- 59 McNaughton L, Morgan R, Smith P, et al. An investigation into the fitness levels of Tasmanian primary schoolchildren. ACHPER Healthy Lifestyles Journal 1996;43:4–10.
- 60 Vandongen R, Jenner DA, Thompson C, *et al*. A controlled evaluation of a fitness and nutrition intervention program on cardiovascular health in 10- to 12-year-old children. *Prev Med* 1995;24:9–22.

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EXHIBIT 17

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European Journal of Sport Science

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ISSN: 1746-1391 (Print) 1536-7290 (Online) Journal homepage: https://www.tandfonline.com/loi/tej-20

Physical fitness normative values for 6–18-yearold Greek boys and girls, using the empirical distribution and the lambda, mu, and sigma statistical method

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To cite this article: Konstantinos D. Tambalis, Demosthenes B. Panagiotakos, Glykeria Psarra, Stelios Daskalakis, Stavros A. Kavouras, Nickos Geladas, Savas Tokmakidis & Labros S. Sidossis (2016) Physical fitness normative values for 6–18-year-old Greek boys and girls, using the empirical distribution and the lambda, mu, and sigma statistical method, European Journal of Sport Science, 16:6, 736-746, DOI: <u>10.1080/17461391.2015.1088577</u>

To link to this article: https://doi.org/10.1080/17461391.2015.1088577

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Routledge Taylor & Francis Group

ORIGINAL ARTICLE

Physical fitness normative values for 6–18-year-old Greek boys and girls, using the empirical distribution and the lambda, mu, and sigma statistical method

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Abstract

The aim of the this study was to establish age- and gender-specific physical fitness normative values and to compare percentiles and Z scores values in a large, nationwide sample of Greek children aged 6–18 years. From March 2014 to May 2014, a total of 424,328 boys and girls aged 6–18 years who attended school in Greece were enrolled. The studied sample was representative, in terms of age-sex distribution and geographical region. Physical fitness tests (i.e. 20 m shuttle run test (SRT), standing long jump, sit and reach, sit-ups, and 10×5 m SRT) were performed and used to calculate normative values, using the percentiles of the empirical distributions and the lambda, mu, and sigma statistical method. Normative values were presented as tabulated percentiles for five health-related fitness tests based on a large data set comprising 424,328 test performances. Boys typically scored higher than girls on cardiovascular endurance, muscular strength, muscular endurance, and speed/agility, but lower on flexibility (all p values <0.001). Older boys and girls had better performances than younger ones (p < 0.001). Physical fitness tests' performances tended to peak at around the age of 15 years in both sexes. The presented population-based data are the most up-to-date sex- and age-values for the health-related fitness scores of children from other countries similar to Greece. Schools need to make efforts to improve the fitness level of the schoolchildren through the physical education curriculum to prevent cardiovascular risk.

Keywords: Physical fitness, normative values, performance, children

Introduction

Physical fitness refers to "the ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample energy to enjoy leisure-time pursuits and to meet unforeseen emergencies" (Caspersen, Powell, & Christenson, 1985, p. 128). Fitness status has long been associated with age, from youth to the middle years of lifespan and even older. However, there is a lack of data in teenagers and younger children. It is crucial to know the fitness level of children as it has been suggested that fitness level in childhood is essential to carry forward favourable behavioural and biological effects into later life (Ortega, Ruiz, Castillo, & Sjöström, 2008). Accumulating epidemiologic evidence reveals that improvement in physical fitness, mainly aerobic fitness, is related to better health in children (Andersen, Wedderkopp, Hansen, Cooper, & Froberg, 2003; Ekelund et al., 2007; Hurtig-Wennlof, Ruiz, Harro, & Sjöström, 2007; Tambalis,

Correspondence: L. S. Sidossis, Department of Internal Medicine, Shriners Hospital for Children, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-0177, USA, E-mail: lasidoss@utmb.edu Physical fitness normative values for 6-18-year-old Greek boys and girls 737

Panagiotakos, Psarra, & Sidossis, 2011), in a dose response manner (Anderssen et al., 2007). Moreover, subjects with high physical fitness during adolescence may have lower levels of body fatness as adults (Eisenmann, Wickel, Welk, & Blair, 2005). In contrast, low levels of physical fitness in children have been associated with a number of cardiometabolic risk factors, such as hypertension, hyperlipidaemia, and obesity (Anderssen et al., 2007). To prevent early development of cardiovascular disease risk factors, among other significant interventions (e.g. increased physical activity levels, decreased obesity levels), preventive strategies must incorporate ageand sex-specific physical fitness assessment, even from childhood.

To make useful recommendations as regards to physical fitness, percentiles and Z scores have been used to assess children's and adolescent's growth and fitness status (Eisenmann, Laurson, & Welk, 2011). Percentiles are easier to understand and utilize in practice. In contrast, Z scores are more complicated and may be of limited use in clinical settings, but are more useful in research. However, it has been suggested that the lambda, mu, and sigma (LMS) method, a technique that uses Zscores and is based on smoothed percentile distribution curves over ages, better fits the data than the empirical percentile method (Wang, Moreno, Caballero, & Cole, 2006). These different methodologies may affect the cut-off points for the evaluation of fitness status and may lead to different results.

Numerous data sets on physical fitness levels among children are available worldwide; moreover age-, gender-, as well as region-specific values are essential for a variety of health-risk measurements (i.e. not only for fitness), in order to develop effective public health strategies (Tambalis et al., 2011; Tambalis, Panagiotakos, Arnaoutis, & Sidossis, 2013; Tokmakidis, Kasambalis, & Christodoulos, 2006). In addition, a comparison of both methodologies may prove useful for future researchers who would also like to calculate country-specific normative curves for fitness levels, as it would allow them to select the most appropriate procedure.

Therefore, the aim of the present work was to present the Greek region-specific distribution of age and sex physical fitness test measurements for 6-18-year-old children and adolescents and to evaluate sex- and age-related differences, using both percentiles and Z scores values in a nationwide sample of schoolchildren. These distributions (percentiles and Z scores) may also be valid for other similar populations around the world for comparisons among the same health-related fitness scores of children similar to Greece (i.e. Caucasians), and guide preventive strategies to better prevent cardiometabolic disorders in the future.

Methods

Study design and participants

Population-based, representative data were derived from a nationwide school-based survey under the auspices of the Ministry of Education. Specifically, anthropometric, physical activity, nutrition, and physical fitness data along with information on age and sex were collected from March 2014 to May 2014. In total, 424,328 (51% boys and 49% girls) children aged 6–18 years from Greek public and private schools agreed to participate in the study (participation rate was 40% of the total population). The working sample was representative of the entire Greek population (chi-square p value as compared with the current sample with the age–sex distribution of all Greek areas = 0.93).

Study approval

Ethical approval for the survey was granted by the Review Board of the Ministry of Education and the Ethical Committee of Harokopio University.

Assessment of fitness status

The Euro-fit physical fitness test battery was used to evaluate children's physical fitness levels; it is a set of nine physical tests covering flexibility, speed, endurance, and strength. The standardized test battery was proposed by the Council of Europe for children of school age and has been used in many European countries schools since the 1980s. Five fitness tests of the Euro-fit Battery, representative of flexibility, explosive strength, speed/agility, and aerobic performance, were administered by trained physical education professionals during the physical education classes. Specifically, measurements were performed by one teacher of physical education in each class. All physical education professionals were instructed through a detailed and extended manual of operations and followed a standardized procedure of measurements in order to minimize the potential inter-rate variability among schools. The physical education teachers were first trained by a school advisor of physical education for accurate anatomical landmarks, subject positioning, and measurement techniques. Verbal informed consent for the child to participate in the measurements was taken from physical education teachers. As the measurements were included in an obligatory school programme, verbal informed consent was considered sufficient.

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Briefly, the test assessed: (a) standing long jump (SLJ; jump as far as possible from a standing position at the start) to evaluate lower body explosive power; (b) sit and reach (SR; this test involves sitting on the floor with legs stretched out straight ahead without shoes. The soles of the feet are placed flat against the box. With the palms facing downwards, and the hands on top of each other or side by side, the participant reaches forward along the measuring line as far as possible. The score is recorded to the nearest centimetre as the distance reached by the hand, using 15 cm at the level of the feet) to measure flexibility; (c) sit-ups in 30 s (SUs; lie on the mat with the knees bent at right angles, with the feet flat on the floor and held down by a partner), to measure the endurance of the abdominal and hip-flexor muscles; (d) 10×5 m shuttle run test (10×5 m SRT; from a standing start), to evaluate speed and agility; and (e) multi-stage 20 m SRT, to estimate aerobic performance. The 20 m SRT test consists of measuring the number of laps completed by participants running up and down between two lines in groups, set 20 m apart, at an initial speed of 8.5 km/ h which increases by 0.5 km/h every minute, using a pre-recorded audio tape (Leger, Lambert, Goulet, Rowan, & Dinelle, 1984; Leger, Mercier, Gadoury, & Lambert, 1988). Repeat tests (two trials) were allowed for the SLJ, SR, SU, and 10×5m SRT, with the best performance of each recorded.

Statistical analysis

Descriptive statistics (mean \pm standard deviation) for boys and girls were calculated. Comparisons of the physical fitness tests' performances data between boys and girls were performed using the independent samples t test, after testing for equality of variances using the Levene test. Comparisons between percentile values of the physical fitness tests' performance data of both calculation methods were performed using the paired samples t test.

Age- and sex-specific distributions and percentiles were calculated using two methods: through the empirical distribution of the data, the 3th, 10th, 25th, 50th, 75th, 90th, and 97th percentiles were calculated; also, the LMS method was used (Cole & Green, 1992). The LMS method was used in order to smooth the age-dependent skewness usually observed in fitness values. Based on this method, the data were normalized using the Box-Cox power transformation. The Box-Cox λ -power transformation of the variable y_i has the following form:

$$y_i^{(\lambda)} = \frac{y_i^{\lambda} - 1}{\lambda(\operatorname{gm}(y))^{\lambda - 1}} \quad \text{if } \lambda \neq 0$$

or

$$y_i^{(\lambda)} = \operatorname{gm}(y_i) \log y_i \quad \text{if } \lambda \neq 0$$

where gm is the geometric mean of y_i . The power transformation was calculated from the raw data and skewness in the distribution where y was removed. The principle idea of the LMS method is to power transform the measurement, i.e. SLJ here, and to use the coefficient of variation (CV = standard deviation/mean) of the raw data. The optimal Box-Cox power λ is the one that gives the lowest CV (Cole & Green, 1992). Thus, the LMS method calculates the best power (L), the best mean (M), and CV (S) in each series of measurements at a specific age. The degrees of freedom used to determine the L, M, and S were chosen on the basis of that which achieved the smallest difference in the penalized deviance $(-2 \times \log\{\text{penalised likelihood}\})$ statistic, as well as the Schwarz Bayesian Criterion (both goodness-of-fit measures) between the estimated models. Then, centiles of physical fitness tests were calculated as follows:

Centile(
$$\alpha$$
) = $M_{age} \times (1 + L_{age} \times S_{age} \times Z_{\alpha})^{1/L_{age}}$, (1)

where Z_{α} is the Z score (i.e. (variable – mean)/standard deviation) corresponding to the required centile (e.g. Z = -0.67 gives the 25th centile, Z = 0 gives the median M, Z = 0.67 gives the 75th centile). The gender-specific cut-offs of fitness tests for each age group were calculated for various centiles by solving the previous equation. Particularly, from Equation (1) we have:

$$\begin{split} \left(\frac{\mathrm{Centile}(\alpha)}{M_{\mathrm{age}}}\right)^{L_{\mathrm{age}}} &= 1 + L_{\mathrm{age}} \times S_{\mathrm{age}} \times Z_{\alpha}, \\ \left(\frac{\mathrm{Centile}(\alpha)}{M_{\mathrm{age}}}\right)^{L_{\mathrm{age}}} - 1 &= L_{\mathrm{age}} \times S_{\mathrm{age}} \times Z_{\alpha}. \end{split}$$

Thus,

$$Z_{\alpha} = \left(\left(\frac{\text{Centile}(\alpha)}{M_{\text{age}}} \right)^{L_{\text{age}}} - 1 \right) \times \left(L_{\text{age}} \times S_{\text{age}} \right)^{-1}.$$
(2)

The LMS values can be used to calculate Z scores and therefore percentile values by looking up a Z

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table, using the following formula:

$$z = \frac{(x/M) - 1}{L \times S},$$

where x is performance, L is the gender- and agespecific L value, M is the gender- and age-specific M value, and S is the gender- and age-specific S value. All statistical analyses were performed using the SPSS program (Release 18; SPSS Inc., Chicago, IL, USA). The LMSchartmaker (Pan & Cole, 2010) and the LMSgrowth (Pan & Cole, 2011) freeware packages were used to calculate L, M, and S values at ages 6-18 based on Greek reference values.

Results

In Tables I and II, normative physical fitness data for 6-18-year-old children in Greece, by gender and age as tabulated critical percentiles and LMS values from 3 to 97 (P_3 , P_{10} , P_{25} , P_{50} , P_{75} , P_{90} , P_{97}), are presented. Also presented are the gender- and age-specific LMS values for all fitness tests.

For each of the fitness tests, performance was better in boys compared with girls (p < 0.001), except for the SR test (p < 0.001). Moreover, older boys and girls had better performances than younger ones (p < 0.001). Physical fitness tests' performances also tended to peak at about the age of 15–16 years in both sexes.

In order to investigate potential differences between percentile values from the two methods, comparisons by physical fitness test and gender were performed. Data analysis revealed no significant differences between critical percentiles and LMS percentiles in SR, SLJ, SUs, 20 m SRT, and 10×5 m SRT values in either sex (all *p* values >0.05).

Discussion

The aim of the present work was to develop up-todate age- and sex-specific physical fitness normative values for Greek children aged 6–18 years and to compare specific percentile values from two widely applied estimation methods: the frequency percentiles and the LMS smoothed percentiles. This study provides information relating to normative values across a range of health-related fitness tests. These values could be used as approximate indicative values for comparisons among the same healthrelated fitness scores of children (according to National Statistical Services the vast majority of children population in Greece are Caucasians) from other countries similar to Greece: i.e. a developed country with population predominately Caucasian. Moreover, these data can be used as benchmark values for health-related fitness screening and surveillance of children 6–18 years in Greece.

The presented data could be useful in a crude classification of how well children in Greece perform on the specific health-related fitness tests relative to their age- and sex-matched peers and to identify those children with specific physical fitness characteristics that could be considered essential for sporting success. Moreover, our findings facilitate the recognition of children and adolescents with low physical fitness aiming to set appropriate goals in the future and to promote encouraging health behaviours. Previous studies in children from Greece have linked low cardiorespiratory fitness with increased metabolic score risk and inflammation (Christodoulos, Douda, & Tokmakidis, 2012; Flouris, Bouziotas, Christodoulos, & Koutedakis, 2008). According to the presented percentile classification from both methods, and for a practical use of these data, children could be classified as having a performance score: very poor $(\langle P_{10} \rangle)$, in the poor quartile (1st), in the good quartiles (2nd-3rd), in the very good quartile (4th), and excellent $(X > P_{90})$ of the distribution. Although this classification is not criterion-referenced, Looney and Plowman (1990) suggested that test scores above the 25th percentile (the poor quartile) in fitness tests should be considered acceptable from a health perspective. On the contrary, low levels of physical fitness have been associated with many serious health problems in childhood, while sufficient levels of physical fitness may have an important cardio-protective role in children. According to Anderssen et al. (2007), there was a strong association between aerobic fitness and the clustering of cardiovascular disease risk factors. Specifically, the odds ratios for clustering in each quartile of fitness, using the quartile with the highest fitness as reference, were 13.0 [95% confidence interval (CI) 8.8-19.1] 4.8 [95% CI 3.2-7.1], and 2.5 [95% CI 1.6-3.8], respectively, after adjusting for several confounding factors (Andersen et al., 2003; Anderssen et al., 2007; Ekelund et al., 2007; Hurtig-Wennlof et al., 2007). Future studies need to examine which specific childhood and/or adolescence thresholds for aerobic fitness are significantly associated with clustered cardiovascular disease risk factors.

The presented results revealed that boys consistently scored higher than girls in almost all fitness tests (with the exception of the SR test of flexibility). Moreover, older boys and girls performed better than younger ones (p < 0.001). Our findings are in accordance with recent studies from Latvia (Sauka et al.,

				H	Percentiles			Ì					ILMS n	LMS method				Ĩ
Age	Ν	P_3	P_{10}	P_{25}	P_{50}	P_{75}	P_{90}	P_{97}	P_3	P_{10}	P_{25}	P_{50}	P_{75}	P_{90}	P_{97}	Г	Μ	S
20 m SF	20 m SRT (completed laps)	d laps)		ć	0 F.	0.00	5	4	0		0			i.	2	0		
5 1	00/T	0.1	4.0	0. ⁴	14.0	0.22	0.16	7.44	7.7	4.9	0.6	14.0	1.22	0.15	45.2	0.38	14,04	, 0, U
1	15,196	5.0	8.0	12.0	18.0	28.0	38.0	50.0	3.2	6.7	11.8	18.9	28.0	39.4	53.2	0.39	18.86	0.64
ø	20,774	6.0	9,0	14.0	23.0	34.0	47.0	59.0	4.2	8.6	14.8	23.1	33.6	46.6	62.1	0.41	23.06	0.61
9	20,149	7.0	11.0	17.0	28.0	41.0	53.0	64.0	5.2	10.4	17.6	27.1	38.9	53.0	69.7	0.44	27.09	0.59
10	19,279	8.0	12.0	20.0	31.0	45.0	58.0	70.0	0.0	12.1	20.5	31.1	44.1	59.3	76.8	0.49	31.13	0.57
11	18,793	9.0	15.0	23.0	36.0	51.0	64.0	78.0	6.6	13.8	23.4	35.4	49.5	65.8	84.1	0.54	35.36	0.55
12	11,456	8.0	15.0	24.0	39.0	54.0	69.0	82.0	6.8	15.4	26.4	39.7	55.1	72.4	91.6	0.60	39.71	0.54
13	15,555	7.0	16.0	28.0	44.0	61.0	76.0	91.0	6.8	16.7	29.3	44.0	60.7	79.0	98.9	0.66	44.04	0.54
14	13,681	7.0	19.0	32.0	50.0	0.69	85.0	100	6.4	17.8	31.8	47.8	65.4	84.5	105	0.73	47.79	0.53
15	10,135	0.0	20.0	34.0	53.0	72.0	0.06	103	5.5	18.3	33.5	50.4	68.6	87.9	108	0.79	50.37	0.52
16	1862	7.0	20.0	36.0	54.0	75.0	0.06	103	4.4	18.4	34.5	51.9	70.2	89.3	109	0.85	51.87	0.52
17	670	7.0	18.0	33.0	50.0	68.0	86.0	100	3.9	18.3	35.1	52.8	71.0	89.7	109	0.91	52.76	0.51
18	305	7.4	13.0	28.0	48.0	66.0	87.0	115	3.2	18.0	35.6	53.5	71.6	89.9	108	0.97	53.48	0.51
SLJ (cm)	0																	
, 9	17,494	64.0	76.0	90.0	102	115	128	140	59.0	75.0	89.7	103	116	129	141	1.42	103.4	0.19
5	23,994	71.0	85.0	100	113	127	140	151	66.0	83.1	98.7	113	127	141	153	1.42	113.4	0.19
00	23,836	80.0	94.0	108	123	138	150	162	72.8	90.7	107	123	137	151	165	1.43	122.7	0.18
6	22,667	85.0	100	116	131	147	160	172	79.0	<i>L'L6</i>	115	131	146	161	175	1.44	131.2	0.18
10	21,886	90.06	108	123	140	155	169	181	84.8	104	122	139	155	171	185	1.44	139.2	0.18
11	21,241	0.79	113	130	147	163	177	190	90.3	111	130	147	164	180	196	1.45	147.3	0.18
12	12,961	100.0	119	135	154	171	187	201	95.9	117	137	156	174	191	207	1.45	~156.0	0.17
13	8683	105.0	125	144	164	183	200	217	101	124	145	165	184	202	219	1.46	165.0	0.17
14	7231	112.0	133	153	175	195	212	228	106	130	152	173	193	212	230	1.48	173.3	0.17
15	2633	118	140	160	183	204	220	240	110	135	159	180	201	220	239	1.51	180.3	0.17
16	7729	122	145	167	188	209	225	240	112	139	163	186	207	226	245	1.55	185.6	0.17
17	2737	120	146	167	188	210	228	245	113	142	167	190	211	231	250	1.60	189.7	0.17
18	375	110	137	160	187	222	244	250	114	144	169	193	215	235	254	1.66	193.0	0.17
JK (cm)		0	C L	0		0 0 7	0.00	0	ç	C 4		1 7	001		0.00		02.61	24.0
0 1	21,440	0.2	5 0 6 4	0.01	0.61	10.01	0.22	0.02	1.2	u	רים מים	7.CT	10.01	4.77	0.12	10.0	01.01	01.0
- 0	0000000	0.2		0.0	0.01	10.01	0.22	0.02	1 6	200	7.6	13.1	5-11 17 7	5.00	1		13 14	0120
0 0	26,607	2.0	7 C F	0.6 0	14.0	18.0	0.77	25.5	1.4	7. 6 7	n ur o or	10.8	17.4	0.22 0.22	07.6	10.0	12.78	0 53
10	25,017	2.0	4.0	0.8	13.0	17.0	21.5	26.0	1.3	6.4	1.8	12.4	17.1	22.2	27.5	0.76	12.44	0.54
11	24,418	1.0	3.0	7.0	13.0	17.0	21.0	26.0	1.2	4.2	8.0	12.3	17.1	22.2	27.7	0.74	12.29	0.56
12	15,636	1.0	3.0	7.0	13.0	17.0	22.0	27.0	1.2	4.2	8.0	12.5	17.4	22.8	28.5	0.72	12.47	0.57
13	14,613	1.0	3.0	8.0	14.0	18.0	23.0	28.0	1.3	4.4	8.4	13.0	18.2	23.8	29,9	0.71	12.99	0.57
14	12,871	2.0	4.0	9.0	15.0	20.0	25.0	30.0	1.4	4.7	8.9	13.8	19.3	25.3	31.7	0.71	13.80	0.57
15	9537	2.0	5.0	10.0	16.0	21.0	27.0	31.0	1.6	5.1	9.5	14.8	20.6	27.0	33.8	0.71	14.77	0.56
16	7291	2.0	5.0	11.0	16.5	22.0	28.0	32.0	1.8	5.5	10.2	15.8	21.9	28.7	35.9	0.70	15.76	0.56
17	2573	2.0	5.0	11.0	17.0	22.0	28.0	33.0	2.0	5,9	10.9	16.7	23.2	30.3	37.8	0.70	16.72	0.55
18	352	1.0	5.0	10.0	16.0	22.0	29.0	33.0	2.2	6.4	11.6	17.7	24.4	31.8	39.7	0.70	17.68	0.55

							\boldsymbol{P}	ny.	sic	al .	fiti	nes	S 7	wr	mc	un	ve	va	uu	zs J	or	0-	-10	у -у	ea	r=0	nu	Greek
	0.38	0.36	0.33	0.31	0.29	0.28	0.27	0.26	0.25	0.25	0.25	0.25	0.26		0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	
	13.46	15.27	16.94	18.40	19.62	20.62	21.47	22.20	22.76	23.11	23.31	23.40	23.40		24.04	23.32	22.62	21.96	21.37	20.86	20.45	20.13	19.91	19.77	19.68	19.64	19.61	
	1.34	1.29	1.25	1.21	1.17	1.14	1.09	1.03	0.97	0.91	0.84	0.77	0.70		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
	22.8	25.3	27.5	29.3	30.6	31.7	32.7	33.6	34.4	35.0	35.5	35.9	36.2		16.3	15.9	15.4	15.0	14.6	14.2	14.0	13.7	13.6	13.5	13.5	13.5	13.5	
	19.9	22.1	24.1	25.8	27.0	28.1	29.0	29.8	30.5	31.0	31.3	31.6	31.7		18.9	18.3	17.8	17.3	16.8	16.4	16.1	15.9	15.7	15.6	15.6	15.6	15.6	
	16.8	18.8	20.6	22.1	23.4	24.4	25.2	26.0	26.6	27.0	27.3	27.4	27.5		21.4	20.8	20.2	19.6	1.9.1	18.6	18.3	18.0	17.8	17.7	17.7	17.6	17.6	
	13.5	15.3	16.9	18.4	19.6	20.6	21.5	22.2	22.8	23.1	23.3	23.4	23.4		24.0	23.3	22.6	22.0	21.4	20.9	20.4	20.1	19.9	19.8	19.7	19.6	19.6	
	9.8	11.5	13.1	14.5	15.7	16.8	17.6	18.4	18.9	19.3	19.4	19.5	19.5		26.5	25.7	25.0	24.2	23.6	23.1	22.6	22.3	22.1	21.9	21.9	21.8	21.8	ycars.
	5.7	7.3	8.9	10.3	11.7	12.8	13.7	14.5	15.1	15.5	15.7	15.8	15.8		29.0	28.2	27.4	26.6	25.9	25.3	24.8	24.4	24.2	24.0	24.0	23.9	23.9	.00-6.99
	1.0	2.0	4.0	5.8	7.3	8.6	9.7	10.6	11.3	11.8	12.1	12.3	12.4		31.6	30.6	29.7	28.9	28.1	27.5	27.0	26.6	26.3	26.1	26.1	26.0	26.0	5 years = (
	23.0	25.0	27.0	29.0	30.0	31.0	32.0	33.0	34.0	35.0	34.0	35.0	36.0		19.5	18.6	18.0	17.4	17.0	16.4	16.1	15.7	15.3	15.0	15.0	5.0	15.0	age, e.g. (
	19.0	21.0	23.0	25.0	26.0	27.0	28.0	29.0	30.0	30.0	30.0	30.0	30.0		21.0	20.0	19.2	18.7	18.1	17.7	17.3	16.9	16.5	16.4	16.4	16.4	16.7	ation; age: completed age, e.g. 6 years = 6.00–6.99 years.
	16.0	18.0	20.0	22.0	23.0	24.0	25.0	26.0	27.0	27.0	27.0	27.0	26.0		22.3	21.3	20.5	20.0	19.4	19.0	18.7	18.3	17.8	17.8	17.9	17.8	18.2	tion; age: o
	13.0	15.0	17.0	18.0	20.0	21.0	21.0	22.0	23.0	23.0	23.0	23.0	22.0		24.4	23.0	22.1	21.5	21.0	20.4	20.1	19.8	19.3	19.2	19.3	19.4	19.9	nt of varia
	10.0	12.0	14.0	15.0	16.0	17.0	18.0	19.0	19.0	20.0	19.0	19.0	18.0		26.1	25.0	23.9	23.1	22.6	22.00	21.8	21.3	21.0	21.0	21.1	21.2	22.1	S, coefficier
	6.0	8.0	10.0	12.0	13.0	14.0	15.0	15.0	16.0	17.0	16.0	16.0	15.0		28.4	27.0	26.0	25.0	24.3	23.8	23.4	23.2	23.0	23.0	23.5	23.9	25.8	, median; .
	2.0	4.0	6.0	7.0	8.0	10.0	10.0	12.0	12.0	13.0	13.0	12.0	10.0		32.3	30.0	29.0	27.9	27.0	26.1	26.0	26.3	26.0	26.5	27.8	29.4	31.0	, skew; M
t 30 sec)	7,381	3,866	3,782	2,608	1,770	21,159	2,941	5,555	3,681	0,135	688	:753	567	RT (sec)	21,133	27,340	23,231	22,143	25,561	29,745	12,695	15,168	13,244	9528	7146	2487	345	Note: P , percentile; L_2 skew; M , median; S , coefficient of vari
SUs (no. in 30 sec)	6 Ì	7	00	, ,	10 2	11 2	12 1	13 1	14 1	15	16	17 2	18	$10 \times 5 m S$	9	7	00	6	10	11	12	13	14	15	16	17	18	Note: P, I

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				-	Percentiles								LMS r	LMS method				
		P_3	P_{10}	P_{25}	P_{50}	P_{75}	P_{90}	P_{97}	\mathbf{P}_3	P_{10}	P_{25}	P_{50}	P_{75}	P_{90}	P_{97}	T	W	S
	0 m SRT (compli	sted laps)		6	c c	c c	2	0	ı c	I L	c c	0 7	c F	c L	- L (L C C	00	L L
	1040 I	2.0	4,0	0.0	12.0	16.0	20.U	⊃č.U 2∂ ∂	0.0		0.0 -	0.01	0.81	P.02	1.00	CZ.U	70.CI	00.0
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	14,777	0.5	8.0	11.0	15.0	21.0	29.0	39.0	4.1	6.8	10.5	15.4	21.9	30.2	40.6	0.28	15.45	0.55
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	20,084		0.6	12.0	18.0	25.0	34.0	45.0	4.9	8.0	12.4	18.1	25.6	35.0	46.5	0.30	18.14	0.54
	19,806		10.0	14.0	20.0	29.0	40.0	51.0	5.6	9.2	14.3	20.9	29.3	39.8	52.6	0.32	20.87	0.54
			11.0	16.0	23.0	33.0	43.0	55.0	6.1	10.3	16.0	23.4	32.8	44.3	58.2	0.35	23.42	0.54
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	1 18,290		12.0	18.0	26.0	36.0	48.0	61.0	6.4	11.1	17.3	25.4	35.6	48.0	62.8	0.37	25.44	0.54
670 60 120 180 260 371 507 561 512 562 512 562 512 561 512 561 512 561 512 561 512 561 512 512 512 512 512 512 512 512 512 512 512 512 512 512 512 512 512 512 523 525 512 523 <th< td=""><td></td><td></td><td>12.0</td><td>18.0</td><td>26.0</td><td>37.0</td><td>49.0</td><td>62.0</td><td>6.4</td><td>11.3</td><td>17.9</td><td>26.5</td><td>37.2</td><td>50.1</td><td>65.4</td><td>0.40</td><td>26.49</td><td>0.54</td></th<>			12.0	18.0	26.0	37.0	49.0	62.0	6.4	11.3	17.9	26.5	37.2	50.1	65.4	0.40	26.49	0.54
566 50 120 180 260 57 506 57 506 501 505 501 501 501 501 501 501 501 501 501 501 501 501 501 501 601 521 602 501 601 501 601 521 641 501 651 631 632 543 631 632 543 631 632 543 631 632 543 631 632 543 634 632 543 634 632 543 634 632 543 634 632 543 634 632 543 634 632 543 632 543 632 543 633			12.0	18.0	26.0	37.0	50.0	62.0	6.0	11.0	17.9	26.7	37.6	50.7	66.2	0.42	26.71	0.55
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		5.0	12.0	18.0	26.0	37.0	50.0	63.0	5.5	10.6	17.5	26.4	37.4	50.5	62.9	0.45	26.40	0.56
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		7.0	12.0	17.0	25.0	35.0	47.0	62.0	4.9	9.9	16.9	25.8	36.8	49.9	65.1	0.47	25.82	0.58
40 5.0 100 160 230 500 67.0 35.7 85.7 46.7 55.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 53.7 66.7 110 122 132 132 133 130 133 140 137 132 133 130 133 130 137 131 137 131 137 131 137 133 140 137 133 143 137 133 143 137 137 133 136 137 137 133 136 137 137 137 133 136 137 137 133 136 137 137 137 137 133 136 137 137 137		7.0	11.0	16.0	24.0	33.0	45.0	60.0	4.2	9.2	16.2	25.2	36.2	49.2	64.3	0.49	25.21	0.60
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		5.0	10.0	16.0	23.0	36.0	50.0	67.0	3.5	6.8	15.6	24.6	35.7	48.7	63.6	0.52	24.63	0.61
		3.0	8.0	14.0	23.0	28.0	39.5	63.7	2.8	7.8	14.8	23.9	34.9	47.8	62.5	0.54	23.92	0.63
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$L_{f}^{f}(cm)$																	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	17,152		70.0	80.0	93.0	105	116	128	55.3	68.4	81.0	93.2	105	117	128	1.22	93.2	0.19
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	23,303		78.0	0.06	101	115	126	140	61.5	75.6	89.1	102	115	128	140	1.19	102.3	0.19
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	23,099		85.0	97.0	110	125	138	150	67.7	82.6	97.1	111	125	139	152	1.16	111.3	0.19
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	22,427		92.5	105	120	134	148	160	73.6	89.3	105	120	135	149	164	1.13	119.8	0.19
$ \begin{array}{llllllllllllllllllllllllllllllllllll$			0.99	111	127	142	156	170	78.5	95.0	111	127	143	158	174	1.10	127.1	0.19
			103	118	134	150	165	180	82.1	99.1	116	133	149	165	182	1.08	132.6	0.19
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		90.0	105	120	136	153	170	185	83.8	101	119	136	153	170	187	1.07	135.8	0.19
6454 89.0 103 120 135 154 170 188 83.1 101 119 137 155 172 190 1.05 136.9 7677 86.0 100 116 133 152 170 187 79.9 98.7 116 135 154 172 190 1.06 135.7 7677 86.0 100 115 132 152 170 187 79.9 98.7 116 135 154 172 190 1.06 135.7 240 81.0 99.0 112 130 150 169 197 76.2 95.8 115 117 160 107 135.0 240 81.0 99.0 112 130 150 169 197 76.2 95.8 115 117 190 1.07 135.0 $20,463$ 3.0 7.0 120 16.0 21.0 24.0 28.0 3.3 7.4 11.7 160 20.7 29.6 0.93 16.04 $25,849$ 3.0 7.0 120 16.0 21.0 25.0 29.0 30.7 20.7 25.4 30.1 0.92 16.04 $25,849$ 3.0 7.0 11.6 12.0 16.0 21.0 21.0 20.7 25.4 30.1 0.91 16.17 $25,833$ 2.0 50 21.0 21.0 21.0 21.0 21.0 21.0 16.04 <		90.06	104	120	137	154	170	187	84.0	102	119	137	154	172	189	1.06	137.0	0.19
$ \begin{array}{llllllllllllllllllllllllllllllllllll$		89.0	103	120	135	154	170	188	83.1	101	119	137	155	172	190	1.05	136.9	0.19
7677 860 100 115 132 150 170 187 79.9 98.7 117 136 154 172 190 1.06 135.7 2637 83.0 100 116 133 152 170 190 107 135.0 240 81.0 99.0 116 133 152 170 190 107 135.0 240 81.0 91.0 116 133 152 170 190 107 135.0 $20,463$ 3.0 7.0 12.0 16.0 21.0 24.0 28.0 3.3 7.4 11.7 16.0 20.5 29.0 107 135.0 $20,463$ 3.0 7.0 12.0 16.0 21.0 25.0 39.0 3.3 7.4 11.7 16.0 20.7 29.6 99.7 $25,849$ 3.0 7.0 12.0 16.0 21.0 25.0 39.0 2.9 7.1 11.6 16.1 20.7 25.4 30.1 0.92 16.10 $25,849$ 3.0 7.0 11.0 17.0 22.0 29.0 29.0 29.7 21.1 20.7 25.4 30.1 0.91 16.17 $25,849$ 3.0 7.0 11.0 17.0 22.0 29.0 29.0 29.7 21.1 20.7 25.4 30.1 0.91 16.17 $25,633$ 2.0 6.0 11.0 17.0 22.0 29.0		86.0	100	116	134	151	170	186	81.6	100	118	136	154	172	190	1.05	136.3	0.20
2637 83.0 100 116 133 152 170 190 7.1 97.3 116 135 154 172 190 1.07 135.0 240 81.0 99.0 112 130 150 169 197 76.2 95.8 115 134 153 172 190 1.07 135.0 $20,463$ 3.0 7.0 12.0 16.0 20.0 24.0 28.0 3.3 7.4 11.7 16.0 20.5 29.6 0.93 16.04 $25,851$ 3.0 7.0 12.0 16.0 21.0 25.0 29.0 3.1 7.3 11.6 16.2 29.7 29.1 0.92 16.04 $25,849$ 3.0 7.0 12.0 16.0 21.0 25.0 30.0 2.9 7.1 11.6 16.2 20.9 23.1 29.7 29.7 29.7 29.7 29.7 29.7 29.7 29.7 29.7 29.7 28.7 27.8 27.1 16.7 22.7 30.7 29.7 28.7 </td <td></td> <td>86.0</td> <td>100</td> <td>115</td> <td>132</td> <td>150</td> <td>170</td> <td>187</td> <td>79.9</td> <td>98.7</td> <td>117</td> <td>136</td> <td>154</td> <td>172</td> <td>190</td> <td>1.06</td> <td>135.7</td> <td>0.20</td>		86.0	100	115	132	150	170	187	79.9	98.7	117	136	154	172	190	1.06	135.7	0.20
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		83.0	100	116	133	152	170	190	78.1	97.3	116	135	154	172	190	1.07	135.0	0.21
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		81.0	0.06	112	130	150	169	197	76.2	95.8	115	134	153	172	190	1.08	134.3	0.2
26,851 3.0 7.0 12.0 16.0 21.0 25.0 29.0 3.1 7.3 11.6 16.1 20.7 25.4 30.1 0.92 16.10 $25,849$ 3.0 7.0 12.0 16.0 21.0 25.0 30.0 2.9 7.1 11.6 16.2 20.9 25.7 30.7 0.91 16.17 $25,623$ 2.0 6.0 11.0 17.0 22.0 30.0 2.8 7.0 11.7 16.5 21.1 26.1 31.2 0.90 16.27 $24,913$ 2.0 6.0 11.0 17.0 22.0 30.0 2.8 7.0 11.7 16.5 21.1 26.7 32.0 0.99 16.27 $24,913$ 2.0 7.0 12.0 17.0 22.0 30.0 2.8 7.0 11.7 16.5 21.1 26.7 32.0 0.90 16.27 $24,913$ 2.0 7.0 12.0 17.0 23.0 28.0 32.0 2.8 7.0 11.7 16.5 21.6 20.0 $24,913$ 2.0 7.0 12.0 17.1 22.0 28.0 32.0 28.0 32.0 28.0 28.7 21.1 17.1 22.4 27.8 33.3 0.87 17.18 $24,915$ 3.0 8.0 $14,014$ 3.0 8.0 $14,01$ 29.0 25.0 30.0 28.0 32.6 17.6 17.2 22.4 27.8 36.7			7.0	12.0	16.0	20.0	24.0	28.0	3.3	7.4	11.7	16.0	20.5	25.0	29.6	0.93	16.04	0.4
$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$			7.0	12.0	16.0	21.0	25.0	29.0	3.1	7.3	11.6	16.1	20.7	25.4	30.1	0.92	16.10	0.42
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	25,849		7.0	12.0	16.0	21.0	25.0	30.0	2.9	7.1	11.6	16.2	20.9	25.7	30.7	0.91	16.17	0.4
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	25,623		6.0	12.0	16.0	21.0	26.0	30.0	2.8	7.0	11.6	16.3	21.1	26.1	31.2	06'0	16.27	0.4°
$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$			6.0	11.0	17.0	22.0	26.0	30.0	2.8	7.0	11.7	16.5	21.6	26.7	32.0	0.89	16.54	0.4
$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$			7.0	12.0	17.0	23.0	28.0	32.0	2.8	7.3	12.1	17.1	22.4	27.8	33.3	0.88	17.11	0.4
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			7.0	13.0	18.0	24.0	29.0	34.0	3.0	7.6	12.6	17.9	23.4	29.0	34.8	0.87	17.88	0.4
$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$			8.0	14.0	19.0	25.0	30.0	35.0	3.2	8.0	13.2	18.6	24.3	30.2	36.2	0.87	18.63	0.4
9135 3.0 9.0 15.0 20.0 26.0 31.0 36.0 3.6 8.6 14.0 19.7 25.6 31.6 37.8 0.88 19.68 7539 4.0 9.0 15.0 20.0 26.0 31.0 36.0 3.9 8.9 14.3 19.9 25.8 31.8 38.0 0.88 19.93 2597 4.0 10.0 15.0 20.0 26.0 30.0 35.0 4.0 9.1 14.5 20.1 25.9 31.9 38.0 0.89 20.09			0.6	15.0	20.0	26.0	30.0	36.0	3.4	8.3	13.6	19.3	25.1	31.1	37.3	0.87	19.25	0.4
7539 4.0 9.0 15.0 20.0 26.0 31.0 36.0 3.9 8.9 14.3 19.9 25.8 31.8 38.0 0.88 19.93 2597 4.0 10.0 15.0 20.0 26.0 30.0 35.0 4.0 9.1 14.5 20.1 25.9 31.9 38.0 0.89 20.09 20.		3.0	9.0	15.0	20.0	26.0	31.0	36.0	3.6	8.6	14.0	19.7	25.6	31.6	37.8	0.88	19.68	0.4°
4.0 10.0 15.0 20.0 26.0 30.0 35.0 4.0 9.1 14.5 20.1 25.9 31.9 38.0 0.89 20.09		4.0	9'0	15.0	20.0	26.0	31.0	36.0	3,9	8.9	14.3	19.9	25.8	31.8	38.0	0.88	19.93	0.4
	7 2597	4.0	10.0	15.0	20.0	26.0	30.0	35.0	4.0	9.1	14.5	20.1	25.9	31.9	38.0	0.89	20.09	0.4

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							P	hy	sic	al .	fiti	nes	51	ior	m	atu	ve	va	lu	25 J	for	6-	-18	¥-J	pea	r-0	id	Greel
	0.41	95.0	0.35	0.33	0.31	0.29	0.28	0.28	0.27	0.27	0.28	0.28	0.28		0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	Ĩ
	15.24	14.80	16.35	17.61	18.58	19.21	19.52	19.57	19.42	19.09	18.66	18.18	17.65		24.99	24.10	23.27	22.56	22.01	21.64	21.44	21.39	21.45	21.56	21.70	21.85	22.02	
	00.1	1.35	1.29	1.26	1.22	1.17	1.12	1.07	1.01	0.96	0.91	0.87	0.82		0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	
	6.77	25.1	27.0	28.4	29.4	30.0	30.2	30.2	30.0	29.6	29.2	28.7	28.1		18.5	17.9	17.3	16.7	16.3	16.1	15.9	15.9	15.9	16.0	16.1	16.2	16.3	
0	19,9 24 5	21.9	23.6	25.0	25.9	26.5	26.7	26.7	26.5	26.1	25.6	25.1	24.5		20.67	19.8	19.2	18.6	18.1	17.8	17.7	17.6	17.7	17.7	17.9	18.0	18.1	
	10.7	18.5	20.1	21.4	22.3	22.9	23.1	23.1	23.0	22.6	22.1	21.6	21.0		22.7	21.9	21.2	20.5	20.0	19.7	19.5	19.4	19.5	19.6	19.7	19.9	20.0	
((2.01	14.9	16.4	17.6	18.6	19.2	19.5	19.6	19.4	19.1	18.7	18.2	17.7		25.0	24.1	23.3	22.6	22.0	21.6	21.4	21.4	21.4	21.5	21.7	21.8	22.0	
L C	C.Y.	10.9	12.4	13.6	14.7	15.4	15.8	16.0	15.9	15.6	15.3	14.8	14.4		27.4	26.4	25.5	24.7	24.1	23.7	23.5	23.4	23.5	23.6	23.8	23.9	24.1	years.
c L	0.0	0.5	7.9	9.3	10.5	11.4	12.0	12.3	12.3	12.2	11.9	11.6	11.2		29.9	28.9	27.9	27.0	26.3	25.9	25.7	25.6	25.7	25.8	26.0	26.2	26.3	6 years = 6.00-6.99 years.
0	Z.U	2.1	2.5	4.4	5.9	7.2	8.0	8.5	8.8	8.8	8.6	8.4	8.2		32.6	31.4	30.3	29.4	28.7	28.2	27.9	27.9	28.0	28.1	28.3	28.5	28.7	
0	0.22	24.0	26.0	28.0	28.0	30.0	30.0	30.0	30.0	30.0	29.0	28.0	28.0		20.2	19.2	18.5	18.0	17.5	17.0	17.0	16.9	17.0	16.8	16.8	16.8	16.5	d age, e.g.
0	16.0	21.0	22.0	24.0	25.0	25.0	26.0	26.0	26.0	26.0	25.0	25.0	24.0		21.8	20.9	20.0	19.4	18.9	18.4	18.3	18.2	18.2	18.3	18.4	18.5	18.7	complete
¢ L	1).C1	18.0	19.0	21.0	22.0	22.0	22.0	22.0	23.0	22.0	21.0	21.0	20.0		23.2	22.2	21.4	20.7	20.2	19.7	19.6	19.7	19.6	19,9	20.0	20.0	20.2	iation; age
0	12.0	14.0	16.0	17.0	18.0	19.0	19.0	19.0	19.0	19.0	18.0	18.0	17.0		24.9	24.0	23.0	22.3	21.8	21.2	21.2	21.3	21.2	21.5	22.0	22.0	22.4	ient of var
0	9.V	0.11	13.0	14.0	15.0	16.0	16.0	16.0	16.0	16.0	15.0	15.0	14.0		26.9	25.8	24.8	24.0	23.4	22.9	22.9	23.0	23.0	23.4	24.0	24.0	24.7	S, coeffic
C L	0.0	8.U	0.0	10.0	12.0	13.0	13.0	13.0	13.0	13.0	12.0	12.0	10.0		29.0	27.8	26.7	25.8	25.2	24.6	24.6	24.9	25.0	25.4	26.1	26.1	27.7	4, median
0	4.U	4.0	5.0	6.0	8.0	0.6	10.0	10.0	10.0	10.0	9.0	0.0	7.0		32.0	30.3	29.7	28.3	27.8	27.1	27.0	27.8	28.0	28.3	30.0	30.1	32.0	L, skew; A
SUs (no. in 30 sec)	1 (,044	20,158	23,030	22,368	21,511	20,612	15,324	14, 181	12,637	9264	7686	2634	242	< 5 m SRT (sec)	20,030	26,271	25,450	25,394	24,749	23,862	14,920	13,569	12,088	8575	7004	2392	204	Note: P, percentile; L, skew; M, median; S, coefficient of variation; age: completed age, e.g.
SUs (no. é		1	80	6	10	11	12	13	14	15	16	17	18	$10 \times 5 m$	9	7	00	9	10	11	12	13	14	15	16	17	18	Note: P,

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2011), Portugal (Santos et al., 2014), and Australia (Catley & Tomkinson, 2013) that have examined similar physical fitness tests in children aged 6-18 years. In all the aforementioned studies, boys performed better than girls in cardiorespiratory endurspeed/agility, muscular strength, and ance, muscular endurance tests, while older ages, in both sexes, have incorporated higher percentile values in comparison with younger ones (Catley & Tomkinson, 2013; Santos et al., 2014; Sauka et al., 2011). Moreover, it seems that physical fitness test performance tends to peak from about the age of 15 years, especially in girls. This finding is in accordance with results from a previous large European epidemiological study (the HELENA study) which found stability in girls' performance in aerobic fitness, speed/agility, and flexibility tests after about the age of 15 years (Ortega et al., 2011).

A finding that deserves attention is the lack of significant differences between the two methods used to create the percentiles in 20 m SRT, SLJ, SR, SUs, and 10×5 m SRT, in both sexes. The LMS method uses smoothed curves to estimate the critical centiles and has been extensively been used to provide a way of obtaining normalised growth centiles standards (Cole, 1990). To the best of our knowledge, this is one of the few times that the LMS method has been used to calculate fitness centiles in children and adolescents (Bustamante, Beunen, & Maia, 2012; Catley & Tomkinson, 2013; Eisenmann et al., 2011; Gulías-González, Sánchez-López, Olivas-Bravo, Solera-Martínez, & Martínez-Vizcaíno, 2014; Santos et al., 2014; Silva, Aires, Mota, Oliveira, & Ribeiro, 2012). None of the above-mentioned studies have made comparisons between two methods. In our study, for at first time we have made comparison between the LMS method and the empirical method which showed that the results can be trusted, at least in large data analysis.

School in Greece, as in most developed countries, is the first institution providing opportunities for physical activity through the pursuit of the physical education curriculum (Cavill, Kahlmeier, & Racioppi, 2006). Given that our study was based on a yearly nationwide school-based survey programme under the auspices of the Ministry of Education, our findings may be of assistance to physical fitness educators and policy-makers when assessing schools' physical education programmes. In fact, it is an opportunity for a significant intervention focusing on elementary and secondary school children physical fitness through the relatively easy access of schools, where changes in physical fitness can be easier implemented and monitored, and affect almost all schoolchildren.

Strengths and limitations

The present study has several strengths. The sample is representative of the national gender and geographical representation, as we studied almost 40%of the total population of 6–18-year-old children in Greece. The study was performed in children aged 6–18 years. This age range is an advantageous period in the life of children and adolescents at which to apply effective prevention strategies to improve physical fitness levels. Finally, the presented data were derived using the same standardized procedures in all schools.

There are also limitations in our study design. Although a common, validated protocol was used to evaluate fitness tests in all schools, a large number of experienced, professional physical educators participated as evaluators in the study. In order to miniamong the the variability different mize experimenters, all educators were instructed through a detailed and extended manual of operations and followed a standardized procedure of measurements. Even so, some variability in measurement will still exist. Moreover, the 20 m SRT is an objective test of aerobic fitness which indirectly infers peak VO₂ and does not directly measure aerobic fitness. In order to fully understand the development of VO_{2max} during adolescence, longitudinal studies are required to take into consideration the tempo and timing of growth and maturation, particularly in girls (Eisenmann, Laurson, & Welk, 2011). Finally, the cross-sectional design of our study cannot provide causal relationships, but only provides hypotheses for further research.

Conclusions

In conclusion, we established sex- and age-specific normative physical fitness values for children aged 6–18 years living in Greece. Boys performed better in all measurements except flexibility than girls of the same age and older children performed better than younger ones. These findings may help policymakers to design appropriate health-related educational and physical fitness programmes for the young, to facilitate future more detailed epidemiology research on this topic and for comparisons among the same health-related fitness scores of children from other countries similar to Greece.

Acknowledgements

The authors would like to thank the physical education professionals who organized the projects and Physical fitness normative values for 6-18-year-old Greek boys and girls 745

performed the evaluations and the students for agreeing to participate. Also, we are grateful to Mrs Sarah Toombs Smith, Ph.D., for assistance with manuscript preparation.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Graduate Program, Department of Nutrition and Dietetics of Harokopio University, the Hellenic Ministry of Education and Religions, the Hellenic Ministry of Culture and Athletics, the Secretariat General of Sports, and OPAP SA.

References

- Andersen, L. B., Wedderkopp, N., Hansen, H. S., Cooper, A. R., & Froberg, K. (2003). Biological cardiovascular risk factors cluster in Danish children and adolescents: The European youth heart study. *Preventive Medicine*, 37, 363–367. doi:10. 1016/S0091-7435(03)00145-2
- Anderssen, S. A., Cooper, A. R., Riddoch, C., Sardinha, L. B., Harro, M., Brage, S., & Andersen, L. B. (2007). Low cardiorespiratory fitness is a strong predictor for clustering of cardiovascular disease risk factors in children independent of country, age and sex. European Journal of Cardiovascular Prevention & Rehabilitation, 14, 526-531. doi:10.1097/HJR. 0b013e328011efc1
- Bustamante, A., Beunen, G., & Maia, J. (2012). Evaluation of physical fitness levels in children and adolescents: Establishing percentile charts for the central region of Peru. *Revista Peruana de Medicina Experimental y Salud Publica*, 29, 188–97.
- Caspersen, C. J., Powell, K. E., & Christenson, G. M. (1985). Physical activity, exercise, and physical fitness: Definitions and distinctions for health-related research. *Public Health Representative*, 100, 126-131.
- Catley, M. J., & Tomkinson, G. R. (2013). Normative healthrelated fitness values for children: Analysis of 85347 test results on 9-17-year-old Australians since 1985. British Journal of Sports Medicine, 47, 98-108. doi:10.1136/bjsports-2011-090218
- Cavill, N., Kahlmeier, S., & Racioppi, F. (2006). What can the health sector and others do to increase physical activity? In N. Cavill, S. Kahlmeier, & F. Racioppi (Eds.), *Physical activity and health in Europe: Evidence for action* (pp. 15–23). Copenhagen: World Health Organization Regional Office for Europe.
- Christodoulos, A. D., Douda, H. T., & Tokmakidis, S. P. (2012). Cardiorespiratory fitness, metabolic risk, and inflammation in children. *International Journal of Pediatrics*, 12, Article no. 270515. doi:10.1155/2012/270515
- Cole, T. J. (1990) The LMS method for constructing normalized growth standards. *European Journal of Clinical Nutrition*, 44, 45–60. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/ 2354692
- Cole, T. J., & Green, P. J. (1992). Smoothing reference centile curves: The LMS method and penalized likelihood. *Statistics*

in Medicine, 11, 1305–1319. Retrieved from http://www.ncbi. nlm.nih.gov/pubmed/1518992

- Eisenmann, J. C., Laurson, K. R., & Welk, G. J. (2011). Aerobic fitness percentiles for U.S. adolescents. American Journal of Preventive Medicine, 41, S106–S110. doi:10.1016/j.amepre. 2011.07.005
- Eisenmann, J. C., Wickel, E. E., Welk, G. J., & Blair, S. N. (2005). Relationship between adolescent fitness and fatness and cardiovascular disease risk factors in adulthood: The aerobics center longitudinal study (ACLS). American Heart Journal, 149, 46– 53. doi:10.1016/j.abj.2004.07.016
- Ekelund, U., Anderssen, S. A., Froberg, K., Sardinha, L. B., Andersen, L. B., Brage, S., & European Youth Heart Study Group. (2007). Independent associations of physical activity and cardiorespiratory fitness with metabolic risk factors in children: The European youth heart study. Diabetologia, 50, 1832– 1840. doi:10.1016/S0140-6736(06)69075-2
- Flouris, A. D., Bouziotas, C., Christodoulos, A. D., & Koutedakis, Y. (2008). Longitudinal preventive-screening cutoffs for metabolic syndrome in adolescents. *International Journal of Obesity*, 32, 1506–1512. doi:10.1038/ijo.2008.142
- Gulías-González, R., Sánchez-López, M., Olivas-Bravo, Á., Solera-Martínez, M., & Martínez-Vizcaíno, V. (2014). Physical fitness in Spanish schoolchildren aged 6–12 years: Reference values of the battery EUROFIT and associated cardiovascular risk. Journal of School Health, 84, 625–635. doi:10. 1111/josh.12192
- Hurtig-Wennlof, A., Ruiz, J. R., Harro, M., & Sjöström, M. (2007). Cardiorespiratory fitness relates more strongly than physical activity to cardiovascular disease risk factors in healthy children and adolescents: The European youth heart study. European Journal of Cardiovascular Prevention & Rehabilitation, 14, 575– 581. doi:10.1097/HJR.0b013e32808c67e3
- Leger, L. A., Lambert, J., Goulet, A., Rowan, C., & Dinelle, Y. (1984). Aerobic capacity of 6 to 17-year-old Quebecois-20 meter shuttle run test with 1 minute stages. *Canadian Journal* of Applied Sport Sciences, 9, 64–9. Retrieved from http://www. ncbi.nlm.nih.gov/pubmed/6733834.
- Leger, L. A., Mercier, D., Gadoury, C., & Lambert, J. (1988). The multistage 20 metre shuttle run test for aerobic fitness. *Journal of* Sports Sciences, 6, 93–101. doi:10.1080/02640418808729800
- Looney, M. A., & Plowman, S. A. (1990). Passing rates of American children and youth on the FITNESSGRAM criterion-referenced physical fitness standards. *Research Quarterly* for Exercise and Sport, 61, 215-223. doi:10.1080/02701367. 1990.10608682
- Ortega, F. B., Artero, E. G., Ruiz, J. R., España-Romero, V., Vicente-Rodríguez, G., Molnar, D., ... Gutiérrez, A., HELENA Study Group. (2011). Physical fitness levels among European adolescents: The HELENA study. *British Journal of* Sports Medicine, 45, 20-29. doi:10.1136/bjsm.2009.062679
- Ortega, F. B., Ruiz, J. R., Castillo, M. J., & Sjöström, M. (2008). Physical fitness in childhood and adolescence: A powerful marker of health. *International Journal of Obesity*, 32(1), 1–11. doi:10.1038/sj.ijo.0803774
- Pan, H., & Cole, T. J. (2010). LMS Chartmaker, a program to construct growth references using the LMS method. Version 2.43. Retrieved from http://www.healthforallchildren.co.uk/
- Pan, H., & Cole, T. J. (2011). LMS growth, a Microsoft Excel addin to access growth references based on the LMS method. Version 2.71. Retrieved from http://www.healthforallchildren. co.uk/
- Santos, R., Mota, J., Santos, D. A., Silva, A. M., Baptista, F., & Sardinha, L. B. (2014). Physical fitness percentiles for Portuguese children and adolescents aged 10–18 years. *Journal of Sports Sciences*, 13, 1–3. doi:10.1080/02640414. 2014.906046

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- Sauka, M., Priedite, I. S., Artjuhova, L., Larins, V., Selga, G., Dahlström, O., & Timpka, T. (2011). Physical fitness in northern European youth: Reference values from the Latvian physical health in youth study. *Scandinavian Journal of Public Health*, 39, 35-43. doi:10.1177/1403494810380298
- Silva, G., Aires, L., Mota, J., Oliveira, J., & Ribeiro, J. C. (2012). Normative and criterion-related standards for shuttle run performance in youth. Pediatric *Exercise Science*, 24, 157-69.
- Tambalis, K. D., Panagiotakos, D. B., Arnaoutis, G., & Sidossis, L. A. (2013). Endurance, explosive power, and muscle strength in relation to body mass index and physical fitness in Greek children aged 7–10 years. Pediatric *Exercise Science*, 25, 394–406. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23877385
- Tambalis, K. D., Panagiotakos, D. B., Psarra, G., & Sidossis, L. A. (2011). Inverse but independent trends in obesity and fitness levels among Greek children: A time-series analysis from 1997 to 2007. Obesity Facts, 4, 165–174. doi:10.1159/000327994
- Tokmakidis, S. P., Kasambalis, A., & Christodoulos, A. D. (2006). Fitness levels of Greek primary schoolchildren in relationship to overweight and obesity. *European Journal of Pediatrics*, 165, 867– 874. doi:10.1007/s00431-006-0176-2
- Wang, Y., Moreno, L. A., Caballero, B., & Cole, T. J. (2006). Limitations of the current world health organization growth references for children and adolescents. *Food and Nutrition Bulletin*, 27, S175–S188. Retrieved from http://www.ncbi.nlm. nih.gov/pubmed/17361655

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EXHIBIT 18



Thousands of Complaints Filed After Trans YouTuber Allowed To Play On Women's Football League, Reportedly Injured Players



An Australian amateur football league is receiving backlash from parents and players who are voicing frustrations after learning that a man who identifies as a woman has been dominating the female soccer league and injuring female players.

Football New South Wales' League One reportedly has five trans-identified male players, but one player in particular has led the 1st Grade goal kickers table, with seven goals.

Initial reports from *The Daily Mail Australia* had chosen not to identify the player, censoring images of him and declining to provide his name. However, *Reduxx* can confirm the player is trans activist YouTuber Riley J. Dennis.



A censored photo included in The Daily Mail Australia article [L] versus one which can be found through Riley Dennis' Instagram account [R].

It has been alleged that Dennis, 30, who reportedly plays for Inter Lions FC, injured women from an opposing side in a match last weekend. Reports that a female player had to seek hospital attention as a result of her injury are as of yet unconfirmed.

Football NSW appears to have scrubbed Dennis from their website, replacing his name with "Inter Player," due to the backlash they have received, but screenshots from the past iterations of the site exist, showing where Dennis was clearly anonymized.

earch:		
Member Name	♥ Team	
Riley Dennis	Inter Lions FC	7
Elin Rosenberg Cemazar	SD Raiders FC	4
Samantha Cefai	Camden Tigers FC	4
	Nepean FC	3
ISW League One Womens - 1st Grade	Nepean FC	3
ISW League One Womens - 1st Grade Search:	Nepean FC • Team	3 ¢ Goals
ISW League One Womens - 1st Grade iearch: Member Name		
Atorete Connolly ISW League One Womens - 1st Grade ieerch: Member Name Inter Player Elin Rosenberg Cemazar	e Team	e Goals

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Dennis' name visible in a past archive of the Football NSW website [top], compared to how it currently displays [bottom].

On Facebook, some football fans have taken to their platforms to support the female athletes competing against Dennis, condemning Inter Lions FC for allowing Dennis to play amongst women.

"Biological adult males and it's not just one, it is several, are playing in NSW women's state league competitions, which contain teenage girls and young women. Shame on those clubs, who have discarded any morals they might have had, just to sign these things, so as to be able to win, where's the dignity? You put winning above respect for the competition," the NSW NPL Banter Page wrote in a post today.

Women's rights groups are similarly condemning Football NSW for placing female players in harm's way.

Kirralie Smith, a spokeswomen from Binary Australia, a organization set up to combat gender ideology, condemned the football league in a statement to Daily Mail.

"Football NSW has failed to answer the simple questions, 'What is a woman?' and 'Why have a woman's division if men can play in it.' They have failed in safeguarding fairness and safety for girls and women."

Binary Australia are said to have organized a complaint-writing campaign to Football NSW, which reportedly received over 12,000 submissions.

Riley Dennis was a popular trans activist YouTuber who has 113,000 subscribers. He has since stopped creating content but, during the height of his internet career, he received backlash for a controversial video in which he called "genital preferences" transphobic.

The video, titled Your Dating 'Preferences' Might Be Discriminatory and released in 2017, prompted outrage from lesbians who felt Dennis was attempting to guilt them into having sexual relationships with males.

In the video, Dennis, who identifies as a "transgender lesbian," implied that sexual orientation is caused by societal prejudices and that it is discriminatory to exclude members of the opposite sex from your dating preferences if you are homosexual. Dennis deleted the video following widespread criticism.

Addressing the backlash Dennis said: "These accusations of homophobia make it sound like I'm trying to convince lesbians to like men, but I'm not. I'm trying to show that preferences for women with vaginas over women with penises might be partially informed by the influence of a cissexist society."

Dennis was also a writer for the self-described "feminist" publication *Everyday Feminism* where he has published pieces such as "Here's Why Misgendering Trans People Is an Act of Violence" and "Some Children Are Transgender – 5 Explanations for Why That's Perfectly Okay."

After his controversial 2017 video, a petition was launched calling for him to be removed as a writer from *Everyday Feminism*. The petition gained 1,389 signatures.

"We, the 'exclusionary' and 'discriminatory' homosexuals and heterosexuals, want to see homophobic and rapist rhetoric such as this removed from the *Everyday Feminism* platform," the petition announced. "We want people like Riley to consider that our preferences (read: sexualities) are innate and not up for challenge. We want Riley J. Dennis dropped from the *Everyday Feminism* team."

Dennis stopped creating content shortly after and relocated to Australia from the United States to live with his Australian fiancée, Fiona also known as neonfiona.

Women's athletic competitions and fairness in sport have become a major issue in the debate on gender ideology and its impact on women.

The issue mounted to public attention after a trans-identified male swimmer, Lia Thomas, began breaking women's records and winning medals intended for female athletes in 2021. Since then, there have been several instances of trans-identified male athletes taking the podium in women's sporting events.

Most recently, *Reduxx* reported on the case of a 50-year-old trans-identified male runner who seized his eighth championship title in a women's category after smashing the competition at the Italian Indoor Masters Championship in Ancona on March 12. Valentina Petrillo had broken multiple women's running records in Italy, but had failed to earn even a single title when competing as a male.

Despite a global trend of males dominating women's sport, there has been ample pushback from athletes, activists, and coaches concerned with fairness.

On February 28, a young women's basketball team made international headlines after withdrawing from their state tournament in protest of a trans-identified male being on the opposing team. The Mid Vermont Christian School Eagles (MVCS) forfeited their playoff game against the Long Trail School Mountain Lions (LTS) after learning that LTS star player Rose Johnson is male, and have since been banned from participating in state competitions.

Earlier this week, a male powerlifting coach's protest against "trans inclusive" powerlifting policies in Canada went viral after he entered a women's competition and smashed the women's benchpress record.

Avi Silverberg, a powerlifting coach who has worked with Team Canada, self-identified as a woman last week to participate in the women's category at the Heroes Classic Powerlifting Meet held in Lethbridge, Alberta. Silverberg was attempting to highlight the unfair advantage males have when competing in women's athletics. While participating in the Saturday event, Silverberg unofficially broke the Alberta women's bench press record for the 84+ kilograms category.

Silverberg performed the defiant act while the current record holder, a trans-identified male, was in attendance.

As a response to the backlash against Dennis participating in the women's leagues, Football NSW have released a statement on their transgender inclusion policy, writing on their website that they would expedite the development of transgender sports teams but would also introduce LGBTQI training.

"To support the inclusion of transgender and gender-diverse people in Football, Football Australia has initiated the development process for a High Performance Inclusion Policy, specifically balancing the needs and inclusion of transgender and gender-diverse people in the sport, whilst ensuring we maintain the integrity of elite competitive football."

Reduxx is your source of pro-woman, pro-child safeguarding news and commentary. We're 100% independent! Support our mission by joining our Patreon, or consider making a one-time donation.

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EXHIBIT 19



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Front Endocrinol (Lausanne), 2018; 9: 410. Published online 2018 Jul 23. doi: <u>10.3389/fendo.2018.00410</u> PMCID: PMC6070773 PMID: <u>30093882</u>

Up-To-Date Review About Minipuberty and Overview on Hypothalamic-Pituitary-Gonadal Axis Activation in Fetal and Neonatal Life

Lucia Lanciotti, Marta Cofini, Alberto Leonardi, Laura Penta, and Susanna Esposito

Abstract

Minipuberty consists of activation of the hypothalamic-pituitary-gonadal (HPG) axis during the neonatal period, resulting in high gonadotropin and sex steroid levels, and occurs mainly in the first 3–6 months of life in both sexes. The rise in the levels of these hormones allows for the maturation of the sexual organs. In boys, the peak testosterone level is associated with penile and testicular growth and the proliferation of gonadic cells. In girls, the oestradiol levels stimulate breast tissue, but exhibit considerable fluctuations that probably reflect the cycles of maturation and atrophy of the ovarian follicles. Minipuberty allows for the development of the genital organs and creates the basis for future fertility, but further studies are necessary to understand its exact role, especially in girls. Nevertheless, no scientific study has yet elucidated how the HPG axis turns itself off and remains dormant until puberty. Additional future studies may identify clinical implications of minipuberty in selected cohorts of patients, such as premature and small for gestational age infants. Finally, minipuberty provides a fundamental 6-month window of the possibility of making early diagnoses in patients with suspected sexual reproductive disorders to enable the prompt initiation of treatment rather than delaying treatment until pubertal failure.

Keywords: gonadotropin, hypothalamic-pituitary-gonadal, minipuberty, oestradiol, testosterone

Puberty is the period of life in which a child develops secondary sexual characteristics and reproductive function. Puberty requires activation of the hypothalamic-pituitary-gonadal (HPG) axis, resulting in secretion of hypothalamic gonadotropin-releasing hormone (GnRH), which in turn stimulates secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the pitu-

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itary gland and the consequent maturation of gametogenesis as well as secretion of gonadal hormones. Before the onset of puberty, the HPG axis is temporary activated in two other periods of life, i.e., in the midgestational fetus and in the newborn. In recent years, many studies in the literature have referred to this latter period as minipuberty.

Minipuberty was first described in the 1970s (1, 2), but its role is still not well understood. The aim of this review is to analyse the impact and the clinical role of minipuberty. PubMed was used to search for all relevant studies published over the last 25 years using the key words "minipuberty," "mini-puberty," "HPG axis," "gonadotropins," and "sexual hormones," combined with "fetal life," "newborn," "preterm," "small for gestational age," "growth," "congenital hypogonadotropic hypogonadism," "Turner syndrome," "Klinefelter syndrome" and "CAIS". Additional sources were found from the references of the publications that were obtained from the search. The data obtained from studies published between 1973 and 2017 are included in this review, and the most recent research is dated March 2017.

Hypothalamic-pituitary-gonadal (HPG) axis activation in fetal life

During embryogenesis, neurons that produce GnRH develop from the epithelium of the medial olfactory pit and move to the fetal hypothalamus by migrating along nerve fibers (3). This process occurs at ~40 days of gestation (4). Simultaneously, the pituitary gland develops and begins synthesizing both LH and FSH at 9 weeks of gestation (WG) (5), although the hormones appear in the fetal blood by 12–14 WG (6). Kisspeptin and KISS1R are factors that are involved in the regulation of fetal GnRH neuron activity. However, serum LH and FSH levels are independent of GnRH and kisspeptin at midgestation, but they become GnRH-induced after 30-31 WG (7).

The gonadotropin levels peak at midgestation in both the pituitary gland and the serum and subsequently decrease toward birth and are suppressed at term $(\underline{8}, \underline{9})$. This pattern is probably caused by the gradual increase in the production of placental estrogens toward the end of gestation (10) that suppresses the activity of the fetal HPG axis.

Additionally, female fetuses produce higher LH and FSH levels than male fetuses (6, 11). Indeed, Debieve et al. $(\underline{12})$ measured LH and FSH at midpregnancy (the group had median ages of 23.8 WG for the females and 22.6 WG for the males) and at term (median ages: 39.2 WG for the females and 38.9 WG for the males). Both gonadotropins were present in the first group and exhibited a clear difference between the females and males; the girls exhibited much higher levels (33.0 ± 23.2 vs. 4.4 ± 3.3 mIU/mL for LH and 54.4 ± 27.7 vs. 0.77 ± 0.49 mIU/mL for FSH). In contrast, in the term female fetuses, both LH and FSH were undetectable, and only very low FSH levels were observed in the term male fetuses. The midpregnancy gonadotropin peak coincides with the first ovarian follicle or seminiferous tubule maturation. The difference between genders is probably caused by the negative feedback that results from the higher concentrations of fetal testicular hormones (6, 13, 14). Another marked difference between the sexes is that the LH levels overcome the FSH levels in male fetuses (15), whereas the opposite situation occurs in females.

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During fetal life, the masculinization of genitalia depends on the production of testosterone (T) by the Leydig cells of the fetal testicles and on its action on target organs. During the first trimester of gestation, placental human chorio-gonadotropin (hCG) induces the differentiation of testicular mesenchymal cells into Leydig cells and stimulates T production through the activation of the LH/CG receptors expressed on their surfaces (16). Indeed, mutations of the LH/CG receptor can cause the absence of virilization and feminization of the external genitalia (17). Thus, the fetal testicular T is secreted first under the control of placental hCG, and only after the 9th WG, T secretion comes under the control of pituitary LH. A clear increase in T concentration occurs between 8 and 11 WG and reaches a maximum between 11 and 14 WG. The peak level (40–580 ng/dL) is similar to the adult value (14), whereas T levels in the fetal testes can reach \sim 1.9–2.1 ng/mg of tissue (16). After the 20th WG, T decreases toward term (8, 14). The fetal testicles also express FSH receptors that probably control Sertoli cell proliferation, although only few studies exist regarding the effects of FSH (<u>18–20</u>). Anti-mullerian hormone (AMH) that is produced by the Sertoli cells in the fetal testes causes the regression of the mullerian ducts, which prevents the formation of internal feminine genitalia. T favors the development of male urogenital structures from the wolffian duct, such as the vas deferens, epididymis, and seminal vesicles, while the formation of the prostate, penis and scrotum is due to the active metabolite of T (dihydrotestosterone).

In female fetuses, the lack of AMH allows the mullerian ducts to develop into the fallopian tubes, uterus and upper part of the vagina (21). The development of the primordial follicle in the fetal ovaries begins before 13 WG, but the follicles are more rapidly created after 14–15 WG. The pool of primordial follicles is ~100,000 at 15 WG and then rapidly increases to reach a higher number at 34 WG (680,000). Subsequently, the pool remains stable, at least until 8 months after birth (22). This pool, which represents the foundation of female fertility, is formed when estrogen levels are high in the fetal circulation. However, the majority of estrogen production during fetal life is due to the placenta, and ovarian production can be considered irrelevant. Additionally, the roles of FSH and LH in ovarian development during pregnancy are not completely understood. It seems that normal development occurs until the 34th WG even in anencephalic fetuses. In contrast, during the last part of gestation, a marked difference can be found; in anencephalic fetuses small and growing antral follicles cannot be observed as they can in normal fetuses (23), which suggests that hypothalamic stimulation is necessary to ensure physiological ovarian development after the 7th month of gestation.

Minipuberty and its implications in healthy infants

At delivery, in healthy infants the LH and FSH levels are low in the cord blood in both sexes (24) due to the inhibitory effect of the high levels of placental estrogens. In boys, the LH level increases by ~10-fold in the few minutes after delivery, and this increase is followed by a concomitant rise in the T levels that lasts for ~12 h (25, 26). In girls, this increase does not occur.

In the first few days after birth, the fall in circulating placentally produced steroids causes a progressive lack of negative feedback on the neonatal GnRH pulse generator. In this manner, the activity of the HPG axis is reinitiated, and gonadotropin levels begin rising between days 6 and 10 after birth (<u>27</u>, <u>28</u>). In infant boys, the serum LH level reaches the pubertal range by 1 week of age, the peak is detected between the 2nd and 10th weeks of life, and the level then decreases to the pre-

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pubertal range by 4–6 months (27-30) (Figure 1). Female infants have lower peak LH values, but the pattern is similar (Figure 2) (27-29). In contrast, the FSH levels are higher in females than in males and peaking between 1 week and 3 months. Subsequently, in males, the FSH values gradually decrease to the prepubertal range within 4 months of age, whereas in females, these values remain elevated until to 3–4 years of age (27, 29, 31).

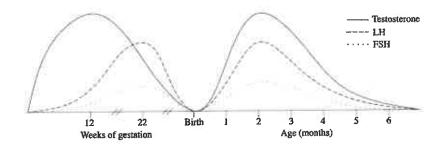


Figure 1

Patterns of fetal and postnatal luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (T) secretion in males.

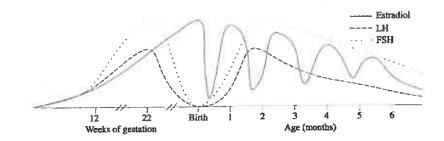


Figure 2

Patterns of fetal and postnatal luteinizing hormone (LH), follicle stimulating hormone (FSH) and oestradiol secretion in females.

Table 1 summarizes sex differences and the patterns of basal LH/FSH in the first month of life. In male neonates, the pattern of T secretion is similar to that of LH secretion: T is low in the cord blood, gradually increases to peak at 1–3 months and then declines to prepubertal values by 6–9 months of age (2, 27, 28, 30, 32). Similarly, the number of Leydig cells in the testicular tissue increases considerably until the third month and then gradually decreases due to apoptosis of the fetal Leydig cells (<u>33–36</u>). Sertoli cells also develop in the first months after birth under the stimulation of FSH (<u>37</u>), but they do not express androgen receptors during infancy, and therefore spermatogenesis does not occur (<u>38</u>). These cells secrete AMH in male neonates: the highest levels are observed at 3 months, and the levels subsequently decline and remain at relatively stable levels throughout childhood until puberty at which point AMH progressively decline to the adult

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level of 3–4% of the infant level (39). The absence of androgen receptors explains why AMH levels remain elevated in the presence of the high T levels during early infancy (38, 40). Additionally, in prepubertal boys, Sertoli cells secrete inhibin B, which represents a marker of Sertoli cell function. This hormone, which is already present in the cord blood, increases soon after birth, peaks at 3–6 months of age, reaches greater values then those observed in adults (mean +/- SD: 378 +/- 23 pg/mL), and remains elevated until at least the age of 15 months (28, 32, 41).

Table 1⁴

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) values (means ± SD) in neonates.

Age (days)	LH in males	LH in females	FSH in males	FSH in females
1-5	0.39 ± 0.48	0.48 ± 0.66	0.96 ± 0.60	2.00 ± 1.37
6-10	2.31 ± 2.29	0.45 ± 0.33	2.91 ± 4.38	2.44 ± 2.52
11-15	3.55 ± 2.84	1.58 ± 1,28	3.71 ± 2.69	8,16 ± 4,27
16-20	4.13 ± 2.76	1.03 ± 1.39	2.63 ± 1.45	1.62 ± 1.05
21-25	2.86 ± 1.51	0.46 ± 0.25	2.50 ± 1.51	7.07 ± 5.92
26-28	2.22 ± 2.37	2.75 ± 2.39	2.25 ± 0.81	9.74 ± 9.89

Values of LH and FSH are in mIU/mL.

Adapted from Schmidt and Schwarz HP (27).

Minipuberty has been associated with physiological gonadal development processes, such as penile and testicular growth and the proliferation of gonadic cells. Cortes et al. (42) found that penile length is positively correlated with serum T and increases from birth (mean +/– SD, 3.49 +/– 0.4 cm) to 3 years of age with the highest growth velocity occurring from birth to 3 months (1 mm/month). In contrast, testicular volume increases significantly in the first 5–6 months of life, from 0.27 to 0.44 cm³, and the volume subsequently decreases to 0.31 cm³ at ~9 months (43). This pattern is positively correlated with FSH levels (30), which is probably due to the proliferation of Sertoli cells in the seminiferous tubules (37). Additionally, Hadziselimovic et al. proposed that germ cells can differentiate into adult (Ad) spermatogonia due to the transient activation of the HPG axis during the first months of life (44).

Thus, the first months of life are fundamental for the development of the male reproductive organs. In contrast, it is not completely clear whether these months are also important for the reproductive functions of girls. At birth, oestradiol levels are high in the cord blood of both sexes. Umbilical cord estrogen concentrations depend on gestational age, the mode of delivery, pregnancy complications, and twinning, but not on infant sex (45). In a study conducted by Troisi et al. (46), the mean oestradiol values measured in cord blood were found to be 11,941 pg/ml in females and 12,782 pg/ml in males, and the difference between gender was not significant. During

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the first postnatal days, oestradiol levels gradually decrease, but after 1 week of age, they increase in girls only and remain high in the subsequent period (47-49) until at least the 6th month of life. Similar observations were made by Bidlingmaier et al. (50), who found the higher oestradiol concentration in the ovaries of 1- to 6-month-old girls compared with those at the end of the first year in postmortem samples. At 3 months of age, the median serum oestradiol level in girls is 30.0 pmol/L (range < 18–100) (47). However, individual oestradiol levels in girls exhibit considerable fluctuation in the first months of life, which may reflect the cycles of maturation and atrophy of the ovarian follicles. Indeed Kuiri-Hanninen et al. (31) reported increased numbers of antral follicles on ovarian ultrasonography in infant girls, and this fact corresponds to parallel elevations of oestradiol (48, 49) and AMH levels (31, 51). Indeed, in infant girls, there is a marked rise of AMH levels at 3 months of age (15 pmol/L; 4.5–29.5 pmol/L) compared with the levels found in cord blood (2 pmol/L; 2–15.5 pmol/L) and at 1 year of age (51). This fact may demonstrate the postnatal proliferation of granulosa cells, which produce AMH, and the contemporary development of the ovarian follicles, which probably occurs in response to the parallel FSH surge (31, 51).

The mammary glands and the uterus are also certainly estrogen target tissues in the fetus and in newborn. At birth, most full term babies of both sexes have palpable breast tissue (52) that probably results from in-uterus stimulation from placental estrogens. However, in the following months, the breast tissue in females remains larger and persists longer (52). In boys, the mammary gland diameter gradually decreases until the 6th month, whereas in full-term girls, it remains large, reflecting the activity of endogenous estrogens (48). In contrast, the uterine length increases primarily during pregnancy due to the hormones that cross the placental barrier, and after birth, it is longest at day 7 in full-term babies and then steadily decreases toward the third month and remains fairly unchanged until the second year (48). Therefore, the role of minipuberty in girls is still controversial and partially unknown.

Minipuberty in premature infants and in those small for gestational age (SGA)

Postnatal HPG axis activation also occurs in premature infants and is even stronger and more prolonged in time than in full-term infants (53, 54). Kuiri-Hanninen et al. (30) recently compared fullterm (FT) and preterm (PT) males by measuring urinary gonadotropins and T in serial urine samples and comparing the results with testicular and penile growth. The trends of LH and T secretion are similar among the groups, but the levels are significantly higher in PT than in FT males when measured at 7 and 30 days of age. These levels then decline in both groups, but a significant difference can still be observed at month 6. Additionally, a positive association between the level of HPG axis activation and the grade of prematurity has been found, and PT males have been associated with faster penile and testicular growth after birth, which suggests that HPG axis activation plays a role in completing genital development.

In PT females, the FSH and LH values are higher than those in FT girls (55) and exhibit a more elevated and more prolonged peak; these patterns might reflect the expression of the immaturity of a negative feedback system in the HPG axis of PT girls. The greater levels of FSH in PT females are probably due to a delay in ovarian folliculogenesis; the ovaries are still immature and do not seem to be able to produce sufficient estrogens that may inhibit gonadotropin secretion. Additionally, the FSH peak is followed by transient ovarian stimulation (which reaches a maximum at ~4 weeks

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of age) that results in the presence of antral follicles on ultrasonography and increases in the levels of granulosa cell-derived AMH and oestradiol (31), which are higher in the serum of PT than FT girls (49). Estrogen receptor alpha is expressed in the fetal mammary glands from ~30 weeks of gestation (56), which might explain the absence of breast development in PT infants. However, in these girls, there is a stronger association between the postnatal oestradiol surge and the growth of the mammary gland diameter and uterine length (48).

A possible clinical consequence of this intensive stimulation on the genital organs that occurs in premature infants is ovarian hyperstimulation syndrome. First described in 1985 by Sedin et al. (57) in 4 very preterm neonates, ovarian hyperstimulation syndrome is characterized by oedema of the vulva, solitary or multiple cysts in the ovaries on ultrasonography, breast growth, occasional vaginal bleeding and high serum gonadotropin and oestradiol levels. This syndrome is probably the extreme consequence of the immaturity of the negative feedback mechanisms that act on the HPG axis, and this absence of feedback results in hyperstimulation of the target organs. Several cases have been reported in the literature (58-62), and all cases indicate that it is a self-limiting disease that does not require treatment if there are no complications, but follow-up until clinical resolution is necessary.

Infants born small for gestational age (SGA) are at risk of developing metabolic and endocrinological disorders (<u>63</u>). It is well known that SGA children are at greater risk of type 2 diabetes and cardiovascular diseases, expecially those with high catch up growth. In fact, the fetus in nutritional deficiency constantly replans his metabolism to slow growth with relative resistance to insulin, IGF-1 and GH, that persists in childhood and adult life too (<u>64</u>). Besides, lower insulin sensitivity has been also associated to an increased incidence of adrenal and ovarian hyperandrogenism, clinically evident as precocious pubarche and reduced ovulation rate (<u>65</u>).

In females, being SGA has been associated with reduced uterine and ovary size (<u>66</u>), whereas, in males, SGA has been linked to infertility and reduced testicular volume and T concentrations in adult life (<u>67</u>). Minipuberty in SGA infants is still not well defined, and the data reported in the literature are controversial. Recently, Nagai et al. (<u>68</u>) conducted a longitudinal study and reported lower FSH and T, as well as higher LH concentrations, in SGA infants compared with appropriate for gestational age (AGA) infants. In contrast, in previous studies, elevated serum FSH concentrations have been detected in SGA infant girls and boys (<u>69</u>); similarly, higher T levels have been found in SGA than in AGA boys (<u>70</u>). Further studies are necessary to definitely clarify the patterns of minipuberty in SGA infants and their clinical implications.

Minipuberty and growth

Growth is influenced by different hormones depending on the period of life. In fact, in the first years, thyroid hormones play the main role, together with insulin and glucocorticoids. By contrast, GH becomes predominant during infancy until the period of puberty, when the rise of levels of sexual hormones results in the growth spurt, which is essential for final growth and bone maturation.

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Recently, new studies have described an association between minipuberty and growth, particularly in males. Indeed, sex steroids and gonadotropic hormones in the first 5 months of life seem to influence somatic development in boys during the following 6 years. Becker et al. (71) conducted a prospective study of 35 healthy infants (17 males) and reported that the surge in T during the first months of life has an influence on human somatic and adipose tissue development in childhood. Indeed, boys exhibit faster increases in weight and BMI at the age of 8 weeks than do girls. At this age, the median T level has been found to be 7.37 nmol/L, which corresponds to pubertal male values. Subsequently, other trials have been conducted with greater numbers of participants. Kiviranta et al. (72) studied 84 healthy neonates (45 of which were males). The linear growth velocity was significantly faster from birth to 6 months of age in boys than in girls, and the greatest growth velocity difference, i.e., 4.1 cm per year, was observed at 1 month of age, which is simultaneous with the peak of the postnatal gonadal activation, especially in terms of T level.

Minipuberty and hypogonadism

In the last 20 years, studies have been conducted to establish an association between minipuberty and hypogonadotropic hypogonadism (HH), especially in males, and have found that the disease is characterized by the absence of the postnatal FSH, LH, and T surge. For this reason, minipuberty provides a short-time window of opportunity to make an early diagnosis (41). At birth, HH can be revealed by a micropenis with or without associated cryptorchidism, and male neonates exhibiting these "red flags" should undergo a single serum sample examination to identify congenital gonadotropin deficiency (73, 74). A novel study conduced in a large cohort of HH patients, in fact, confirmed the importance of identifying male genital tract anomalies in prepubertal age, in particular showing that micropenis or cryptorchidism are significantly more represented in HH resulting from Kallman syndrome (75). The best period for the measurement of the serum concentrations of reproductive hormone is between 4 and 8 weeks of life, but it can be practically performed until 6 months of age (73). Besides, a recent study suggested that testicular position increases from birth to 3 months of age and decreases thereafter, overlapping with the period of minipuberty. Therefore, testicular distance to pubic bone may be a useful biomarker of postnatal testicular function, both for Leyding and Sertoli cell activity (76).

Hadziselimovic et al. identified impaired minipuberty as the main reason for cryptorchidism-induced infertility (77). Indeed, the lack of secretion of LH and T in the perinatal period prevents the differentiation of germ cells, which results in infertility after puberty (78).

Furthermore, the importance of minipuberty has been highlighted by evidence that orchiopexy alone does not necessary improve fertility in cryptorchid males, whereas in 6-month-olds, long targeted therapy with LH-Rh analogs following successful surgery results in the normalization of the sperm parameters in adult life (79). Table 2 presents case reports and descriptions of small trials of patients with HH who were treated during the first year of life with recombinant human LH and FSH or with T in attempts to imitate physiological minipuberty. In all cases, the effects were beneficial. The substitutive therapy with T resulted in a marked increase in T level and penile length, whereas recombinant gonadotropin administration caused increase not only in T level, but also in LH, FSH, as well as inhibin B and AMH. This hormonal pattern results both in the increase of penile length and testicular volume and in fertility potential later in life. The administration of

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gonadotropins is safe, well tolerated and effective. Finally, new evidences suggest also the possibility of treatment with a gonadotropin-releasing hormone agonist, which induces gonocytes to differentiate into Ad spermatogonia and rescues fertility (84). As regards hypergonadotropic hypogonadism, one of the most frequent causes is Turner syndrome (TS). In these patients, perinatal FSH secretion is similar to that in healthy girls (85), however, during infancy, the pattern of FSH secretion is strictly related to karyotype. Young girls with monosomy TS exhibit a persistent elevation of FSH up to 6 years, whereas those with 45,X/46,XX mosaicism have only minimally elevated FSH values, which suggests the presence of feedback effects on the HPG axis due to retained ovarian function (86, 87).

Table 2

References	Year	N.	Hormonal	Clinical and hormonal outcome	
		cases	therapy		
Main et al. (<u>80</u>)	2000	3	Т	↑ T levels and penis length	
Main et al. (<u>81</u>)	2002	1	rLH and rFSH	↑ inhibin B levels; ↑ testicular volume and penis length	
Bougnères et al. (82)	2008	2	rLH and rFSH	↑ T, inhibin B and AMH levels; ↑ testicular volume and penis length	
Stoupa et al. (<u>83</u>)	2017	6	rLH and rFSH	↑ T, inhibin B and AMH levels; ↑ penis length	

Replacement therapy for hypogonadotropic hypogonadism in the first year of life.

AMH, anti-mullerian hormone; rFSH, recombinant stimulating hormone; rLH, recombinant luteinizing hormone; T, testosterone.

Contrasting data can be found in the literature about males with Klinefelter syndrome (KS). Lahlou et al. (88) found that, in their cohort of KS patients 0-3 years old, the FSH, LH, and inhibin B levels were similar to those in healthy controls with the exception of T, which exhibited a physiological increase during the first trimester, but always remained at a lower level thereafter when compared with controls. Similarly, Ross et al. underlined that the neonatal surge in T is attenuated in the KS population (89). In contrast, Aksglaede et al. (90) found elevated LH levels and high-normal serum T levels in KS infants, as well as evidence of subtle Sertoli cell dysfunction with low-normal inhibin B levels. This situation may predict the postpubertal resistance of Sertoli cells to FSH action in subjects with KS in adult life (91). Table 3 summarizes studies on minipuberty in KS. 5/18/23, 3:48 PM

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Table 3

Minipuberty in Klinefelter syndrome (KS).

References	Year	N. cases	N. controls	Age of population	Hormonal findings	
Lahlou et al. (<u>88</u>)	2004	18	215	0–3 years	FSH, LH, inhibin B, and AMH not different between groups; T \downarrow in KS	
Ross et al. (<u>89</u>)	2005	22	-	1–23 months	T↓in KS	
Aksglaede et al. (<u>90</u>)	2007	10	613	3 months	↑ T, LH and FSH; inhibin B not different between groups	
Cabrol et al. (<u>91</u>)	2011	68	215	2–750 days	T, LH, inhibin B and AMH not different between groups; normal or ↑ FSH levels in KS	

AMH, anti-mullerian hormone; LH, uteinizing hormone; rFSH, recombinant stimulating hormone; T, testosterone.

Finally, androgen receptors also play an important role in HPG axis activity. Indeed, infants with complete androgen insensitivity who present with a mutation in the androgen receptor do not exhibit the physiological peaks in LH and consequently T during minipuberty, whereas neonates with partial androgen insensitivity exhibit high-normal levels of postnatal T and LH (92).

Conclusions

The HPG axis is physiologically activated in the fetus during midgestation and gradually turns off toward term due to the negative feedback of placental hormones on the fetal hypothalamus. At birth, when the restriction is removed, the HPG axis reactivates, which results in a T peak in males between months 1 and 3; by contrast, the oestradiol levels in females fluctuate until 6 months of age. Minipuberty allows for the development of the genital organs and creates the basis for future fertility, but further studies are necessary to understand its exact role, especially in girls. Nevertheless, no scientific study has yet elucidated how the HPG axis turns itself off and remains dormant until puberty. Additional future studies may identify clinical implications of minipuberty in selected cohorts of patients, such as premature and small for gestational age infants. Finally, minipuberty provides a fundamental 6-month window of the possibility of making early diagnoses in patients with suspected sexual reproductive disorders to enable the prompt initiation of treatment rather than delaying treatment until pubertal failure.

Author contributions

LL drafted the manuscript. MC and AL performed the literature review. LP supervised the project. SE revised the manuscript and made substantial scientific contributions.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Footnotes

Funding. This review was partially supported by the World Association for Infectious Diseases and Immunological Disorders (grant n. WAidid2017_06). WAidid has no role in literature analysis and manuscript preparation, but only gave an unrestricted grant for covering expenses.

References

1. Forest MG, Cathiard AM. Pattern of plasma testosterone and A4 androstenedione in normal newborns: evidence for testicular activity at birth. *J Clin Endocrinol Metab*. (1975) 1:977–80. 10.1210/jcem-41-5-977 [PubMed] [CrossRef] [Google Scholar]

2. Forest MG, Cathiard AM, Bertrand JA. Evidence of testicular activity in early infncy. *J Clin Endocrinol Metab.* (1973) 1:148–51. 10.1210/jcem-37-1-148 [PubMed] [CrossRef] [Google Scholar]

3. Schwanzel-Fukada M, Pfaff D. Origin of luteinizing hormone-releasing hormone neurons. *Nature* (1989) 338:161–4. 10.1038/338161a0 [PubMed] [CrossRef] [Google Scholar]

4. Crossin KL, Pfaff DW, Bouloux PMG, Pasteur I, France JH. Migration of luteinizing hormone-releasing hormone (LHRH) neurons in early human embryos. *J Clin Invest.* (1996) 1996:547657. [<u>Google Scholar</u>]

5. Hagen C, McNeilly AS. The gonadotrophins and their subunits in foetal pituitary glands and circulation. *J Steroid Biochem*. (1977) 8:537–44. 10.1016/0022-4731(77)90259-X [PubMed] [CrossRef] [Google Scholar]

6. Clements JA, Reyes FI, Winter JSD, Faiman C. Studies on human sexual development. III. Fetal pituitary and serum, and amniotic fluid concentrations of LH, CG, and FSH. *J Clin Endocrinol Metab*. (1976) 42:9. 10.1210/jcem-42-1-9 [<u>PubMed</u>] [CrossRef] [Google Scholar]

7. Guimiot F, Chevrier L, Dreux S, Chevenne D, Caraty A, Delezoide AL, et al.. Negative fetal FSH/LH regulation in late pregnancy is associated with declined Kisspeptin/KISS1R expression in the tuberal hypothalamus. *J Clin Endocrinol Metab*. (2012) 97:2221–9. 10.1210/jc.2012-2078 [PubMed] [CrossRef] [Google Scholar]

8. Takagi S, Yoshida T, Tsubata K, Ozaki H, Fujii TK, Nomura Y, et al.. Sex differences in fetal gonadotropins and androgens. *J* Steroid Biochem. (1977) 8:609–20. 10.1016/0022-4731(77)90270-9 [PubMed] [CrossRef] [Google Scholar]

9. Massa G, de Zegher F, Vanderschueren-Lodeweyckx M. Serum levels of immunoreactive inhibin, FSH, and LH in human infants at preterm and term birth. *Biol Neonate*. (1992) 61:150–5. 10.1159/000243737 [PubMed] [CrossRef] [Google Scholar]

10. Troisi R, Potischman N, Roberts JM, Harger G, Markovic N, Cole B, et al. Hoover RN. Correlation of serum hormone concentrations in maternal and umbilical cord samples. *Cancer Epidemiol Biomarkers Prev*. (2003) 12:452–6. [PubMed] [Google Scholar]

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5/18/23, 3:48 PM Up-To-Date Review About Minipuberty and Overview on Hypothalamic-Pituitary-Gonadal Axis Activation in Fetal and Neonatal Life...

11. Kaplan SL, Grumbach MM. The ontogenesis of human foetal hormones. II. Luteinizing hormone (LH) and follicle stimulating hormone (FSH). *Acta Endocrinol.* (1976) 81:808–29. 10.1530/acta.0.0810808 [PubMed] [GrossRef] [Google Scholar]

12. Debieve F, Beerlandt S, Hubinont C, Thomas K. Gonadotropins, prolactin, inhibin A, inhibin B, and activin A in human fetal serum from midpregnancy and term pregnancy. *J Clin Endocrinol Metab*. (2000) 85:270–4. 10.1210/jcem.85.1.6249 [PubMed] [CrossRef] [Google Scholar]

13. Winter JS. Hypothalamic-pituitary function in the fetus and infant. *Clin Endocrinol Metab.* (1982) 11:41-55. 10.1016/S0300-595X(82)80037-6 [PubMed] [CrossRef] [Google Scholar]

14. Reyes FI, Borodits RS, Winter JSD, Faiman C. Studies on human sexual development .2. Fetal and maternal serum gonadotropin and sex steroid concentrations. *J Clin Endocrinol Metab*. (1974) 38:612–7. 10.1210/jcem-38-4-612 [PubMed] [CrossRef] [Google Scholar]

15. Beck-Peccoz P, Padmanabhan V, Baggiani AM, Cortelazzi D, Buscaglia M, Medri G, et a. Maturation of hypothalamicpituitary-gonadal function in normal human fetuses: circulating levels of gonadotropins, their common a-subunit and free testosterone, and discrepancy between immunological and biological activities of circulating follicle-stimu. *J Clin Endocrinol Metab.* (1991) 73:525–32. 10.1210/jcem-73-3-525 [PubMed] [CrossRef] [Google Scholar]

16. Tapanainen J, Kellokumpu-lehtinen P, Pelliniemi L. Age-related changes in endogenous steroids of human. J Clin Endocrinol Metab. (1981) 52:98–102. 10.1210/jcem-52-1-98 [PubMed] [CrossRef] [Google Scholar]

17. Latronico AC, Anasti J, Arnhold IJ, Rapaport R, Mendonca BB, Bloise W, et al.. Testicular and ovarian resistance to luteinizing hormone caused by inactivating mutations of the luteinizing hormone. *N Engl J Med.* (1996) 334:507–12. 10.1056/NEJM199602223340805 [PubMed] [CrossRef] [Google Scholar]

18. Zhang S, Li W, Zhu C, Wang X, Li Z, Zhang J, et al.. Sertoli cell-specific expression of metastasis-associated protein 2 (MTA2) is required for transcriptional regulation of the follicle-stimulating hormone receptor (FSHR) gene during spermatogenesis. *J Biol Chem*. (2012) 287:40471–83. 10.1074/jbc.M112.383802 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

19. Plant TM, Marshall GR. The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. *Endocr Rev.* (2001) 22:764–86. 10.1210/edrv.22.6.0446 [PubMed] [CrossRef] [Google Scholar]

20. O'Shaughnessy PJ, Baker PJ, Monteiro A, Cassie S, Bhattacharya S, Fowler PA. Developmental changes in human fetal testicular cell numbers and messenger ribonucleic acid levels during the second trimester. *J Clin Endocrinol Metab*. (2007) 92:4792–801. 10.1210/jc.2007-1690 [PubMed] [CrossRef] [Google Scholar]

21. Bowles J, Koopman P. Sex determination in mammalian germ cells: extrinsic versus intrinsic factors. *Reproduction* (2010) 139:943–58. 10.1530/REP-10-0075 [PubMed] [CrossRef] [Google Scholar]

22. Forabosco A, Sforza C. Establishment of ovarian reserve: a quantitative morphometric study of the developing human ovary. *Fertil Steril*. (2007) 88:675–83., 10.1016/j.fertnstert.2006.11.191 [PubMed] [CrossRef] [Google Scholar]

23. Baker TG, Scrimgeour JB. Development of the gonad in normal and anencephalic human fetuses. *J Reprod Fertil.* (1980) 60:193–9. 10.1530/jrf.0.0600193 [PubMed] [CrossRef] [Google Scholar]

24. Varvarigou AA, Liatsis SG, Vassilakos P, Decavalas G, Beratis NG. Effect of maternal smoking on cord blood estriol, placental lactogen, chorionic gonadotropin, FSH, LH, and cortisol. *J Perinat Med*. (2009) 37:364–9. 10.1515/JPM.2009.028 [PubMed] [CrossRef] [Google Scholar]

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5/18/23, 3:48 PM Up-To-Date Review About Minipuberty and Overview on Hypothalamic-Pituitary-Gonadal Axis Activation in Fetal and Neonatal Life...

25. Corbier P, Dehennin L, Castanier M, Mebazaa A, Edwards DA, Roffi J. Sex differences in serum luteinizing hormone and testosterone in the human neonate during the first few hours after birth. *J Clin Endocrinol Metab*. (1990) 71:1344–8. 10.1210/jcem-71-5-1344 [PubMed] [CrossRef] [Google Scholar]

26. de Zegher F, Devlieger H, Veldhuis JD. Pulsatile and sexually dimorphic secretion of luteinizing hormone in the human infant on the day of birth. *Pediatr Res.* (1992) 32:605–7. 10.1203/00006450-199211000-00025 [PubMed] [CrossRef] [Google Scholar]

27. Schmidt H, Schwarz HP. Serum concentrations of LH and FSH in the healthy newborn. *Eur J Endocrinol*. (2000) 143:213– 5. 10.1530/eje.0.1430213 [PubMed] [CrossRef] [Google Scholar]

28. Bergadá I, Milani C, Bedecarrás P, Andreone L, Ropelato MG, Gottlieb S, et al.. Time course of the serum gonadotropin surge, inhibins, and anti-Müllerian hormone in normal newborn males during the first month of life. *J Clin Endocrinol Metab.* (2006) 91:4092–8. 10.1210/jc.2006-1079 [PubMed] [CrossRef] [Google Scholar]

29. Winter JSD, Faiman C, Hobson WC, Prasad AV, Reyes FI. Pituitary-gonadal relations in infancy. I. patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab*. (1975) 40:545–51. 10.1210/jcem-40-4-545 [PubMed] [CrossRef] [Google Scholar]

30. Kuiri-Hänninen T, Seuri R, Tyrväinen E, Turpeinen U, Hämäläinen E, Stenman UH, et al.. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. *J Clin Endocrinol Metab.* (2011) 96:98–105. 10.1210/jc.2010-1359 [PubMed] [CrossRef] [Google Scholar]

31. Kuiri-Hänninen T, Kallio S, Seuri R, Tyrväinen E, Liakka A, Tapanainen J, Sankilampi U, Dunkel L. Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. *J Clin Endocrinol Metab*. (2011) 96:3432–9. 10.1210/jc.2011-1502 [PubMed] [CrossRef] [Google Scholar]

32. Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, et al.. Longitudinal reproductive hormone profiles in infants: Peak of inhibin B levels in infant boys exceeds levels in adult men. *J Clin Endocrinol Metab.* (1998) 83:675–81. 10.1210/jc.83.2.675 [PubMed] [CrossRef] [Google Scholar]

33. Chemes HE. Infancy is not a quiescent period of testicular development. *Int J Androl.* (2001) 24:2-7. 10.1046/j.1365-2605.2001.00260.x [PubMed] [CrossRef] [Google Scholar]

34. Codesal J, Regadera J, Nistal M, Regadera-Sejas J, Paniagua R. Involution of human fetal Leydig cells. An immunohistochemical, ultrastructural and quantitative study. *J Anat*. (1990) 172:103–14. [PMC free article] [PubMed] [Google Scholar]

35. Lejeune H, Habert R, Saez JM. Origin, proliferation and differentiation of Leydig cells. *J Mol Endocrinol*. (1998) 20:1–25. 10.1677/jme.0.0200001 [PubMed] [CrossRef] [Google Scholar]

36. Nistal M, Paniagua R, Regadera J, Santamaria L, Amat P. A quantitative morphological study of human Leydig cells from birth to adulthood. *Cell Tissue Res.* (1986) 246:229–36. 10.1007/BF00215884 [PubMed] [CrossRef] [Google Scholar]

37. Cortes D, Muller J, Skakkebaek NE. Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. *Int J Androl.* (1987) 10:589–96. 10.1111/j.1365-2605.1987.tb00358.x [PubMed] [CrossRef] [Google Scholar]

38. Chemes HE, Rey RA, Nistal M, Regadera J, Musse M, González-Peramato P, et al., Physiological androgen insensitivity of the fetal, neonatal, and early infantile testis is explained by the ontogeny of the androgen receptor expression in sertoli cells. *J Clin Endocrinol Metab.* (2008) 93:4408–12. 10.1210/jc.2008-0915 [PubMed] [CrossRef] [Google Scholar]

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5/18/23, 3:48 PM Up-To-Date Review About Minipuberty and Overview on Hypothalamic-Pituitary-Gonadal Axis Activation in Fetal and Neonatal Life...

39. Aksglaede L, Sørensen K, Boas M, Mouritsen A, Hagen CP, Jensen RB, et al.. Changes in anti-mullerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. *J Clin Endocrinol Metab.* (2010) 95:5357–64. 10.1210/jc.2010-1207 [PubMed] [CrossRef] [Google Scholar]

40. Boukari K, Meduri G, Brailly-Tabard S, Guibourdenche J, Ciampi ML, Massin N, et al.. Lack of androgen receptor expression in sertoli cells accounts for the absence of anti-Mullerian hormone repression during early human testis development. *J Clin Endocrinol Metab*. (2009) 94:1818–25. 10.1210/jc.2008-1909 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

41. Grumbach MM. A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. *J Clin Endocrinol Metab.* (2005) 90:3122–7. 10.1210/jc.2004-2465 [PubMed] [CrossRef] [Google Scholar]

42. Boas M, Boisen KA, Virtanen HE, Kaleva M, Suomi AM, Schmidt IM, et al.. Postnatal penile length and growth rate correlate to serum testosterone levels: a longitudinal study of 1962 normal boys. *Eur J Endocrinol.* (2006) 154:125–9. 10.1530/eje.1.02066 [PubMed] [CrossRef] [Google Scholar]

43. Kuijper EA, van Kooten J, Verbeke JI, van Rooijen M, Lambalk CB. Ultrasonographically measured testicular volumes in 0- to 6-year-old boys. *Hum Reprod*. (2008) 23:792–6. 10.1093/humrep/den021 [PubMed] [CrossRef] [Google Scholar]

44. Hadziselimovic F, Hadziselimovic NO, Demougin P, Krey G, Oakeley E. Piwi-pathway alteration induces LINE-1 transposon derepression and infertility development in cryptorchidism. *Sex Dev.* (2015) 9:98–104. 10.1159/000375351 [PubMed] [CrossRef] [Google Scholar]

45. Hickey M, Hart R, Keelan JA. The relationship between umbilical cord estrogens and perinatal characteristics. *Cancer Epidemiol Biomarkers Prev.* (2014) 23:946–52. 10.1158/1055-9965.EPI-13-1321 [PubMed] [CrossRef] [Google Scholar]

46. Troisi R, Potischman N, Roberts J, Siiteri P, Daftary A, Sims C, et al., Associations of maternal and umbilical cord hormone concentrations with maternal, gestational and neonatal factors (United States). *Cancer Causes Control* (2003) 14:347–55. [PubMed] [Google Scholar]

47. Schmidt IM, Chellakooty M, Haavisto AM, Boisen KA, Damgaard IN, Steendahl U, et al.. Gender difference in breast tissue size in infancy: correlation with serum estradiol. *Pediatr Res.* (2002) 52:682–6. 10.1203/00006450-200211000-00012 [PubMed] [CrossRef] [Google Scholar]

48. Kuiri-Hänninen T, Haanpää M, Turpeinen U. Postnatal ovarian activation has effects in estrogen target tissues in infant girls. J Clin Endocrinol Metab. (2013) 98:4709–16. 10.1210/jc.2013-1677 [PubMed] [CrossRef] [Google Scholar]

49. Chellakooty M, Schmidt IM, Haavisto AM, Boisen KA, Damgaard IN, Mau C, et al.. Inhibin A, inhibin B, folliclestimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels in 473 healthy infant girls. *J Clin Endocrinol Metab*. (2003) 88:3515–20. 10.1210/jc.2002-021468 [PubMed] [CrossRef] [Google Scholar]

50. Bidlingmaier F, Strom TM, Dorr HG, Eisenmenger W, Knorr D. Estrone and estradiol concentrations in human ovaries, testes, and adrenals during the first two years of life. *J Clin Endocrinol Metab*. (1987) 65:862–7. 10.1210/jcem-65-5-862 [PubMed] [CrossRef] [Google Scholar]

51. Hagen CP, Aksglaede L, Sørensen K, Main KM, Boas M, Cleemann L, et al., Juul A. Serum levels of anti-müllerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 turner syndrome patients. *J Clin Endocrinol Metab.* (2010) 95:5003–10. 10.1210/jc.2010-0930 [PubMed] [CrossRef] [Google Scholar]

52. Jayasinghe Y, Cha R, Horn-Ommen J, O'Brien P, Simmons PS. Establishment of normative data for the amount of breast tissue present in healthy children up to two years of age. *J Pediatr Adolesc Gynecol*. (2010) 23:305–11. 10.1016/j.jpag.2010.03.002 [PubMed] [CrossRef] [Google Scholar]

Case 4:23-cv-00185-JGZ Document 92-23 Filed 06/29/23 Page 16 of 18

5/18/23, 3:48 PM Up-To-Date Review About Minipuberty and Overview on Hypothalamic-Pituitary-Gonadal Axis Activation in Fetal and Neonatal Life...

53. Tapanainen J, Koivisto M, Vihko R, Huhtaniemi I. Enhanced activity of the pituitary-gonadal axis in premature human infants. J Clin Endocrinol Metab. (1981) 52:235–8. 10.1210/jcem-52-2-235 [PubMed] [CrossRef] [Google Scholar]

54. Shinkawa O, Furuhashi N, Fukaya T, Suzuki M, Kono H, Tachibana Y. Changes of Serum Gonadotropin Levels and Sex Differences in Premature and Mature Infant during Neonatal Life. *J Clin Endocrinol Metab.* (1983) 56:1327–31. 10.1210/jcem-56-6-1327 [PubMed] [CrossRef] [Google Scholar]

55. De Jong M, Rotteveel J, Heijboer AC, Cranendonk A, Twisk JWR, Van Weissenbruch MM. Urine gonadotropin and estradiol levels in female very-low-birth-weight infants. *Early Hum Dev*. (2013) 89:131–5. 10.1016/j.earlhumdev.2012.09.007 [PubMed] [CrossRef] [Google Scholar]

56. Keeling JW, Ozer E, King G, Walker F. Oestrogen receptor alpha in female fetal, infant, and child mammary tissue. *J* Pathol. (2000) 191:449–51. 10.1002/1096-9896(2000)9999:9999<::AID-PATH661>3.0.CO;2-# [PubMed] [CrossRef] [Google Scholar]

57. Sedin G, Bergquist C, Lindgren PG. Ovarian hyperstimulation syndrome in preterm infants. *Pediatr Res.* (1985) 19:548– 52. 10.1203/00006450-198506000-00009 [<u>PubMed</u>] [<u>CrossRef</u>] [<u>Google Scholar</u>]

58. Marinkovic M, Rasmussen M, Jones K. Feminizing changes in a prematurely born infant. *Clin Pediatr (Phila)*. (2010) 49:188–9. 10.1177/0009922809337624 [PubMed] [CrossRef] [Google Scholar]

59. Starzyk J, Wojcik M, Wojtys J, Tomasik P, Mitkowska Z, Pietrzyk JJ. Ovarian hyperstimulation syndrome in newborns-a case presentation and literature review. *Horm Res.* (2009) 71:60–4. 10.1159/000173743 [PubMed] [CrossRef] [Google Scholar]

60. Altuntas N, Turkyilmaz C, Yuce O, Kulali F, Hirfanoglu IM, Onal E, et al.. Preterm ovarian hyperstimulation syndrome presented with vaginal bleeding: a case report. *J Pediatr Endocrinol Metab*. (2014) 27:355–8. 10.1515/jpem-2013-0166 [PubMed] [CrossRef] [Google Scholar]

61. Durst MA, Wicklow B, Narvey M. Atypical case of preterm ovarian hyperstimulation syndrome. *BMJ Case Rep*. (2017) 1:2017 10.1136/bcr-2016-217517 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

62. Vochem M. [Ovarian hyperstimulation syndrome in preterm infants]. *Z Geburtshilfe Neonatol*. (2002) 206:156–60. 10.1055/s-2002-33669 [PubMed] [CrossRef] [Google Scholar]

63. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science*. (2004) 305:1733–6. 10.1126/science.1095292 [PubMed] [CrossRef] [Google Scholar]

64. Mericq V, Ong KK, Bazaes R, Peña V, Avila A, Salazar T, et al.. Longitudinal changes in insulin sensitivity and secretion from birth to age three years in small- and appropriate-for-gestational-age children. *Diabetologia* (2005) 48:2609–14. 10.1007/s00125-005-0036-z [PubMed] [CrossRef] [Google Scholar]

65. Ibáñez L, de Zegher F. Puberty after prenatal growth restraint. *Horm Res.* (2006) 65(Suppl. 3):112–115. [PubMed] [Google Scholar]

66. Ibanez L, Potau N, Enriquez G, Marcos MV, de Zegher F. Hypergonadotrophinaemia with reduced uterine and ovarian size in women born small-for-gestational-age. *Hum Reprod.* (2003) 18:1565–9. 10.1093/humrep/deg351 [PubMed] [CrossRef] [Google Scholar]

67. Cicognani A, Alessandroni R, Pasini A, Pirazzoli P, Cassio A, Barbieri E, Cacciari E. Low birth weight for gestational age and subsequent male gonadal function. *J Pediatr*. (2002) 141:376. 10.1067/mpd.2002.126300 [<u>PubMed</u>] [<u>CrossRef</u>] [<u>Google Scholar</u>]

Case 4:23-cv-00185-JGZ Document 92-23 Filed 06/29/23 Page 17 of 18

5/18/23, 3:48 PM Up-To-Date Review About Minipuberty and Overview on Hypothalamic-Pituitary-Gonadal Axis Activation in Fetal and Neonatal Life...

68. Nagai S, Kawai M, Myowa-Yamakoshi M, Morimoto T, Matsukura T, Heike T. Gonadotropin levels in urine during early postnatal period in small for gestational age preterm male infants with fetal growth restriction. *J Perinatol.* (2017) 37:843–7. 10.1038/jp.2017.55 [PubMed] [CrossRef] [Google Scholar]

69. Ibáñez L, Valls C, Cols M, Ferrer A, Marcos MV, De Zegher F. Hypersecretion of FSH in infant boys and girls born small for gestational age. *J Clin Endocrinol Metab*. (2002) 87:1986–8. 10.1210/jcem.87.5.8459 [PubMed] [CrossRef] [Google Scholar]

70. Forest MG, de Peretti E, Bertrand J. Testicular and adrenal androgens and their binding to plasma proteins in the perinatal period: developmental patterns of plasma testosterone, 4-androstenedione, dehydroepiandrosterone and its sulfate in premature and small for date infants as compared wit. *J Steroid Biochem*. (1980) 12:25–36. 10.1016/0022-4731(80)90247-2 [PubMed] [CrossRef] [Google Scholar]

71. Becker M, Oehler K, Partsch CJ, Ulmen U, Schmutzler R, Cammann H, et al. Hormonal "minipuberty" influences the somatic development of boys but not of girls up to the age of 6 years. *Clin Endocrinol*. (2015) 83:694–701. 10.1111/cen.12827 [PubMed] [CrossRef] [Google Scholar]

72. Kiviranta P, Kuiri-Hänninen T, Saari A, Lamidi M-L, Dunkel L, Sankilampi U. Transient postnatal gonadal activation and growth velocity in infancy. *Pediatrics* (2016) 138:e20153561. 10.1542/peds.2015-3561 [PubMed] [CrossRef] [Google Scholar]

73. Quinton R, Mamoojee Y, Jayasena CN, Young J, Howard S, Dunkel L, et al.. Society for Endocrinology UK guidance on the evaluation of suspected disorders of sexual development: emphasizing the opportunity to predict adolescent pubertal failure through a neonatal diagnosis of absent minipuberty. *Clin Endocrinol*. (2017) 86:305–6. 10.1111/cen.13257 [PubMed] [CrossRef] [Google Scholar]

74. Dwyer AA, Jayasena CN, Quinton R. Congenital hypogonadotropic hypogonadism: implications of absent mini-puberty. Minerva Endocrinol. (2016) 41:188–95. [<u>PubMed</u>] [<u>Google Scholar</u>]

75. Bonomi M, Vezzoli V, Krausz C, Guizzardi F, Vezzani S, Simoni M, et al. Italian Network on Central Hypogonadism. Characteristics of a nationwide cohort of patients presenting with isolated hypogonadotropic hypogonadism (IHH). *Eur J Endocrinol*. (2018) 178:23–32. 10.1530/EJE-17-0065 [PubMed] [GrossRef] [Google Scholar]

76. Koskenniemi JJ, Virtanen HE, Wohlfahrt-Veje C, Löyttyniemi E, Skakkebaek NE, Juul A, et al.. Postnatal changes in testicular position are associated with IGF-I and function of Sertoli and Leydig cells. *J Clin Endocrinol Metab*. (2018) 103:1429–37. 10.1210/jc.2017-01889 [PubMed] [CrossRef] [Google Scholar]

77. Hadziselimovic F, Zitvkovic D, Bica DTG, Emmons LR. The importance of mini-puberty for fertility in Cryptorchidism. *J* Urol. (2005) 174:1536–9. 10.1097/01.ju.0000181506.97839.b0 [PubMed] [CrossRef] [Google Scholar]

78. Hadziselimovic F, Emmons LR, Buser MW. A diminished postnatal surge of Ad spermatogonia in cryptorchid infants is additional evidence for hypogonadotropic hypogonadism. *Swiss Med Wkly*. (2004) 134:381–4. [PubMed] [Google Scholar]

79. Hadziselimovic F, Gegenschatz-Schmid K, Verkauskas G, Demougin P, Bilius V, Dasevicius D, et al.. Gene expression changes underlying idiopathic central hypogonadism in cryptorchidism with defective mini-puberty. *Sex Dev*. (2016) 10:136–46. 10.1159/000447762 [PubMed] [CrossRef] [Google Scholar]

80. Main KM, Schmidt IM, Skakkebæk NE. A possible role for reproductive hormones in newborn boys: Progressive hypogonadism without the postnatal testosterone peak. *J Clin Endocrinol Metab.* (2000) 85:4905–7. 10.1210/jcem.85.12.7058 [PubMed] [CrossRef] [Google Scholar]

Case 4:23-cv-00185-JGZ Document 92-23 Filed 06/29/23 Page 18 of 18

5/18/23, 3:48 PM Up-To-Date Review About Minipuberty and Overview on Hypothalamic-Pituitary-Gonadal Axis Activation in Fetal and Neonatal Life...

81. Main KM, Schmidt IM, Toppari J, Skakkebæk NE. Early postnatal treatment of hypogonadotropic hypogonadism with recombinant human FSH and LH. *Eur J Endocrinol*. (2002) 146:75–9. 10.1530/eje.0.1460075 [PubMed] [CrossRef] [Google Scholar]

82. Bougnères P, François M, Pantalone L, Rodrigue D, Bouvattier C, Demesteere E, et al.. Effects of an Early Postnatal Treatment of Hypogonadotropic Hypogonadism with a Continuous Subcutaneous Infusion of Recombinant Follicle-Stimulating Hormone and Luteinizing Hormone. *J Clin Endocrinol Metab.* (2008) 93:2202–5. 10.1210/jc.2008-0121 [PubMed] [CrossRef] [Google Scholar]

83. Stoupa A, Samara-Boustani D, Flechtner I, Pinto G, Jourdon I, González-Briceño L, et al.. Efficacy and safety of continuous subcutaneous infusion of recombinant human gonadotropins for congenital micropenis during early infancy. *Horm Res Paediatr.* (2017) 87:103–10. 10.1159/000454861 [PubMed] [CrossRef] [Google Scholar]

84. Gegenschatz-Schmid K, Verkauskas G, Demougin P, Bilius V, Dasevicius D, Stadler MB, et al.. Curative GnRHa treatment has an unexpected repressive effect on Sertoli cell specific genes. *Basic Clin Androl.* (2018) 28:2. 10.1186/s12610-018-0067-1 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

85. Heinrichs C, Bourdoux P, Saussez C, Vis HL, Bourguignon JP. Blood spot follicle-stimulating hormone during early postnatal life in normal girls and Turner's syndrome. *J Clin Endocrinol Metab*. (1994) 78:978–81. [PubMed] [Google Scholar]

86. Fechner PY, Davenport ML, Qualy RL, Ross JL, Gunther DF, et al. Toddler Turner Study Group. Differences in folliclestimulating hormone secretion between 45,X monosomy Turner syndrome and 45,X/46,XX mosaicism are evident at an early age. J Clin Endocrinol Metab. (2006) 91:4896–902. 10.1210/jc.2006-1157 [PubMed] [CrossRef] [Google Scholar]

87. Chrysis D, Spiliotis BE, Stene M, Cacciari E, Davenport ML. Gonadotropin secretion in girls with Turner syndrome measured by an ultrasensitive immunochemiluminometric assay. *Horm Res.* (2006) 65:261–6. 10.1159/000092516 [PubMed] [CrossRef] [Google Scholar]

88. Lahlou N, Fennoy I, Carel J-C, Roger M. Inhibin B and anti-müllerian hormone, but not testosterone levels, are normal in infants with nonmosaic Klinefelter syndrome. *J Clin Endocrinol Metab*. (2004) 89:1864–8. 10.1210/jc.2003-031624 [PubMed] [CrossRef] [Google Scholar]

89. Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF, Zinn A. Early androgen deficiency in infants and young boys with 47,XXY Klinefelter syndrome. *Horm Res.* (2005) 64:39–45. 10.1159/000087313 [PubMed] [CrossRef] [Google Scholar]

90. Aksglaede L, Petersen JH, Main KM, Skakkebæk NE, Juul A. High normal testosterone levels in infants with non-mosaic Klinefelter's syndrome. Eur J Endocrinol. (2007) 157:345–50. 10.1530/EJE-07-0310 [PubMed] [CrossRef] [Google Scholar]

91. Cabrol S, Ross JL, Fennoy I, Bouvattier C, Roger M, Lahlou N. Assessment of leydig and sertoli cell functions in infants with nonmosaic klinefelter syndrome: Insulin-like peptide 3 levels are normal and positively correlated with lh levels. *J Clin Endocrinol Metab*. (2011) 96:746–53. 10.1210/jc.2010-2103 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

92. Bouvattier C, Carel J-C, Lecointre C, David A, Sultan C, Bertrand AM, et al.. Postnatal changes of T, LH, and FSH in 46,XY infants with mutations in the AR gene. *J Clin Endocrinol Metab*. (2002) 87:29–32. 10.1210/jcem.87.1.7923 [PubMed] [CrossRef] [Google Scholar]

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EXHIBIT 20



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WATCH: Transgender Soccer Player Injures Female Opponent

A male soccer player who identifies as transgender injured a female player during a semi-professional women's league game in Australia last month.

The transgender player's aggressive shoulder check sent the female player to the ground where she lay unmoving. She was "unable to train until later in the week," sources told *Reduxx*, and "when she did, it was only very lightly."

The transgender player is former YouTube personality Riley J. Dennis, who amassed 113,000 subscribers by creating videos on LGBTQ topics.

The injury is the latest in a series of incidents that include transgender athletes injuring female players.

Dennis has allegedly injured female players in the league before, according to media reports. Dennis is tied for most goals in the league, with 11 on the season.

A high school volleyball player in North Carolina urged the state in April to ban transgender athletes from women's sports after she suffered a concussion and neck injury when a transgender athlete spiked a ball in her face.

The injuries have strengthened support around the world for the protection of women's sports for biological females. In 2020, World Rugby banned transgender athletes from participating in women's elite leagues due to significant "safety" concerns.

Kirralie Smith, a spokeswoman for activist group Binary Australia, told the *Daily Mail* the league remains unwilling to take action to protect biological female players, despite "warnings of injuries."

"They have failed in safeguarding fairness and safety for girls and women," Smith said.

As of March 30, 2023, at least five men who identified as transgender participated in the women's league, according to the *Daily Mail*.

At least 21 U.S. states have banned transgender-identifying boys from playing on school sports teams for girls, according to the Movement Advancement Project. Sixteen states and the District of Columbia, however, have transgender-"friendly state guidance" for high school sports, according to the Transathlete website.

House Republicans passed a bill in April banning transgender athletes from competing in women's sports, but President Joe Biden has promised to veto the bill in the unlikely event it reaches his desk.