Ontogénesis postnatal de la extremidad inferior basada en telemetrías y morfometría geométrica. Aplicación en paleoantropología y antropología forense

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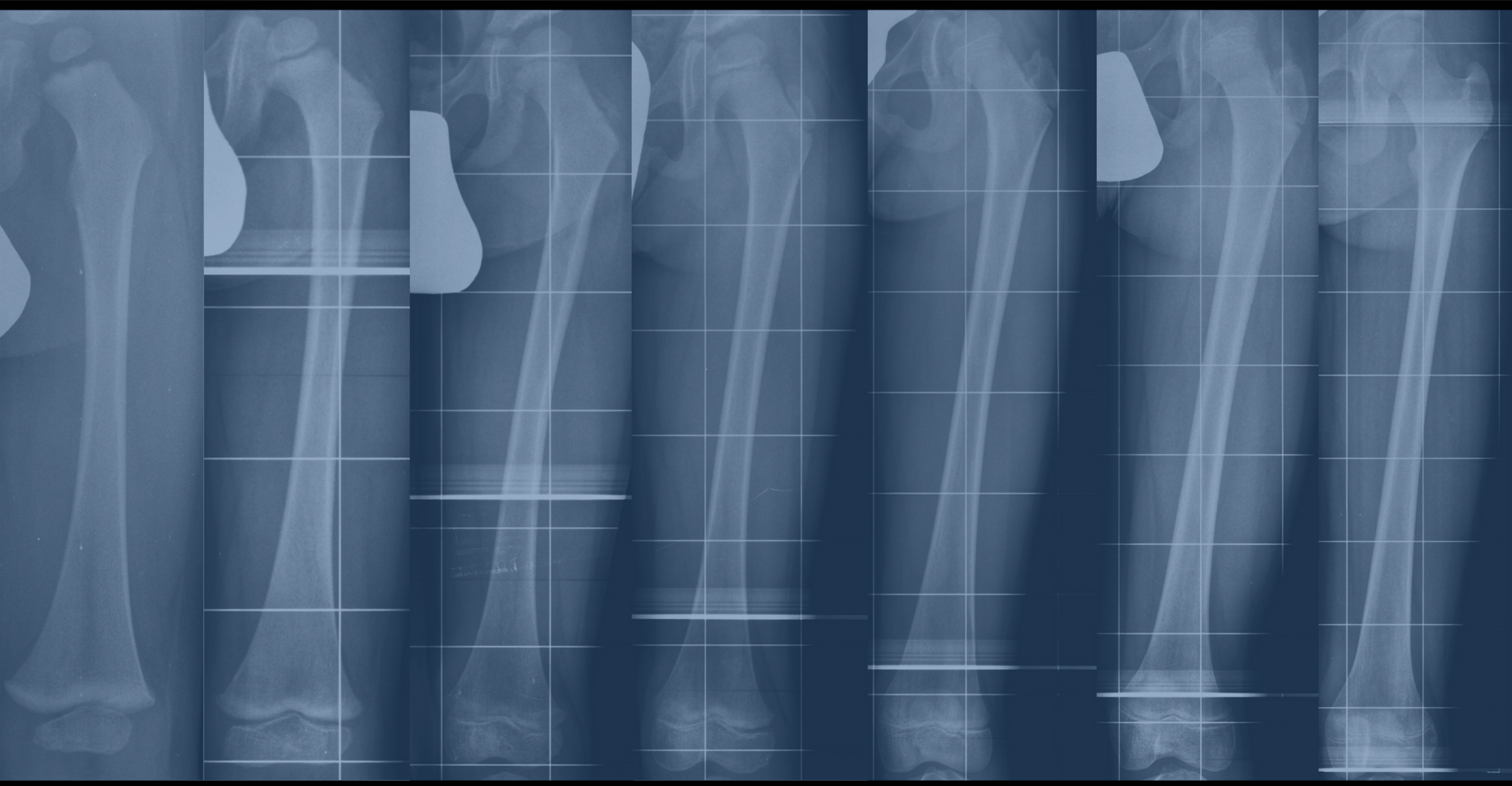

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Ontogénesis postnatal de la extremidad inferior basada en telemetrías y morfometría geométrica. Aplicación en paleoantropología y antropología forense

Memoria presentada por

Aniol Pujol Bayona

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Director / Tutor

Daniel Turbón Borrega
Departamento de Biología Animal
Facultad de Biología
Universidad de Barcelona

Codirectora

Carme Rissech Badalló
Departamento de Ciencias Médicas
Facultad de Medicina
Universidad de Girona

A mis padres

A Anna

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1. Introducción

1. INTRODUCCIÓN

En el desarrollo físico de un niño destacan dos procesos diferentes pero, a la vez, muy relacionados: el crecimiento y la maduración. El crecimiento es un proceso cuantitativo, que viene determinado por un incremento de los valores corporales, peso y volumen (Thompson, 1917; Carrió, 1984). A nivel celular, se corresponde con un aumento del número de células, cuyo efecto se traduce en una modificación del volumen corporal. Este proceso se produce de forma continua en los individuos, aunque su velocidad cambia de ritmo a lo largo del desarrollo. A nivel individual también se observan diferencias en la velocidad de crecimiento, aspecto que queda reflejado en las diferencias de estatura que se observan en los adultos (Thompson, 1917).

La maduración es un proceso cualitativo que se expresa por las modificaciones de la estructura, composición y funcionamiento de las células, de los órganos o del cuerpo en su conjunto y que se puede observar a nivel morfológico, funcional y de complejidad (Todd, 1937). Se trata de un fenómeno continuo que difícilmente modifica su velocidad (Carrió, 1984).

Clínicamente se distinguen dos tipos de factores que afectan al desarrollo: 1) los factores genéticos, que vendrían dados por la información genética del individuo o del grupo poblacional al que pertenecen, y 2) los factores ambientales, que, a su vez podrían ser de dos tipos: biológicos (nutrición, enfermedades, etc.) y culturales.

Así pues, dada la sensibilidad que presenta el desarrollo a las condiciones ambientales (biológicas y no biológicas) se considera un indicador idóneo del estado de salud y bienestar del individuo. El desarrollo de los individuos se mide mediante indicadores de crecimiento y maduración. Entre los indicadores de crecimiento se encuentran la estatura, el peso y las dimensiones de los huesos. Entre los indicadores de maduración están las modificaciones del tejido óseo (fusión de las epífisis y la morfología de los diferentes elementos esqueléticos), los caracteres sexuales secundarios y la maduración psicomotriz.

1.1. Importancia de los estudios de crecimiento y maduración esquelética

Los estudios sobre el desarrollo esquelético son de suma importancia para: 1) la valoración del estado de maduración del individuo en casos de ortodoncia, cirugía ortopédica y tratamientos hormonales; 2) para la estimación de la edad en casos judiciales donde esté implicado un menor; 3) para la identificación personal de individuos vivos o muertos en casos forenses; y 4) para la estimación de la edad de los restos esqueléticos de origen arqueológico, lo cual permite la reconstrucción de los perfiles demográficos y la interpretación de muchos factores incluyendo los indicadores de salud y las condiciones de vida de las poblaciones pasadas (véase Scheuer y Black, 2000). La fiabilidad y la precisión de los métodos de identificación biológica (edad, sexo, estatura, etc.) de los individuos inmaduros dependen de los modelos de desarrollo en los que se basen.

1.2. Modelos de crecimiento y maduración ósea

Para que los modelos de desarrollo óseo sean de aplicación eficaz en los diferentes individuos, es necesario que las medidas realizadas estén basadas en colecciones esqueléticas documentadas (edad, sexo, origen biológico conocidos), o bien tomadas de material radiográfico de alta resolución (no distorsionado por el mismo proceso). También es primordial que la población utilizada como referencia sea próxima biológicamente a la población de estudio tanto en el tiempo como en el espacio (Komar y Grivas, 2008), para así evitar errores debidos a las diferencias poblacionales o al efecto secular.

Las colecciones osteológicas documentadas son de vital importancia para testar y desarrollar las diferentes metodologías osteológicas, para estudiar el dimorfismo sexual y el envejecimiento en los diferentes grupos poblacionales (Alemán et al., 1997, Hunt et al., 2005, Eliopoulus et al., 2007, Landa et al., 2009, Rissech et al., 2011). Su mayor problema radica en que todas estas poblaciones por numerosa que sea la muestra se encuentran sesgadas, limitadas, son parciales y muchas de ellas carecen de contextualización socioeconómica, temporal y demográfica en la que vivieron los individuos de la colección (Rissech et al., 2011, Komar et al., 2008).

Actualmente, el estudio sobre el desarrollo de los individuos inmaduros queda enriquecido por una multitud de nuevas técnicas de imagen, entre ellas el material radiográfico de alta resolución (las tomografías computarizadas multicorte y telemetrías), que no distorsiona las medidas reales, lo que proporciona un potente impulso al estudio del desarrollo óseo (García et al., 2010).

Actualmente, los estándares de crecimiento y maduración postnatal se basan en material radiográfico tradicional procedente de niños de América del Norte de origen europeo (Reynolds, 1945, 1947; Ghantus, 1951; Maresh, 1955; Coleman, 1969;

Gindhart, 1973). Aunque se han realizado estudios de crecimiento y maduración a partir de material osteológico, la mayoría proceden de poblaciones eslavas (Stloukal y Hanáková, 1978), germánicas (Sundick, 1978), esquimales (Stewart, 1976) y amerindias (Merchant y Ubelaker, 1977; Sundick, 1978; Jantz y Owsley, 1984), aún son muy pocos los estudios basados en niños de Europa Occidental (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles y Bulman, 1994, 1995; Majó 2000, Rissech et al., 2001, 2003; Rissech y Malgosa, 2005, 2007; Rissech y Black, 2007; Rissech et al., 2008; López-Costas et al., 2011); y aún menos los basados en niños de la Península Ibérica (Rissech et al., 2001, 2003; Rissech y Malgosa, 2005, 2007; Rissech y Black, 2007; Rissech et al., 2008; López-Costas et al., 2011). La mayoría de estos estudios se basan en material arqueológico de edad y sexo determinado en el laboratorio (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles y Bulman, 1994; 1995; Majó, 2000). Los escasos trabajos que existen, utilizan colecciones documentadas que tratan sobre el hueso coxal, la escápula, el fémur, la tibia y el húmero (Rissech et al., 2001, 2003; Rissech y Malgosa, 2005, 2007; Rissech y Black, 2007; López-Costas et al., 2011; Rissech et al., 2008, 2012). Además, los estudios mencionados, tanto los basados en colecciones esqueléticas documentadas como en material radiográfico tradicional, se basan en individuos inmaduros de los años 50.

Como es sabido, la población occidental europea ha experimentado un aumento en la estatura a partir de la segunda mitad del siglo XX debido a la mejora de las condiciones de vida, llegando a alcanzar un incremento promedio de 10 cm en la población española (Spijker et al., 2008). Por ello se hace imprescindible completar y detallar el desarrollo esquelético postnatal de la población de Europa Occidental actual, y en especial de la Península Ibérica, para su posterior aplicación en Antropología Forense.

1.3. Importancia anatómica y antropológica de la extremidad inferior

En anatomía humana la extremidad inferior es cada uno de los 2 miembros que se encuentran unidos al tronco a través de la pelvis mediante la articulación de la cadera. Cada miembro inferior se compone de varios segmentos correspondientes a: cintura pélvica, muslo, rodilla, pierna, tobillo y pie. La cintura pélvica es una estructura ósea en forma de embudo formada por los dos coxales y el sacro, la cual transmite el peso del cuerpo a las extremidades inferiores, soporta el peso de las vísceras y proporciona puntos de anclaje a los músculos para mover las extremidades inferiores y mantener en posición vertical el cuerpo.

El muslo es el segundo segmento de la extremidad inferior situado entre la cintura pélvica y la pierna. El muslo consta de un solo elemento esquelético, el fémur (Figura 1A) que se articula con la cintura pélvica en la articulación coxofemoral. La pierna es el tercer segmento de la extremidad inferior, compuesta por dos elementos esqueléticos: la tibia y el peroné; y se articula con el muslo a través de la rodilla y con el pie a través del tobillo. La tibia es el hueso principal de la pierna (Figura 1B), articulada con el fémur en la rodilla. El hueso restante implicado en esta articulación es la rótula, un hueso de origen sesamoideo dentro del tendón del *cuádriceps femoris*. El peroné es el segundo hueso de la pierna situado lateralmente a la tibia y se articula superior e inferiormente con ella (articulaciones tibio-peroneales). Tibia y peroné conforman una superficie articulan en forma de “U” que engloba el estrágalo, formando una bisagra estable que restringe el rango de movimientos del pie en el tobillo.

El pie que es la porción más distal del cuerpo (Figura 1C). Está constituido de 26 huesos pequeños que se dividen en tres grupos: el tarso con siete huesos siendo, de atrás a delante el calcáneo, el astrágalo, el escafoides, el cuboides y tres cuñas (primera o medial, segunda o intermedia y tercera o lateral); el metatarso con cinco huesos largos, que se disponen de dentro afuera con los nombres de primero, segundo, tercero, cuarto y quinto; y las falanges con catorce huesos.

Figura 1: Miembros de la extremidad inferior A) Fémur B) Tibia y peroné C) El pie

Se conocen con los nombres de primera o proximal, segunda o media y tercera o distal o ungueal. El pie en los humanos ha sacrificado sus capacidades prensiles de los primates no humanos en favor de la estabilidad que es esencial en la locomoción bípeda terrestre.

La extremidad inferior está conectada al esqueleto axial mediante la cintura pélvica a través de la cual se transmite todo el peso del cuerpo. Por esta razón, la función de la extremidad inferior es la de sustentar el peso del cuerpo en la posición bípeda y hacer posible los desplazamientos. La extremidad inferior es el órgano más importante relacionado con la locomoción. A causa de estas dos funciones los huesos y las articulaciones de la extremidad inferior presentan unas características morfológicas de robustez y anclaje de músculos, que le confieren menos movilidad en relación a la extremidad superior (Williams et al., 2001).

La extremidad inferior es importante en antropología física y medicina desde diferentes puntos de vista. La extremidad inferior es un elemento básico para la comprensión de la evolución humana por su relación con la locomoción, pues su estudio morfológico comparado aporta mucha información sobre la aparición de la locomoción bípeda en el linaje humano (Straus, 1926; Keith, 1929; Elftman y Manter, 1935; Sigmon, 1971; Jenkins, 1972; Zihlman y Brunker, 1979; Lewis, 1983; Susman, 1983; Suzuki, 1985, Day, 1991). Por otro lado los elementos esqueléticos de la pierna, en concreto el fémur y la tibia, son importantes por su robustez y resistencia post-deposicional, y por tanto útiles para la identificación biológica de los restos humanos (Reynolds, 1987; Aiello y Dean, 1990). Además de su importancia clínica para el estudio de anomalías en el crecimiento, en la marcha así como problemas de escoliosis (columna torcida), o de *genu valg*o (Ellis, 1889; Morton, 1922; Morton, 1942; Lake, 1943; Eberhart et al., 1954; Hicks, 1955; Bowden, 1967; Stott y Stokes, 1973; Sarrafian, 1983; Tardieu y Trinkaus, 1994; Tardieu, 1998).

Nuestro interés se ha centrado exclusivamente en el estudio del fémur y de la tibia por ser dos huesos útiles para la estimación de la edad y el grado de maduración de los restos esqueléticos debido al crecimiento longitudinal que presentan ya que se conservan fácilmente.

1.4. Consideraciones anatómicas

El objeto de nuestro interés en este apartado es la morfología y desarrollo del fémur y de la tibia por ser los objetivos anatómicos de estudio de este trabajo.

1.4.1. El fémur

El fémur (Figura 2) es el hueso más largo del esqueleto humano y el más estudiado de los huesos largos (Humphry, 1889; Parsons, 1914; Pearson y Bell, 1919; Ingalls, 1924; Hrdlicka, 1934a, b, 1938; Backman, 1957; Davivongs, 1963; Trotter et al., 1968; Van Gerven, 1972; Lavelle, 1974). El fémur es un hueso largo, par y asimétrico, dirigido oblicuamente de arriba abajo y de fuera adentro, oblicuidad que resulta más notable en el caso de la mujer por la mayor separación entre las cavidades cotiloideas de los coxales). En él se distinguen tres regiones anatómicas: la región proximal (Figura 1A), la diáfisis (Figura 1B) y la región distal (Figura 1C). La región proximal del fémur está formada por la cabeza, el cuello y dos trocánteres (mayor y menor). La cabeza tiene la forma de alrededor de 2/3 de una esfera y articula con el acetábulo del hueso coxal de forma directa en la articulación coxofemoral. La cabeza del fémur tiene una orientación vertical en sentido medial, y ligeramente hacia delante. Ésta presenta una pequeña concavidad en medio de ella denominada la *fóvea capitis*, donde se inserta el ligamento redondo que une el fémur con el acetábulo, en su interior contiene los vasos sanguíneos que nutren el acetábulo. La cabeza (Figura 3A) está conectada con la diáfisis del fémur a través del cuello, el cual tiene forma de cilindro antero posteriormente. Se orienta hacia abajo y hacia afuera y forma con el cuerpo del hueso, la diáfisis, un ángulo denominado ángulo cuello-diafisario, el cual varía a lo largo de la madurez del individuo, sufriendo más cambios durante la etapa subadulta, y sin casi variación hasta la vejez. Este ángulo será comentado con más detenimiento más adelante.

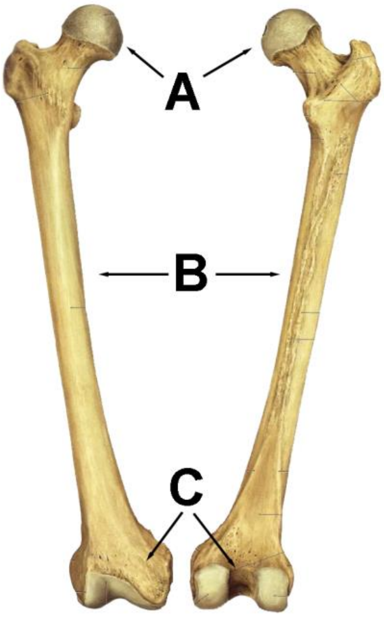


Figura 2: Fémur adulto en visión anterior (izquierda) y posterior (derecha). A) Epífisis proximal. B) Diáfisis. C) Epífisis distal

El trocánter mayor (Figura 3B) es una eminencia ósea en forma cuboide, aplastada en sentido transversal, situada lateralmente y un poco por debajo de la cabeza del fémur. El trocánter mayor tiene dos caras y cuatro bordes que son lugar de inserción de numerosos músculos. Su cara externa, convexa, presenta la cresta del glúteo mediano, dónde se inserta este músculo. La cara interna, por el contrario, está excavada verticalmente. Presenta una depresión profunda, la fosa trocantérica, dónde se insertan los músculos *obturador externo*, *obturador interno* y *géminos*. El borde superior, casi horizontal, presta inserción al músculo *piramidal*. Del borde inferior se inician algunos fascículos del *cuádriceps crural -vasto externo-*. En el borde posterior se inserta el *cuadrado crural*. Finalmente, el borde anterior, muy ancho, presta inserción al *glúteo menor*.

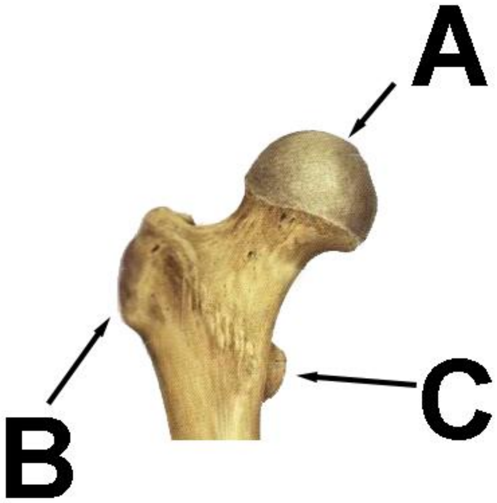


Figura 3: Detalle de la región proximal del fémur adulto: A) Cabeza del fémur B) Trocánter mayor C) Trocánter menor

El trocánter menor (Figura 3C), es una apófisis cónica en la que se inserta el musculo *psoas-ilíaco*. El trocánter menor está unido al trocánter mayor por la línea y la cresta intertrocantérica. En la línea intertrocantérica tiene una posición anterior y se inserta el *ligamento iliofemoral.* La cresta intertrocantérica tiene una posición posterior y se inserta el músculo *cuadrado crural*. En la cara anterior del fémur proximal, la línea intertrocantérica marca el límite entre el cuello y la diáfisis del fémur.

La diáfisis del fémur empieza debajo del trocánter menor, tiene forma aproximadamente prismática triangular y presenta una torsión sobre su eje, de tal manera que el plano transversal de su epífisis inferior forma un ángulo abierto medialmente con el plano transversal de su epífisis superior. El ángulo normalmente oscila entre los 9 y los 15º (Elftman, 1945), aunque puede presentar una gran variación sobre todo debido a la dificultad para su medición (Stirland, 1984).

La forma prismática permite describir tres caras y tres bordes. La cara anterior es ligeramente convexa y lisa, en ella se insertan el *vasto intermedio* y el *músculo articula*r de la rodilla. La cara lateral y medial son también convexas y lisas en sus dos tercios superiores y se estrechan hacia abajo por la parte inferior del borde posterior del hueso, en ellas se insertan los vastos *lateral y medial*. En la cara posterior de la diáfisis del fémur se encuentra la línea áspera que es un área rugosa para la inserción muscular que recorre longitudinalmente la diáfisis. El labio externo de la línea áspera presta inserción al *vasto externo*; el labio interno, *al vasto interno* y en el intersticio de la línea áspera se insertan los *aductores del muslo*, por arriba, y la porción corta del *bíceps crural*, por abajo. La línea áspera termina de modo distinto en la región superior e inferior de la diáfisis. En la región superior de la diáfisis del fémur la línea áspera se divide en tres líneas: una externa, otra media y una interna. En la línea externa, o también llamada cresta del *vasto externo*, se insertan los músculos *vasto externo, abductor mayor y glúteo mayor*. En la línea de trifurcación media o cresta del pectíneo se inserta el músculo *pectíneo* y el *abductor men*or. Por último, en la línea de trifurcación interna o del vasto interno, se inserta el *vasto interno*.

La línea áspera en la región distal de la diáfisis del fémur se bifurca en las líneas supracondíleas lateral y medial, las cuales, junto a los cóndilos femorales, delimitan un espacio triangular: el triángulo poplíteo, que es la pared anterior ósea del hueco poplíteo.

La región distal del fémur (Figura 4) está formada por la epífisis distal del fémur que se organiza en los cóndilos, dos masas laterales respecto al plano sagital de la diáfisis (cóndilo lateral y cóndilo medial, Figura 4 A y B). En ellos se desarrolla la tróclea, superficie lisa para la articulación del fémur con la tibia en la rodilla o articulación femorotibial, constituida por dos vertientes laterales que convergen en un surco anteroposterior, siendo la lateral más ancha. En la región posterior de la epífisis inferior, los cóndilos medial y lateral se extienden más allá del triángulo poplíteo separados por la fosa intercondílea. Las superficies articulares de los cóndilos medial y lateral tienen una forma semicircular así como caras cutáneas rugosas y prominentes debido a las inserciones de los ligamentos de la articulación de la rodilla. El cóndilo medial es mayor que el lateral y está más alineado a la diáfisis, soportando de forma directa el peso del cuerpo. Presenta un tamaño mayor para permitir apoyar el cóndilo lateral en la meseta tibial, debido a la inclinación de la diáfisis femoral respecto a la vertical del cuerpo.

En la parte anterior de la epífisis distal encontramos la superficie articular de la rótula, de forma asimétrica, donde la región lateral se caracteriza por un abultamiento y altura mayores que los de la región medial. Este hecho parece ser el causante de que la rótula no se disloque de su región de forma lateral, sobre todo en mujeres en quienes el ángulo bicondilar es mayor y, por tanto, las fuerzas de desplazamiento de la rótula también lo son.

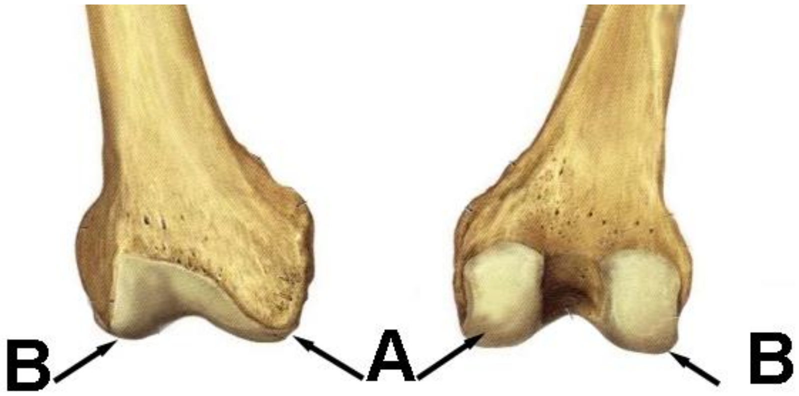


Figura 4: Detalle de la región distal del fémur A) Cóndilo medial B) Cóndilo lateral

1.4.1.1. Desarrollo del fémur

El desarrollo del fémur es el propio de un hueso largo y empieza con un esbozo cartilaginoso (osificación endocondral) donde aparecen cinco puntos de osificación: un punto primario, el de la diáfisis; tres puntos secundarios en la región superior pertenecientes a la cabeza, el trocánter mayor y menor; y un punto distal perteneciente a la epífisis distal. Su desarrollo se produce, siempre, antes en chicas que en chicos, siendo las diferencias, en los primeros meses, de un par de semanas. En la adolescencia, edad en la que termina el crecimiento de este elemento anatómico, las diferencias entre ambos sexos son de 2 años.

El centro primario de osificación de la diáfisis aparece a las 7-8 semanas del desarrollo embrionario en el centro de la diáfisis (Gardner y Gray, 1970; O'Rahilly y Gardner, 1975); una semana después empieza la osificación, proceso que no puede detectarse radiológicamente hasta dos semanas después.

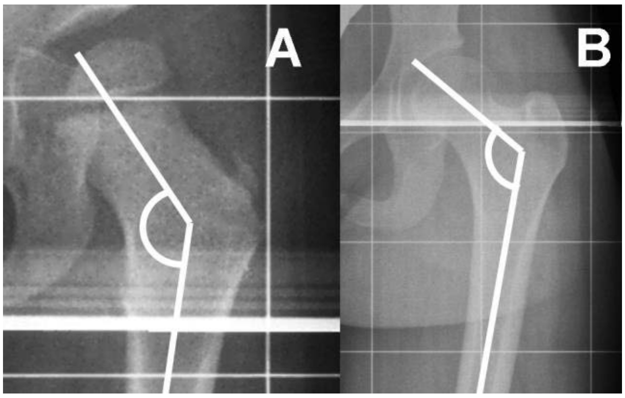
Alrededor de las 12-13 semanas de gestación la osificación de la diáfisis ya alcanza la región del cuello por la parte proximal y la epífisis en la distal. A los 7 meses aproximadamente la región proximal del fémur cambia su forma de cúpula convexa a angular en dos planos, que genera dos regiones donde se encontrarán los centros de osificación secundarios de la cabeza y el trocánter mayor.

La región distal de la diáfisis desarrolla una depresión central. Estos cambios responden a la forma de la epífisis distal. A los 3 años de edad se empiezan a ver diferencias en la producción de hueso entre las regiones de los dos cóndilos de la epífisis distal, y se manifiesta la antes comentada asimetría entre ellos.

El centro de osificación de la epífisis distal es el de crecimiento más rápido de todo el cuerpo. Normalmente proviene de un solo núcleo de osificación. En las epífisis de los huesos largos, el centro de osificación de la epífisis distal del fémur es el primero en aparecer y uno de los últimos en fusionarse. El centro aparece normalmente el mes antes de nacer, teniendo entonces forma oval. A partir de los 7 años en las niñas y a partir de los 9 en los niños, la epífisis ya es tan ancha como la diáfisis (Pyle y Hoerr, 1955), los cóndilos y la fosa intercondilar adquiere ya su forma distintiva. Dado que las cargas de los cóndilos son asimétricas, y por la angulación del fémur, durante el desarrollo el cóndilo medial gana en tamaño para compensar su inclinación respecto al cóndilo lateral.

La angulación cuello-diafisaria (Figura 5) empieza con unos valores aproximados de 141º al nacer, y se va cerrando a medida que se adquiere la bipedestación y durante la adolescencia debido al ensanchamiento de la pelvis, por lo que al final de la pubertad los valores se encuentran entre los 127-123º (Humphry, 1889), con variación entre ambos sexos, presentando las chicas ángulos más cerrados.

Figura 5: Comparación del ángulo cuello diafisario entre una niña de 3 años (A) y una chica de 18 (B)



La fusión de la epífisis distal del fémur coincide con el fin del crecimiento en altura, al igual que el resto de epífisis que se encuentran en la zona de la articulación de la rodilla. El crecimiento del fémur a partir de su región distal corresponde a un 70% del crecimiento en longitud de este hueso (Scheuer y Black, 2000). Según los datos radiográficos esta fusión ocurre entre los 16-19 años en niños y entre los 14-18 años en niñas (Scheuer y Black, 2000).

La región proximal del fémur tiene tres, y a veces 4, centros de osificación secundarios. El centro de osificación de la cabeza es raramente visible en el nacimiento pero está presente en el 60-90% de los niños a los 6 meses de edad, y casi siempre visible al año (Puyhaubert, 1913; Walmsley, 1915; Davies y Parsons, 1927; Paterson, 1929; Flecker, 1932; Menees y Holly, 1932; Francis et al., 1939; Elgenmark, 1946; Ryder y Mellin, 1966). La edad de aparición del centro de osificación de la cabeza del fémur en niños es a los 6 meses de vida intrauterina y en niñas a los 5 meses (Hansman, 1962).

Figura 6: Detalle de la diáfisis de un individuo perinatal, a la derecha vista anterior, a la izquierda vista posterior (según Scheuer y Black, 2000).

La parte osificada de la cabeza del fémur es esférica hasta el año y medio. Posteriormente, adquiere una forma aplanada en la región inferior para acomodarse a la sección media del extremo proximal de la diáfisis del fémur y al establecimiento de la placa metafisaria. A los 3 años de edad ya tiene la forma de la mitad de una esfera con la región lateral ligeramente aplanada. La edad de fusión de la cabeza femoral se encuentra alrededor de los 14 años y dos meses en chicas y de los 16 años y 3 meses en chicos (Scheuer y Black, 2000) (Figura 7). No obstante hay cierta variabilidad entre autores respecto a la edad de fusión, con fases que llegan hasta los 17 o 18 años y medio (McKern y Stewart, 1957).

El centro de osificación del trocánter mayor aparece entre los 2 y 5 años (Puyhaubert, 1913; Davies y Parsons, 1927; Paterson, 1929; Flecker, 1932; Francis et al., 1939; Elgenmark, 1946; Garn et al., 1967). Cuando el trocánter mayor aparece su placa metafisaria ya está separada del de la cabeza femoral, por lo que sus ritmos de desarrollo son diferentes. En la pubertad, el hueso ya tiene su forma adulta y se fusiona entre los 16 y 18 años en chicos y los 14 y 16 en chicas (Figura 7).

En cuanto al trocánter menor, sus descripciones son más variables. Este elemento tiene un rango de aparición que va de los 7 a los 11 años y su fusión se produce hacia los 16-17 años de edad (Scheuer y Black, 2000) (Figura 7). Es el elemento óseo que presenta un tiempo más corto como entidad separada de la diáfisis, y algunas veces ni es visible, habiendo dudado algunos autores de su existencia como tal (Paterson, 1929).

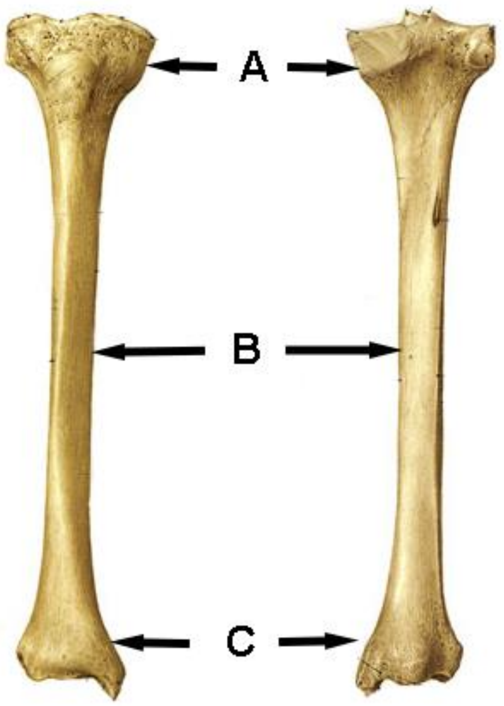
Figura 7: Edad de fusión de los centros de osificación

1.4.2. La tibia

La tibia (Figura 8), es un hueso largo par y no simétrico, situado en la parte anterior e interna de la pierna, en la cara interior del peroné --con el cual se articula por sus dos extremos--, por debajo del fémur que descansa sobre ella, y por encima del conglomerado óseo del tarso, al cual transmite el peso del cuerpo (Testut y Latarjet, 1932).

La tibia, en su correspondiente sitio del esqueleto armado, es vertical y forma con el fémur, un ángulo fuertemente obtuso dirigido hacia fuera. Considerada aisladamente en sí misma, no es exactamente rectilínea, sino que presenta dos curvaturas en sentido contrario: 1) una de ellas se corresponde a la mitad superior del hueso y es convexa; 2) la otra corresponde a su mitad inferior y es cóncava. De esta doble incurvación resulta su forma de *“S”* itálica.

Figura 8: Tibia de adulto, a la izquierda vista anterior, a la derecha vista posterior. A) Región proximal B) Diáfisis C) Región distal.



Al igual que el fémur, está ligeramente retorcida sobre su eje. En la tibia (Figura 1), como en todos los huesos largos, hay tres porciones: el cuerpo (diáfisis) y dos extremos (epífisis distal y epífisis proximal). La diáfisis es prismático-triangular con bastante regularidad, y se distinguen tres caras (interna, externa y posterior) y tres bordes (anterior, interno y externo) (Testut y Latarjet, 1932).

La epífisis proximal de la tibia es muy voluminosa (Figura 9), cuadrangular y prolongada en sentido transversal. Está destinada a articularse con los cóndilos del fémur, por lo que presenta en su cara superior dos superficies articulares horizontales (medial y lateral), ligeramente excavadas, conocidas como cavidades glenoideas de la tibia. Ambas están definidas por un borde externo semicircular y un borde medio (en relación al eje del hueso) casi rectilíneo. Este último borde, a nivel de su parte media, se eleva en dos eminencias óseas en forma de tubérculos (espinas intercondíleas) que sirven de inserción a los ligamentos cruzados de la articulación de la rodilla.

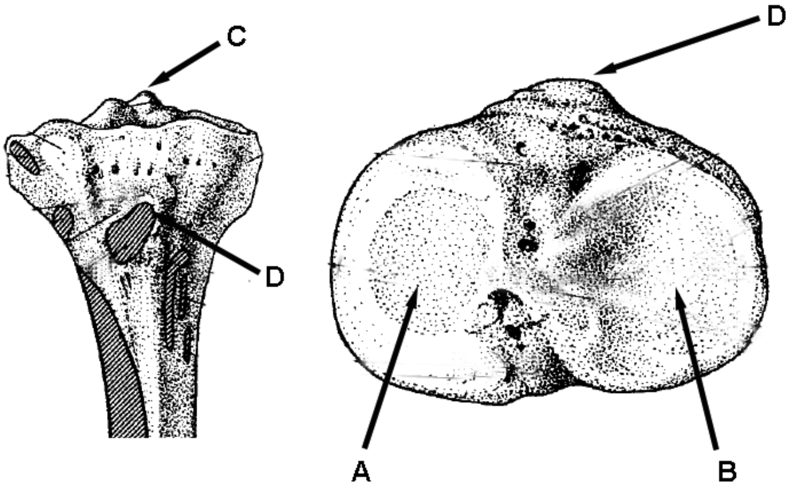


Figura 9: Detalle de la epífisis proximal, a la izquierda visión anterior, a la derecha visión superior. A) Cavidad glenoidea medial B) Cavidad glenoidea lateral C) Eminencia Intercondilar D) Tuberosidad tibial (según Scheuer y Black, 2000)

La epífisis distal está mucho menos desarrollada que la proximal, pero, como esta última, presenta también una forma cuboidal y, por consiguiente, se consideran en ella seis caras (superior, anterior, posterior, externa e interna). La cara superior, cuando la epífisis distal está fusionada con la diáfisis y no existe separación entre ambas, se confunde con el cuerpo del hueso. La cara inferior se articula con la polea astragalina por lo que presenta una extensa superficie cuadrilátera, lisa y uniforme, cóncava de delante a atrás y algo más ancha por fuera que por dentro. La cara anterior, convexa y lisa, es continuación de la cara externa del cuerpo del hueso y está en relación con los tendones de los músculos del pie. La cara posterior, igualmente convexa, presenta por fuera un canal oblicuo, para el paso del tendón del flexor propio del dedo grueso. La cara externa ofrece una excavación de forma triangular, cuyo vértice se continúa con el borde externo del hueso. Esta excavación recibe el extremo inferior del peroné. La cara interna se prolonga hacia abajo en una apófisis voluminosa llamada *maléolo interno*. La cara externa de esta apófisis es convexa y lisa y está en relación directa con la piel (Testut y Latarjet, 1932).

La epífisis superior es voluminosa y maciza, algo proyectada hacia atrás. Tiene forma de pirámide triangular invertida, estando su vértice orientado hacia la diáfisis y su base en la cara superior, llamada meseta tibial, presenta 2 superficies articulares horizontales, ligeramente excavadas, que reciben a los cóndilos femorales. Son las cavidades glenoideas medial y lateral, siendo la medial más larga y excavada, y la lateral más extendida en sentido transversal (Figura 9, A y B). Cada cavidad presenta un borde periférico semicircular. En la parte central de la meseta, ambos bordes se elevan para formar los respectivos tubérculos medial y lateral. En conjunto, ambos tubérculos forman la espina de la tibia, por delante y por detrás de la cual se encuentran las superficies preespinal y retroespinal respectivamente (ambas comprendidas entre las 2 cavidades glenoideas).

Las cavidades glenoideas están apoyadas por las tuberosidades tibiales medial y lateral, que se perciben como salientes en la parte lateral de la epífisis superior.

La tuberosidad lateral presenta postero-lateralmente una cara articular, redondeada u oval, es la cara articular para el peroné.

En la parte anterior se observa una importante prominencia denominada tuberosidad tibial anterior (Figura 9D), lateralmente a la cual se encuentra una pequeña protuberancia, el *tubérculo de gerdy* donde se inserta la *fascia lata*.

Entre las tuberosidades tibiales anterior y medial hay una zona triangular, plana, de textura rugosa, denominada *pata de ganso*, donde se insertan los músculos *sartorio*, *recto interno* y *semitendinoso*.

La diáfisis o cuerpo es de sección transversal triangular. Su cara lateral es cóncava en su parte superior para hacerse convexa en la inferior, en tanto que la cara posterior está atravesada por una saliente afilada que transcurre de arriba hacia abajo y de lateral a medial, es la línea para *el sóleo*, que da inserción al músculo del mismo nombre.

El borde anterior tiene forma de *"S"* itálica, zona muy expuesta a traumatismos debido a su ubicación anterior superficial y subcutánea. El borde medial es poco marcado arriba y más saliente abajo. El borde lateral, llamado borde interóseo, da inserción a la *membrana interósea*. Dicho borde se bifurca en la parte más inferior para circunscribir, ya en la epífisis inferior, a la cara articular para el peroné.

La epífisis inferior es notablemente más pequeña que la superior. Participa en 2 articulaciones: la tibiotarsiana y la tibioperonea inferior. Tiene forma de pirámide cuadrangular en al que se describen su cara inferior o base, y sus 4 caras laterales.

Su cara inferior se articula con la tróclea del astrágalo mediante una superficie cuadrilátera, lisa y uniforme, cóncava de adelante hacia atrás y algo más ancha lateral que medialmente. Una cresta anteroposterior roma la divide en dos vertientes que apoyan en la tróclea astragalina, la cresta se corresponde con la garganta de la tróclea. La cara anterior es convexa y lisa, sin relieves óseos. La cara posterior está marcada por canales oblicuos orientados medialmente para el paso de los tendones de los *músculos flexores plantares* del pie y de los dedos. La cara lateral, orientada algo hacia atrás, presenta la superficie articular para la extremidad inferior del peroné.

La cara medial está prolongada hacia abajo por el maléolo medial, cuya cara medial, convexa y lisa es subcutánea, la cara lateral del maléolo es plana y es la continuación de la cara inferior de la epífisis inferior de la tibia que se articula con la cara medial del astrágalo. Su borde posterior presenta el canal maleolar de la tibia para los *músculos tibial posterior y flexor largo* común de los dedos

1.4.2.1. Desarrollo de la tibia

La tibia se desarrolla desde cuatro puntos de osificación: uno primitivo para el cuerpo y tres complementarios para los extremos. El punto primitivo para el cuerpo aparece en el centro de la diáfisis entre los treinta y cinco y cuarenta días de la vida intrauterina. Se prolonga muy rápidamente y forma, no solo todo el cuerpo del hueso, sino también una buena parte de sus extremos. Produce por sí solo, al menos once de las doce partes de la tibia. A los 6 meses, la región proximal ya tiene una forma aplanada para adaptarse al desarrollo de la epífisis proximal (Figura 10). A los 4-5 años de edad las dos regiones epifisarias ya tienen la forma característica y empiezan a adaptarse a los cambios que sufren las epífisis.

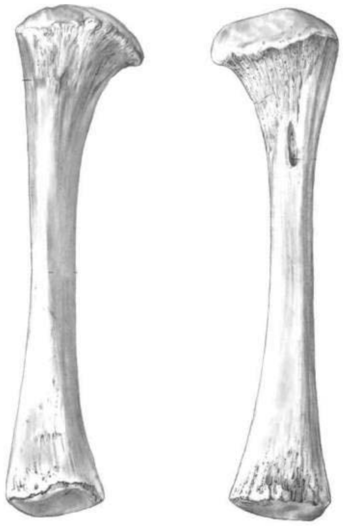


Figura 10: Tibia perinatal, a la izquierda vista anterior, a la derecha vista posterior (según Scheuer y Black, 2000)

De los puntos complementarios, uno está destinado a la epífisis superior (punto epifisario superior), el segundo a la epífisis inferior (punto epifisario inferior) y el tercero a la tuberosidad anterior (punto complementario al punto epifisario anterior). El punto epifisario superior surge en el momento del nacimiento. Está situado por encima de la diáfisis y en forma de lámina horizontal aparece normalmente justo antes del nacimiento (Flecker, 1932); radiográficamente la epífisis es visible a partir de la semana 35 de gestación. (Kuhns y Finnstorm, 1976). La epífisis está siempre presente a partir de la tercera semana de vida (Puyhaubert, 1913; Davies y Parsons, 1927; Paterson, 1929; Hasselwander, 1938; Francis et al., 1939; Christie, 1949; Pyle y Hoerr, 1955; Hansman, 1962). A los 7 años en chicas y a los 9 en chicos la anchura de la epífisis se iguala con el del extremo proximal de la diáfisis (Pyle y Hoerr, 1955). A los 11-13 años el tamaño de la epífisis ya es importante, y las regiones medial y lateral ya empiezan a diferenciarse. La epífisis superior se suelda al cuerpo del hueso entre los 13 y 17 años en individuos femeninos, y entre los 15 y 19 en individuos masculino.

El punto epifisario inferior surge en la mitad del segundo año, formado también por una lámina horizontal, de la cual penderá el maléolo tibial. Aparece sobre los 3-4 meses de vida, aunque normalmente no es visible claramente hasta los 7-8 meses. Inicialmente tiene una forma redondeada (Hoerr et al., 1962). A los 5 años en chicas y a los 6 años y medio en chicos la anchura de la epífisis es aproximadamente igual al de la región distal de la diáfisis de la tibia. Es habitual que el maléolo osifique como un centro separado de la epífisis distal, lo que parece que se da más comúnmente en chicas, manifestándose a los 7-8 años de edad, y en chicos a los 9-10 años (Den Hoed, 1925; Lapidus, 1933; Powell, 1961; Selby, 1961; Coral, 1987; Ogden y Lee, 1990) La epífisis inferior fusiona entre los 14 y 16 en chicas y entre los 15 y 18 en chicos (Scheuer y Black, 2000) (Figura 11).

Figura 11: Edad de fusión de los centros de osificación de la epífisis proximal y distal

El punto de la tuberosidad anterior aparece entre el segundo y cuarto año. Algunos meses más tarde se suelda por su borde superior a la epífisis superior de la tibia y entonces forma una especie de medallón suspendido de la parte inferior de esta epífisis.

La tibia presenta un crecimiento más uniforme durante todo la infancia, en contraposición al fémur que crece más lentamente durante la niñez para después acelerar el crecimiento durante el brote puberal. En la pubertad, los bordes epifisarios de la diáfisis están más definidos, la región de la epífisis distal tiene forma cuadrangular y presenta el surco de la articulación del peroné.

1.4.3. Biomecánica de la extremidad inferior en el ámbito de la locomoción

La marcha humana es un proceso de locomoción en el cual el cuerpo humano, en posición erecta generalmente, se mueve hacia delante, siendo su peso soportado alternativamente por ambos miembros inferiores (Inman et al., 1981). Se caracteriza por el contacto permanente del individuo con el suelo a través de al menos uno de sus pies.

En la marcha humana se distinguen cuatro fases que son: (i) el primer doble apoyo, (ii) el primer apoyo unilateral, (iii) el segundo doble apoyo y finalmente (iv) el segundo apoyo unilateral (Ducroquet et al., 1972; Lehmann y Lateur, 1993; Plas et al., 1984; Viladot y Viladot, 1984). Describiremos cada una de estas fases que corresponden a un ciclo completo de uno de los dos miembros inferiores, teniendo en cuenta que en el miembro inferior contralateral acontece lo mismo, pero trasladado en el tiempo medio ciclo.

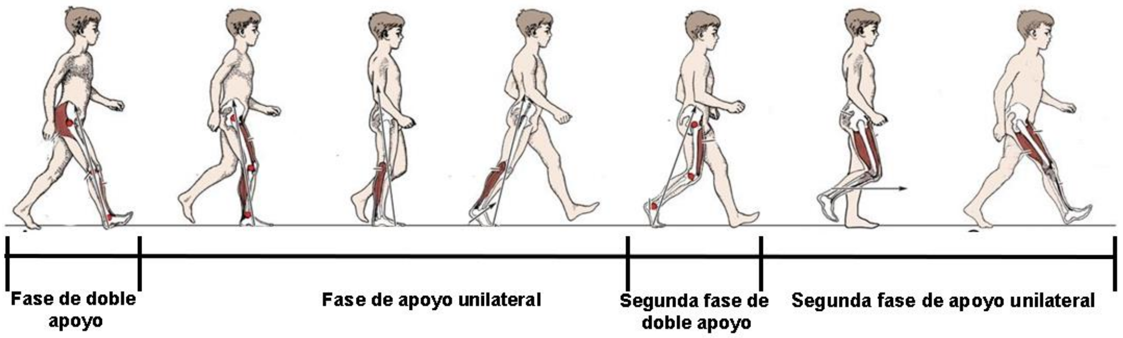


Figura 12: Fases de la marcha en humanos

(i) El «primer doble apoyo» corresponde a la primera fase (Figura 12), de la marcha humana (Viladot y Viladot, 1984). Se caracteriza porque el miembro inferior atrasado se inclina hacia delante por una extensión de cadera, la rodilla se flexiona mientras que la articulación tibiotarsiana se flexiona plantarmente. Hacia el final de esta fase el músculo cuádriceps se contrae, extendiendo prácticamente la rodilla, mientras la articulación tibiotarsiana está en máxima flexión plantar.

(ii) El «primer apoyo unilateral» corresponde a la segunda fase de la marcha humana (Figura 12) (Viladot y Viladot, 1984). El pie que en la fase anterior sólo apoyaba con el primer dedo se despega del suelo, la rodilla y la cadera se flexionan y todo el miembro inferior se desplaza en el plano sagital, adelantándose al resto del cuerpo, siendo el miembro inferior contralateral el que sostiene la totalidad del peso corporal. En esta fase es cuando el miembro inferior alcanza su mínima longitud al producirse la flexión conjugada de cadera, rodilla y flexión dorsal del tobillo.

(iii) El «segundo doble apoyo» corresponde a la tercera fase, de la marcha humana (Figura 12) (Viladot y Viladot, 1984). Se caracteriza porque el miembro inferior oscilante que en la segunda fase cruzaba, toca el suelo por medio del talón, recibiendo parte del peso del cuerpo. Durante esta fase el miembro inferior ha de medir, frenar y regular la progresión hacia delante.

(iv) El «segundo apoyo unilateral» corresponde a la cuarta fase de la marcha humana (Figura 12) (Viladot y Viladot, 1984). Durante esta fase, el miembro inferior apoyado soporta todo el peso del cuerpo a la vez que mantiene el equilibrio en los tres planos y permite la traslación corporal hacia delante.

En el segundo apoyo unilateral (el miembro inferior contrario se encontraría en la fase de oscilación), el miembro inferior que nos ocupa verticaliza su segmento tibial muy rápidamente, mientras que el muslo se mantiene aún en flexión para después enderezarse. Junto a la extensión de rodilla e inclinación anterior de la tibia permiten que todo el miembro inferior se incline hacia delante.

1.4.4. Cambios en la articulación coxo-femoral debido a al desarrollo de la locomoción durante el crecimiento

En el nacimiento, el acetábulo y la cabeza del fémur se encuentran en pleno desarrollo, y este proseguirá hasta la pubertad. Un desarrollo normal de la articulación coxo-femoral se basa fundamentalmente en una perfecta y permanente relación concéntrica femoro-cotiloidea, así como en una integridad anatómica y funcional de las estructuras musculo-esqueléticas de la cadera (Ballerter, 2001).

Al iniciarse la marcha bípeda humana, que es aprendida, se produce un conflicto, una incongruencia articular coxo-femoral (de la articulación de la cadera) debido a que para adoptar una posición erecta es necesaria la extensión excesiva del fémur, produciéndose un mal ensamblaje de la cabeza del fémur dentro de la cavidad acetabular (Llorach, 2006); pero esta inestabilidad articular inicial, se pone de manifiesto cuando el niño adquiere la marcha independiente en torno a los 12-14 meses de vida, y va desapareciendo a medida que las estructuras músculo-ligamentosas del niño van madurando.

En estas condiciones, en las que los elementos esqueléticos constituyen el principal factor de coaptación, la correcta alineación torsional y rotacional de éstos, es fundamental para la normal fisiología y biomecánica articular.

Durante la evolución del desarrollo torsional y rotacional de la cadera, existen factores que condicionan el patrón de la marcha en el niño, que son:

1. El ángulo de inclinación o ángulo cuello-diafisario, del cual se ha hablado anteriormente, que pasa de valores de 140º al nacer a valores de 123º-120º en la edad adulta (Figura 5).
2. El ángulo de anteversión femoral es de unos 40º en el momento del nacimiento, con tendencia a disminuir progresivamente hasta alcanzar la edad adulta (Llorach et al., 2006; Ballerter, 2001), cuando pasa a medir entre 15-18º.

Estudios recientes sobre la formación de la articulación de la cadera, basados en las imágenes de las tomografías axiales computarizadas (Gormedino, 2005), han demostrado que la duración del desarrollo llega, al menos, hasta los 13 años de edad.

1.4.5. Evolución del bipedismo y de la biomecánica de la marcha

Uno de los factores que ha llegado a diferenciarnos más del resto de los primates, es nuestra forma de desplazarnos (Asensio et al., 2002). El bipedismo implicó un cambio estructural total de la región de la cadera en el paso de la marcha cuadrúpeda a la bípeda. Uno de estos cambios reside en la articulación de la cadera, se trata del ángulo cuello-diafisario. Este ángulo es menor en los humanos en relación a los demás primates adultos. El ángulo cuello-diafisario muy abierto, como el de los demás primates, hace que las rodillas estén en posición de abducción, perpetuando un ángulo femoro-tibial aumentado, *genu varo*. En los humanos, la disminución del ángulo cuello-diafisario acerca las rodillas a la línea de gravedad del cuerpo y provoca un ángulo femoro-tibial menor, *genu valgo*, imprescindible para que el miembro inferior pueda mantenerse estable al cargar el peso del cuerpo y para que la rodilla se bloquee en la fase de apoyo unipodal en la que todo el peso del cuerpo recae sobre un solo miembro inferior.

En el momento del nacimiento estas similitudes con nuestros ancestros se manifiestan en el hecho de que durante el primer año de vida, el niño presenta genu *varo* fisiológico que posteriormente evolucionará hacia *genu valgo*, muy influenciado por la adquisición de la marcha bípeda, que es aprendida.

En los humanos adultos el centro de gravedad está localizado en la línea media justo por delante de la segunda vértebra sacra (MacConaill y Basmajian, 1969). Cuando el sujeto está de pie y en reposo, la línea pasa a través de este punto y perpendicular al suelo, y también a través de la corta distancia que hay entre las dos rodillas, las cuales se aproximan a la línea media del centro de gravedad, al igual que los tobillos. De esta forma solo una actividad muscular mínima es necesaria para mantener la postura erguida. El potente ligamento que une el fémur con el ilium y que pasa justo por delante de la articulación de la cadera (*el ligamento iliofemoral*) ayuda mantener el tronco y que éste no caiga hacia atrás y los ligamentos de la rodilla (los *ligamentos cruzados*) ayudan a mantener el cuerpo y que éste no caiga hacia delante a la altura de esta articulación.

Aunque los humanos actuales, comparados con los primeros bípedos guardan muchas similitudes, también hay muchos elementos que se diferencian entre unos y otros debido a los distintos tipos de bipedismos, en función de los entornos ecológicos. Por ello encontramos diferencias entre los individuos más antiguos, *A. afarensis*, H. *habilis, H. erectus* y los *Homo sapiens* actuales.

Las aptitudes para la bipedación se concentran en torno a una pelvis corta y en forma de cubeta. Visto desde arriba, el perfil de las grandes alas –alas ilíacas- sigue una línea recta desde el sacro hasta la espina ilíaca anterior. La presencia de esta espina ósea se vuelve a encontrar en la pelvis del hombre y corresponde a la inserción de un haz del potente músculo cuádriceps. Los huesos púbicos son largos y su unión forma una sínfisis púbica relativamente esbelta. Comparada con la pelvis del hombre, la de los la de los australopitecos se cierra menos hacia delante, pero se abre más a los lados. El sacro es corto y ancho. Se inserta como una moneda entra las dos alas ilíacas, asegurando de este modo la transmisión del peso de la parte superior del cuerpo. La distancia entre la articulación sacroiliaca y la del fémur –acetabulum- es corta, lo que favorece una transferencia de peso eficaz entre el tronco y las extremidades inferiores. La observación radiográfica pone de relieve una organización, en el interior del hueso, de los tramos óseos –los trabéculos- dedicados a reforzar esta región, por lo demás consolidada mediante pilares óseos. El acetabulum está reforzado asimismo en sus márgenes. Los detalles de estos refuerzos difieren de los que sabemos acerca de los chimpancés y de los hombres. Estos caracteres están asociados a una cabeza de fémur relativamente modesta, poco insertada en el acetabulum (Según Brunet y Picq, 2004).

El cuello del fémur –que solo existe en los homínidos bípedos- es largo, más bien esbelto y más desarrollado en sus dimensiones verticales. La parte larga del fémur –la diáfisis- forma un ángulo con la vertical. Esta anatomía en *valgus* hace converger el eje del fémur hacia las rodillas, otra característica compartida con el hombre. Sin embargo, la articulación de la rodilla parece más flexible. En efecto, en el hombre la anatomía de esta articulación presenta una disimetría, pues el desarrollo de sus partes laterales es más pronunciado –meseta tibial ligeramente cóncava y perfil elíptico del cóndilo lateral del fémur- y favorece el bloqueo de la rodilla en extensión en el curso de la marcha.

En los australopitecos, la meseta tibial ligeramente convexa y el cóndilo lateral del fémur denotan un perfil más circular. Estas diferencias –a las que se añaden detalles anatómicos relativos a la rótula, la inserción de los meniscos articulares y el tamaño de la espina tibial- sugieren el mantenimiento de la rodilla en posición flexionada en el curso de la marcha. A partir de la rodilla se encuentran caracteres que recuerdan de nuevo a los monos antropomorfos arborícolas, algo que confirma la anatomía del pie. El tobillo (hueso del tarso) permite movimientos más variados. Los huesos del tarso son largos, sin presentar por ello los caracteres asociados a la presencia de una bóveda plantar. Las primeras falanges son largas y curvadas, mientras que el dedo gordo es divergente. Las proporciones generales del pie revelan una longitud sensiblemente igual de sus tres partes (tarso, metatarso y falange). El metatarso y las falanges son relativamente más largas en los chimpancés, mientras que estas partes experimentan una regresión en el hombre a favor de un tarso alargado. La anatomía de las articulaciones sugiere un apoyo del pie sobre su borde lateral, o apoyo en *varus*, durante la marcha.

Los australopitecos poseían, pues, un conjunto de caracteres que se encuentran, en algunos casos, en los chimpancés, incluso en los hombres, y otros que les son propios. Su aparato masticador es más robusto que el de los chimpancés y el de los hombres, su cerebro es relativamente más desarrollado que el de los chimpancés, su bipedación menos eficaz que la de los hombres. Sin embargo, sería erróneo considerarlos intermedios morfológicos entre los chimpancés –a los que con excesiva frecuencia se considera todavía una imagen del antepasado de los homínidos- y los hombres, forzosamente más evolucionados.

1.5. Morfometría geométrica

La *morfometría geométrica* tiene como objetivo el estudio de la variación de la forma y su relación con otras variables (Bookstein, 1991; Dryden y Mardia, 1998). La morfometría tradicional se basa en la aplicación de análisis estadísticos multivariados de variables cuantitativas como la longitud, la anchura o la altura. No obstante estos métodos normalmente no acababan de eliminar los efectos estrechamente ligados del tamaño (Bookstein et al., 1985) distorsionando las observaciones. Por esta razón, los esfuerzos se centraron en elaborar métodos de corrección del tamaño, a fin de estudiar las variables sin el factor tamaño y así observar los patrones de forma en las muestras analizadas (Sundberg, 1989; Jungers et al.; 1995).

David Kendall y otros estadísticos desarrollaron una rigurosa teoría estadística para el análisis de la forma a través de la geometría que hizo posible el uso combinado de estadística multivariante y de métodos para la visualización directa de la forma biológica del objeto estudiado. Bookstein se refirió a ello como la “síntesis morfométrica” (Bookstein, 1996a).

La geometría morfométrica empieza con la recolección de coordenadas bi o tridimensionales de hitos definibles biológicamente también denominados landmarks. Analizar estos hitos de forma directa como variables no es posible debido a que los efectos de variación en la posición, orientación y escala de los especímenes aún está presente. Es por ello que después de obtener los landmarks (o hitos) se procede a la eliminación de la variación no asociada a la forma matemáticamente antes de analizar las variables.

El *método de superposición* elimina la variación no asociada a la forma a través del solapamiento, mediante algún criterio de optimización. Se han propuesto numerosos métodos, como, por ejemplo, el de registro de dos puntos de una superposición simple creado por Bookstein. El análisis generalizado de Procrustes (GPA, también denominado mínimos cuadrados generalizados o GLS) superpone las configuraciones de los landmarks usando la estimación de mínimos cuadrados para los parámetros de translación y rotación. En primer lugar, se traslada al origen el centroide de cada configuración, y se escalan las configuraciones de los diferentes especímenes respecto a un único y común tamaño entro todos ellos (dividiéndolo por el centroide, Bookstein 1986). Finalmente, las configuraciones se rotan de forma óptima para minimizar las diferencias cuadradas entre los diferentes landmarks (Gower, 1975; Rohlf y Slice, 1990). El proceso es iterado para computar la forma media, la cual no se puede estimar antes de la superposición (Figura 13).

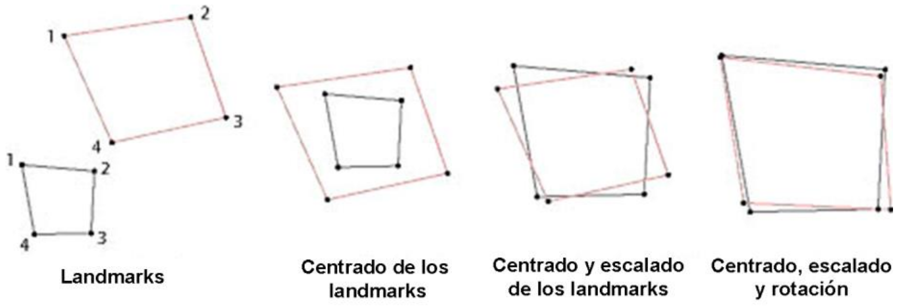


Figura 13: Procedimiento del GPA

Tras la superposición, las diferencias de forma pueden describirse como las diferencias en las coordenadas de los correspondientes landmarks entre los objetos. Estas diferencias también se pueden usar como datos para comparaciones mediante análisis multivariantes de la variación de la forma (Bookstein 1996b). A parte de los análisis multivariantes, también se pueden observar los cambios de forma en las rejillas de deformación (thin-plate spline o TPS), las cuales sirven como mapas de deformación de un espécimen a otro (Bookstein, 1991). Las diferencias de forma representadas mediante este método son rigurosas representaciones matemáticas de las rejillas de deformación de Thompson (1917), en las que un objeto es deformado (*warped*) en otro. De este modo las diferencias de forma entre objetos pueden ser así descritas en términos de diferencias en la deformación de las rejillas pertenecientes a cada objeto. Los parámetros que describen estas deformaciones (*partial warp scores*) pueden ser usados como variables de forma para comparaciones estadísticas de la variación de la forma en la población y entre poblaciones. Una de estas aproximaciones que ganó importancia en los años 90 fue el *relative warp analysis*, un análisis de componentes principales de los *partial warps*.

Hoy en día casi todos los estudios morfométricos basados en landmarks analizan las variaciones de forma a través del espacio de forma de Kendall, las distancias Procrustes o sus aproximaciones tangenciales a dicho espacio. Todo esto se debe a que cada vez se ha hecho más patente que todos los estudios basados en el especio de forma de Kendall (Figura 14) muestran el mejor potencial estadístico, así como el mínimo error en la media de cuadrados, imponiendo mínimas restricciones en los patrones de variación detectables (Kent, 1994; Rohlf, 1999; 2000a; 2000b). Además, estos métodos están basados en la teoría estadística de cómo se define la forma (Kendall, 1977) y como los patrones de variación de forma pueden analizarse (Kendall, 1984; 1985; Small, 1996).

Utilizando esta aproximación, el análisis de los landmarks puede ser resumido en un **Análisis Generalizado de Procrustes** (GPA), seguido por una **proyección** de estas coordenadas alineadas en un espacio tangencial lineal para un análisis multivariado, y una **visualización** gráfica de los resultados en términos de la configuración de los landmarks. El GPA es un procedimiento importante debido a que elimina la variación en la digitalización de los landmarks, en la orientación y en la escala, además de superponer los objetos en un sistema de coordenadas común.

Además los especímenes alineados a través del espacio tangente lineal del GPA proveen puntos que pueden proyectarse sobre el espacio tangencial al espacio de forma de Kendall (Kendall, 1984; Rohlf, 1999; Slice, 2001). En este último espacio tangencial lineal (Figura 14), las distancias entre pares de puntos (de especímenes) se aproximan a las distancias Procrustes entre las correspondientes configuraciones de pares de landmarks.

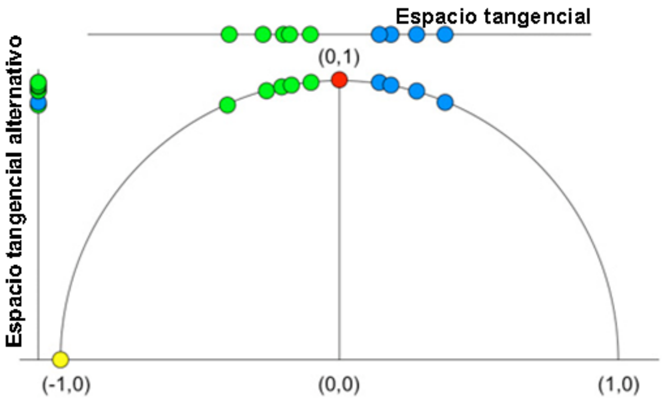


Figura 14: Proyección de los landmarks en el espacio tangencial de Kendall

El análisis de los *partial warps* de la rejilla de deformación (Thin-plate spline) (el cual describe los cambios de forma que pueden ser expresados por deformaciones locales (Bookstein, 1991)) más los componentes de forma uniformes (que describen los cambios de forma que pueden ser descritos por una escala infinita de estrechamiento o compresión (Bookstein, 1996c; Rohlf y Bookstein, 2003), son un conveniente **conjunto de variables de forma** que pueden ser interpretadas como ejes para el espacio lineal tangente. Los valores en estos ejes pueden ser tratados como valores multivariables que representan **forma**, y pueden utilizarse en análisis mutivariantes convencionales (Caldecutt y Adams, 1998; Bookstein et al., 1999; Adams y Rohlf, 2000; Gharaibeh al., 2000; Rüber y Adams, 2001; Klingenberg y Leamy, 2001). Cabe destacar que las rejillas de deformación, ampliamente asociadas a los estudios de morfometría geométrica no son un requisito indispensable para esta metodología, cualquier proyección ortogonal al espacio tangente es suficiente (Rohlf, 1999).

El paso final de un análisis de morfometría geométrica es la visualización en los gráficos de los resultados de los análisis estadísticos. Uno de los puntos más fuertes y característicos de la morfometría geométrica es que pueden representarse gráficamente los resultados, en términos de las configuraciones de los puntos más que como diagramas de dispersión estadísticos habituales. Ello es posible porque la morfometría geométrica **conserva la geometría de la forma** durante todo el análisis. Típicamente, las diferencias en forma se presentan de una manera análoga a las rejillas de deformación de D'Arcy Thompson (1917), en donde un objeto, por lo general, se deforma en otro usando la rejilla de deformación (Thin-plate spline). Así las diferencias en la forma entre los objetos se pueden describir en términos de diferencias en las rejillas de deformación que representan los objetos.

1.6. La radiografía digital y las imágenes de alta resolución

Willhelm Roentgen, profesor de física experimental en Alemania, descubrió los rayos X en 1895 mientras trabajaba en las emisiones de la corriente eléctrica en el vacío. A lo largo de los años la técnica se ha ido refinando al igual que la maquinaria utilizada para ello. En la actualidad se encuentran instalaciones de radiología en cualquier hospital y unidades de emergencias.

Uno de estos grandes cambios a lo largo de la historia de la radiografía ha sido la fase computacional. La aplicación de ordenadores al ámbito de la radiografía y de las técnicas de imagen diagnóstica ha sido algo inevitable. La radiografía digital se empezó a introducir en el mundo médico a mediados de los 80, y su aumento en su popularidad se debió al incremento de la calidad obtenida que ha desplazado prácticamente a la radiografía tradicional en todo el sistema sanitario.

Las radiografías son el punto de partida para el diagnóstico de gran variedad de situaciones clínicas. Tiene claras ventajas respecto a otras técnicas, como son su fácil accesibilidad, familiaridad para los profesionales de la medicina, son poco invasivas y la rapidez de obtención de las imágenes de gran resolución y contraste. Es por ello que son una de las técnicas más usadas en la actualidad.

La radiografía clásica, ampliamente utilizada hasta hace relativamente pocos años, tenía numerosos inconvenientes: como la dosis de radiación fija para cada determinado caso o región anatómica, la escala de grises de las imágenes que no podían modificarse, y la limitada posibilidad de reducir la radiación al paciente. Además, una vez obtenida la placa, ésta ya no puede ser modificada, la gestión de los residuos (papel radiográfico), además del coste del papel, y el almacenaje a largo plazo era costoso y espacioso además de que las placas perdían calidad con el paso de los años. Añádase que la radiografía clásica no es compatible con los nuevos sistemas de almacenamiento de imágenes PACS.

La radiografía digital ha evolucionado ampliamente desde sus inicios y en diferentes vías. En la radiografía digital o computarizada, una placa de fósforo fotosensible es usada para la detección de los rayos X en vez de las películas convencionales. La placa expuesta es escaneada por un láser de helio-neón que provoca que ésta emita una luz que es capturada por un tubo fotomultiplicador y convertida a un sistema electrónico analógico que genera la digitalización. Otra técnica se basa en un sensor que capta la radiación directamente y convierte los rayos X en señal eléctrica, eliminando de esta forma el paso intermedio de la imagen latente y del escaneo.

Los rayos X son producidos al bombardear un objetivo metálico con electrones de alta energía. En la radiografía convencional los rayos X pasan a través del cuerpo y estos son absorbidos, lo cual causa atenuación de la placa en la que inciden posteriormente. Son los cambios de radiación del haz de rayos X los que se imprimen en la placa posteriormente. El contraste en una imagen de rayos X depende de la atenuación diferencial de éstos rayos al pasar a través de los diferentes órganos y tejidos.

En la radiografía digital (Figura 15), la producción de la imagen está compuesta por 4 fases, **la adquisición de la imagen**, **el procesamiento de ésta**, **el**

almacenamiento y **la proyección**. Cada uno de estos pasos puede ser optimizado de forma aislada al resto y obtenerse así una eficacia y definición mucho mayor.

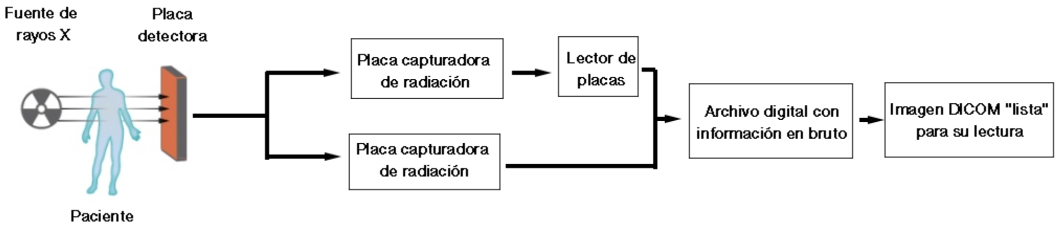


Figura 15: Esquema de funcionamiento de la radiografía digital

En la radiografía digital el detector debe ser capaz de captar pequeñas cantidades de energía así como tener un amplio rango dinámico, para detectar sutiles cambios sin añadir artefactos a la imagen. Las placas de fósforo utilizadas en la radiografía digital son de 2 a 4 veces más rápidas que los films clásicos. **Una eficacia mayor implica una dosis de radiación inferior para el paciente en contraste con la** calidad similar o superior obtenida. La calidad en el sistema digital depende de la calidad del equipo de rayos X, de la dosis aplicada, y adicionalmente del tamaño y profundidad del píxel, y el ratio de ruido de la señal y del rango dinámico.

El hecho de que la radiografía digital tenga fases separadas conlleva ventajas, como la reutilización de las placas, la posibilidad de procesar una imagen después de su adquisición, sin importar el tiempo, y poder compartir las imágenes a través de la red. Además, puede almacenarse gran cantidad de información en un espacio relativamente más pequeño que en el caso de las radiografías clásicas, además de un acceso al material mucho más rápido

La imagen digital cuenta con una calidad igual o superior a la calidad de la radiografía clásica (Swee, 1997). Las técnicas de ampliación en la radiografía computarizada pueden superar las limitaciones impuestas por la resolución espacial limitada en ésta (Nakano et al., 1987).

El sistema de almacenamiento de imágenes electrónicas o PACS (Arenson, 1988), se ha desarrollado al mismo tiempo que la radiografía digital, desde mediados de los 80 y ha ido evolucionando desde entonces. La popularidad de la radiografía digital, así como de otras técnicas digitales, como las tomografías computarizadas (TACS) o las resonancias magnéticas, ha provocado una expansión del uso de los

PACS como estándar para el almacenamiento de gran cantidad de información gráfica.

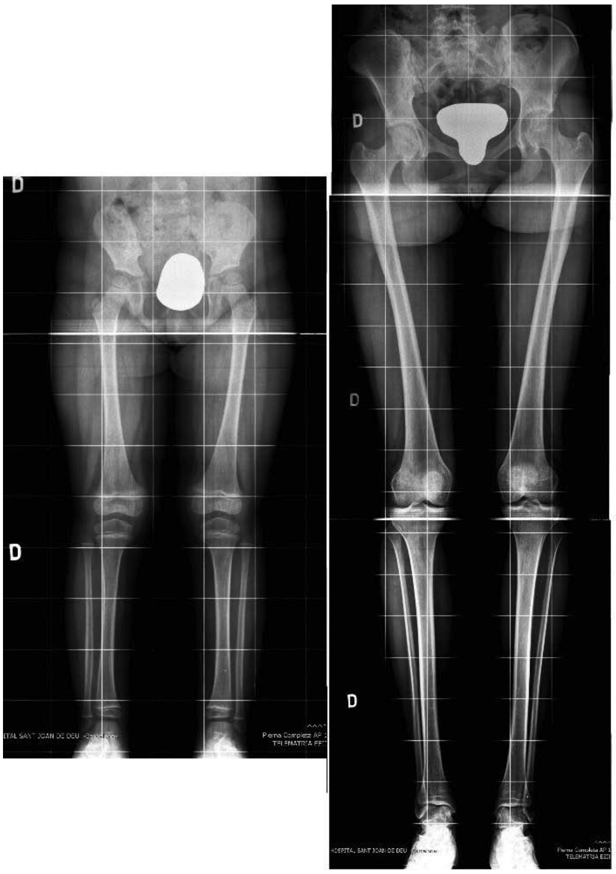
Figura 16: Máquina de rayos X utilizada para la realización de telemetrías

Para poder trabajar con PACS el hospital requiere de ciertas instalaciones para poder aprovechar todas las posibilidades que ofrecen, desde la adquisición de las imágenes, la proyección de copias en el monitor, transmisión de las imágenes a la red, almacenamiento de las imágenes, acceso a la información radiológica del hospital, y finalmente creación de copias físicas. Para todo ello se utilizan los potentes ordenadores (*workstations*).

Para trabajar con las PACS se ha procedido a generar un estándar para que cualquier ordenador de condiciones adecuadas (*workstation*), pueda acceder y trabajar con estas imágenes. Este estándar es el denominado DICOM (Digital imaging and communications in Medicine) (Spilker, 1989).

Las telemetrías utilizadas en este estudio son imágenes radiográficas en las que el paciente se encuentra de pie, en contacto con la placa fotosensible, perpendicular a los rayos y a una distancia de dos metros del aparato emisor. De esta forma la imagen que se obtiene no presenta ningún tipo de deformación ya que los rayos que recibe la placa son perpendiculares, no llegando a ella los rayos con diferente inclinación y que provocan opacidades y deformaciones en la imagen (Figura 16). Los diferentes fines clínicos para los que se obtienen las imágenes radiológicas requieren o permiten diferentes grados de calidad en las imágenes finales y, en consecuencia, hay que ajustar la dosis.

Figura17: Telemetrías de la extremidad inferior, izquierda niño de 2 años, en la derecha niña de 17 años



Los estudios de medición morfológica, como la telemetría de extremidades inferiores (Figura 17), son buenos candidatos para aplicar métodos de reducción de dosis al paciente.

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2. Objetivos

2. OBJETIVOS

Los objetivos de esta Tesis Doctoral son:

1. Aportar información sobre el desarrollo postnatal del fémur y la tibia, tanto en individuos masculinos como femeninos, desde el nacimiento hasta el final del brote puberal.

2. Describir los patrones diferenciales de los cambios en la forma y el tamaño entre chicos y chicas mediante morfometría geométrica.

3. Aportar información del fémur y la tibia para la estimación de la edad de individuos actuales vivos de la población mediterránea, especialmente de la población española.

4. Elaborar una base de datos amplia y representativa de los individuos subadultos de la población actual viva española.

5. Comprobar la utilidad de las técnicas de morfometría geométrica para el estudio de los cambios de forma durante el crecimiento de un hueso largo como es el fémur, mediante la aplicación de hitos (landmarks) en determinadas regiones del hueso. Además, comprobar si la ubicación de estos landmarks suministran información suficiente que permitan apreciar los cambios que se dan durante el desarrollo humano.

INFORME DE LOS DIRECTORES

Dr. Daniel Turbón y Dra. Carme Rissech, supervisores de la tesis doctoral titulada "*Ontogénesis postnatal de la extremidad inferior basada en telemetrías y morfometría geométrica. Aplicación en paleoantropología y antropología forense*", certifican que la disertación presentada aquí ha sido desarrollada completamente por Aniol Pujol Bayona, y garantizan su derecho a defender su tesis doctoral en frente de un comité científico.

Como supervisores, han contribuido en el diseño, la guía y la corrección de los borradores de los diferentes capítulos así como de los artículos escritos por el doctorando. Esta tesis consta de 4 artículos científicos, presentados como apartados de esta tesis. Cada artículo ha sido publicado, o será publicado en un corto periodo de tiempo, en una revista científica con Factor de Impacto determinado por el JCR. La contribución del doctorando a cada manuscrito se relata a continuación, así como el nombre de la revista y el factor de impacto de ésta.

Apartado 3.1:

Pujol A, Rissech C, Ventura J, Badosa Q, Turbón D. 2014. Ontogeny of the female femur: geometric morphometric analysis applied on current living individuals of a Spanish population. *Journal of Anatomy* 225:346-57.

Factor de Impacto: 2.097 (2014)

El doctorando ha contribuido en este artículo en los siguientes campos: I) Diseño del estudio; II) Recolección de los datos; III) Análisis de los datos; IV) Redacción del artículo.

Apartado 3.2:

Pujol A, Rissech C, Ventura J, Badosa Q, Turbón D. 2015. Ontogeny of the male femur: Geometric morphometric analysis applied to a contemporary Spanish population. *American Journal of Physical Anthropology*. DOI: 10.1002/ajpa.22846.

Factor de Impacto: 2.379 (2014)

El doctorando ha contribuido en este artículo en los siguientes campos: I) Diseño del estudio; II) Recolección de los datos; III) Análisis de los datos; IV) Redacción del artículo.

Apartado 3.3:

Pujol A, Rissech C, Turbón D. 2015. Postnatal development of the femur based on a Spanish sample of living individuals. Implications for age estimation. *International Journal of Legal Medicine* (sometido)

Factor de Impacto: 2.714 (2014)

El doctorando ha contribuido en este artículo en los siguientes campos: I) Diseño del estudio; II) Recolección de los datos; III) Análisis de los datos; IV) Redacción del artículo.

Apartado 4.1:

Pujol A, Rissech C, Turbón D. Preliminar study of tibial development in a subadult Spanish living sample. *Forensic Science International* (por someter)

Factor de Impacto: 2.140 (2014)

El doctorando ha contribuido en este artículo en los siguientes campos: I) Diseño del estudio; II) Recolección de los datos; III) Análisis de los datos; IV) Redacción del artículo.

Además certifico que ninguno de los manuscritos utilizados en esta tesis se han usado para ninguna otra tesis.

Barcelona, 2 de Noviembre de 2015

Daniel Turbón Borrega

Departamento de Biología Animal
Facultad de Biología
Universidad de Barcelona

Carme Rissech Badalló

Departamento de Ciencias Médicas
Facultad de Medicina
Universidad de Girona

3. Estudio de la ontogenia del fémur

3.1. ONTOGENY OF THE FEMALE FEMUR: GEOMETRIC MORPHOMETRIC ANALYSIS APPLIED ON CURRENT LIVING INDIVIDUALS OF A SPANISH POPULATION

Aniol Pujol,[1] Carme Rissech,[1] Jacint Ventura,[2] Joaquim Badosa[3] and Daniel Turbón[1]

[1]Unitat d'Antropologia Física, Departament de Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

[2]Unitat de Zoologia, Departament Biologia Animal, de Biologia Vegetal i d'Ecologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Barcelona, Spain

[3]Unitat de Radiogiagnòstic de l'Hospital Sant Joan de De'u de Barcelona, Barcelona, Spain

ABSTRACT

In this study we describe the development of the female femur based on the analysis of high-resolution radiographic images by means of geometric morphometrics, while assessing the usefulness of this method in these kinds of studies. The material analysed consisted of digital images in DICOM format (telemetries), corresponding to 184 left femora in anterior view, obtained from the database of the Hospital Sant Joan de Deu of Barcelona (Spain). Bones analysed corresponded to individuals from 9 to 14 years old. Size and shape variation of the entire femur was quantified by 22 two-dimensional landmarks. Landmark digitisation errors were assessed using Procrustes ANOVA test. Centroid size (CS) variation with age was valuated by an ANOVA test. Shape variation was assessed by principal component analysis. A MANCOVA test between the first five principal components and age, using the CS as covariable, was applied. Results indicated that both size and shape vary significantly with age. Several age-related shape changes remained significant after removing the allometric effect. In general, an increase in the robustness of the bone and noticeable phenotypic changes in certain areas of the femur were observed. During growth in the proximal region of the femur, the collo-diaphyseal angle decreases, the neck of the femur widens and the fovea moves to a lower position, standing more in line with the plane of the neck. Likewise, the size of the greater and lesser trochanters increase. In the distal region, a significant increase of epiphyseal dimensions was recorded, mainly in the medial condyle. The angular remodelling of the neck and the bicondylar region of the femur in females continues until 13 years old. The information provided in the present study increases our knowledge on the timing and morphology of the femur during development, and in particular the morphology of the different femoral ossification centres during development.

Key words: development; female femur; geometric morphometric; lower limb; ontogeny; sub-adult individuals.

INTRODUCTION

Developmental studies on the human skeleton are very important for: (i) reconstructing demographic profiles in past populations; (ii) estimating sub-adult age in skeletal remains; (iii) the biological identification of living individuals in legal proceedings where a minor is involved; and (iv) the determination and interpretation of many factors, including indicators of health status and living conditions. Methods of age estimation in infant and juvenile individuals rely on the degree of skeletal and dental development. Based on the skeletal age of the individual, these methods provide a specific age range based on the degree of bone growth and development. Therefore, to estimate the biological age of living sub-adult individuals or in a sub-adult skeletal sample, it is essential to understand the normal pattern of growth and maturation of every skeletal element, and to develop the corresponding growth model. These growth models must be devised from two fully documented sources: (i) skeletal collections provided with information on age, sex, ancestry, pathology and cause of death; or (ii) on radiographic material of high resolution, which does not distort the actual measurements. It is even better if the demographic, socioeconomic and temporal contexts in which the individuals lived are also known (López-Costas et al. 2012; Rissech et al. 2013a). It is essential that the origin of the reference sample used in the creation of the method is biologically comparable to the population under study, because the accuracy of the estimations depends on the application of appropriate data relating to the growth and maturation of the skeletal elements with regard to genetic, environmental and cultural factors (Biewener & Bertram, 1993; Slemenda et al. 1994; Arden & Spector, 1997; McGuigan et al. 2002).

However, despite the importance of having a register of growth and developmental data for different skeletal elements and populations, there is still a gap in the studies based on direct or radiographic material from which actual measurements can be taken. In fact, these studies are scarce because of the lack of sub-adult skeletal collections (Rissech, 2008), and because until recently there has not been appropriate radiographic material of high resolution, such as multislice computerised tomographies and telemetries (García et al. 2010).

Although there is a relatively large number of studies on human skeletal growth (Greulich & Thoms, 1938; Reynolds, 1945, 1947; Ghantus, 1951; Maresh, 1955; Greulich, 1960; Garn, 1962; Tanner, 1962; Coleman, 1969; Pyle et al. 1971; Gindhart, 1973; Tanner et al. 1976; Hoffman, 1979; Alduc-le Bagousse, 1988; Gasser et al. 1985, 1991; Hoppa, 1992; Miles & Bulman, 1994, 1995; Majó, 2000; Rissech et al.

2001, 2003, 2008, 2013a,b; Rissech & Malgosa, 2005, 2007; Rissech & Black, 2007; Schillaci et al. 2011, 2012; López-Costas et al. 2012 among others), very few works have been based on the study of material from Western European children (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles & Bulman, 1994, 1995; Majó, 2000; Rissech et al. 2001, 2003, 2008, 2013a,b; Rissech & Malgosa, 2005, 2007; Rissech & Black, 2007; López-Costas et al. 2012), and particularly on children of Spanish populations (Rissech et al. 2013b). Most of these latter studies have been based on archaeological material, in which age and sex were estimated in the laboratory (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles & Bulman, 1994, 1995; Majó, 2000). Analyses on documented skeletal collections are scarce and restricted to specific skeletal elements, such as the pelvic bone (Rissech et al. 2001, 2003; Rissech & Malgosa, 2005, 2007), scapula (Rissech & Black, 2007), tibia (López-Costas et al. 2012) and humerus (Rissech et al. 2013a). The femur is a lower limb skeletal element that is anthropologically important for its locomotor function (Scheuer & Black, 2000), and because of its robustness and post-depositional resistance. Despite the anthropological and forensic interest of the femur and the amount of research pertaining to this bone (Scheuer & Black, 2000), only a study of its developmental osteology has been conducted on documented skeletal individuals originating from Western Europe (Rissech et al. 2008). We have also encountered no femoral growth studies based on telemetries and geometric morphometric analysis, and even less femoral growth studies based on the current Spanish living population. For all this, it is important to complete and detail the postnatal development of the skeleton in Western European populations, and specifically in those from the Iberian Peninsula. Additionally, this information would be useful in palaeoanthropology and forensic anthropology.

Landmark-based geometric morphometrics (Bookstein et al. 1985; Bookstein, 1991; Reyment, 1991; Rohlf, 1999, 2000a,b) is a powerful tool because it permits the discrimination of anatomical differences through detailed shape analysis. Statistical analyses applied to landmark-based data allow testing hypotheses about the factors that affect the phenotype. Although geometric morphometrics is an appropriate procedure to analyse shape differences in skeletal structures (Lockwood et al. 2002; Pretorius et al. 2006; Scholtz et al. 2010; Toro-Ibacache et al. 2010), so far there have been no studies applying their method to track the development of human postcranial skeletal elements.

Our aim is to provide information on the postnatal development of the femur in the current living Spanish population. However, because of the extent of the subject we divided the study into two sub-studies, one for female and one for male femurs. For this reason, this first study will focus exclusively on the female femur, and we evaluate the morphological changes of the femur in females between 9 and 14 years old by applying landmark-based geometric morphometric techniques.

MATERIALS AND METHODS

High-resolution digital radiological images (telemetries) in Digital Imaging and Communication in Medicine (DICOM) format from the archives of Hospital Sant Joan de Déu de Barcelona were used for this analysis. All personal data were omitted with the exception of the demographic information (sex and age) following the Spanish Organic Law of Data Protection (Ley Orgánica de Protección de Datos; for details see García et al. 2010). This study has the approval of the Bioethical Committee of the Hospital Sant Joan de Déu de Barcelona (Ref. number 2938). The telemetries were chosen for this study due to the absence of a significant image deformation involved. This improvement of the image quality is the result of both the distance at which telemetries are taken (more than 180 cm from the centre of the X-ray focus and the subject) and the contact that the subject has with the radiographic film (it is in the bucky mural in telemetries). The bucky is a grid used in conventional radiology that selectively filters the radiation produced by the X-ray machine and eliminates non-perpendicular rays to the chassis that produce radiographic opacities that corrupt the image. For these reasons, these distant types of high-resolution digital radiographies are routinely used for medical purposes of evaluation and accurate measurement of the limbs (telemetries) and rachis (vertebral column radiographies, also known as scoliograms).

All the telemetries were taken in the Hospital Sant Joan de Déu following the standardised procedure of the hospital. The subject was made to undress from the waist down and to stand upright with the back of his body on the bucky mural for the purpose of telemetry, with arms extended along the body, knees and feet together, and hips and knees in full extension, the patellae facing forward with tibiae vertical and femora with slight internal rotation as is the natural anatomical position in this vertical position. There was equal weight bearing on both limbs. The tube was focused perpendicularly at the inferior angle of the patellae in the knee; the film-focus distance was 200 cm. Exposure was 120 Kvat 50–60 mA s^{-1}.

The left femora in anterior view of living girls aged from 9 to 14 years were analysed. This age range was selected because the female growth spurt takes place in this age interval (Tanner et al. 1981; Tanner, 1986). Those individuals displaying any type of pathology or anatomical deformation that could affect the analysis were excluded. Femora of 184 girls were analysed (girls of 9 years old: n=30; 10 years: n=30; 11 years: n=30; 12 years: n=32; 14 years: n=30).

Twenty-two bidimensional landmarks were digitised using the Thin Plate Spine program series (Rohlf, 2006). Landmarks were placed in the femoral regions that show greater changes during growth, such as the head, the fovea, the neck, the trochanter, and the distal diaphysis and epiphysis. These landmarks (Fig. 1) were the following: (1) the most medial point of the growth plate between the head and neck; (2) the most angulated point of the growth plate between the head and neck; (3) the most lateral point of the growth plate between the head and neck; (4) the lowest point of the fovea; (5) the most upper point of the fovea; (6) the lowest point of the narrowest region of the neck; (7) the most upper point of the narrowest region of the neck; (8) the most proximal point of the growth plate between the greater trochanter and the shaft; (9) the midpoint of the growth plate between the greater trochanter and the shaft; (10) the most distal point of the growth plate between the greater trochanter and the shaft; (11) the tip of the greater trochanter; (12) the most proximal point of the growth plate between the lesser trochanter and the shaft; (13) the tip point of the lesser trochanter; (14) the most distal point of the growth plate between the lesser trochanter and the shaft; (15) the midpoint of the medial aspect of the shaft; (16) the midpoint of the lateral aspect of the shaft; (17) the most medial point of the growth plate between the diaphysis and distal epiphysis; (18) the midpoint of the growth plate between the diaphysis and distal epiphysis; (19) the most lateral point of the growth plate between the diaphysis and distal epiphysis; (20) the most distal point on the medial condyle; (21) the intercondylar superior angle of distal epiphysis in the articular margin; and (22) the most distal point of the lateral condyle.

Measurement error is an important source of variation affecting morphometric data that can increase the likelihood of type II errors and lead to biased results (Arnqvist & Martensson, 1998; Bailey & Byrnes, 1990). In order to evaluate the impact of error on the current set of landmarks, each landmark was located on each radiograph 10 times over two separate days. A Procrustes ANOVA comparing variation in landmark location both among and within femora was performed (Klingenberg & McIntyre, 1998; Klingenberg et al. 2002). Because the variation between femora clearly exceeded that of the measurement error, we considered the effect of the measurement error in the landmark location process negligible (see Results).

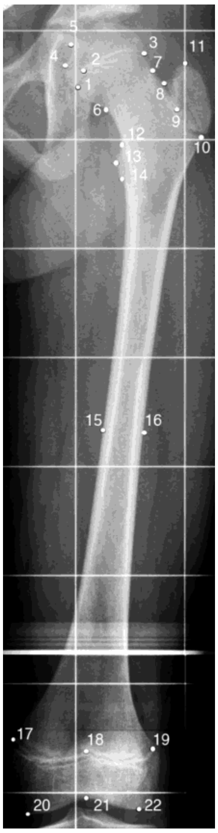


Figure 1: Landmark locations on the anterior aspect of the left femur in telemetry. For the definition of each landmark, see the text.

To test size-related differences during growth, an analysis of variance (ANOVA test) on centroid size (CS) was applied. In order to determine changes in femur shape related to age, a principal component analysis (PCA) of the covariance matrix of the partial warps scores (tpsRelw program, version 1.46; see also Rohlf, 1999) was performed. Allometry, assumed as size-dependent shape variation, was evaluated through a multivariate analysis of covariance (MANCOVA) on the first five principal components, using CS as covariate. Statistical analyses were performed using SPSS 16.

RESULTS

The Procrustes ANOVA showed high significant differences between femora, both in size and shape ($P < 0.0001$). The mean squares for femur size variation (MS femur = 115603.84) exceeded the mean squares for the replicates (MS error = 12.49) by 9254.66-fold. For shape, mean squares for femur variation (MS femur=0.000022) exceeded the mean squares for the replicates (MS error = 0.000011) by 1.92-fold. These results indicate low measurement error and consequently strong repeatability of the landmark location of the femur.

The ANOVA test showed that CS increased significantly with age (F = 4.496, *P = 0.032). This size variation was related to the growth spurt that takes place between 9 and 13 years old in the girls studied (Fig. 2). MANCOVA test revealed that, after removing the allometric effect, shape variation related to age was statistically significantly (Lambda de Wilks = 0.093, F = 2.652, *P = 0.003).

In the PCA, the first (PC1) and second (PC2) principal components explained 63.3% and 9.2% of the shape variation, respectively. Distribution of the different individuals in the shape space defined by these components (Fig. 3) showed that femur shape varied progressively according to age. The traits that accounted for this variation along PC1 were the robustness of the femur (Fig. 4), the verticality of the head with respect to the femur (Fig. 5), the angle of the neck of the femur with respect to its diaphysis (Fig. 5), the size of the distal epiphysis (Fig. 6), and the position of the greater trochanter (Fig. 5). The second principal component allowed the separation of the different age groups by the increase in size of the lesser and greater trochanter (Fig. 5), the increase of the diaphysis width (Fig. 4), and the increase of width of the femoral neck (Figs 4 and 5).

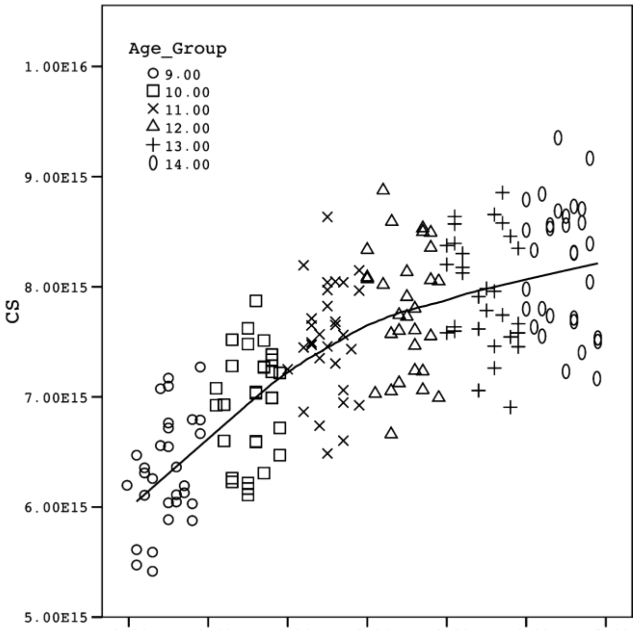


Figure 2: Scatter plot of age and CS, including the Lowess regression line.

In general terms, in younger individuals the head of the femur tends to be in a more vertical position in relation to the diaphysis (Fig. 4), the lesser trochanter is unfused and the greater trochanter is relatively large; meanwhile, the distal and proximal epiphysis are well developed but have not reached the final size. In older individuals, the whole femur gains robustness. The diaphysis and the femoral neck increase their diameter, and the femoral head assumes a more horizontal position relative to the shaft. The lesser and the greater trochanter enlarge considerably and, in turn, the greater trochanter holds a superior position in relation to the juvenile femur. The distal epiphysis also grows in size, but mainly focuses on increasing the medial condyle, giving rise to a greater femoral angle in relation to the central axis of the body, and the angle between the femoral neck and the diaphysis becomes smaller. All these changes were related to the angular femoral remodelling that occurs during growth, and according to our results they finish at the end of puberty (Figs 4–6).

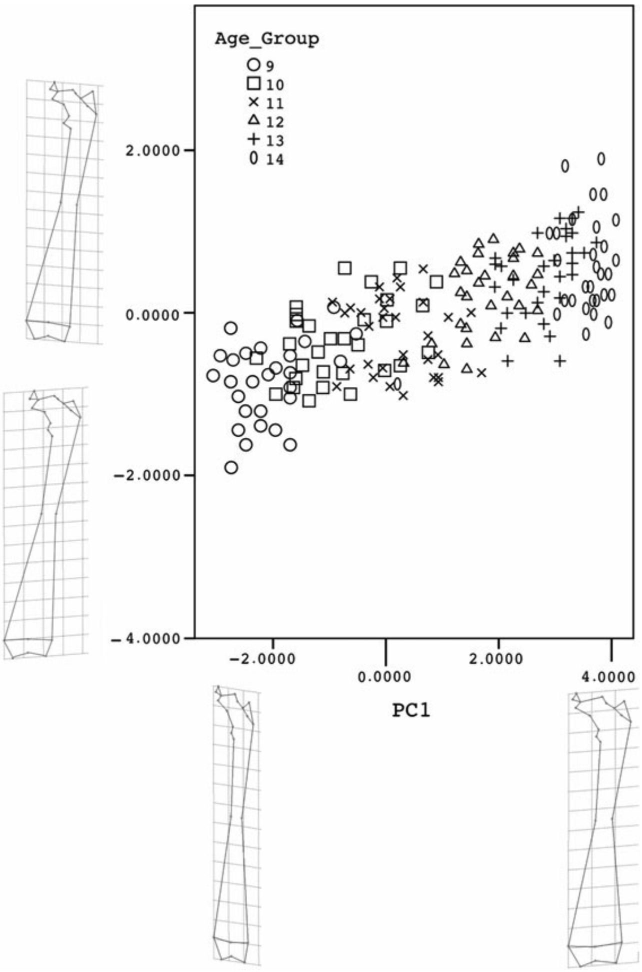


Figure 3: Ordination of the individuals in the space of the two first principal components (PC1, PC2) based on the weight matrix for the anterior aspect of the femur.

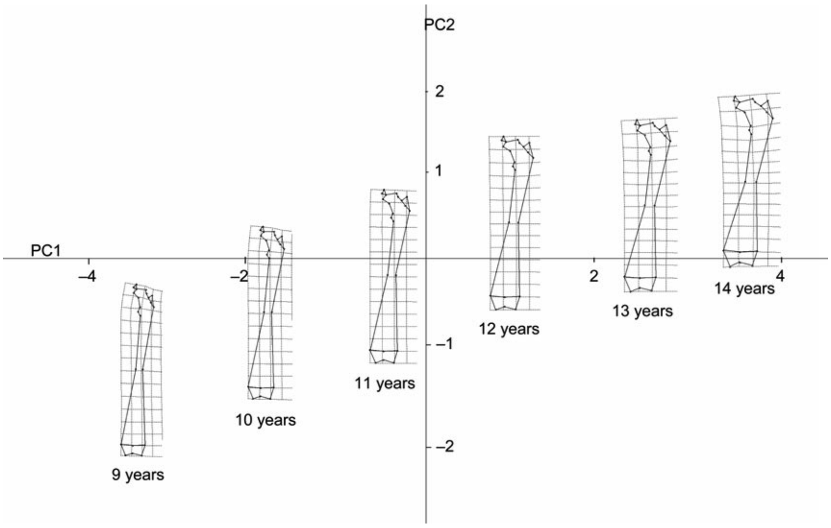


Figure 4: Graphic representation of the consensus form in each age of the analysed interval of age (from 9 to 14 years) based on the space of the two first principal components

In order to quantify the changes in the angulation of both femoral epiphyses, the collo-diaphyseal and bicondylar angles were measured and graphically represented in relation to the individual age (Table 1; Figs 7 and 8). The former is the angle formed by the intersection between the longitudinal axis of the femoral diaphysis and the longitudinal axis of the neck (Tardieu & Damsin, 1997). The bicondylar angle is that between the longitudinal axis of the diaphysis and a perpendicular line to the infracondylar plane of the femur (Shefelbine et al. 2002). The longitudinal axis of the femoral diaphysis is the midline along the diaphysis, which links the middle of the infra-condylar segment and the middle of the proximal segment of the femur, located 2 cm below the great trochanter. The longitudinal axis of the neck is the midline between the distal and proximal borders of the neck. Results reveal that both angles clearly change until 13 years old, when they tend to stabilise, and show divergent trends. Thus, during this period, whereas the collo-diaphyseal angle decreases, the bicondylar angle shows a substantial increase.

DISCUSSION

Geometric morphometrics and associated statistical analyses allowed us to describe the morphological changes in the female femur between 9 and 14 years old. Results revealed that both the size and the shape of the femur vary significantly during this period. Due to the presence of the pubertal growth spurt within this age interval, the size increase of the femur is one of the most significant changes observed in this bone, increasing first in length and later in robustness. These results are in accordance with those by other authors who suggest that the elongation of the femur precedes its diameter (robustness) and mass increases (Bass et al. 1999; Bradney et al. 2000; Rauch et al. 2001). In fact, mass and muscle strength are important for bone development in robustness (Van der Meulen et al. 1993, 1996, 2002; Moro et al. 1996; Schoenau, 1998, 2005; Schoenau et al. 2000). By raising the muscular load, biomechanical forces increase, leading to the development of higher robustness (Schiessl et al. 1998; Schoenau et al. 2000; Ruff, 2003). The gain in muscle mass is generally higher in boys than in girls due to the increase of testosterone during male growth (Round et al. 1999). Consequently, the ratio of bone to muscle is higher in females than in males (Hogler et al. 2008), and hence the femur of the girls is less robust (Ruff, 2003).

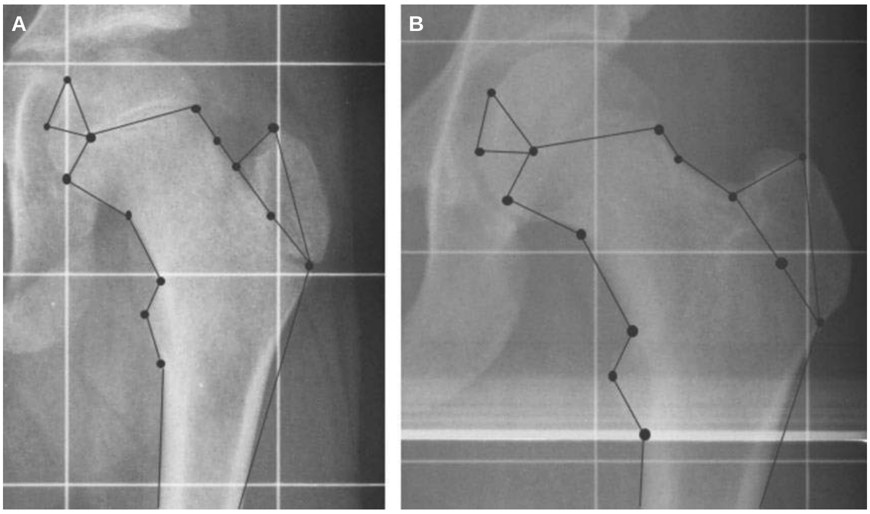


Figure 5: Shape changes in the proximal femur between the individuals who were (A) 9 years old and (B) 14 years old.

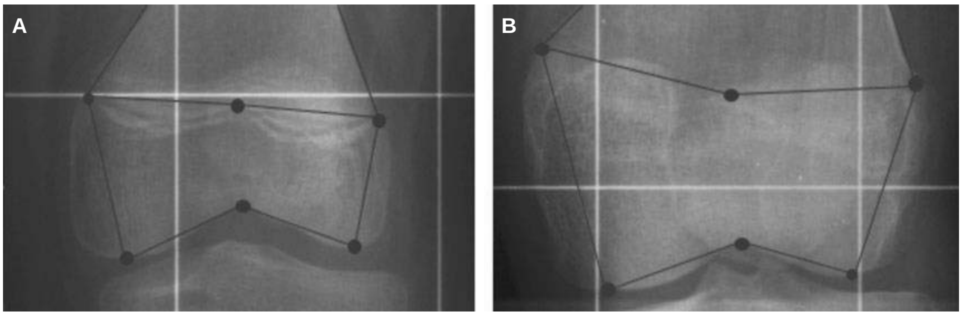


Figure 6: Shape changes in the distal epiphysis between individuals who were (A) 9 years old and (B) 14 years old.

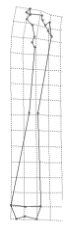
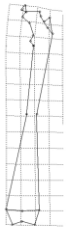
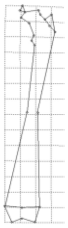
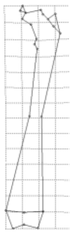
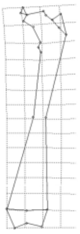
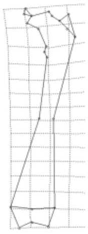
Age		Bicondylar angle	Collo-diaphyseal angle	Consensus Form in each specific age
9				
	Mean	6.92	137.00	
	n	30	30	
	DS	0.53	3.52	
10				
	Mean	8.72	133.36	
	n	30	30	
	DS	0.50	3.24	
11				
	Mean	9.51	130.35	
	n	30	30	
	DS	0.33	4.68	
12				
	Mean	10.48	127.39	
	n	30	30	
	DS	0.57	3.51	
13				
	Mean	11.09	125.92	
	n	30	30	
	DS	0.42	3.70	
14				
	Mean	10.80	123.95	
	n	31	31	
	DS	0.45	2.77	

Table 1: Descriptive statistics of the bicondylar and collo-diaphysal angles by age with the corresponding consensus form in each age.

The shape changes associated with growth were significant even after removing the allometric effect. Among these changes, those in both proximal and distal epiphyses are outstanding, due to the angular remodelling of the femur determined by a more horizontal position of the femoral neck (angular decrease of the femoral neck) and the size increase of the medial condyle of the distal epiphysis (bicondylar angular increase).

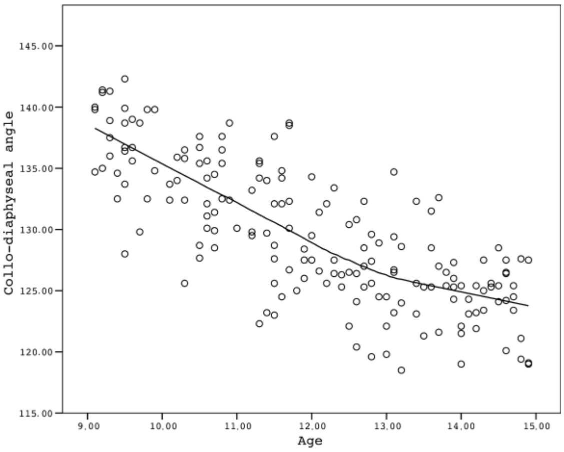


Figure 7: Collo-diaphyseal angle variation regarding age. The curve was calculated using the Lowess method.

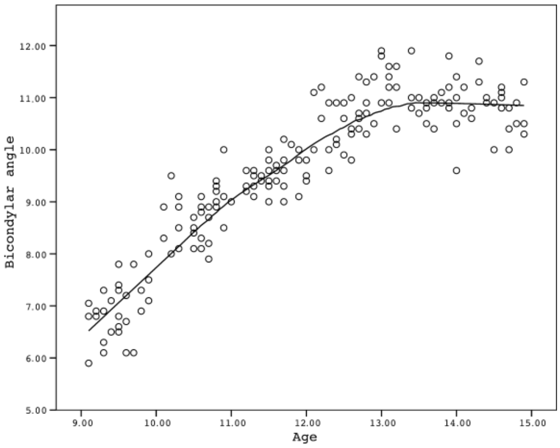


Figure 8: Bicondylar angle variation regarding age. The curve was calculated using the Lowess method.

Results reported here indicate that the angular remodelling of the femur (collo-diaphyseal and bicondylar angles) takes place until approximately 13 years old in females, which corresponds to the mean age of the end of Spanish female puberty (12.8 years old according to Fernández-Méndez & Seara-Aguilar, 1994). This age falls in the middle of the normal age range (9.5–14.5 years) of growth spurt in girls in the current living population (Tanner, 1962; Gasser et al. 1991). The obtained average for collo-diaphyseal angle in each age group of our study is inside the normal range of variability given in current literature on the growth process of this variable (Billing, 1954; Muñóz-Gutiérrez, 2001). Our sample starts with a value of 137° at 9 years old, and reaches 123.95° at 14 years old, which falls within the normal range of variation (122.3°-135.9º) for adult European women (Anderson & Trinkaus, 1998). Beyond 13 years old, the collo-diaphyseal angle stabilises in our sample, which is congruent with the results obtained in other studies that have shown that the values of this angle are very stable from mid-adolescence throughout most adulthood (Humphry, 1889; Yamaguchi, 1993; Anderson & Trinkaus, 1998). The morphology of the femoral diaphysis changes considerably throughout growth (Scheuer & Black, 2000). The proximal epiphysis, the neck, the head and both trochanters increase in size. During the early stages of life, the angle of the femoral neck with the shaft is more obtuse, namely the neck has a more vertical position, as the abductor muscle develops in response to the start of locomotion. The collo-diaphyseal angle decreases from an average of 150° at birth to approximately 127° at the end of growth after the end of the pubertal growth spurt (Humphry, 1889; Keats et al. 1966; Hefti, 2000; Igbigbi, 2003). Previous studies have identified considerable populational variability in the collo-diaphyseal angle (Anderson & Trinkaus, 1998; Igbigbi, 2003). This variability is related to both individual stature (Lofgren, 1956; Lusted & Keats, 1966; Singh & Singh, 1975; Igbigbi, 2003) and differential economic activity (Anderson & Trinkaus, 1998). This variable increases significantly across populations with an increasingly sedentary existence and with mechanisation (Anderson & Trinkaus, 1998). High angles commonly encountered in modern industrial societies cluster at the high end of normal recent human ranges of variation. Lower values, even below 120°, are by no means unusual in medically normal individuals in non-industrial societies (Anderson & Trinkaus, 1998). More recently, some authors have pointed out the correlation of this angle with climate (Gilligan et al. 2013).

Furthermore, females generally have smaller collo-diaphyseal angles than males because of their wider pelvis and shorter femur (Singh & Singh, 1975; Gulan et al. 2000; Igbigbi, 2003). In fact, the change in angulation of the femoral neck is not only associated with an increased mechanical load generated by the muscles and ligaments, but it is also depends on the widening of the pelvis, which is important in females at this age (Tague, 2005; Birkenmaier et al. 2010). With regard to the trochanters, size and shape are also conditioned by the increase of the mass of the gluteus maximus and minimus (greater trochanter), and pectineus (lesser trochanter) muscles.

The bicondylar angle is the skeletal feature that permits the adduction of the lower limb in humans. This is different from the tibio-femoral angle, which is the angle between the tibial and femoral diaphyseal axes in the coronal plane of the leg. Conversely, the tibio-femoral angle measures the evolution of a physiological phenomenon and not an osteological angular remodelling (Tardieu & Damsin, 1997).

The bicondylar angle allows the knee and ankle joints to be placed almost directly under the centre of gravity of the body, making the human displacements more economical, as the load transmitted by the lower limbs is close to the vertical axis of gravity of the body during the phases of walking and running. Due to its implication of bipedal walking, the bicondylar angle was one of the most important characteristics that permitted the attribution of some fossils to human lineage (LeGros Clark, 1947; Johanson & Coppens, 1976; Tardieu & Trinkaus, 1994), as it indicates a clear adaptation to bipedal locomotion (Tardieu & Damsin, 1997). Although this has palaeoanthropological significance and there is great angular remodelling that implies femur growth in humans (changing from a varus position in newborns to a valgus position in adults), only an extremely few studies have focused on the development of the bicondylar angle (Salenius & Vankka, 1975; Tardieu & Damsin, 1997). Our results are in agreement with those obtained previously in girls from 0 to 14 years old (Tardieu & Damsin, 1997), which propose a regression function to calculate the female bicondylar angle by age with 97% expressed variability (bicondylar angle = 4.1244 + 0.0396 x age in months). This function indicates a bicondylar angle of 6° at 10 months of post-natal life, an angle of 8° at 9 years old, and an angle of 10.8° at 14 years old.

The distal epiphysis reacts to the mechanical loads caused by the change of angulation of the femur. This shape modification results in pressures generated in the bicondylar region. The stress exerted by the different muscle groups as well as the patella generates an asymmetrical load in the condyles, focusing the force mainly on the medial condyle. This leads to a reaction in the endochondral cartilage of the medial condyle, causing a greater development of that condyle and consequently the angulation of the distal femur (Shefelbine et al. 2002). The bicondylar angle undergoes a major transformation from the beginning of locomotion to 8 years old and, according to our results, it continue after the femoral growth spurt before the completion of epiphyseal closure, which is in accordance with the observations of Tardieu & Damsin (1997). The age of stabilisation of this variable has remained an open question (Tardieu & Damsin, 1997) because of the scarcity of studies on this subject, and because the few studies that exist (Tardieu & Trinkaus, 1994; Tardieu & Damsin, 1997) do not have enough information to show it clearly. Most of the studies are based on the tibio-femoral angle (Salenius & Vankka, 1975; Cahuzak et al. 1995; Mehmet-Arazi et al. 2001) and they do not agree in relation to the stabilisation age of this variable. Selenius and Vankka stated that the tibio-femoral angle would stabilise at about 6 or 7 years old, and from this age the femur would grow in length maintaining a constant angle. Other authors affirm that this would take place at about 10 years old (Cahuzak et al. 1995), and others at about 12 years (Mehmet-Arazi et al. 2001). But the fact is that these studies are based on the development of the tibio-femoral angle not the bicondylar angle. In relation to the development of the bicondylar angle, Tardieu &

Damsin (1997) observed that in females it could stabilise at 13 years old. Our study clearly shows that the age of stabilisation in the Spanish female bicondylar angle is about 13 years old, after the female growth spurt and coinciding with the observations of Tardieu & Damsin (1997). In the present study, the average bicondylar angle in older individuals is 10.8°, which falls into to the current normal range of variation, between 8° and 15° (Pearson & Bell, 1919; Keats et al. 1966; Singh & Singh, 1975; Pandya et al. 2008), around the given value from the London population and higher than that given from the Paris population (London = 10.2°, Paris = 7.9°; Tardieu & Trinkaus, 1994). Like the collo-diaphyseal angle, the bicondylar angle varies between populations (Pandya et al. 2008) as a consequence of mechanical loading experienced by the femur due to diverse physical activity coupled with several genetic, cultural and environmental processes that take place in different individuals and populations (Tardieu & Trinkaus, 1994).

In summary, the angular remodelling of the femoral neck causes a displacement of the axis load, which results in it not coinciding with the central axis of the femoral diaphysis but intercepting it in the distal epiphysis. This change causes the knee to come nearer to the midline of the pelvis. This phenomenon is associated with an increase of the bicondylar angle and coincides with the development of a more efficient locomotion, which is given by the knee, lower leg and foot being nearer to the medial axis body (Aiello & Dean, 1990). Consequently, the centre of gravity of the body needs to move only a short lateral distance to stay on the supporting leg during walking (Aiello & Dean, 1990).

It is true that all human populations grow and develop similarly under healthy conditions. However, it is also true that the existence of specific standards for individuals of specific regions is necessary and important in both anthropological and forensic fields (Cameriere & Ferrante, 2008; Boccome et al. 2010; Charisi et al. 2011; Rissech et al. 2013b). Most of the current standards are based primarily on North American (USA) skeletal or radiographic samples, and the magnitude of the error in its application to European populations is unknown. For example, the method for calculating adult stature based on USA reference samples fails in the estimation of living height in Spain and Italy (Formicola, 1993; Formicola & Franceschi, 1996; Lalueza-Fox, 1998). In Spain and Italy, the use of the formulae proposed by Pearson (1899) at the end of the 19th century, based on a French sample, performs better (Formicola, 1993; Formicola & Franceschi, 1996; Lalueza-Fox, 1998) than the Trotter and Gleser formulae for Whites, because of the intertwined biological population history of French, Spanish and Italian populations (Formicola, 1993; Formicola & Franceschi, 1996; Lalueza-Fox, 1998), and because they are populations of medium stature (Formicola, 1993; Formicola & Franceschi, 1996). In contrast, the equations of Trotter and Gleser for Whites systematically overestimate stature in both female and male skeletons of Spanish and Italian origin (Formicola & Franceschi, 1996; Lalueza-Fox, 1998). In sub-adult age estimation, for example, according to the data given by Gindhart (1973) and Hoffman (1979) studies based on a White American sample belonging to the middle of the 20th century of North Western European descent, a tibial diaphyseal length of 203 mm from a Portuguese individual aged 7 years from the same historical period is situated in the lower limit of the expected normal variation range of growth (200–260 mm) given by these authors for individuals of 7 years old (Rissech et al. 2013). For this reason, the estimated age (which is an average age of the normal range interval) for this individual is 4.5 years (Rissech et al. 2013b). To assume that all populations grow like those from the USA would not be exactly correct. It is well known that the USA population is taller than most European populations (Komolos, 2001; Komlos & Baur, 2004; Smith & Norris, 2004). Although in recent years stature has increased in Europeans due to improvements in living conditions, some differences still exist (Pebles & Norris, 2011). Forensic age estimation of unidentified corpses and skeletons for the purpose of identification has been a traditional feature of forensic science. Successfully determining the identity of a decedent is of considerable significance from the ethical, legal and criminal perspective; not only is it the prerequisite for officially declaring an individual dead, but it is also the basis for investigating crimes, mass disasters or war crimes. There is a pressing need for accuracy and reliability of the methods in the field of Forensic Anthropology. In this way, the data presented in this study are appropriate for current living populations from the 21st Century, especially for those belonging to Western Europe and more specifically from the Iberian Peninsula.

The information provided in the present study increases our knowledge of the timing and morphology of the femur during development, and in particular the morphology of the different femoral ossification centres during development. Improvement of our knowledge of bone development helps in age estimation, which will be determined with greater accuracy in both the clinical context, and in archaeological and forensic assemblages. In a clinical context it can help to increase the accuracy in assessing maturity. In archaeological and forensic assemblages, the data of the present study may be useful in age estimation of juveniles. Furthermore, it can be of utility in some palaeonthological cases, as for example the given values for the bicondylar angle by age, which can be extremely useful in determining bipedal locomotion in young individual fossils. However, further research on the development of the femur is necessary to obtain better information for skeletal diagnosis. In particular, it is necessary to study male femur development in order to compare it with the data presented here.

CONCLUSIONS

The findings in this study constitute the first approach to elucidate the size and shape changes in the female human femur during puberty by using geometric morphometrics of landmark-based data. Furthermore, it is also the first skeletal growth study based on the Spanish population, and more specifically on the current living Spanish population from the 21st Century. The results obtained agree with the development patterns observed in previous studies on the development of the femur during puberty. Furthermore, they bring new information on the timing of the angular remodelling of the neck and bicondylar region during postnatal growth of the female femur based on a considerable sample (184 females). Results indicate continuous remodelling until 13 years old for both variables (collo-diaphyseal and bicondylar angle), and agree with the high variability demonstrated by different studies among different regions. The angular remodelling of the neck and the bicondylar region of the femur is determined by the increase in the mechanical load generated by muscles and ligaments, and by the widening of the pelvis, which is important in females (Tague, 2005; Birkenmaier et al. 2010). Widening of the pelvis does not finish until the end of the female juvenile stage (Rissech et al. 2003; Rissech & Malgosa, 2005, 2007). These results reveal that geometric morphometric analyses can be very useful for the understanding of skeletal development. Furthermore, it could be useful as a method for detecting morphological criteria of differentiation between the different ages in sub-adult individuals, which could be useful for sub-adult age estimation. Results reported here can also be an interesting resource for palaeoanthropological studies on immature individuals from the fossil record, especially those in which it is important to detect bipedalism.

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3.2. ONTOGENY OF THE MALE FEMUR: GEOMETRIC MORPHOMETRIC ANALYSIS APPLIED TO A CONTEMPORARY SPANISH POPULATION

Aniol Pujol[1], Carme Rissech[1], Jacint Ventura[2], and Daniel Turbón[1]

[1]Departament de Biologia Animal, Facultat de Biologia, Unitat d'Antropologia Física, Universitat de Barcelona, Barcelona, Spain

[2]Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Unitat de Zoologia, Facultat de Biociències, Universitat Aut'onoma de Barcelona, Barcelona, Spain

ABSTRACT

Objective: To describe the morphological changes of the male femur during the adolescent growth spurt and to compare the pattern obtained with that reported previously for females.

Material and Methods: Two hundred and forty males from a Spanish population aged between 9 and 16 years were analysed, based on telemetries. Size and shape variation of the femur was quantified by 22 2D-landmarks and analysed using geometric morphometric methods. Likewise, the variation of neck–shaft and bicondylar angles were also determined and evaluated by Student's t-test. Sexual differences were analysed by comparing results here obtained on boys with those corresponding to girls reported in a previous study.

Results: In males, both size and shape varied significantly with age, with males having larger dimensions than females. In general terms, these changes are generally characterised by an increase in robustness of the femur and shape modifications in the epiphyses. During growth, the neck–shaft angle decreases and the size of the greater and lesser trochanters increase. A significant increase of distal epiphyseal dimensions was recorded, mainly in the medial condyle. The angular remodeling of both the neck and the bicondylar regions of the male femur continues until 16 and 15 years, respectively. Female and male femur each followed divergent growth trajectories. Males showed a greater variability in neck–shaft and bicondylar angles than females.

Discussion: The timing, morphology and growth trajectories provided on the femur during development can be very helpful in anthropological, paleoanthropological and evolution studies.

Key words: development; male femur; geometric morphometric; lower limb; ontogeny; subadult individuals

INTRODUCTION

Research on human development has a long tradition in the field of physical anthropology, dating the first longitudinal growth study from 17th century (Bogin, 1999). At present, growth and maturation processes, specifically developmental studies on the human skeleton, are considered essential for resolving different medical and anthropological questions, such as: i) establishing the degree of growth and maturation of subadult individuals in cases of orthopaedic surgery or growth hormone treatment; ii) establishing the health status and living conditions of children; iii) reconstructing demographic profiles in past populations; iv) estimating the age of skeletal remains of subadult individuals; v) the biological identification of living individuals in legal proceedings where a minor is involved; vi) understanding the current human variability and vii) understanding the evolution of human ontogeny which will ultimately provide a framework for reliable interpreting the evolution in fossil hominins (Neubauer et al., 2009).

Basically, these estimations rely on the relationship observed between chronological age and the degree of bone growth and maturation for infant and juvenile individuals. Taken into account the skeletal age of one individual, the methods for subadult age estimation provide a specific age range based on the degree of growth and maturation. Each ageing method is based on a model of the bone development process based on a specific reference sample. These models can be based on documented skeletal collections (with known sex, age, biological origin and cause of death) or high-resolution digital radiographies, ranging from telemetries (accurate radiographies of the limbs) and scoliograms (vertebral column radiographies; also known as rachis) to multislice computerised tomographies, which do not distort the actual measurements. Furthermore, it is essential that the reference population used to create the model be biologically comparable to the analysed sample, since the accuracy of the estimations depends on the application of appropriate data relating to the development of the skeletal elements with regard to genetic, environmental and cultural factors (Biewener and Bertram, 1993; McGuigan et al., 2002; Rissech et al., 2008). However, in spite of the importance of having a register of growth and maturation values from different skeletal elements and populations, and the large amount of studies on human skeletal development (Greulich and Thoms, 1938; Greulich, 1960; Garn, 1962; Tanner, 1962, 1986; Coleman, 1969; Pyle et al., 1971; Gindhart, 1973; Tanner et al., 1976, 1981; Hoppa, 1992; Miles and Bulman, 1994, 1995; Rissech et al., 2013a,b; Frelat and Mitteroecker, 2011; Schillaci et al., 2011, 2012; López-Costas et al., 2012), there is still a serious lack of information regarding the development of many of the elements of the human skeleton in different populations (Rissech et al., 2013a,b).

Of the growth standards currently available for osteological studies of Western European individuals, many are based on radiological images and skeletal assemblages from white North American children from the 1950s and beforehand (Greulich and Thoms, 1938; Reynolds, 1945, 1947; Ghantus, 1951; Maresh, 1955; Coleman, 1969; Pyle et al., 1971; Gindhart, 1973; Hoffman, 1979; Gasser et al., 1985, 1991). The few studies which focus on Western European children (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles and Bulman, 1994, 1995; Majó, 2000; Rissech et al., 2001, 2003, 2008, 2013a, 2013b; Rissech and Malgosa, 2005, 2007; Rissech and Black, 2007; Frelat and Mitteroecker, 2011; López-Costas et al., 2012) and particularly on Spanish children are mostly based on: i) archaeological material with age and sex estimated in the lab (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles and Bulman, 1994, 1995; Majó, 2000); and ii) documented skeletal remains from the late 19th and first half of the 20th century (Rissech et al., 2001, 2003, 2013a, 2013b; Rissech and Malgosa, 2005, 2007; Rissech and Black, 2007; Frelat and Mitteroecker, 2011; López-Costas et al., 2012; Cardoso et al., 2014). Furthermore, these last studies are restricted to specific skeletal elements such as the pelvic bone (Rissech et al., 2001, 2003; Rissech and Malgosa, 2005, 2007), scapula (Rissech and Black, 2007), tibia (Frelat and Mitteroecker, 2011; López-Costas et al., 2012; Cardoso et al., 2014) and humerus (Rissech et al., 2013a; Cardoso et al., 2014).

For identification and age estimation purposes, the existence of developmental models for individuals of specific populations is necessary and important. However, because most of the current standards are based primarily on North American (USA) samples, the magnitude of error when applied to European populations is unknown. For example, the method for calculating adult stature (it is necessary to remember that the stature results from the growing process) based on White American reference samples systematically overestimates stature in both female and male skeletons of Spanish and Italian origin (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998). Because of the intertwined biological population history of French, Spanish and Italian populations, and because they are populations of medium stature, the use of the formulae proposed by Pearson (1899) at the end of 19th century, based on a French sample performs better than the Trotter and Gleser (1952) formulae for “Whites” (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998). Similarly, the data given by Gindhart (1973) and Hoffman (1979), based on a White Americans of North Western European descent, also underestimate ages in subadult individuals from the

Iberian Peninsula, even though both the reference sample and children derive from the mid-20th century (Rissech et al., 2013b). One reason of these errors in stature calculation and subadult age estimation is that the current North American population is taller than most European populations (Komlos, 2001; Komlos and Baur, 2004; Smith and Norris, 2004), and even though the stature has increased in Europeans recently, due to the improvements in health and living conditions, some differences still exist (Pebles and Norris, 2011).

Age estimation is basic in any osteological study and biological identification, being a characteristic of forensic anthropology. The success in determining the identity of a deceased is not only a requisite for officially declaring an individual dead but it is also the basis for investigating interpersonal and war crimes and mass disasters. Therefore, an increase in the accuracy and reliability of the ageing methods is urgent in the field of Forensic Anthropology. This becomes even more evident considering the noticeable height increase (10 cm) seen in Spain in the second half of the 20th century as a result of improved living conditions (Spijker et al., 2012). Moreover, the need for studies on skeletal development based on different populations becomes more important when we take into account their broad relevance in biological anthropology and human evolution. The study of changes in size increase, shape and form of different skeletal elements in different populations during growth can help to elucidate, for example, how patterns of bone growth have changed in recent generations of children and how selective forces influence the development of sexual dimorphism in the different anatomical regions of the skeleton (Bulygina et al., 2006; Coquerelle et al., 2011; Crespo et al., 2015).

In light of this background, there is an obvious need for studies regarding the developmental osteology of individuals from contemporary populations, as has been recommended by different authors (Martrille et al., 2007; Cameriere and Ferrante, 2008; Boccone et al., 2010; Charisi et al., 2011; López-Costas et al., 2012; Rissech et al., 2012; San Millán et al., 2013) and demonstrated in several studies (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998; Bulygina et al., 2006; Rissech et al., 2013b; Crespo et al., 2015).

Given the anthropological importance of the femur as a result of its locomotory function (Aiello and Dean, 1990; Anderson and Trinkaus, 1998; Andriacchi and Alexander 2000) and the postdepositional resistance exhibited by this element due to its structural density, we analysed femoral development during puberty in a contemporary Spanish population. This research was divided into two studies, one for the female and another for the male femur. Results from the study of the female femur (Pujol et al., 2014) revealed an overall increase in the robustness and length of the female femur, together with marked morphological changes in specific regions of this bone with age. In general, an increase in the robustness of the bone and noticeable phenotypic changes in certain areas of the femur were observed. The aims of this study are twofold: i) to determine the morphological changes during the adolescent growth spurt of the femur in boys and youths between 9 and 16 years of age in the current living Spanish population and ii) to evaluate the development of the sexual dimorphism in femoral morphology by the comparison of the results here obtained in boys with our previous findings for girls. For these purposes, we applied landmark-based geometric morphometric techniques (GMM) to high-resolution radiographic images.

MATERIALS AND METHODS

High-resolution radiographic images of the lower half of the body (telemetries), in DICOM (Digital Imaging and Communication in Medicine) format, were obtained from the database at the Hospital Infantil Sant Joan de Déu de Barcelona (Spain). In accordance with current Spanish data protection legislation (García et al., 2010), all personally identifying information other than age and sex was deleted from these images prior to use. This study was approved by the Bioethics Committee at the Hospital Sant Joan de Déu, Barcelona (Ref.: 2938).

The suitability of using telemetries (distant type of digital radiography of the limbs which lacks of significant image deformation) and the radiography protocols followed by the Hospital Sant Joan de Déu are explained in detail by Pujol et al. (2014). However, it is worth clarifying that in the radiographic procedure of the Hospital, the knee joints were always aligned to the center of the body gravity to permit the comparison of both femoral angles between individuals (Pujol et al., 2014).

In brief, the left femur of male children from 9 to 16 years old born in the 21st century was analysed in anterior view. This age range was selected to include the onset of the male pubertal growth spurt (Tanner et al., 1981; Tanner, 1986). Individuals presenting disease or anatomical variation that could affect the ability to determine “normal” development osteology were discarded. A total of 240 male femora were analysed and distributed into groups of 30 individuals per year. We did not have access to the entire clinical information of each individual, since the radiographic images were separated from the clinical histories due to the law of individual data protection.

Table 1: Height (in cm) of a sample of the analysed individuals

	Males		Females	
Age (years)	*n*	Height Mean (SD)	*n*	Height Mean (SD)
9	10	135.6 (15.3)	11	134.3 (14.3)
10	12	138.9 (16.4)	9	139.4 (14.9)
11	15	144.2 (17.5)	10	146.7 (15.9)
12	12	148.3 (17.3)	14	153.9 (15.3)
13	17	156.6 (18.1)	18	157.1 (16.9)
14	19	163.7 (17.4)	20	160.3 (17.5)
15	22	172.4 (17.5)	20	163.4 (15.8)
16	22	174.9 (16.1)	21	164.1 (13.6)

The data of the female series were obtained from the study by Pujol et al. (2014). SD, standard deviation.

However, some of the radiographs included the height of the individual. To offer information on the general body size variation related to age in the samples analysed, in Table 1 we summarised the mean values of the height obtained for the boys here considered and for the girls analysed in our previous study (Pujol et al., 2014). All the individuals come from the subadults' radiological collection of the Universitat de Barcelona, recently created by one of the authors of this study (A.P.) for his doctoral thesis under the supervision of D.T. and C.R. This collection comprises radiological images obtained from 1080 individuals (540 boys and 540 girls) of Spanish origin, born between 1991 and 2010, ranging from birth to 18 years of age. All of these individuals come from middle social-economic class, showing the boys of 18 years of age a mean height of 177.5 cm and the girls a mean height of 164.3 cm. These values correspond to the expected male and female adult mean height in current living Spanish population: 174.20 cm (SD: 7.08) for males and 162.09 cm (SD: 6.37) for females (Spijker et al., 2012).

GMM: landmarks selection and location

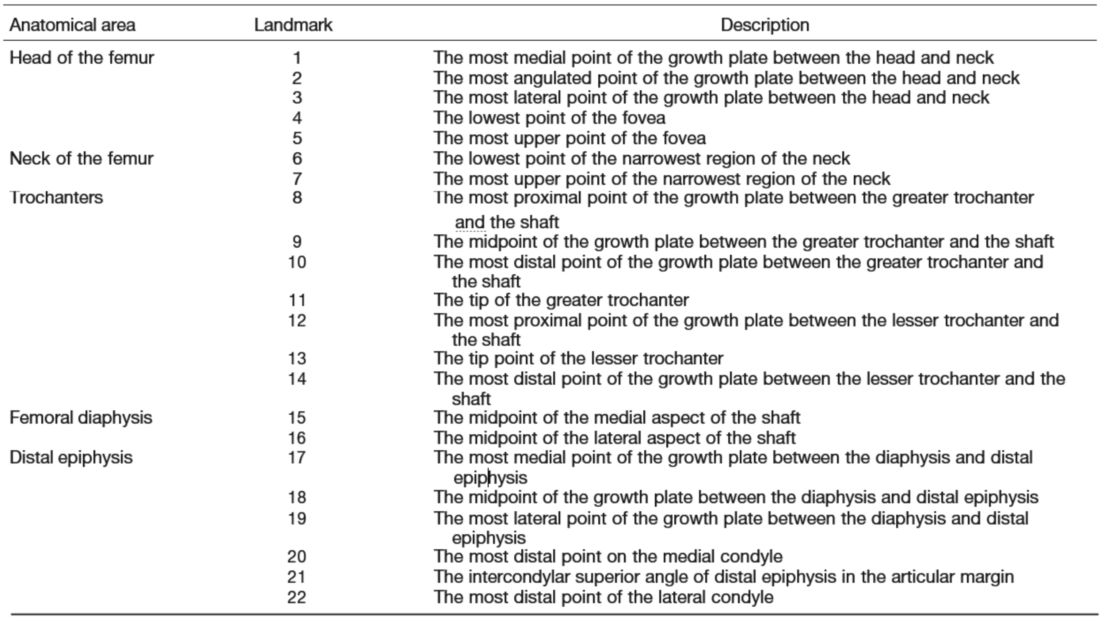
The landmark-based GMM procedure is an effective method for capturing information on the shape of an individual and useful for analysing statistically shape differences between individuals or groups (Bookstein et al., 1985; Rohlf 2000a,b; Scholtz et al., 2010). Furthermore, it is a potent and intuitive tool for visualising shape, and shape differences between the analysed individuals and groups because data are recorded to capture the geometry of the analysed structure (Bookstein et al., 1985; Rohlf 2000a,b; Zelditch et al., 2004; Toro-Ibacache et al., 2010). Landmarks can be bidimensional or threedimensional (2D or 3D) coordinates of morphological points fitted by superimposition methods, which pair homologous landmarks as closely as possible by minimizing the summed squared distances of the Procrustes between corresponding landmarks. According to this, shape changes may be defined as the residuals of the superimposition given as transformation vectors (Rohlf and Marcus, 1993).

A total of 22 bidimensional landmarks (Fig. 1) were digitised using the thin plate spline (TPS) program series (Rohlf, 2006). These landmarks were the same as those we defined and used in the study by Pujol et al. (2014) to ensure that the data obtained in both series were fully compatible and comparable. These 22 landmarks were defined on the femoral regions which displayed greater changes during growth, specifically (see Table 2 and Fig. 1): the head, the fovea, the neck, the trochanter and the distal diaphysis and epiphysis. Taking into account the results obtained in females (Pujol et al., 2014) and the fact that analysis was undertaken by the same person (A.P.), the measurement error in the landmark location was considered negligible.

Figure 1: Landmark locations on the anterior aspect of the left femur. For definitions of the landmarks see Table 2.

Data analyses: femur development in boys

In males, after digitising the 240 configurations of 22 landmarks, we used Procrustes-based geometric morphometrics (Rohlf and Slice, 1990; Bookstein, 1991) to obtain shape variables for statistical analyses. For this purpose, we first performed a generalised procrustes analysis (GPA; Rohlf and Slice, 1990) to compute shape coordinates. This implies translation to a common centroid, scaling to unit centroid size (CS: square root of the summed squared Euclidean distances from all landmarks to their centroid), and rotation of the configurations to minimize the overall sum of squared distances between pairs of corresponding landmarks. Then, the coordinates of superimposed configurations were orthogonally projected to the tangent space to obtain shape variables for statistical analyses. This resulted in a vector of 44 shape variables and a CS for each individual. Procrustes superimposition and projection to the tangent space were performed using the TPS programs series (Rohlf, 2006).



Anatomical area	Landmark	Description
Head of the femur	1	The most medial point of the growth plate between the head and neck
	2	The most angulated point of the growth plate between the head and neck
	3	The most lateral point of the growth plate between the head and neck
	4	The lowest point of the fovea
	5	The most upper point of the fovea
Neck of the femur	6	The lowest point of the narrowest region of the neck
	7	The most upper point of the narrowest region of the neck
Trochanters	8	The most proximal point of the growth plate between the greater trochanter and the shaft
	9	The midpoint of the growth plate between the greater trochanter and the shaft
	10	The most distal point of the growth plate between the greater trochanter and the shaft
	11	The tip of the greater trochanter
	12	The most proximal point of the growth plate between the lesser trochanter and the shaft
	13	The tip point of the lesser trochanter
	14	The most distal point of the growth plate between the lesser trochanter and the shaft
Femoral diaphysis	15	The midpoint of the medial aspect of the shaft
	16	The midpoint of the lateral aspect of the shaft
Distal epiphysis	17	The most medial point of the growth plate between the diaphysis and distal epiphysis
	18	The midpoint of the growth plate between the diaphysis and distal epiphysis
	19	The most lateral point of the growth plate between the diaphysis and distal epiphysis
	20	The most distal point on the medial condyle
	21	The intercondylar superior angle of distal epiphysis in the articular margin
	22	The most distal point of the lateral condyle

Table 2: Description of the 22 bidimensional landmarks used in this study

First of all and to test femoral size-related differences during growth, an analysis of variance (ANOVA) was performed on the CS and age. Secondly, to evaluate age-related changes in the femoral shape of the boys sample a principal components analysis (PCA in shape space or relative warp analysis) was applied (tpsRelw program, version 1.46; see also Rohlf, 1999). This analysis reduces the complex multidimensional data into a few eigenvectors that were linear combinations of the landmark displacements (Zelditch et al., 2004). A multivariate analysis of covariance (MANCOVA) was performed with the first five components, using the CS as covariable, to evaluate the effect of the allometric factor on shape changes. Furthermore, plots of the corresponding PC scores of this analysis were complemented by the results of applying a multivariate regression of shape on lnCS to evaluate allometry (Mitteroecker et al., 2013). Additionally, and to better evaluate the allometric relation between shape and size of the femur during growth, we applied a PCA in size–shape space (Mitteroecker et al., 2004a,b). This is a composite approach which consists of a relative warp analysis of the usual matrix of Procrustes shape coordinates (Bookstein, 1991; Rohlf, 1993) augmented by one single additional column for the natural logarithm of CS (Mitteroecker et al., 2004a,b). The resulting low-dimensional eigenspace allows comparisons of size and shape in one analysis (the form), whereas the classical PCA of the Procrustes coordinates allows the analysis of the shape only. In addition, plots of the corresponding size–shape PC scores of this analysis were complemented by the results of applying a multivariate regression of size and shape coordinates on lnCS to evaluate allometry (Mitteroecker et al., 2013). The study and comparison of both generated spaces (shape space and size–shape space) allow a better comprehension of differences and dissociations of the femur size–shape relationship between ages during the individual development.

Changes in femur shape were visualised in all cases using TPS deformation grids (Bookstein, 1991). All the statistical analyses of this study were performed using SPSS 20 and R v2.15.3.

Data analyses: boys–girls femur variation during development

To determine possible sexual differences of the femur during growth we first performed a single GPA, using our boys sample together with the girls sample analysed in the study by Pujol et al. (2014). The resulting GPA displacement vectors were ordinated by PCA of shape coordinates to explain shape variation among samples. Taking into account the lower age range considered for girls (Pujol et al., 2014), only individuals between 9 and 14 years of age were analysed. To evaluate the size effect on shape changes, a MANCOVA was performed with the three first components, using CS as co-variable. We considered only the three first PCs because of the low percentage of variation explained by the remaining PCs. As in boys, to better assess the differences and dissociations of the size–shape relationship in the femur, a size–shape PCA was performed.

To compare sexual differences in femoral shape (shape space) and form (size–shape space) at the different ages, we calculated sex-specific mean shapes for 9, 10, 11, 12, 13 and 14 years of age by a moving average algorithm (Bulygina et al., 2006; Coquerelle et al., 2011). This method calculates the mean shape and form change in the analysed period of time by a quadratic regression of all the shape PC, in the case of shape space, and size–shape PC, in the case of size–shape space.

To visualize the sexual dimorphism in shape during femoral development, TPS deformation grids (Bookstein, 1991) were obtained in each age group to its next older stage for males and females separately.

Metrical analysis

As in the study of the female femur (Pujol et al., 2014), development of the male neck–shaft and bicondylar angles was quantified and graphically represented in relation to individual chronological age. The neck–shaft angle (Fig. 2) is the angle between the longitudinal axis of the femoral diaphysis and the longitudinal axis of the neck (Tardieu and Damsin, 1997). The bicondylar angle (Fig. 2) is located between the longitudinal axis of the diaphysis and a line perpendicular to the infracondylar plane of the femur (Shefelbine et al., 2002). The longitudinal axis of the femoral shaft is the midline along the shaft which connects the middle of the infracondylar and proximal segments of the femur. The longitudinal axis of the neck is the midline along the neck which connects the distal and proximal limits of this anatomical region. To graphically locate these axes on the radiological images, two perpendicular lines to the shaft and neck directions were plotted on the middle of these anatomical regions (Fig. 2). The line which crosses perpendicularly the middle of these two lines was considered the shaft and neck axes,

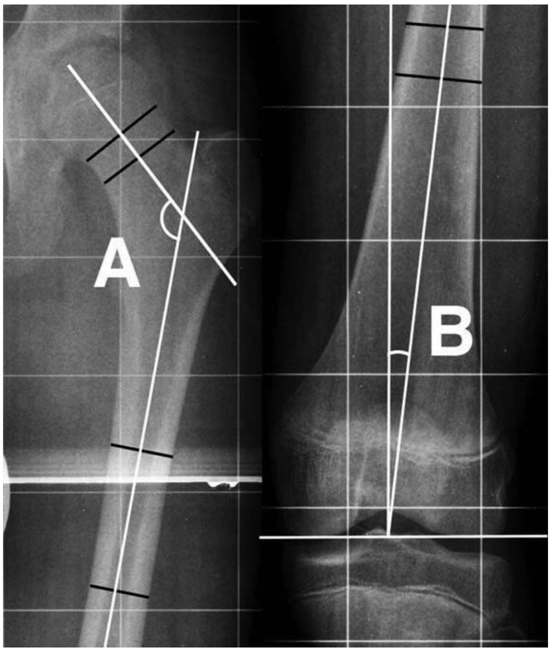


Figure 2: Measurement process of the neck–shaft angle (A) and bicondylar angle (B). White lines indicate the diaphyseal and neck axes. Black lines are the lines drawn to locate the neck and shaft axes, see the text.

respectively (Fig. 2). To evaluate the sexual differences in femoral size (CS) and in the neck–shaft and bicondylar angles, Student's t-test was applied.

RESULTS

Size variation

The ANOVA test showed that male CS increased significantly with age (F = 6.689, *P = 0.0000). The MANCOVA test performed on the male sample indicated that the effect of size on shape was significant (Wilks' Lambda = 0.925, F = 3.777, *P = 0.012) and that after removing the effect of allometry, age-related shape changes were significant (Wilks' Lambda = 0.767, F = 4.655, *P = 0.0000). Likewise, the MANCOVA test performed on both sexual series together also showed a significant effect of size on shape (Wilks' Lambda = 0.933, F = 3.411, *P = 0.031) and showed that after removing the allometry effect, age-related (Wilks' Lambda = 0.096, F = 10.266, *P = 0.0000) and sex-related shape changes (Wilks' Lambda = 0.632, F = 72.641, *P = 0.0000) were significant.

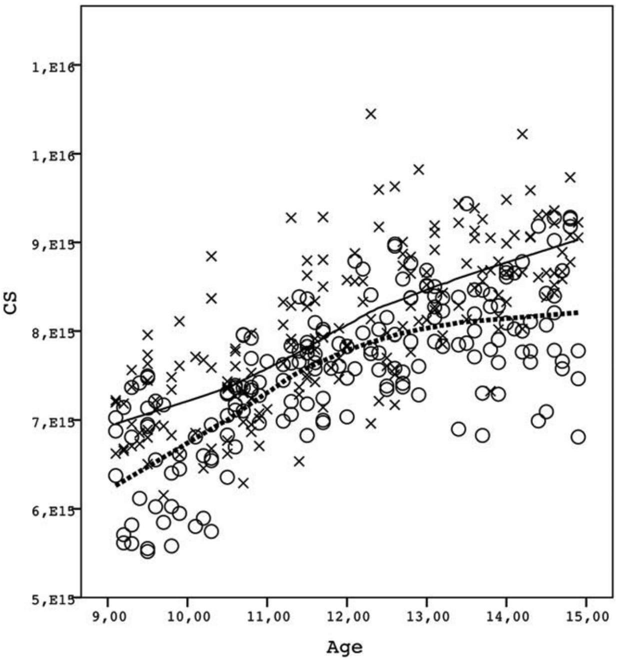


Figure 3: Centroid size (CS) variation regarding age and sex. Males (X); females (O). The data of the female series were obtained from Pujol et al. (2014).

A comparison between both sexual series (Fig. 3) showed that the mean CS was higher for males at all ages (9–14 years; see Table 3). These differences were significant from 11 years upwards (Table 3). The mean CS value in males at 15 years of age was similar to that obtained at 16 years (Table 3), which indicates a possible stabilisation of this variable from 15 years of age. This happens before in females, at around 13 years of age (Pujol et al., 2014).

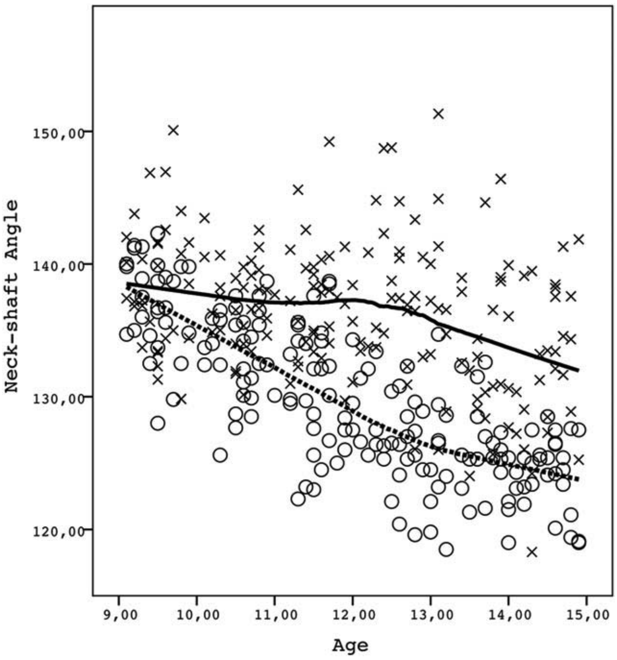


Figure 4: Neck–shaft angle variation in males (X) and females (O) according age. The data of the female series were obtained from Pujol et al.

In males, the neck–shaft angle undergoes major changes between 9 and 16 years of age (Fig. 4, Table 3). Although a constant decrease of this angle occurs during this period, the most dramatic reduction occurs from approximately 12 until around 16 years of age (Table 3). The decrease in neck–shaft angle averaged 10º between 9 and 16 years of age (Table 3, Fig. 4). A comparison of the results obtained in this study with those corresponding to the female series (Pujol et al., 2014) showed that males had larger neck–shaft angles (Table 3, Fig. 4) and that these differences were significant from 10 years of age upward (Table 3). Males exhibited a greater variability in neck–shaft angles than females, particularly from 12 years of age upward (Table 3, Fig. 4). The variability in the neck–shaft angle increased more with age in males than in females (Table 3).

As expected, the bicondylar angle in the male series increased with age (Fig. 5), although the most marked increase was detected at around 11 years of age, with values tending to stabilize at around 15 years of age (Table 3). These ages are slightly earlier (by around 1 year) than those observed for the neck–shaft angle. The bicondylar angle increased by around 2–3º on average over the period considered, from 10 years old (Table 3, Fig. 5). Males consistently exhibited smaller bicondylar angles than females (Table 3, Fig. 5). These differences were statistically significant from 10 years of age upward (Table 3). Likewise, males showed greater variability in bicondylar angle than females at all ages (Fig. 5, Table 3). These sex-related differences in bicondylar angle are much more marked than those observed for the neck–shaft angle. The female bicondylar angle increases within a very narrow range over the period

Variables	Age (years)							
	9	10	11	12	13	14	15	16
CS								
Males *n*	30	30	30	30	30	30	30	30
Mean	$7.100\ 3\ 10^{15}$	$7.300\ 3\ 10^{15}$	$7.940\ 3\ 10^{15}$	$8.380\ 3\ 10^{15}$	$8.600\ 3\ 10^{15}$	$8.940\ 3\ 10^{15}$	$9.520\ 3\ 10^{15}$	$9.260\ 3\ 10^{15}$
SD	$4.586\ 3\ 10^{14}$	$5.572\ 3\ 10^{14}$	$6.340\ 3\ 10^{14}$	$8.315\ 3\ 10^{14}$	$5.118\ 3\ 10^{14}$	$4.689\ 3\ 10^{14}$	$5.944\ 3\ 10^{14}$	$7.851\ 3\ 10^{14}$
Females *n*	30	30	30	30	30	30	30	30
Mean	$6.490\ 3\ 10^{15}$	$7.040\ 3\ 10^{15}$	$7.610\ 3\ 10^{15}$	$7.940\ 3\ 10^{15}$	$8.080\ 3\ 10^{15}$	$8.230\ 3\ 10^{15}$	–	–
SD	$6.693\ 3\ 10^{14}$	$5.808\ 3\ 10^{14}$	$4.008\ 3\ 10^{14}$	$5.461\ 3\ 10^{14}$	$5.386\ 3\ 10^{14}$	$6.946\ 3\ 10^{14}$	–	–
P	0.081	0.087	0.021*	0.019*	0.000*	0.000*	–	–
Neck–shaft angle								
Males *n*	30	30	30	30	30	30	30	30
Mean	138.65	137.11	137.41	137.63	135.13	132.56	129.68	129.49
SD	4.94	3.25	4.25	5.23	6.51	5.72	5.33	5.19
CV	3.56	2.37	3.09	3.80	4.82	4.32	4.11	4.01
Females *n*	30	30	30	30	30	31	–	–
Mean	137.00	133.36	130.35	127.39	125.92	123.95	–	–
SD	3.52	3.24	4.68	3.51	3.70	2.77		
CV	2.57	2.43	3.59	2.76	2.94	2.23		
P	0.140	0.000	0.000	0.000	0.000	0.000		
Bicondylar angle								
Males *n*	30	30	30	30	30	31	31	31
Mean	6.93	7.21	7.40	7.51	7.99	8.03	8.20	8.11
SD	1.32	1.25	1.33	1.07	0.89	1.40	1.32	1.23
CV	19.05	17.34	17.97	14.25	11.14	17.44	16.10	15.17
Females *n*	30	30	30	30	30	31	–	–
Mean	6.92	8.72	9.51	10.48	11.09	10.80	–	–
SD	0.53	0.50	0.33	0.57	0.42	0.45	–	–
CV	7.66	5.73	3.47	5.44	3.79	4.17		
P	0.977	0.000	0.000	0.000	0.000	0.000		

The data of the female series were obtained from the study of Pujol et al (2014).

Table 3: Sexual differences in the collo-diaphysal and bicondylar angles

considered, with the variability remaining unchanged. In contrast, this range was greater in males (standard deviation range for males: 0.87–1.4; for females: 0.33– 0.57; see Fig. 5).

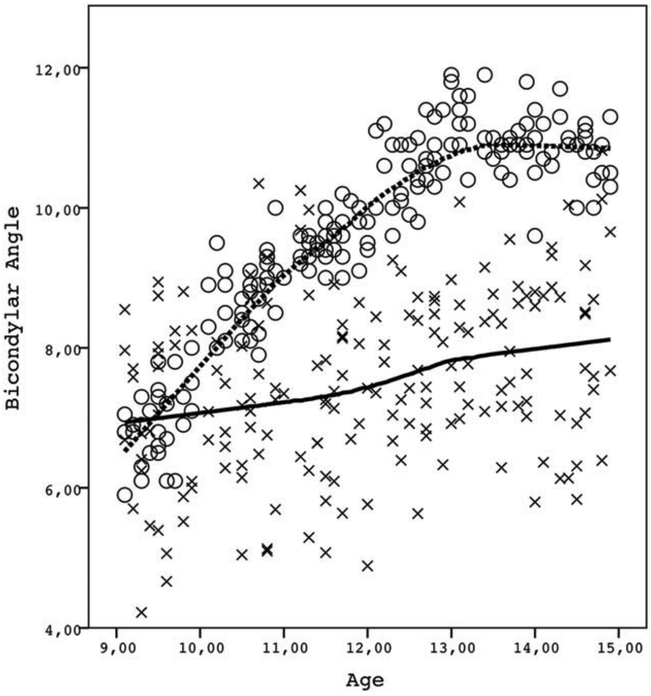


Figure 5: Bicondylar angle variation between males (X) and females (O) according age. The data of the female series were obtained from Pujol et al. (2014).

Change of shape and form in boys

The first two components (PC1 and PC2) in the PCA in shape space (Fig. 6) explained 74.3% of the male femur shape variation (PC1: 65.3%; PC2: 9%). The most dominant factor of shape variation was the shape difference between young and old individuals, which varied progressively with age (Fig. 6). The arrow represents the coefficient vector of the regression of shape on LnCS (r = 0.81), indicating the actual ontogenetic allometry.

This arrow is positively correlated with PC1 and PC2 and indicates similar significant correlation of both PC to the CS during growth (Fig. 6). As in girls (Pujol et al., 2014), the shape changes associated with the increase in PC1 score were the following: i) increase of the robustness of the femur in both the diaphyseal and neck regions; ii) vertical decrease of the femoral head in relation to the overall femur, revealed by the decrease of the neck–shaft angle (Fig. 6); and iii) increase of the proportions of the distal epiphysis in relation to the overall femur (Fig. 6). An increase in PC2 scores was related to a change in the proportions between the upper (greater and lesser trochanters) and the lower epiphyses in relation to the diaphysis (Figs. 6 and 7).

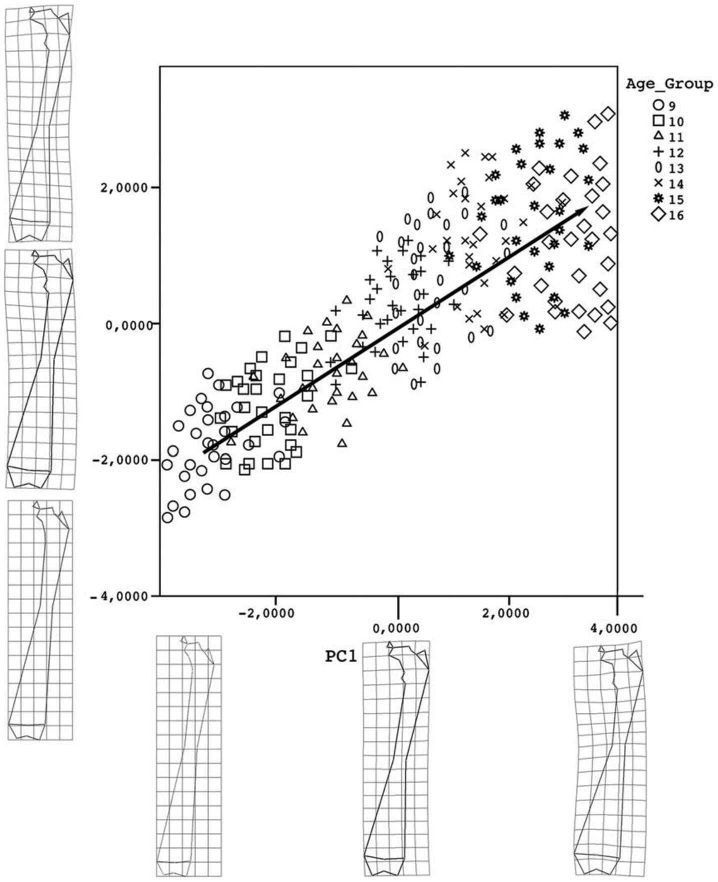


Figure 6: Ordination of the male individuals in the space of the two-first principal components (PC1, PC2) based on the weight matrix for the anterior aspect of the femur. The arrow represents ontogenetic allometry (allometric shape and size)—the regression of shape on ln CS. Grids show the shape changes throw the negatives values (untransformed shape) to positive values of the axis.

In general, the femoral head showed a much more vertical position in the youngest male individuals (9 years of age; Fig. 8), with both trochanters already present, albeit small, and the distal epiphysis well-developed (Fig. 9). Femora from the older males (15 and 16 years) mainly presented changes in the femoral head region (Fig. 8), which takes a much more horizontal position, resulting in a reduction in the neck–shaft angle (Fig. 8). The greater trochanter increased in size with respect to the lesser trochanter and the femur as a whole. In addition, the angle of the greater trochanter was located in a relatively higher position than in the femora of subjects aged 9 years (Fig. 8). The distal epiphysis reached approximately its final shape at 15–16 years, which was indicated by fusion of this epiphysis to the shaft at 17 years of age in the great majority of the individuals, which in turn indicated the ending of male growth of this anatomical area. This observation is consistent with the given standards of union of the knee epiphyses for the current male population (Scheuer and Black, 2000), and more specifically for the current male population of the Iberian Peninsula (Rissech et al., 2008;

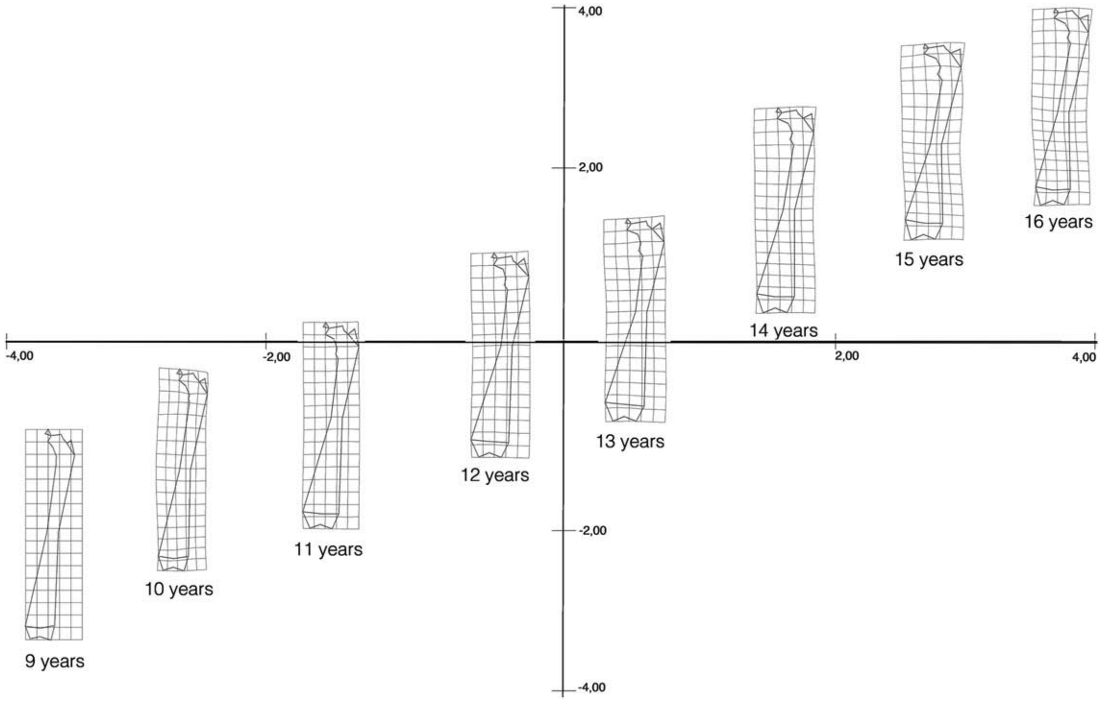


Figure 7: Distribution of the consensus form of each age [from 9 (untransformed shape) to 16 years] in the space defined by the two first PCs in males.

López-Costas et al., 2012). At 15 and 16 years, the distal epiphysis presented proportionally larger condyles (especially the medial condyle) in relation to the femur as a whole. This modification is associated with the adjustment of the femur to the change in neck angle (Fig. 9). These changes in the bicondylar region and in the femoral neck are related to the angular remodeling that occurs during male femoral development.

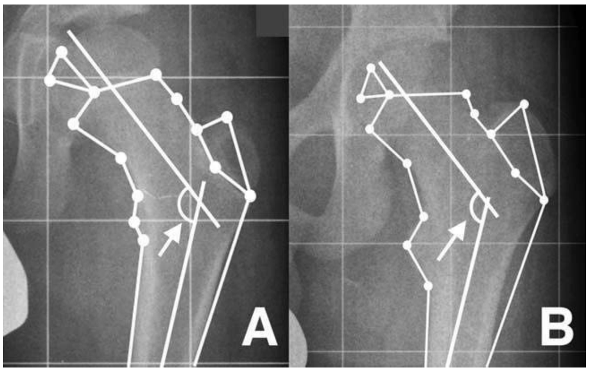


Figure 8: Consensus forms of the proximal male femur for individuals aged 9 (A) and 16 years (B). The arrow shows the neck–shaft angle.

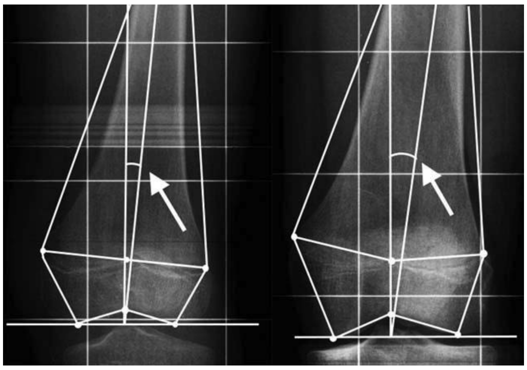


Figure 9: Consensus forms of the distal male femur for indi- viduals aged 9 (A) and 16 (B). The arrow shows the bicondylar angle.

The first two components (PC1 and PC2) of the PCA in size–shape space (Fig. 10) explained 87% male femur form variation (PC1: 82%; PC2: 5%). Femoral growth includes changes in both shape and size, because of this femoral form (size–shape space) closely reflects an individual's age femoral than femoral shape. The arrow represents the coefficient vector of the regression of size– shape on LnCS (r = 0.802). Consequently PC1 represents the axis of allometric development of these individuals. Shape differences corresponding to PC1 are similar to the PC1 in shape space (Fig. 6), but PC1 in form space additionally comprises differences in size, and because of this the regression vector (the arrow) was more stretched along PC1. It indicates the significance of the increasing in size of the male femur during development. The increase in PC1 score was associated with: the increased robustness of the diaphysis and distal epiphysis; the decreased verticality of the femoral head in relation to the overall femur (decreasing in neck–shaft angle); and the gained size in distal epiphysis in relation to the overall femur (Fig. 10). The increase in PC2 score is associated with the change in proportions between the upper and lower epiphyses in relation to the diaphysis.

Change of shape and form between boys and girls

Figure 11 shows the distribution of the male and female individuals on shape space. The first two components accounted for 72% of shape variation (PC1 = 65%, PC = 57%). Female and male lines, computed via local linear regression of the PC scores on age, show the ontogenetic trajectories of the femur in each sex on the shape space (Fig. 11). Results indicated that the male and female ontogenetic trajectories diverged progressively from 9 to 14 years of age, with boys having higher values on PC2 than girls. Specifically, sexual shape differences in the average scores estimated at each age were significant from 11 to 14 years ($P < 0.001$). In particular, these differences increase much more after approximately 12 years of age, around the time of the female pubertal growth spurt (12.5 years). After this age the female ontogenetic trajectory tends towards a more horizontal slope, indicating that in this period the most evident age-related changes in girls are associated exclusively with the angular remodeling of the femur, which corresponds to the main changes along PC1. Contrarily, in boys, from 9 to 14 years of age, the most evident age-related modification correspond to the increase in diaphyseal length and size of both trochanters, although angular remodeling of the femur is still evident. These changes correspond to the main variation along PC2.

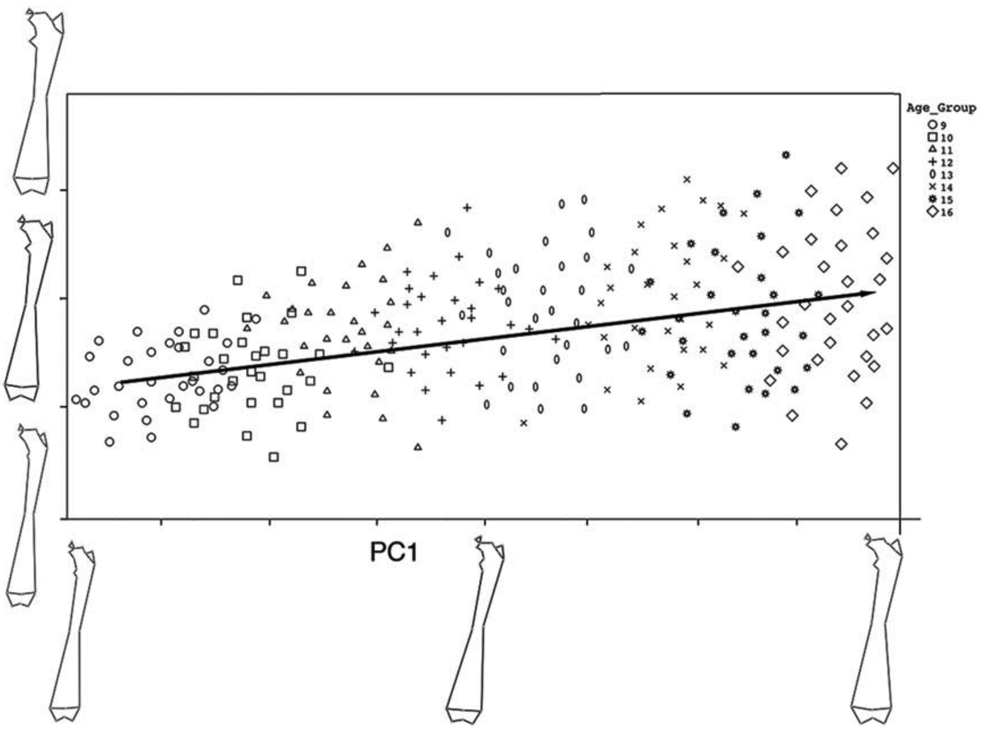


Figure 10: Scatter plot of the first two PCs of male femur form (Procrustes shape coordinates augmented by ln CS). The arrow represents ontogenetic allometry (allometric shape and size)—the regression of form on ln CS. Grids show the shape–size changes throw the negatives values (untransformed shape) to positive values of the axis.

Figure 12 shows sexual dimorphism in femur shape between 9 and 14 years. It is visualised as a series of six TPS deformation grids displaying the different consensus shape age stages for males and females separately. Results indicate that, in early stages, 9 and 10 years of age, girls and boys have similar femur shapes; however, at those ages, the girls' neck–shaft angle is reduced in relation to those of boys. Clear differences between the shapes of both femora appear from 11 years of age upwards, probably because of the proximity of the female growth spurt. In general, during the pubertal period, the female femur "seems" to increase its diaphyseal thickness in relation to the total femur, although in absolute values the male femora are somewhat broader and more robust than female femora at the same age (Fig. 12). This is because of the great increase in length of the male femur in relation to the female one. Furthermore, the female greater and lesser trochanters have smaller dimensions than those of boys; the femoral neck becomes more horizontal and the medial condyle of the distal epiphysis increases in size due to the angular remodeling of the femur, which is higher in girls than in boys.

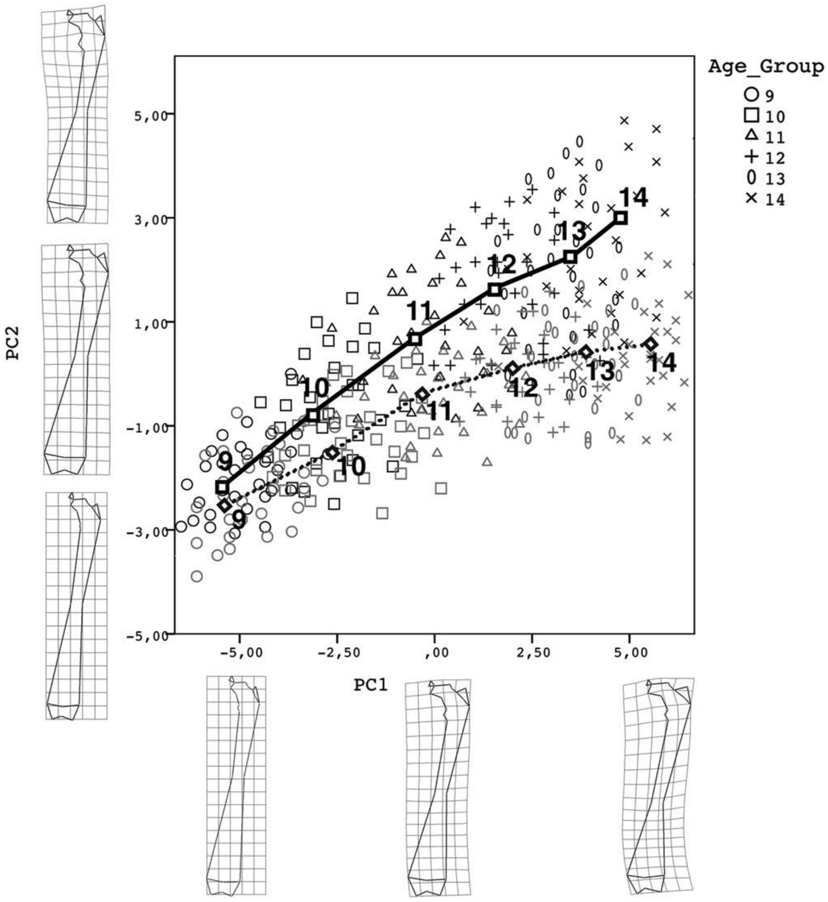
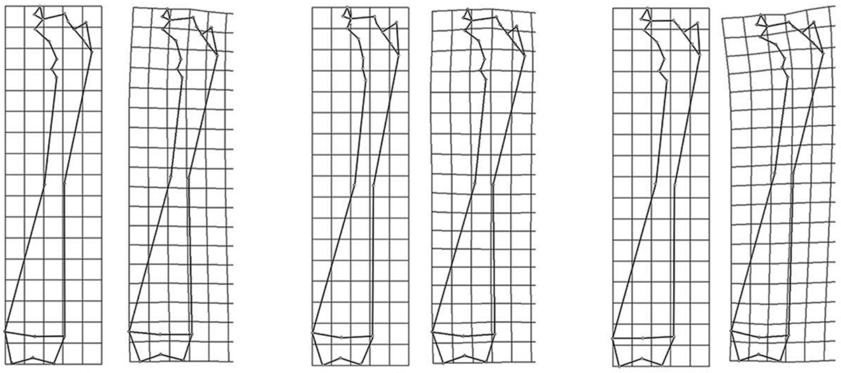
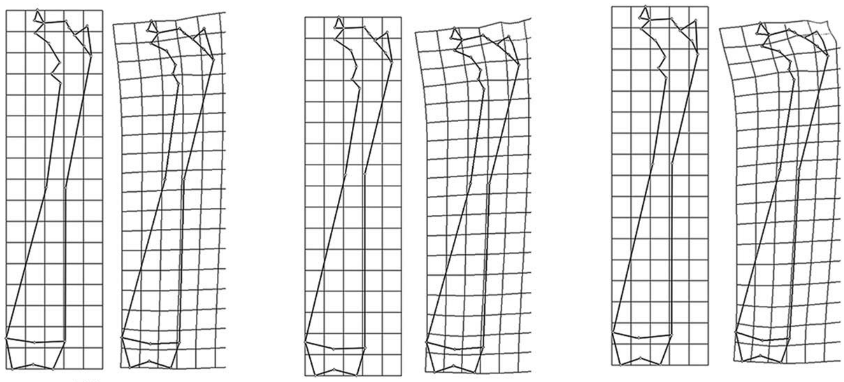


Figure 11: Scatter plot of males and females in shape space PCA and ontogeny trajectories established from the average PC scores at different ages in both sexes. Continuous line for boys and discontinuous line for girls. Grids show the shape changes throw the negatives values (untransformed shape) to positive values of the axis.

Figure 13 shows the ontogenetic trajectories (Fig. 13) of females and males in the size–shape space (Fig. 13). The first two components of the PCA in this space account for 88% of the size–shape variation (PC1 = 83%, PC2 = 5%). The ontogenetic trajectories are slightly different from those obtained in shape space. In this case the ontogenetic trajectories of both sexes run closely parallel (with boys having higher values in PC2) until approximately 12 years of age, from which they start to diverge significantly ($P < 0.001$). These differences between trajectories in shape space and size–shape space indicate that the amount of shape change per size change differs between sexes depending on age. From 9 to 11 years of age the size–shape relationship is more or less similar between males and females, however from the age of 12 years significant differences in shape occur (Fig. 12). In addition, in all ages from 10 years of age, male size–shape average grows faster than that of females (Fig. 13), due to the significant size increase of the male femur related to female femur during development. However, at 9 years this situation is reversed.



9 years 10 years 11 years



12 years 13 years 14 years

Figure 12: TPS deformation grids in shape for the age stages of 9, 10, 11, 12, 13 and 14 years for males and females separately.

DISCUSSION

The results obtained in this study allowed us to describe the variation in the size and shape of the male femur between the ages of 9 and 16 years. The maximum rate of increase in femoral dimensions is around 15 years, coinciding with the age range of the Spanish male pubertal growth spurt (10–15 years of age according to Ferrández et al., 2009) and before the age at which most Spanish males reach adult size which is 17 years (Fernández-Méndez and Seara-Aguilar, 1994; Carrascosa et al., 2008; Ferrández et al., 2009; García Cuartero et al., 2010). This is concordant with our observation on the fusion of the distal epiphysis in boys. These values are within the normal age range for the male pubertal growth spurt (12.5–17.5 years) in the current living male population (Tanner, 1962; Marshall and Tanner 1970; Gasser et al., 1991). Our findings indicate that the size differences between male and female femora can be detected from 11 years of age, when most Spanish females are entering or have already entered the pubertal growth spurt. The girls growth spurt starts about 2 years earlier (8–13 years according to Ferrández et al., 2009) than in males. As is well known, the male femur can grow for longer, thereby leading to longer femora (Bachrach and Smith, 1996; Scheuer and Black, 2000; Seeman, 2003). These femora and the fact that the elongation of the bone precedes the thickening (Bass et al., 1999; Bradney et al., 2000; Rauch et al., 2001;

Pujol et al., 2014) probably make the male femur appear in our study less robust (although it is not) than that of females of the same age (Pujol et al., 2014). In fact, mass and muscle strength are important for the development of bone robustness (Van der Meulen et al., 1993, 1996, 2002; Moro et al., 1996; Schoenau, 1998, 2005; Schoenau et al., 2000). By raising the muscular load, biomechanical forces increase, leading to the development of higher robustness (Schiessl et al., 1998; Rauch and Schoenau, 2001; Ferretti et al., 2003; Ruff, 2003). Males generally show a greater increase in muscle mass than females due to the increase in testosterone levels during growth (Round, 1999). Consequently, the muscle/bone ratio is higher in females than in males (Hogler et al., 2008) and the female femur is less robust (Ruff, 2003).

The shape variation in the male femur varied significantly between the ages of 9 and 16 years. Among these changes, those in both proximal and distal epiphysis are particularly striking (see below).

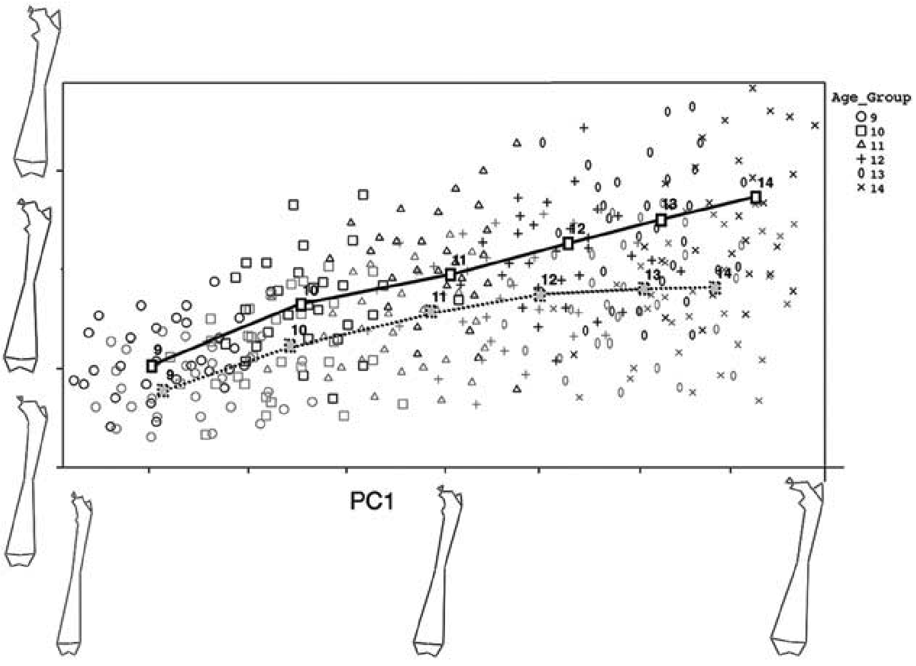


Figure 13: Distribution of males and females in size–shape space PC with ontogeny trajectories established from the average PC scores at different ages in both sexes. Continuous line for boys and discontinuous line for girls. Grids show the shape– size changes throw the negatives values (untransformed shape) to positive values of the axis.

Proximal epiphysis: Neck–shaft angle and trochanters

The obtained average for neck–shaft angle in each age group falls within the normal range of variability for each of these ages (Billing, 1954; Tardieu and Damsin, 1997; Muñoz-Gutiérrez, 2001). The youngest individuals of our sample (9 years of age) showed a mean value of 138º, and the oldest (16 years of age) an average of 129º. This last value is inside the normal range of variation (121.7º–136.5º) reported for adult European males (Anderson and Trinkaus, 1998). After 16 years of age, the neck–shaft angle became stable, agreeing with observations of previous authors who indicated that the values of this angle are stable from mid-adolescence throughout most adult life (Humphry, 1889; Yamaguchi, 1993), and with previous study on female femur (Pujol et al., 2014). During growth, there is a great increase in size in the proximal epiphysis, neck, head and both trochanters. The neck–shaft angle is more obtuse during the first years of individual life. Namely, the neck has a more vertical position, because the abductor muscle only develops as response to the onset of locomotion (Lovejoy, 2005). The neck–shaft angle decreases during growth, starting from an average of 150º at birth to approximately 127º at the end of the pubertal growth spurt, (Humphry, 1889; Keats et al., 1966; Hefti, 2000; Igbigbi, 2003).

Considerable population-based variability in the neck–shaft angle has been highlighted in the literature (Anderson and Trinkaus, 1998; Igbigbi, 2003). This variability seems to be related to physical activity (Anderson and Trinkaus, 1998), individual high (Lofgren, 1956; Lusted and Keats, 1966; Singh and Singh, 1975; Igbigbi, 2003) and climate (Gilligan et al., 2013). In mechanised societies with sedentary habits, the neck–shaft angle acquires greater values (Anderson and Trinkaus, 1998), which are located in the upper end of normal recent human ranges of variation. Lower values, which sometimes can be smaller than 120º, are not rare in clinically normal individuals of non industrialised societies (Anderson and Trinkaus, 1998). According to our results, sex-based differences in the neck–shaft angle are not significant during the early years of life and do not exceed 2º at 9 years of age.

However, sexual differences increase markedly with the onset of puberty, reaching an average difference between sexes of 10º at 14 years of age and statistically significant from 10 years of age onwards, age of the onset of the pubertal growth spurt in the female pubis (Rissech and Malgosa, 2007). The presence of sexual differences in the neck–shaft angle in the older age groups was concordant with the observations on adult femur by Walmsley (1933), Igbigbi (2003), and Bernard et al., (2012), and disagrees with the results by Otsianyi et al., (2011) and Gilligan et al., (2013). However, it must be taken into account that these two last studies mixed together individuals of different populations and ethnicities, with different levels and types of physical activities that could mask sexual differences. Likewise, the study of Otsianyi does not show a strong lack of sexual differences. Probably, the reason of these discordant results between authors could also be related to the possible different growth pattern of the neck–shaft angle in girls and boys. As can be seen from Figure 4, while girl values of neck–shaft angle are more or less constrained to a specific region of the graph, boys have a larger variability that in some cases overlaps with female values. Actually, the decreasing angulation of the femoral neck depends on both mechanical load generated by the muscles and ligaments (Serafimov, 1974; Tompkins et al., 1988) and the widening of the pelvis, which is important in females at this age (Tague, 2005; Birkenmaier et al., 2010). The broadening of the pelvis in females plays a key role in decreasing the neck–shaft angle (Singh and Singh, 1975; Gulan et al., 2000; Igbigbi, 2003; Tague, 2005; Birkenmaier et al., 2010) and could explain the lower variability observed for this angle in the female series. The lower values of some boys could be explained by their higher physical activity.

The size and shape of the trochanters are also related to the increase in mass of the gluteus maximus, gluteus minimus (greater trochanter) and pectineus (lesser trochanter) muscles, which actively participate in locomotion and in the stabilization of the femoro-pelvic and femoro-tibial joints. As expected, the comparison of the results of this study with those obtained in the female series (Pujol et al., 2014) shows that the increase in size and robustness of this region is greater in males (Fig. 13).

Distal epiphysis: bicondylar angle

The bicondylar angle is associated with the abduction movement of the lower limb. It is important to note that the bicondylar and the tibio-femoral angles must not be confused; the latter is the angle between the tibial and femoral diaphyseal axes in the coronal plane of the leg. The tibio-femoral angle measures a physiological phenomenon and the bicondylar angle measures an osteological one (Tardieu and Damsin, 1997). The bicondylar angle permits the location of the knee and ankle joints almost directly under the body's center of gravity to generate biomechanically efficient bipedal locomotion (Aiello and Dean, 1990). This makes the load transmitted through the lower limbs to be close to the vertical axis of gravity of the body during the phases of walking and running. Consequently, the center of gravity of the body needs to move only a short distance laterally to lie above the standing leg during walking (Aiello and Dean, 1990). The bicondylar angle has been key of the assignation of fossils to human lineage (LeGros Clark, 1947; Johanson and Coppens, 1976; Tardieu and Trinkaus, 1994) because its implication in bipedal locomotion (Tardieu and Damsin, 1997).

Despite the paleoanthroplogical significance of the bicondylar angle, few studies have focused on its development (Salenius and Vankka, 1975; Tardieu and Damsin, 1997). Our results are consistent with those obtained by Tardieu and Damsin (1997) in boys from 0 to 12 years of age. These authors proposed a function for calculating the male bicondylar angle taking in relation to age. According to this study, at birth the bicondylar angle is 0º; at 9 years of age 6º; and at 12 years 7.1º. In our sample, the angulation was 6.93º at 9 years of age, 7.51º at 12 years, increasing to 8.11º at 16 years. These results contrast with those found in other studies, which show that the male bicondylar angle only varies up to 7 years of age, reaching its adult value at that age (Tardieu et al., 2006). The values of the bicondylar angle in our sample stabilise at around 15 years of age, the mean age at which the male pubertal growth spurt finishes in the population of Barcelona (Ferrández et al., 2009).

The change of angulation of the femur produces specific mechanical loads that in turn pressure in the femoral bicondylar region which makes to react the distal epiphysis. The force produced to the action of the different muscle groups and the patella causes an asymmetrical load in the condyles, which is greater on the medial condyle. This generates a reaction in the endochondral cartilage of the medial condyle, which lead to a greater size increase of this condyle which in turn produces the angulation of the distal femur (Shefelbine et al., 2002). The greatest changes of the bicondylar angle take place from the beginning of locomotion to 8 years of age and, according to our results, keep on until the femoral growth spurt (15 years), before the epiphyseal closure. These findings are in agreement with the variation observed during puberty in the female Spanish series analysed by Pujol et al. (2014). The growth cessation of the bicondylar angle is an open question (Tardieu and Damsin, 1997) due to the paucity of studies on this subject and the scarcity of available studies (Tardieu and Trinkaus, 1994; Tardieu and Damsin, 1997) do not furnish enough information to clarify the question. Most of these studies are based on the tibio-femoral angle (Salenius and Vankka, 1975; Cahuzak et al., 1995; Mehmet-Arazi et al., 2001), and they do not offer concordant results on the age of growth cessation for the bicondylar angle. Salenius and Vankka (1975) claim that the tibio-femoral angle would stabilise at 6 or 7 years of age, and thus the femur would grow in length keeping this angle constant from this age. In contrast, other authors have suggested that the values for this angle stabilise at around 10 (Cahuzak et al., 1995) or 12 years of age (Mehmet-Arazi et al., 2001). But the point is, that all this studies and given ages of growth cessation are built on the development of the tibio-femoral angle and not on the bicondylar angle. In relation to the increase of the bicondylar angle, Tardieu and Damsin (1997) and Pujol et al. (2014) observed that in females it finishes at around 13 years of age, which is around the feminine growth spurt in height. This study suggests that the age of growth cessation of the bicondylar angle in males is about 15 years, around the male growth spurt and concordant with the observations of Tardieu and Damsin (1997) and Pujol et al. (2014). In this study, the mean bicondylar angle at 16 years of age is 8.11º, value that falls within the normal range of variation for the current population (8º–15º; Pearson and Bell, 1919; Keats et al., 1966; Singh and Singh, 1975; Pandya et al., 2008). This value is also close to that reported for a sample of Parisians (7.9º) but slightly lower than that obtained for a sample of Londoners (10.2º; Tardieu and Trinkaus, 1994). As the neck–shaft angle, the bicondylar angle shows great variation between different populations (Pandya et al., 2008). This variability is the result of different mechanical loads supported by the femur, due to the different physical activities performed by the individuals and the influence of different genetic, cultural and environmental factors (Tardieu and Trinkaus, 1994).

In females, the bicondylar angle (Pujol et al., 2014) is higher than in males. This result is concordant with previous observations (Tardieu and Damsin, 1997; Scheuer and Black, 2000, Igbigbi and Sharrif, 2005; Pandya et al., 2008). These sexual differences are statistically significant from 10 years of age, when the pubertal growth spurt in the female pubis occurs (Rissech and Malgosa, 2007) and agree with the significant sexual differences found in adults (Igbigbi and Sharrif, 2005; Pandya et al., 2008). This finding further supports the idea that the distal region of the femur has a higher sexual discrimination power for adult individuals (Gill, 2001; Holliday and Ruff, 2001). The bicondylar angle in males is more variable than in females, which exhibit a greater canalised growth. Canalization in human growth is a widely understood phenomenon and extended concept in pediatrics, indicating that children stay in one or two adjacent growth channels following a parallel-to-centile pattern in growth (Hermanussen et al., 2001). This idea of growth canalization was described by Tanner (1962) and it is considered an evidence of the robust control that genes exert over body size—See Nelson, Text book of Pediatrics pp. 58 (Behrman et al., 1999). Canalisation means that children normally grow with remarkable fidelity relative to the normal growth curve, and physicians are trained to note abnormal height velocity since it always warrants further evaluation (Wilson et al., 1998). Even when illness or starvation temporally interrupts the process of normal height acquisition, catch up growth usually seems to compensate for these losses (Boersma and Wit, 1997), leading stature back to the original centile. Catch up growth has been considered one of the strongest examples of developmental canalization in humans (Prader et al., 1963).

These findings of different growth patterns of both femoral angles between boys and girls, specifically the bicondylar, could indicate the importance of pelvic breadth development in females. Given the anatomical and functional relationship femur–pelvis, the former is subjected to two selective pressures, namely locomotory needs and the interacetabular distance in females, a variable that is related to the width of the birth canal (Parsons, 1914; Pearson and Bell, 1919; Walmsley, 1933; Heiple and Lovejoy, 1971; Tardieu, 1981, 1983; Berge, 1993; Tardieu and Trinkaus, 1994). It is not possible to compare exhaustively the findings on the canalised growth in the bicondylar angle in girls. Most studies on this variable are based on adults and, as we stated before, there is a lack of information on its changes during growth. Furthermore, the few published studies on this subject based on subadults used small samples which may bias findings. For example, Tardieu and Damsin (1997) used a sample of 77 postnatal individuals between 5 months and 17 years old, and Tardieu et al. (2006) used a sample of 48 individuals between 6 fetal months and 18 years of age. In addition, Tardieu et al. (2006) applied logarithms in their analysis of correlation between this variable and age, which could be another possible reason which obscures the differences in the bicondylar angle pattern of variation between boys and girls detected in our study.

The bicondylar angle arises due to the asymmetric growth of the femoral condyles. The greater size of the medial condyle is related to asymmetric loads due to the decrease in the neck–shaft angle and the action of muscles from the lower limb, as well as the load from the patella. All these forces determine that the metaphyseal cartilage generates more bone tissue in the medial condyle (Shefelbine et al., 2002). In turn, the decrease in the neck–shaft angle is related to the increase in mechanical loads generated by muscles and ligaments, structures that also depend on the increase in pelvic breadth, which is particularly important in young females. Indeed, the appearance of sex-based differences in these two angles occurs at around 10 years of age, when the pubertal growth spurt in the female pubis generally starts (Rissech and Malgosa, 2007).

According to our results, the angular remodeling of the femur is extremely significant in girls, specifically from 12 years of age upward. Conversely in boys, both the increase in size and the angular remodeling of the femur are equal in importance during the age period analysed. These different patterns of femoral development are expressed by divergent trajectories, especially from 12 years of age. The postnatal divergent trajectories during development is an aspect of the process to acquire adult shape differences (Mitteroecker et al., 2004a; Frelat and Mitteroecker, 2011), in this case, sexual differences. The extension or truncation of growth trajectories to generate sex-specific and species-specific differences is currently a subject of discussion. Older and univariate studies presented sex-specific and species-specific differences as extension or truncation of identical developmental trajectories (see Bulygina et al., 2006). However, current studies indicate that this vision is too simplistic (Bulygina et al., 2006). Contrarily, several studies on humans (Bulygina et al., 2006; Coquerelle et al., 2011) and other vertebrates (Hingst-Zaher et al., 2000) demonstrated that developmental trajectories are not frequently the same between species or between sexes of the same species.

The constant size difference between boys and girls in both femoral angles and the constrained values acquired in girls, especially in the bicondylar angle, are new findings, which suggests that the sexual dimorphism in these two angles could be partially prenatally established in girls. Likewise, our results on sexual dimorphism of the femur contribute to the discussion about the underlying factors that affect pelvic morphology. Both angles, neck–shaft and bicondylar, are related with the development of the pelvic breath, specifically with the biacetabular and transverse diameter of the mid plane of the pelvis. The significant sexual differences in this last variable of the birth canal have been found by different authors and types of studies (Kurki, 2013; Crespo et al., 2015), pointing out its importance in the structure of the birth canal and the human parturition process (Kurki, 2013; Crespo et al., 2015). The growth pattern of the girl femur could indicate that the observed constrictions of these two femoral angles are due to the functional need of the female birth canal in Homo sapiens. Quantitative explorations on the development patterns of the femoral sexual dimorphism in other humans and primates will be helpful to elucidate some of these questions and to determine the existence of shared developmental patterns among primates.

CONCLUSIONS

The information provided by this study increases the understanding of femoral development in 21st-century children, especially those from Spain. Specifically, it provides information concerning the period and morphology of the different secondary ossification centers in the male femur during development, which is in agreement with the development patterns observed in previous studies on the development of the femur during puberty. Furthermore, our results provide new information on the patterns of femoral development, the possible factors that contribute to femoral sexual differences and the timing of the angular remodeling of the postnatal male femur. Our analyses also indicate a continuous remodeling until 16 years old for male neck–shaft angle and 15 years old for bicondylar angle and agree with the high variability shown by different studies. Sexual dimorphism analysis showed a divergent pattern of development between boys and girls, especially from 12 years of age. From that age, the angular remodeling of the femur predominates almost exclusively in girls. In boys, the angular remodeling and the increase in size have equal importance during the ages considered. In the female femur, the observation of a canalised growth in both angles, specifically in the bicondylar, is a new finding, which suggests that the sexual dimorphism observed in these angles could be partially established prenatally. Furthermore, our results on sexual dimorphism may contribute to the discussion on the underlying factors that affect pelvic morphology because the close relationship with the pelvic morphology and bipedal locomotion. Our findings may be also useful for helping to determine bipedal locomotion in immature fossil individuals, especially considering the differences in variability of these angles between males and females, and the effect of pelvic breadth on the formation of these angles, especially the bicondylar angle. Sex-related differences here detected should be taken into account in future analyses on these angles (e.g., in studies of human evolution in which the sex of the fossil individuals is unknown). In general, results of this study can be meaningful to improve our understanding of the onset of bipedalism in the human lineage and to refine sub-adult age-estimation methods, both of which are essential to progress in osteoarchaeology, forensic sciences and paleoanthropology.

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3.3. POSTNATAL DEVELOPMENT OF THE FEMUR BASED ON A SPANISH SAMPLE OF LIVING INDIVIDUALS. IMPLICATIONS FOR AGE ESTIMATION.

Aniol Pujol[1], Carme Rissech[1], and Daniel Turbón[1]

[1]Departament de Biologia Animal, Facultat de Biologia, Unitat d'Antropologia Física, Universitat de Barcelona, Barcelona, Spain

ABSTRACT

This study analyses the post-natal development of six femoral variables (maximum length, diaphyseal length, head diameter, bicondylar breath, collo-diaphyseal angle and bicondylar angle) and the epiphyseal fusion of the head and distal epiphysis in order to evaluate their significance and capacity for age and sex estimation in living and death individuals from current Spanish population. The material analysed consisted of digital images in DICOM format (telemetries), corresponding to 1140 left femora in anterior view (570 boys and 570 girls), obtained from the database of the Hospital Sant Joan de Déu in Barcelona (Spain). The samples belonged to individuals from the 21st century whose age ranged from birth to 18 years. Metrical variables were analysed by polynomial analysis and the growth curves were depicted. Concerning the qualitative variables of the stage of union of the epiphyses (O'Connor's 5 phases), we analysed their frequency and possible sexual differences in age at each stage (Student T-test).

None of the curves showed lineal growth. Both male maximum femoral length and diaphyseal length had the most complicated growth, expressed by a six-degree polynomial. All the variables were useful for sub-adult age estimation except the collo-diaphyseal and bicondylar angles, due to their high expressed variability. Significant sexual differences were observed from age 10 onward, with the exception of the head diameter and the bicondylar breath, which were significant from 9 years of age onward.

Regarding the quantitative variables, they followed the same timing as other samples from equal socioeconomic status and time period. They started to fuse at 14.5 years of age in boys and 12.1 years of age in girls, finishing at 17.1 and 16.1 years of age respectively. These results indicate that any of the studied femoral measurements can be useful for age estimation in individuals of Spanish origin under 19 years of age from current forensic cases.

Key words: Femur, Development, Polynomial function, Age, Sex

INTRODUCTION

Sub-adult age estimation based on the stage of skeletal growth and maturation is basic for the diagnostic, prognostic, and therapeutic evaluation in paediatric pathology and in archaeologic and forensic cases. These age estimations depend on the relationship between chronological age and the degree of bone development. According to the degree of growth and maturation of the analysed individual, the methods for sub-adult age estimation give us a specific age range. Each method for sub-adult age estimation is built on a model of bone development (growth and maturation) based on a specific reference sample. These models can be based on documented skeletal collections (with known sex and age) or high-resolution digital radiographies, ranging from telemetries (accurate radiographies of the limbs) and scoliograms (vertebral column radiographies; also known as rachis) to multislice computerised tomographies, which do not distort the actual measurements. In addition, the biological proximity between the reference collection, in which the method is based, and the analysed sample is essential for the accuracy of the estimations. In fact, the accuracy of the estimations depends on the availability of appropriate data relating to the development of the skeletal elements, which we are going to analyse, with regard to genetic, environmental and cultural factors [1-3]

Most of the growth and maturation standards for osteological studies for Western European individuals currently available are based on radiological images and skeletal collections from white North American children from the 1950s and before [4-15]. The few studies which focus on Western European children and particularly Spanish children are mostly based on archaeological material in which the sex and age were estimated in the lab [16-20] and in documented skeletal remains from the late 19th and first half of the 20th century [21-30]. However, the current Western European population has had a noticeable height increase in the second half of the 20th century as a result of improved living conditions (for example, for the Spaniards it has been 10 cm [31]), and the magnitude of error when we apply these methods in current living Spanish children is unknown. For example, the adult stature (it is necessary to remember that the adult stature is the result of the growth) is systematically overestimated in male and female of Spanish and Italian origin when Trotter and Gleser [32] formulae for calculating adult stature based on White American reference samples is applied [33-35]. Because of the intertwined biological population history of French, Spanish and Italian populations, and because they are populations of medium stature, the use of the formulae proposed by Pearson [36] at the end of 19th century, based on a French sample performs better than the Trotter and Gleser method [33-35]. Likewise, the data given by Gindhart [12] and

Hoffman [13], based on White Americans of North-Western European descent, underestimate ages in sub-adult individuals from the Iberian Peninsula, even though both the reference sample and children derive from the mid-20th century [24]. In fact, North American populations are taller than most European populations [36-38], and even though the stature has increased in Europeans recently, due to the improvements in health and living conditions, some differences still exist [39].

Given the anthropological importance of the femur as a result of its locomotory function [40-42] and its postdepositional resistance, we analysed femoral development during the entire postnatal period in a contemporary Spanish population.

MATERIAL AND METHODS

Sample and data acquisition

High-resolution digital radiological images (telemetries) in Digital Imaging and Communication in Medicine (DICOM) format from the archives of Hospital Sant Joan de Déu de Barcelona were used for this analysis. All personal data were omitted with the exception of the demographic information (sex and age) following the Spanish Organic Law of Data Protection (*Ley Orgánica de Protección de Datos*; for details see García et al [43]), and this study has the approval of the Bioethical Committee of the Hospital Sant Joan de Déu de Barcelona (Ref. number 2938). This type of material (telemetries) was chosen because of the absence of any type of image deformation in it, which is due to both the distance at which telemetries are taken (more than 180 cm from the centre of the x-ray focus and the object to be radiographed) and the contact that the subject has with the chassis or radiographic film (it is in bucky mural in telemetries). For this reason these distant type of high-resolution digital radiographies are fully used for medical purposes of evaluation and real mensuration of the extremities (telemetries) and rachis (scoliograms).

The left femora in anterior view of a total of 1140 living boys and girls aged from 0 to 18 years and born between 1991 and 2010 were analysed (in groups of 30 individuals for each group of age and sex). Those individuals displaying any type of pathology or anatomical deformation that could affect the analysis were excluded.

All the telemetries were taken in the Hospital Sant Joan de Deu following the standardized procedure of the Hospital. The subject was made to undress from the waist down, and to stand in a bipedal anterior position, supporting the back of the body on a bucky mural special for telemetry, with the arms extended along the body, feet together and hip and knee in full extension: the patellae facing forward with the femora and tibiae vertical with a slight internal rotation, just like the natural anatomical position would be in this vertical position. There was an equal weight bearing on both limbs. The tube was focused perpendicularly at the inferior angle of the platellae in the knee; there was a film-focus distance of 200 cm. Exposure was 120 Kv y 50 – 60 mA per second.

All the individuals come from the subadults' radiological collection of the Universitat de Barcelona, recently created by one of the authors of this study (A.P.) for his doctoral thesis under the supervision of D.T. and C.R. This collection comprises radiological images obtained from 1140 individuals (570 boys and 570 girls) of Spanish origin, born between 1991 and 2010, ranging from birth to 18 years. All of these individuals belong to a middle socioeconomic class: the 18-year-old boys show a mean height of 177.5 cm, and the girls a mean height of 164.3 cm. These values correspond to the expected male and female adult mean height in current living Spanish population: 174.20 cm (SD: 7.08) for males and 162.09 cm (SD: 6.37) for females [31]. More information concerning these individuals can be found in Pujol et al. [44].

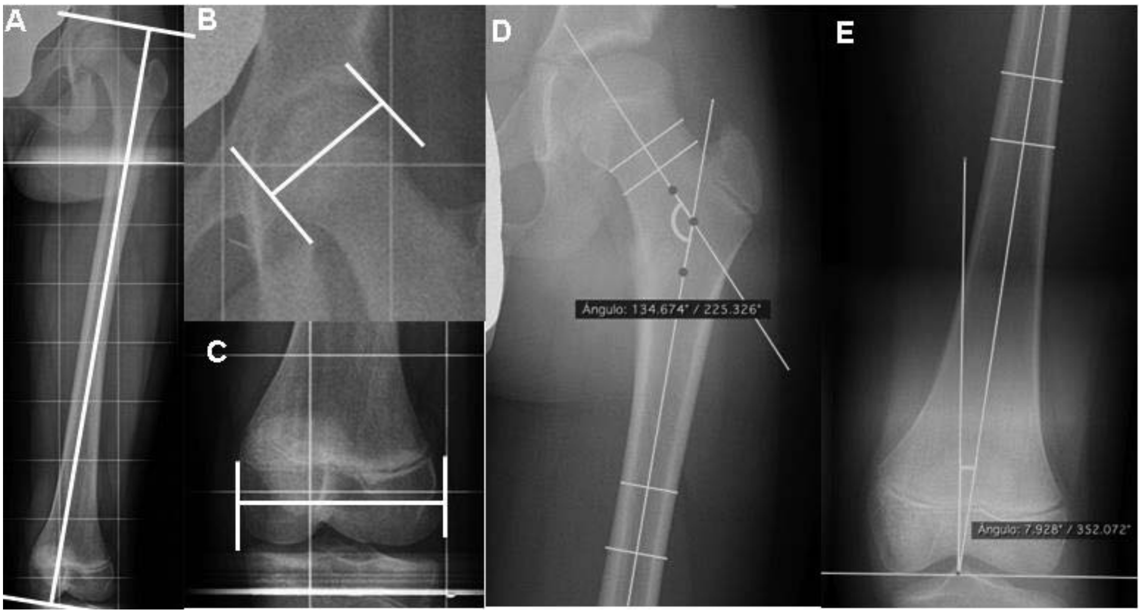


Figure 1: Analyzed measures: A) Maximum length of femur; B) Diaphyseal length; C) Head diameter; D) Bicondylar breadth; E) Collo-diaphyseal Angle; F) Bicondylar Angle

In this study 6 metrical and 2 qualitative variables of the femur were evaluated. The qualitative variables describe the state of union of epiphyseal fusion process, in one side of femoral head with the diaphysis, and in the other side distal epiphysis to the diaphysis. The union of the greater and lesser trochanters was not measured because the difficulty to observe this process in them made their evaluation impossible. The 6 metrical variables taken were the following:

1) Maximum length of the femur (Figure 1A): Maximum distance between the femoral head and the medial condyle of the femur [45, 46]. When epiphysis were not fusioned yet, the length was measured including the epiphysis as one unique unit, without considering the space between diaphysis and epiphysis.

2) Diaphyseal length (Figure 1B): Maximum distance between the proximal and the distal region of the diaphysis, without both epiphysis. More specifically were the methapisarial lines are.

3) Vertical diameter of the head (Figure 1C): Diameter perpendicular to the anteroposterior diameter of the femoral head [45, 46].

4) Distal epiphysis width (Figure 1D): Maximal width between the distal and medial condyle [46].

5) Collo-dyaphiseal angle (Figure 1E): It is the angle between the longitudinal axis of the femoral diaphysis and the longitudinal axis of the neck [47]. The longitudinal axis of the femoral shaft is the midline along the shaft which connects the middle of the infra-condylar and proximal segments of the femur. The longitudinal axis of the neck is the midline along the neck which connects the distal and proximal limits of this anatomical region. In order to graphically locate these axes on the radiological images, two perpendicular lines to the shaft and neck directions were plotted in the middle of these anatomical regions (Figure 1E). The line which crosses the middle of these two lines perpendicularly was considered the shaft and neck axes, respectively (Figure 1E).

6) Bicondylar angle (Figura 1F): It is the angle formed by the longitudinal axis of the diaphysis and a line perpendicular to the infracondylar plane of the femur [48].

With regard to the qualitative variables, to evaluate the stage of union of the head and distal epiphysis of the femur, we considered the 5 phases proposed by O’Connor et al. [49] based on radiographic assessment, which are a modification of those proposed by McKern & Stewart [50] for the knee and which were in turn based on osteological assessment. These 5 phases of union are the following:

Stage 0 – Non-union (Figure 2A): The diaphyseal and epiphyseal bones are adjacent to each other but show no intimate relation as yet. The epiphysis remains separate from the diaphysis due to the presence of the cartilaginous growth plate. This is apparent on the radiograph, in at least one view, as a continuous radiolucent gap between the epiphysis and diaphysis. The terminal plate is a layer of cortical bone, on the metaphyseal aspect of the epiphyseal centre of ossification that is visible radiographically [51].

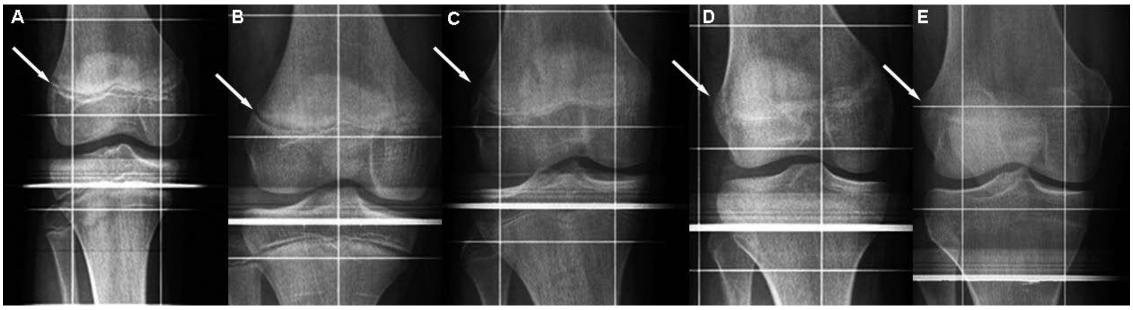


Figure 2: Distal epiphysis region where stage of union of the fusion process can be observed, from stage 0 (A) until stage 4 (E). Arrows signalize metaphyseal plate zone.

Stage 1 – Beginning union (Figure 2B): The epiphyseal and diaphyseal surfaces closely approximate each other. The radiolucent strip between adjacent surfaces of the epiphysis and diaphysis has become narrowed by comparison with the stage of non-union. The radiolucent gap is not continuous from anterior to posterior or medial to lateral. Towards the central region of the growth plate there is a definite breaking up of the adjacent outlines of the epiphyseal and metaphyseal margins. This appears as a hazy area in the centre which is of greater density than the adjacent bone. The radiodense appearance on the radiograph is a consequence of the extension of bone across the intervening gap between shaft and epiphysis. This demonstrates that the union phenomenon has begun, but not in the totality of the metaphyseal plate.

Stage 2 – Active union (Figure 2C): The epiphysis and diaphysis now cap each other. *Capping* refers to the way in which the epiphysis overlaps the metaphysis as maturation proceeds [51]. The terminal plate of the epiphysis is no longer distinguishable. A fusion line or zone of greater density than the adjacent bone replaces the epiphyseal cartilage. The presence of the radiodense region indicates that fusion is actively occurring. Small areas of radiolucency separating the epiphysis and diaphysis may be evident towards the margins of the bone. Less than half the growth plate is judged to be radiolucent. This indicates that union is actively occurring but has not yet reached the margins of the bone.

Stage 3 – Recent union (Figure 2D): The epiphysis and diaphysis have united to form a single unit of bone, i.e. there is complete capping. The position of the former epiphysis and diaphysis can still be distinguished. A fine line of fusion of greater density may remain between the epiphysis and diaphysis. There is discontinuity of trabeculae between the former epiphysis and diaphysis. Although the epiphysis caps the diaphysis there may still be a slight notch at the margin of the growth plate (less than 2 mm) that has not yet completely calcified. The combination of discontinuity of trabeculae between the epiphysis and diaphysis and the notches at the peripheral margins of the bone indicate that the bone is recently united.

Stage 4 – Complete union (Figure 2E): The epiphysis and diaphysis are united as a single unit of bone. Remodelling has taken place and there is continuity of trabeculae from shaft to former epiphysis. This presents as a uniformity of internal bone pattern throughout the end of the long bone up to the articular surface. All trace of epiphyseal differentiation has been lost. There are no radiolucent notches evident at the peripheral margin of the bone, indicating that the growth plate has now completely ossified and the bone is fused in its entirety. A thin terminal line, the epiphyseal scar, which marks part of the location of the former epiphyseo-metaphyseal junction, may remain in some cases.

Statistical analisis

To analyse each of the six metrical variables, we divided the study in 3 parts. Firstly, we made a first approximation to the sexual dimorphism of the analysed variables by a Student T-test in each age group. Secondly, in order to describe the growth behaviour of each variable a polynomial regression up until the tenth degree, treating age as continuous, was applied. Regression analysis was selected based on the assumption that the dynamics of growth can be described by an incremental continuous function [10, 52]. The most appropriate statistical model was then selected on the basis of three factors: (1) the strength of the correlation coefficient (R^2); (2) the significance of the function expressed by the F value; and (3) the significance of the coefficients of the function obtained by the ANOVA test.

Finally, in order to permit age prediction, inverse regression analysis was performed for age and each metrical variable of the femur using age as dependent variable. In other words, each metrical variable of the femur (x) was regressed on age (y). This method was selected because generally when the individual for study comes from the same distribution as a referenced sample (same or similar population from a nearby geographical area), the inverse regression is the correct methodology to use [53]. Polynomial regression was calculated for both sexual series separately, but in the series without sexual differences, calculus was applied to the whole data (female and male together). This latter case enables the application of the results in archaeological and anthropological remains when the sex is unknown.

Concerning the epiphyses stage of union of the epiphyseal fusion, in order to evaluate the existence of possible differences in the mean age of each stage of union between boys and girls, we applied a Student t test.

RESULTS

Tables 1 (A/B) and 2 show the results for the metrical variables. Table 3 and 4 show the results of the epiphysis state of union. For the sake of clarity, the results for each variable will be presented separately.

Maximum length of the femur

Results in table 1 (A) show that in boys, the average maximum length of the femur was 134.44 mm at birth and 475.36 at 18 years of age, being constant from 17 years onwards. In girls, the average maximum length of the femur was 135.43 mm at birth and 443.63 at 18 years of age, being more or less constant from 15 years of age onwards. From birth up until 11 years of age, the average values for females were always greater than in males. Beyond this age, the average values for males were always higher. However, these differences were statistically significant from 10 years of age onwards.

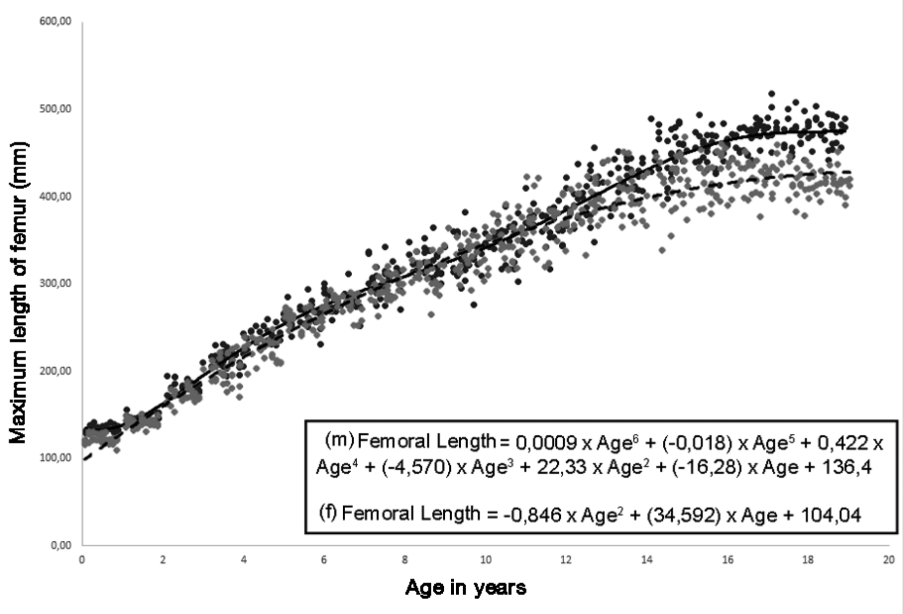
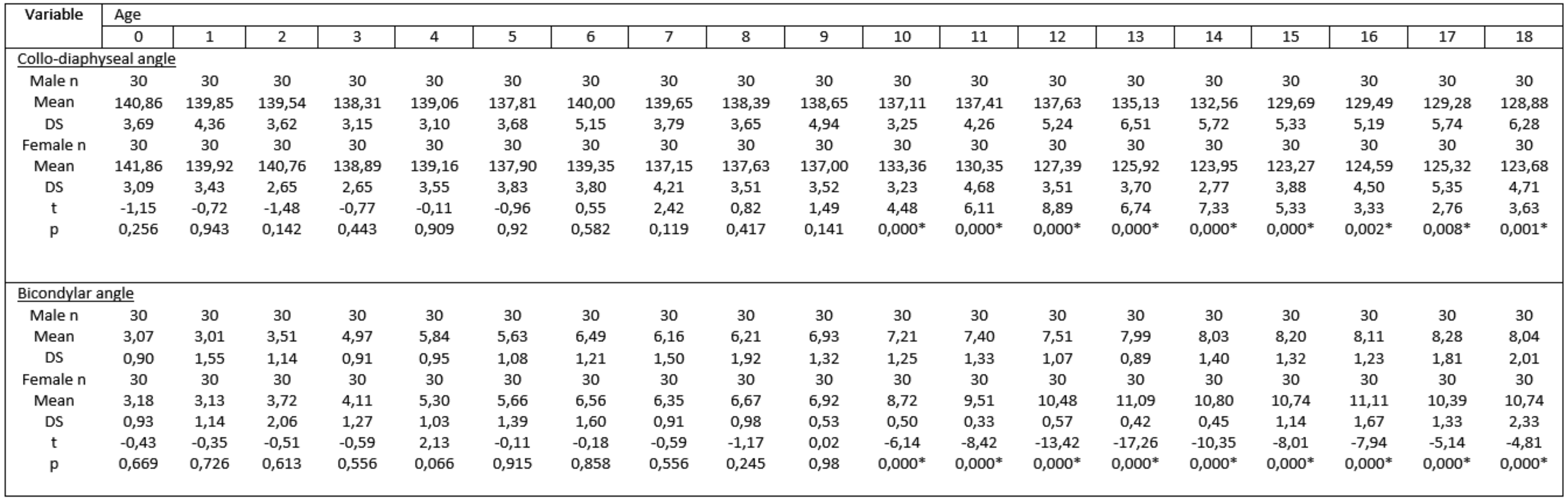


Figure 3: Polynomial regression line of the maximum length of the femur for both sexes (continuous line for boys, discontinuous for girls), from 0 to 18 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

Variable	Age																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Max. length femur																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	134,44	143,42	180,82	209,57	235,43	149,54	276,11	301,6	318,43	340,54	359,76	382,3	403,65	426,76	450,90	452,22	466,65	475,28	475,36
DS	2,36	5,11	7,21	6,41	10,65	13,42	12,43	18,35	16,54	20,99	20,01	18,65	24,65	22,43	24,84	20,25	13,87	18,67	14,97
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	135,43	147,65	178,65	210,32	235,65	265,76	283,44	301,54	326,43	345,65	369,87	376,43	391,65	412,21	437,77	442,56	445,78	445,83	443,63
DS	4,23	3,54	7,87	13,25	13,65	11,65	9,89	12,87	19,01	18,46	24.65	23,65	25,43	21,42	29,01	23,78	17,32	19,60	12,74
t	1,28	0,78	0,78	0,97	0,67	-0,36	-1,34	-1,61	-1,14	1,77	-4,15	-4,15	-1,801	1,26	1,88	1,69	5,15	5,96	8,84
p	0,079	0,145	0,143	0,345	0,555	0,675	0,132	0,232	0,343	0,324	0,000*	0,000*	0,077	0,046*	0,032*	0,000*	0,000*	0,000*	0.000*
Diaphyseal length																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	-	-	-	-	-
Mean	133,92	146,80	182,27	213,73	237,53	262,58	280,68	304,98	325,10	330,14	347,51	365,95	401,24	418,90	-	-	-	-	-
DS	3,76	4,12	6,51	8,71	11,16	12,62	11,78	17,56	15,23	21,69	19,87	19,16	25,92	21,73	-	-	-	-	-
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	-	-	-	-	-
Mean	131,39	150,33	182,73	210,78	238,61	271,99	291,76	311,51	330,05	351,60	372,19	389,67	412,88	422,70	-	-	-	-	-
DS	5,33	4,87	7,66	14,35	12,13	10,18	10,26	13,51	18,24	18,58	25,83	24,81	24,10	21,72	-	-	-	-	-
t	1,28	0,78	0,78	0,97	0,67	-0,36	-1,34	-1,61	-1,14	1,77	-4,15	-4,15	-1,801	1,26	-	-	-	-	-
p	0,089	0,122	0,122	0,338	0,567	0,723	0,238	0,112	0,259	0,281	0,000*	0,000*	0,077	0,046*	-	-	-	-	-
Head diameter																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	6,33	10,85	15,79	20,27	27,20	29,06	29,99	29,56	31,77	35,99	38,62	41,79	42,69	44,66	46,74	50,30	50,52	52,00	52,06
DS	1,14	1,47	1,79	1,44	1,72	1,73	1,13	1,74	1,88	2,02	2,39	2,17	1,81	1,64	1,45	2,63	2,44	3,30	3,29
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	8,55	18,86	19,16	19,67	24,26	26,49	29,08	30,25	29,88	31,76	33,58	34,74	36,83	37,71	41,03	42,20	41,41	41,22	41,56
DS	1,54	1,17	1,80	1,61	1,43	1,53	1,37	1,66	1,64	1,98	1,87	2,31	2,48	1,93	1,80	3,09	3,14	3,15	2,90
t	-2,65	-5,43	-4,67	1,51	-0,43	-5,43	4,01	-1,59	4,16	8,18	9,08	12,18	10,45	15,01	13,55	10,92	12,55	12,94	13,12
p	0,234	0,545	0,435	0,138	0,099	0,543	0,073	0,118	0,078	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0.000*
Bicondylar breadth																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	21,80	30,90	39,84	42,60	47,40	59,96	60,82	63,64	67,33	68,98	75,34	76,79	81,52	83,43	85,95	85,60	90,36	89,14	90,08
DS	1,40	1,86	1,52	1,34	2,02	2,02	2,20	1,44	1,67	2,50	3,09	3,15	2,67	3,28	3,20	3,78	4,03	3,42	2,96
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	23,46	34,16	36,19	39,49	45,85	55,40	56,00	57,69	63,52	66,31	69,67	69,45	69,61	68,34	70,34	70,38	68,27	69,12	68,47
DS	1,89	1,70	1,39	2,00	2,07	2,03	1,96	2,52	2,06	1,86	2,92	2,54	2,53	3,28	2,34	3,44	3,14	3,90	3,41
t	4,54	6,54	5,43	7,65	4,54	9,78	9,76	2,45	7,87	4,69	7,30	9,94	17,73	17,83	21,58	16,31	23,70	21,13	26,19
p	0,343	0,435	0,564	0,765	0,443	0,232	0,237	0,156	0,059	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*

Table 1A: Descriptive statistics of the first four variables classified according to each age category and sex. Sexual differences by t-Student test. The significance is indicated by asterisk (*).



Variable	Age																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Collo-diaphyseal angle																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	140,86	139,85	139,54	138,31	139,06	137,81	140,00	139,65	138,39	138,65	137,11	137,41	137,63	135,13	132,56	129,69	129,49	129,28	128,88
DS	3,69	4,36	3,62	3,15	3,10	3,68	5,15	3,79	3,65	4,94	3,25	4,26	5,24	6,51	5,72	5,33	5,19	5,74	6,28
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	141,86	139,92	140,76	138,89	139,16	137,90	139,35	137,15	137,63	137,00	133,36	130,35	127,39	125,92	123,95	123,27	124,59	125,32	123,68
DS	3,09	3,43	2,65	2,65	3,55	3,83	3,80	4,21	3,51	3,52	3,23	4,68	3,51	3,70	2,77	3,88	4,50	5,35	4,71
t	-1,15	-0,72	-1,48	-0,77	-0,11	-0,96	0,55	2,42	0,82	1,49	4,48	6,11	8,89	6,74	7,33	5,33	3,33	2,76	3,63
p	0,256	0,943	0,142	0,443	0,909	0,92	0,582	0,119	0,417	0,141	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,002*	0,008*	0,001*
Bicondylar angle																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	3,07	3,01	3,51	4,97	5,84	5,63	6,49	6,16	6,21	6,93	7,21	7,40	7,51	7,99	8,03	8,20	8,11	8,28	8,04
DS	0,90	1,55	1,14	0,91	0,95	1,08	1,21	1,50	1,92	1,32	1,25	1,33	1,07	0,89	1,40	1,32	1,23	1,81	2,01
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	3,18	3,13	3,72	4,11	5,30	5,66	6,56	6,35	6,67	6,92	8,72	9,51	10,48	11,09	10,80	10,74	11,11	10,39	10,74
DS	0,93	1,14	2,06	1,27	1,03	1,39	1,60	0,91	0,98	0,53	0,50	0,33	0,57	0,42	0,45	1,14	1,67	1,33	2,33
t	-0,43	-0,35	-0,51	-0,59	2,13	-0,11	-0,18	-0,59	-1,17	0,02	-6,14	-8,42	-13,42	-17,26	-10,35	-8,01	-7,94	-5,14	-4,81
p	0,669	0,726	0,613	0,556	0,066	0,915	0,858	0,556	0,245	0,98	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*

Table 1B: Descriptive statistics of the two last variables classified according to each age category and sex. Sexual differences by t-Student test. The significance is indicated by asterisk (*).

The best growth model for the male maximum femoral length was a six-degree polynomial (Figure 3) with a 97% explained variability, whereas for the female it was a second-degree polynomial (Figure 3) with a 97% explained variability. In the male fitted curve a growth restraint is observed between 10-11 years of age, before the male growth spurt takes place. In the female fitted curve it is not possible to observe any growth restraint because of the type of growth model in which its growth behaviour is explained. It is a second-degree polynomial, which is a half parabola. Although it is difficult to observe the beginning of the growth spurt in males and females due to the nature of the data, in table 1 (A) it can be observed that the greater increase in maximum length of the femur takes place in boys at around 14 years of age (between 13 and 14 years it increases 24.14 mm) and in girls at around 10 years of age (between 9 and 10 years it increases 24.22 mm), indicating that the boys and girls growth spurt of this variable takes place at approximately at around 14 years in boys and at around 10 years in girls. This is in agreement with Tanner [52, 54] and Gasser et al. [15].

	R2	Age limit
Maximum length of femur		
Male		
Age = 0,0484 x Femoral maximum length – 6,479	0,961	Up to 19 years
Female		
Age = 0,049 x Femoral maximum length – 6,949	0,923	Up to 19 years
Combined		
Age = 0,039 x Femoral maximum length – 4,625	0,950	Up to 9 years
Diaphyseal length		
Male		
Age = 0,0284 x Femoral diaphyseal length – 4,243	0,931	Up to 13 years
Female		
Age = 0,0274 x Femoral diaphyseal length – 2,543	0,964	Up to 13 years
Combined		
Age = 0,0154 x Femoral diaphyseal length – 3,321	0,921	Up to 9 years
Femoral Head Diameter		
Male		
Age = 0,383 x Femoral Head diameter – 3,742	0,936	Up to 19 years
Female		
Age = 0,539 x Femoral head diameter – 7,221	0,901	Up to 19 years
Combined		
Age = 0,317 x Femoral Head diameter – 2,521	0,923	Up to 8 years
Bicondylar breadth		
Male		
Age = 0,252 x Bicondilar breadth – 7,265	0,921	Up to 19 years
Female		
Age = 0,325 x Bicondilar breadth – 9,373	0,812	Up to 19 years
Combined		
Age = 0,237 x Bicondilar breadth – 5,432	0,896	Up to 8 years

Table 2: Inverse functions for age prediction—coefficient of correlation of the function R^2.

In order to assess age at death, the inverse regression between maximum femoral length and age was calculated (Table 2). A first-degree polynomial for male, female and combined sexual series (unisex series) was selected with a 96.1%, 92.3% and 95.0% explained variability respectively. The unisex series function was calculated only taking into account individuals under 10 years of age, because sexual differences begin at this point.

Diaphyseal length

The average diaphyseal length of the femur in boys was 133.92 at birth and 418.90 mm at 13 years of age (Table 1 A). In girls, the average diaphyseal length at birth was 131.39 mm and 422.70 mm at 13 years of age (Table 1). This length could no longer be measured once the union of the femoral head had begun, impeding the analyses of this variable in posterior ages. Generally speaking, the female average values of this variable were always greater than male averages values. These differences were statistically significant in the age intervals of 10, 11 and 13 years of age (Table 1).

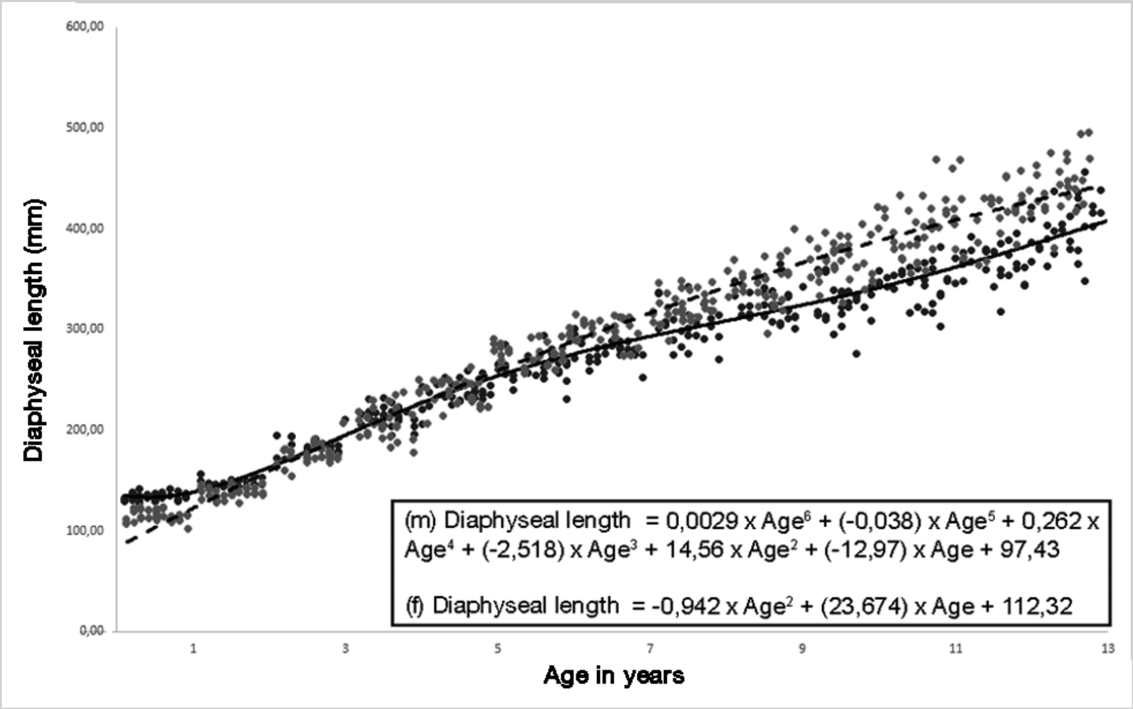


Figure 4: Polynomial regression line of the diaphyseal length of the femur for both sexes (continuous line for boys, discontinuous for girls), from 0 to 13 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

The best growth model for the male diaphyseal length was a six-degree polynomial with a 93.0% explained variability (Figure 4). The best growth model for the female diaphyseal length was a second-degree polynomial with a 95.0% explained variability (Figure 4). In the male fitted curve a growth restraint is observed between 10-11 years of age before the male growth spurt takes place. In the female fitted curve it is not possible to observe any growth restraint because it is a half parabola. The beginning of the growth spurt in both sexual series is difficult to observe due to the nature of the data. However, in table 1, it is possible to observe a greater increase in the diaphyseal length of the femur at around 12 years of age (between 11 and 12 years it increases 35.29 mm) in boys and at around 10 years of age (between 9 and 10 years it increases 20.59 mm) in girls, indicating that boys' and girls' growth spurt of this variable takes place approximately around 12 and 10 years respectively. Ths result is in agreement with Tanner [52, 54] and Gasser et at. [15].

The calculus of the inverse regression between the diaphyseal length of the femur and age (Table 2) gave us a first-degree polynomial for male, female and combined sexual series (unisex series) with a 93.1%, 96.4% and 92.1% explained variability respectively. The unisex series function was calculated taking into account only individuals under 10 years of age, because sexual differences begin at this age.

Head diameter

The average head diameter of the femur in boys measured 6.33 mm at birth and 52.06 mm at 18 years of age (Table 1A), whereas in girls it measured 8.55 mm and 41.56 mm at 18 years of age (Table 1A). Although, at birth girls had higher values for this variable than boys, it is boys who usually had greater values of this variable than girls in all ages. These differences were significant from 9 years of age onwards. However, we can observe that this differences were close to significance at 8 years of age, indicating the usefulness of this variable for sex diagnosis in individuals over 7 years of age.

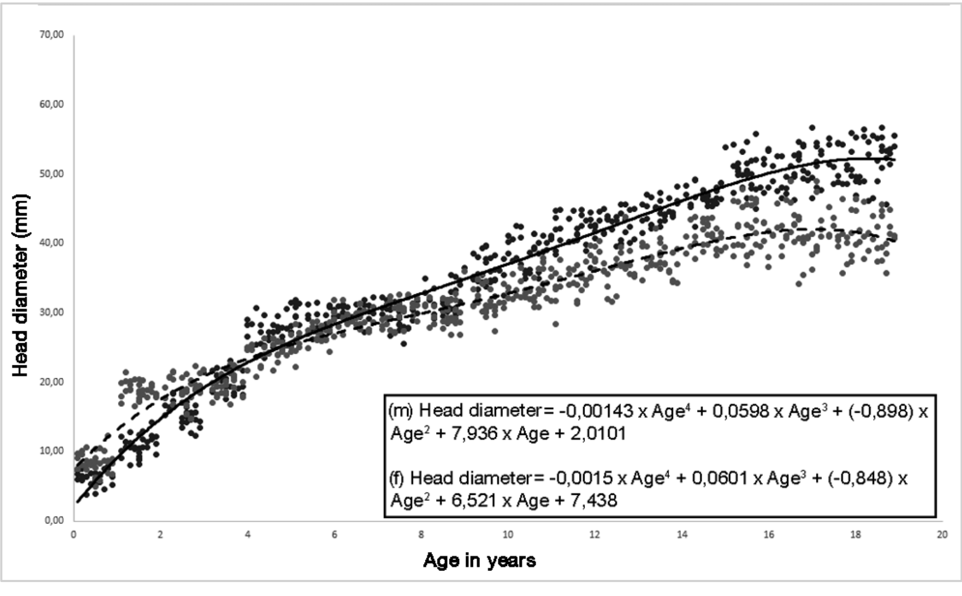


Figure 5: Polynomial regression line of the head diameter of the femur for both sexes (continuous line for boys, discontinuous for girls), from 0 to 18 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

The greater increase of the head of the femur was observed in boys between 2 and 3 years of age and in girls between 0 and 1 year of age (Table 1A). In addition, a pronounced growth restraint is observed at between 5 and 7 years of age in boys and 1 and 3 years in girls (Table 1). At the end of this growth restraint (7 years in boys and 3 years in girls) and in both sexual series there is a growth increase in the head of the femur, but in this case it is more moderate than that observed prior to the growth restraint. The values of this variable start to be constant at 14 years of age in girls, and at 16 years of age in boys.

The best growth models for the head diameter of the femur were a four-degree polynomial for both sexes with a 97.0% explained variability in boys and 93.0% in girls (Figure 5). The fitted curves indicate a great increase of the head of the femur in boys in relation to girls. This is due to both a higher rate of growth in boys and the earlier pronounced growth restraint in girls, resulting in males having greater head diameters than females.

The inverse regression of the diameter of the head of the femur and age (Table 2) indicate that the best function for estimating age with this variable is a first-degree polynomial for the three sexual series (male, female and unisex series) with a 93.6%, 90.1% and 92.3% explained variability respectively. The unisex series function was calculated taking into account only individuals under 8 years of age, because sexual differences begin at this age.

Bicondylar breadth

The average value of the bicondylar breadth in boys was 21.80 mm at birth and 90.08 mm at 18 years years of age. In girls the average value of this variable was 23.46 mm at birth and 68.47 at 18 years of age. From birth up until 2 years of age, the average values of the bicondylar breadth for girls were always greater than in boys. Beyond this age, the average values for boys were always greater than those of the girls. However, these differences were statistically significant from 9 years of age onwards.

The best growth models for the bicondylar breadth were a fourth-degree polynomial for both sexes, with an explained variability for boys of 97%, and 95% for girls (Figure 6). The values in both cases follow a more constant development impeding to observe any growth restraint and any growth spurt. In girls, the values of the bicondylar breadth start to be constant at 12 years of age, and in boys at 18 years (Table 1A).

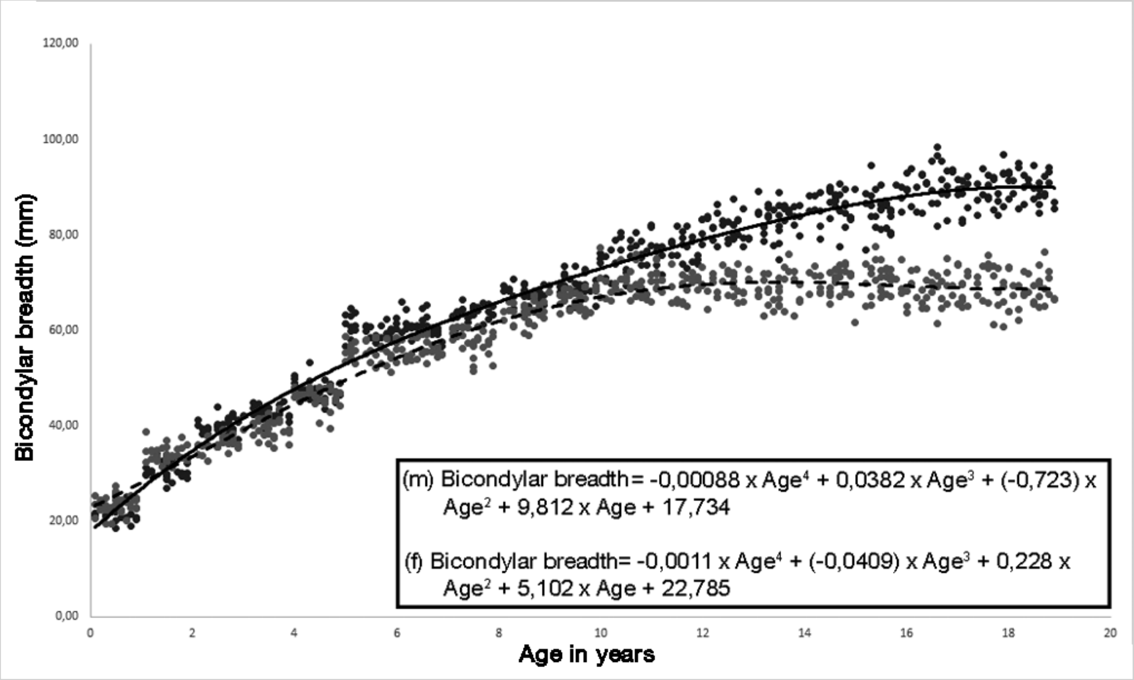


Figure 6: Polynomial regression line of the bicondylar breadth of the femur for both sexes (continuous line for boys, discontinuous for girls), from 0 to 18 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

The inverse regression of the bicondylar breadth of the femur (Table 2) gave us a first-degree polynomial for the three sexual series, male, female and combined sex (unisex series) with 92.3%, 93.6% and 90.1% explained variability respectively. The function of the unisex series was calculated taken into account only individuals under 8 years of age, because sexual differences begin at this age.

Collo-diaphyseal angle

The average value of the collo-diaphyseal angle in boys was 140.86° at birth and 128.88° at 18 years of age (Table 1B). In girls, the average value of this variable was 141.85° at birth and 123.68° at 18 years of age (Table 1B). This variable decreases with age, having a faster decrease at 10 years of age in boys and 8 years of age in girls. The decrease of this variable stays similar between boys and girls until at around 6 years of age. After that, female values for collo-diaphyseal angle decrease faster than those of the males, between 2 and 4 degrees. The observed sexual differences in this variable were significant from 10 years of age onwards (Table 1B). This variable starts to be constant from 15 years and 14 years of age in boys and girls respectively. It is necessary

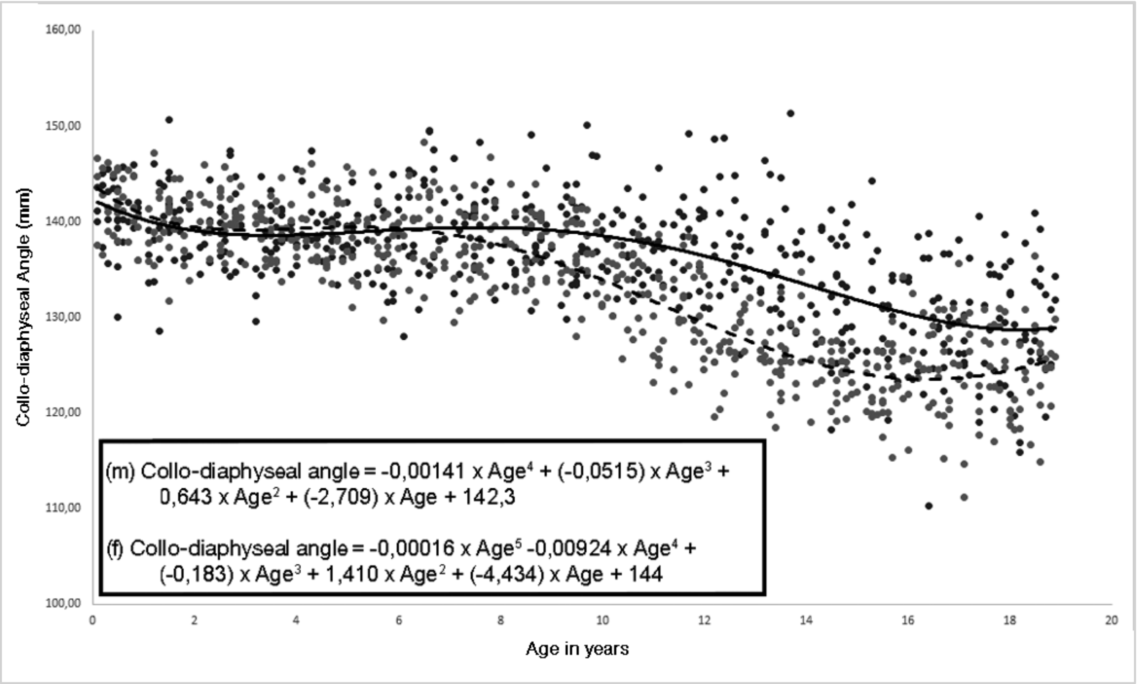


Figure 7: Polynomial regression line of the collo-diaphyseal angle of the femur for both sexes (continuous line for boys, discontinuous for girls), from 0 to 18 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

to highlight the higher standard deviation in males' collo-diaphyseal angle than in females (Table 1B). This standard deviation expresses the variability of the collo-diaphyseal angle in both sexual series, which in most age intervals is more than double in boys in relation to girls. This is more evident from 10 years of age and onwards (Table 1B).

The best growth models for the collo-diaphyseal angle were a four-degree polynomial for both sexes with a 41.0% explained variability in boys and 75.0% in girls (Figure 7). These results indicate a large dispersion of individual values. Obtained curves indicated the presence of a possible growth spurt at around 10 years of age in males and 8 years of age in females.

Due to the low level of information provided by the models, which is indicated by the low explained variability, the calculus of the inverse relation between collo-diaphyseal angle and age at death has not been considered.

Bicondylar angle

The average value for the bicondylar angle was 3.07° for boys at birth and 8.04° at 18 years of age. In girls, the average value of this variable was 3.18° at birth and 10.74° at 18 years (Table 1B). This variable increases with age in both sexes and the average values between males and females become closer; however, boys have always higher values than girls, with the exception of the first two years of life, in which the situation is reversed. These differences were statistically significant from 10 years of age onwards (Table 1B). This variable starts to be constant at 15 years in boys and at 13 in girls. The higher variability of the male bicondylar angle in relation to female one must be highlighted. In most age intervals the variability in boys more than doubles that of the girls, with these differences of variability of angle starting at 10 years (Table 1B), age at which girls' pubertal growth spurt of the pubis has started [55].

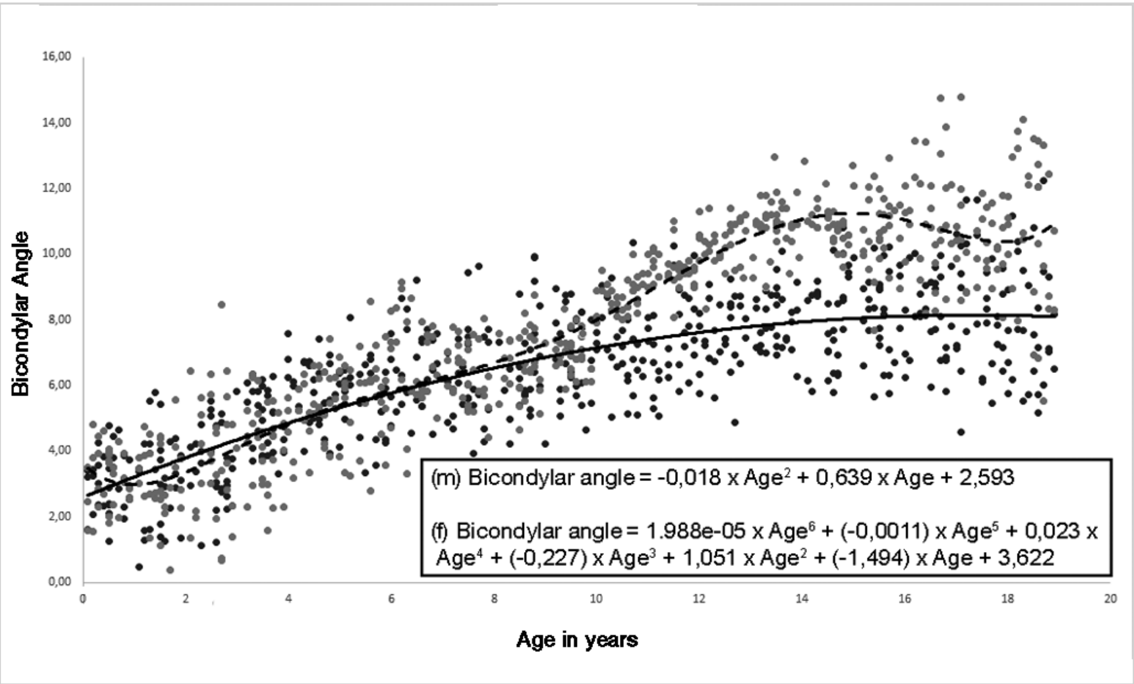


Figure 8: Polynomial regression line of the bicondylar angle of the femur for both sexes (continuous line for boys, discontinuous for girls), from 0 to 18 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

Boys present a more regular growth of this value; moreover, girls present a more complex development, with a restraint growth period just before the initiation of the growth spurt, which seems to be at around 10 years of age. Due to the higher regularity in boys, it is not possible to determine at what age their growth spurt of this variable takes place. The values' dispersion in boys are higher than in girls, especially during puberty.

The best growth models for the bicondylar angle were a second-degree polynomial function for boys, with a variability explained of 60%, and a sixth-degree polynomial for girls, with a variability explained of 84% (Figure 8). These results indicate a large dispersion of individual values. According to these models, the growth of the collo-diapyseal angle in boys is more regular than in girls, who showed a great increase in the rate of growth around 9 – 10 years of age (Figure 8).

Due to the low level information furnished by the models, the calculus of the inverse relation between collo-diaphyseal angle and age at death has not been considered

Epiphyses fusion

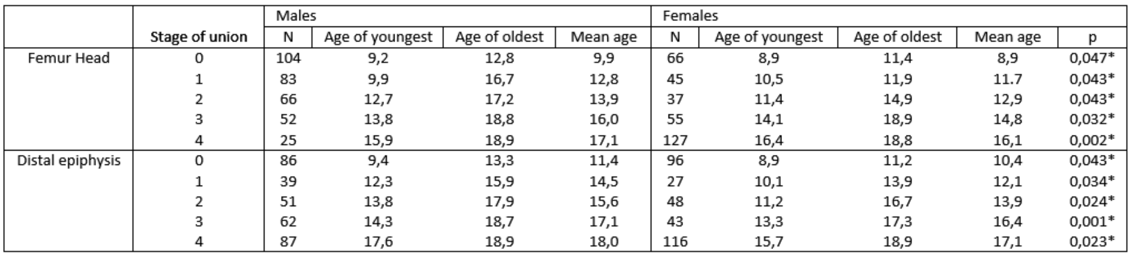
Table 3 shows the frequencies of the degree of union at head and distal epiphysis of the femur by sex and age, and Table 4 shows the mean ages at which each degree of union of the head and distal epiphysis is acquired. It also shows the presence of sexual differences among the mean age of each degree of union. Both tables, table 3 and table 4, also show the range of degree of union variability observed in the sample.

		Male										Female									
		Head of the femur Stage of union					Distal epiphysis Stage of union					Head of the femur Stage of union					Distal epiphysis Stage of union				
Age (years)	n	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
8-8,9	30	30	-	-	-	-	30	-	-	-	-	25	5	-	-	-	30	-	-	-	-
9-9,9	30	28	2	-	-	-	28	2	-	-	-	19	11	-	-	-	27	3	-	-	-
10-10,9	30	27	3	-	-	-	20	10	-	-	-	13	14	3	-	-	24	6	-	-	-
11-11,9	30	14	16	-	-	-	6	14	10	-	-	9	15	4	2	-	15	10	5	-	-
12-12,9	30	5	24	1	-	-	2	9	15	4	-	-	-	17	9	4	-	4	18	8	-
13-13,9	30	-	20	7	3	-	-	2	9	19	-	-	-	10	13	7	-	4	10	15	1
14-14,9	30	-	13	10	7	-	-	2	10	16	2	-	-	3	16	11	-	-	10	12	8
15-15,9	30	-	4	17	8	1	-	-	5	10	10	-	-	-	12	18	-	-	5	4	21
16-16,9	30	-	1	21	6	2	-	-	2	10	18	-	-	-	3	27	-	-	-	4	26
17-17,9	30	-	-	10	10	10	-	-	-	2	28	-	-	-	-	30	-	-	-	-	30
18-18,9	30	-	-	-	18	12	-	-	-	1	29	-	-	-	-	30	-	-	-	-	30

Table 3: Numbers of individuals at each stage of union for each of the proximal and distal femur epiphysis for each age group (years) for male and female.

As it was expected, both tables indicate that the female maturation process is significatively advanced in relation to males (Tables 3 and 4). For example, the mean age at which the union of the femoral head starts in boys is at 9.9 years of age (9.2 y to 12.8 y) and in girls it is at 8.9 years of age (9.8 y to 11.4 y) (Table 4). Regarding the distal epiphysis, boys start to fuse this secondary ossification centre at the mean age of 11.4 years of age (9.4 y to 13.3 y) and girls at 10.4 years of age (8.9 y to 11.2 y) (Table 4). The head of the femur and the diaphysis united to form a single unit bone (stage 3) at the mean age of 15 years (13.8 y. to 18.8 y) in boys and 14.8 years (14.1 y. to 18.9 y.) in girls. The distal epiphysis of the femur and the diaphysis united to form a single unit bone (stage 3) at the mean age of 17.1 years (14.3 y. to 18.7 y) in boys and 16.4 years

(13.3 y. to 17.3 y.) in girls. The process of union of the head of the femur with bone remodelation finishes completely at the mean age of 17.1 years (15.9 y to 18.9 y) in boys and 16.1 years (16.4 y to 18.8 y) in girls (Table 4). In the distal epiphysis, the process of union with bone remodelation finishes completely at the mean age of 18 years (17.6 y. to 18.9 y) in boys and 17.1 years (15.7 y. 18.9 y) in girls (Table 4).



		Males				Females				
	Stage of union	N	Age of youngest	Age of oldest	Mean age	N	Age of youngest	Age of oldest	Mean age	p
Femur Head	0	104	9,2	12,8	9,9	66	8,9	11,4	8,9	0,047*
	1	83	9,9	16,7	12,8	45	10,5	11,9	11.7	0,043*
	2	66	12,7	17,2	13,9	37	11,4	14,9	12,9	0,043*
	3	52	13,8	18,8	16,0	55	14,1	18,9	14,8	0,032*
	4	25	15,9	18,9	17,1	127	16,4	18,8	16,1	0,002*
Distal epiphysis	0	86	9,4	13,3	11,4	96	8,9	11,2	10,4	0,043*
	1	39	12,3	15,9	14,5	27	10,1	13,9	12,1	0,034*
	2	51	13,8	17,9	15,6	48	11,2	16,7	13,9	0,024*
	3	62	14,3	18,7	17,1	43	13,3	17,3	16,4	0,001*
	4	87	17,6	18,9	18,0	116	15,7	18,9	17,1	0,023*

Table 4: Mean (SD) and range in age (years) for each stage of union at distal epiphysis of the femur for males and females. Significance is indicated by asterisk (*) for the t-test.

DISCUSSION

The growth pattern of the 6 metrical variables analysed in this study appears not to be linear. Most of the analysed variables, especially those that have horizontal position, show a growth restraint before the growth spurt, as was indicated by Rissech et al. [22] and Rissech and Malgosa [26]. These variables are male maximum length, male diaphyseal length, male and female collo-diaphyseal angle and female collo-diaphyseal angle. This growth pattern approximately coincides with the observations of Miles and Bulman [18] and Pujol et al. [44, 56]. The obtained polynomial models can be summarised as follows:

- Female maximum femoral length, female femoral diaphyseal length and male bicondylar angle follow a second-degree polynomial;
- Male and female femoral head diameter, male and female distal epiphysis width and male collo-diaphyseal angle follow a four-degree polynomial;
- Female collo-diaphyseal angle follows a five-degree polynomial and male maximum femoral length, male femoral diaphyseal length, and female bicondylar angle follow a six-degree polynomial;
- In general, curves have a good fit and there is not a great deal of scatter, as evidenced by the consistently high correlation and significance of the functions coefficients achieved in the models.

According to our observations, and as it was expected, the growth spurt of each variable occurred at a different time. The growth spurt of the maximum length of the femur was observed at around 14 years and 10 years of age in boys and girls respectively. The growth spurt of the diaphyseal length was observed at around 12 years and 10 years of age in boys and girls respectively. Because of its constant development, it was not possible to observe the growth of the bicondylar breadth. According to the obtained curves, the collo-diaphyseal angle shows a growth spurt at around 11 years of age in boys and 7 years of age in girls. In the bicondylar angle it is only possible to observe the female growth spurt, which is at around 10 years of age. With regard to the head diameter, it exhibited a special behaviour. In general, the head of the femur grew quickly, but at a specific point it underwent a pronounced growth restraint (between 5 and 7 years of age in boys and 1 and 3 years in girls) and then restarted its growth at 7 years of age in boys and at 4 years of age in girls. However, this new period of growth showed a low growth rate, which is the opposite of what is expected for a growth spurt. This special behaviour of the head diameter is probably related to the mechanical needs of the hip-femoral joint, since it is one of the first skeletal areas to mature [22]. In fact, according to Rissech et al. [22] the acetabulum, which is the counterpart of the femoral head, grows quickly and it does not display a growth spurt, because the acetabulum fuses before the body reaches the age at which this growth spurt occurs.

If we take into account that the maximum femoral length is related to the individual stature and that the age of the growth spurt in living individuals is given through the observation of the growth pattern of the stature, we can state that the growth spurt in our sample starts at around 14 years of age for boys and 10 years for girls. These ages match the given ages for the growth spurt in boys (10-15 years) and girls (2-13 years) of Spanish origin [57], and these values are within the normal age range for the male (12.5–17.5 years) and female (9.5–14.5 years) pubertal growth spurt in current living population [15, 52, 58]. In addition, the mean ages of fusion of the distal epiphyses for boys (17.1 years) and girls (16.4 years) of the analysed sample are consistent with the age at which most Spanish males reach adult size, which is at 17 years [57, 59, 60] and at 15.5 years [60, 61]. In this case we have only taken into account stage 3 of epiphyseal fusion because that is exactly when the growth of the femur finishes. In stage 4 only bone remodelation is expressed.

The obtained average values for collo-diapheseal and bicondylar angles in each age group fall within the normal range of variability for each of these ages [47, 62, 63]. The youngest individuals of our sample (0 years) showed a collo-diaphyseal mean value of 140.86° in males and 141.86° in females, and the oldest (18 years) an average of 128.8° and 123.68° in males and females respectively. These last values are inside the normal range of variation reported for adult European males (121.78–136.58) and females (122.3–135.9) by Anderson and Trinkaus [41]. After 16 years of age for males and after 14 years of age for females, the collo-diaphyseal angle became stable, thus agreeing with previous observations made by other authors who indicated that the values of this angle are stable from mid-adolescence throughout most adult life [64, 65], and with previous studies on male and female femurs [44, 56]. During growth, there is a great increase in size in the proximal epiphysis, including the neck and head. The collo-diaphyseal angle is more obtuse during the first years of individual life. Namely, the neck has a more vertical position, because the abductor muscle only develops as a response to the onset of locomotion [66]. The collo-diaphyseal angle decreases during growth, starting from an average of 150.8° at birth to approximately 127.8° at the end of the pubertal growth spurt [64, 67-69].

In our study the youngest individual (0 years) showed a mean value of the bicondylar angle of 3.07° in males and 3.18 in females, and the oldest (18 years) showed and average of 8.04° in males and 10.74° in females. Our results are consistent with those obtained by Tardieu and Damsin [47] in boys and girls from 0 to 12 years of age. These authors proposed two functions for calculating the male and female bicondylar angle taking in relation to age. According to this study, males' bicondylar angle is 0° at birth; 6° at 9 years of age; and 7.1° at 12 years, and females' bicondylar angle is 4.1° at birth, 6° at 10 months of post-natal life, 8° at 9 years of age, and 10.8 at 14 years of age. These results differ from those found in other studies, which show that the male bicondylar angle only increases up to 7 years of age, reaching its adult value at that age [70]. The values of the bicondylar angle in our sample stabilise at around 15 years of age in males and 13 in females, the mean age at which the male and female pubertal growth spurt finishes in the population of Barcelona [57]. The greatest changes on the bicondylar angle take place from the beginning of locomotion to 8 years of age and, according to our results, carry on until the femoral growth spurt in both sexes, before the epiphyseal closure.

Sexual differences. From our results the growth stop of the female sample is in the base of the sexual dimorphism found in the maximum femoral length and diaphyseal length. The sexual dimorphism found in the femoral head diameter and bicondylar breadth is related to a higher rate of growth in these variables in boys, and the sexual dimorphism found in collo-diaphyseal and bicondylar angles is related to a higher growth rate in these variables in girls. The statistical significance of these variables from

9 (femoral head and bicondylar breadth) and 10 years of age (maximum femoral length, diaphyseal length, collo-diaphyseal and bicondylar angle) indicates their importance in sexual determination. However, the head of the femur seems to be one of the most significant variables in sexual determination because of the distance between male and female mean values in this variable, an observation in line with the current research on this variable [46, 71].

According to our results, sex-based differences in the collo-diaphyseal and bicondylar angles are not significant during the early years of life and do not exceed 2° at 9 years of age (bicondylar angle difference is 0º). However, sexual differences increase markedly with the onset of puberty, reaching an average difference between sexes of 8.61° in collo-diaphyseal angle and 2.78° in bicondylar angle at 14 years of age. This difference is statistically significant from 10 years of age onwards on both variables, which is the age of the onset of the pubertal growth spurt in the female pubis [26]. The presence of sexual differences in the collo-diaphyseal angle in the older age groups concurs with the observations on adult femur made by Walmsley [72], Igbigbi [69], and Bernard et al., [73], and disagrees with the results obtained by Otsianyi et al, [74] and Gilligan et al, [75]. However, it must be taken into account that the latter mixed together individuals of different populations and ethnicities with different levels and types of physical activities that could mask sexual differences. Likewise, Otsianyi's study does not show a strong lack of sexual differences. The reason of these discordant results between authors could probably also be related to the possible different growth pattern of the collo-diaphyseal angle in girls and boys observed in our study and the study of Pujol et al. [44].

The sexual differences found in the bicondylar angle are concordant with previous observations [47, 76-78]. These sexual differences are statistically significant from 10 years of age, when the pubertal growth spurt in the female pubis occurs [26] and agree with the significant sexual differences found in adults [77, 78]. This finding further supports the idea that the distal region of the femur has a higher sexual discrimination power for adult individuals [79, 80]. The bicondylar angle in males is more variable than in females, which exhibit a greater canalized growth [44]. The greatest changes of the bicondylar angle take place from the beginning of locomotion to 8 years of age and, according to our results, carry on until the femoral growth spurt in both sexes, before the epiphyseal closure. These findings are in agreement with the variation observed during puberty in the female Spanish series analysed by Pujol et al. [44, 56].

Epiphyses fusion. As in our sample, the age in which the male femoral head started to fuse was at 12.8 years of age and it was totally fused (stage 3) at 16 years of age. Females femoral head started to fuse at 11.7 years of age and it was fused completely (stage 3) at 14.8 years of age. Regarding the fusion of the distal epiphysis, in our male sample it started to fuse at (stage 2) at 14.5 years of age and it fused completely (stage 3) at 17.1 years of age. Females' distal epiphysis started to fuse at 12.1 years of age and was fused completely at 16.4 years of age. As it was expected, the ages in which both epiphyses started to fuse in females were 2 years younger than in males, coinciding with current bibliography [81-86].

Although there exist numerous studies on the age of fusion of the knee epiphyses, the differences on the methodology (specifically on the definition of the stages of fusion on age fusion of the knee), the disparity of methods to define the melt and the variety of ages in the studied samples (most individuals from 17 years), make few comparable to the study presented here, so the results obtained in the region of the distal epiphysis will be compared as far as possible mainly with the study of O'Connor [49], which has the same melting stages and works with X-rays, McKern & Stewart [50] and Schaefer & Black [87], which apply a similar methodology while still working with osteological material; however, O'Connor is the only one with a sample younger than 17 years of age.

The ages of fusion obtained in our study are in accordance with the results presented in O'Connor [49]. According to this author's observations male distal epiphysis stated to fuse at 14.7 and it was completely fused at 17.1, and female distal epiphysis started to fuse at 12.1 and it was fused completely at 16.4.

Comparing these results with those obtained by Cardoso [88], based on the analysis of the documented osteological collection of Lisbon, we observed that in the Lisbon collection the male femoral head started to fuse at 15 years of age and it was totally fused at 19 years of age, whereas the female femoral head started to fuse at 14 years of age and finished at 17 years of age. Regarding the distal epiphysis, in our sample it started to fuse (stage 2) at 14.5 years of age and it fused completely (stage 3) at 17.1 years of age in males and for females it started at 12.1 years of age and it fused completely at 16.4 years of age. However, Cardoso's male sample started to fuse at 16 years of age and the fusion finished at 19 years of age, and for females it started at around 14 years of age and finished at around 20 years of age.

These retarded stages of fusion in Cardoso's population in relation to our results can be attributed to the fact that Cardoso's sample comes from a middle-lower socioeconomic class from the beginning of the 20th century, whereas our sample is a well-nourished sample from the 21st century.

The variation found in the maturation process of the analysed population and the populations with which it has been compared is the result of an enormous variability result of multiple genetic and environmental influences. Specifically, individual nutrition has proven to be a factor significant in growth throughout its development, leading to stunted growth and reduced height [89-93]. It is known that some of the studies, such as Schaefer & Black [87] or McKern & Stewart [50], analyzed populations with lower social groups or with victims of war and its attendant shortages, which is why these series and ours differs. However, when we compare our population to those with a medium socioeconomic class without known deficiencies, the values obtained are similar between them [49]. Even then, the disparity between American values and series (more similar between them) and European series reinforces the theory that the genetic component outweighs environmental factors [94, 95].

Age estimation. It is possible to estimate age at death of sub-adult individuals by using four of the absolute measurements of the femur analysed in this study (namely maximum length, diaphyseal length, head diameter and bicondylar breadth) and the fusion of the head and distal epiphysis of this anatomical element.

The use of the regression equation for these four variables can be applicable until 18 years of age in samples of known sex. In samples where the sex is unknown the unisex functions have a more reduced application time, because they were calculated only until the age in which sexual differences appeared (9 and 10 years).

The regression formulae calculated from living individuals from Barcelona analyzed in this study allow us to predict the age of young human remains from the 21st century with accuracy. In general, calculated curves fit well with our Barcelona series and match the behaviour deduced from the adult bone and other skeletal segments.

The study of the epiphyses fusion of the femur is useful in age estimation of young individuals because it takes place during their puberty. However this technique, as the others, has it own incovenients, such as the inconsistency among authors in providing a range in age for the time of complete union. However, the results of the present study are the first based on a current living Spanish sample and can be of great help in medico-legal cases in which the age estimation of a minor is required.

CONCLUSIONS

The cross-sectional study of 8 femoral measurements, 6 metrical and 2 qualitaive variables related to the head and distal epiphysis fusion, collected from telemetries of 1140 living individuals of the 21st century from Barcelona have provided researchers with information pertaining to the development profile of the femur. Using the metrical data as a basis, calculus was performed to derive formulae that may prove valuable in age at death diagnosis of the skeleton and living individuals of the 21st century from the Iberian Peninsula and Mediterranean area. The analysis has also provided information regarding the time at which sexual differences were present within the metrics of the femur, thus offering indication as to when the variables may be useful in the diagnosis of sex. Furthermore, the data furnished on the fusion of the femoral epiphyses provides information on the development of the distal and femoral epiphyses of the femur based on a sample whose number gives consistence to the results. The results and formulae obtained in this study are useful tools in the diagnosis of age and sex in forensic tasks. Further research on the growth and development of the femur is necessary to obtain better information for skeletal diagnosis, especially within sub-adults.

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4. Estudio de la ontogenia de la tibia

4.1. PRELIMINAR STUDY OF TIBIAL DEVELOPMENT IN A SUBADULT SPANISH LIVING SAMPLE

INTRODUCTION

Studies of skeletal development are of great importance in the archaeological field and in physical and forensic anthropology, since the information they provide is needed in many procedures, such as reliable age estimation of the skeletal remains of immature individuals, reconstruction of the demographic profile of past populations, biological identification of living individuals in legal proceedings when a minor is involved, and interpretation and identification of many factors, including indicators of a population's health and living conditions (Pujol et al. 2014, 2015). More specifically, it is important to have genetic, cultural and economic information and data from populations nearby the individuals under study (Biewner & Bertram, 1993; Slemenda et al. 1994; Arden & Spector, 1997; McGuigan et al 2002).

In order for development models to be effectively applied on the different individuals, the measurements must be based on documented skeletal collections (known age, sex and biological origin) or high resolution radiographic material (undistorted by the same process). It is also essential that the population used as a reference be biologically related to the analysed sample both in time and space (Komar and Grivas, 2008), to avoid errors caused by population differences or the secular effect. Currently, studies on the development of immature individuals are enriched by a multitude of new imaging techniques, including material high resolution radiographic images (computerized tomography and multislice telemetry), which does not distort the actual measurements and provides a powerful tool for the study of bone development (Garcia et al., 2010). However, despite the growing need to register growth and maturation values for different skeletal elements, there are not much data on postnatal growth and maturation based on direct measurements or radiographic material that allows the extraction of actual measurements in current individuals. In fact, this type of work has been limited due to the lack of documented collections of immature skeletons (Rissech, 2008) and because it is only very recently that high-resolution radiographic material suitable for the development of such studies has been made available (Garcia et al., 2010). At present, the standards in postnatal growth and maturation are based on traditional radiographic material from North American children of European origin (Reynolds, 1945, 1947; Ghantus, 1951; Maresh, 1955; Coleman, 1969; Gindhart, 1973;

Moerman, 1981). Although there have been studies of growth and maturation based on osteological material, most of them come from Slavic (Stloukal and Hanáková, 1978), Germanic (Sundick, 1978), Eskimo (Stewart, 1976) and Amerindian populations (Merchant and Ubelaker, 1977; Sundick, 1978; Jantz and Owsley, 1984). There are not many studies based on children in Western Europe (Alduc-le Bagousse, 1988, Hoppa, 1992; Miles and Bulman, 1994, 1995; Majó 2000 Rissech et al, 2001, 2003; Rissech and Malgosa, 2005, 2007; Rissech and Black, 2007; Rissech et al., 2008; Lopez-Costas et al, 2011), and even less based on children of the Iberian Peninsula (Rissech et al, 2001, 2003; Rissech and Malgosa, 2005; 2007; Rissech and Black, 2007; Rissech et al., 2008; Lopez-Costas et al., 2011; Pujol et al., 2104; 2015). Most of these studies are based on archaeological material whose age and sex were determined in the laboratory (Alduc-le Bagousse, 1988, Hoppa, 1992; Miles and Bulman, 1994; 1995; Majó, 2000). The few existing studies that use documented collections focus on the hip bone, the scapula, the femur, the tibia and the humerus (Rissech et al, 2001, 2003; Rissech and Malgosa, 2005, 2007; Rissech and Black, 2007; Lopez-Costas et al., 2011; Rissech et al., 2008, 2012). Besides all these studies, those based on documented skeletal collections and those created with traditional radiographic material are based on immature individuals from the 50s. It has been proved that the western population has experienced an increase in stature in the second half of the twentieth century due to the improvement of living conditions, reaching an average increase of 10 cm in the Spanish population (Spijker et al, 2008). Therefore, it is essential to complete and to detail postnatal skeletal development of the population in Western Europe, and especially in the Iberian Peninsula, for its subsequent application in Forensic Anthropology.

Given the anthropological importance of the femur and tibia as a result of its locomotory function (Aiello and Dean, 1990; Anderson and Trinkaus, 1998; Andriacchi and Alexander 2000) and the postdepositional resistance exhibited by these elements due to their structural density, we first analysed femoral development during puberty in a contemporary Spanish population by means of a Geometric Morphometric analysis (Pujol et al., 2014; 2015) and longitudinal measurements. Results from these studies revealed an overall increase in the robustness and length of the female femur, together with marked morphological changes in specific regions of this bone with age, whereas males showed a greater variability in neck–shaft and bicondylar angles than females. The angular remodeling of both the neck and the bicondylar regions of the male and female femur continues until the end of their growth spurt. This study aims to carry on with the research on lower extremity development, in this case through the analysis of tibial growth in boys and girls between birth and 18 years of age in the current living Spanish population.

MATERIAL AND METHODS

High-resolution digital radiological images (telemetries) in Digital Imaging and Communication in Medicine (DICOM) format from the archives of Hospital Sant Joan de Déu de Barcelona were used for this analysis. All personal data were omitted with the exception of the demographic information (sex and age) following the Spanish Organic Law of Data Protection (*Ley Orgánica de Protección de Datos*; for details see García et al. 2010), and this study was approved by the Bioethical committee of the Hospital Sant Joan de Déu de Barcelona (Ref. number 2938). This type of material (telemetries) was chosen because of the absence of any type of image deformation, which is due to both the distance at which telemetries are taken (more than 180 cm from the centre of the x-ray focus and the object to be radiographed) and the contact that the subject has with the chassis or radiographic film (a bucky mural is used for telemetries). For this reason this distant type of high-resolution digital radiographies are used for medical purposes of evaluation and real mensuration of the extremities (telemetries) and rachis (scoliograms).

The left tibia in anterior view of a total of 1140 living boys and girls aged from 0 to 18 years was analysed (30 individuals per age and sex group). Those individuals displaying any type of pathology or anatomical deformation that could affect the analysis were excluded.

All the telemetries were taken in the Hospital Sant Joan de Deu following its standardized procedure (for further information, see Pujol et al., 2014; 2015). All the individuals come from Universitat de Barcelona's subadult radiological collection, recently created by one of the authors of this study (A. P.) for his doctoral thesis under the supervision of D. T. and C. R. This collection comprises radiological images from 1080 individuals (540 boys and 540 girls) of Spanish origin and from a middle socioeconomic class, born between 1991 and 2010, ranging from birth to 18 years of age. The boys of 18 years of age show a mean height of 177.5 cm, and the girls, a mean height of 164.3 cm. These values correspond to the expected male and female adult mean height in current living Spanish population: 174.20 cm (SD: 7.08) for males and 162.09 cm (SD: 6.37) for females (Spijker et al., 2012)

The program used for the measures' intake was OsiriX. It is an image visualizer created to work with DICOM images, the standard format of high resolution images in medicine, although the images additionally come with metadata such as age, sex, birth date, the machine employed for the telemetry, a short medical history and the reason behind the tests.

A total of 4 measures were taken:

(1) Maximum length of the tibia: Maximum distance between the proximal end of the diaphysis and the distal end of the distal epiphysis (Figure 1A).

(2) Diaphyseal length of the tibia: Maximum distance between the proximal and distal ends of the tibial shaft without taking into account both epiphyses. This measurement could no longer be recorded once the proximal epiphysis had begun to unite (Figure 1B).

(3) Maximum breadth of the proximal epiphysis: Maximum distance between the lateral and medial areas of the epiphysis (Figure 1C).

(5) Maximum breadth of the distal epiphysis: Maximum distance between the two most laterally projecting points on the medial malleolus and the lateral surface of the distal epiphysis (Fig. 1D).

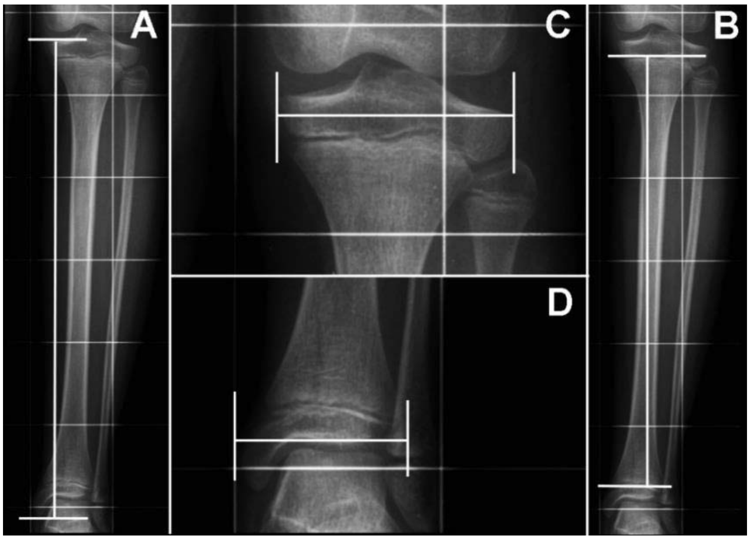

Figure 1: Measures realized:
A) Maximum length
B) Diaphyseal length
C) Proximal epiphysis breadth
D) Distal epiphysis breadth

To analyse each of the five metrical variables, we divided the study in 3 parts:

Firstly, we made a first approximation to the sexual dimorphism of the analysed variables through a Student T-test in each age group. In this first part we divided the sample into groups of 30 individuals per year in one-year age intervals.

Secondly, in order to describe the growth behaviour of each variable a polynomial regression was applied up until the tenth degree, treating age as continuous. Regression analysis was selected based on the assumption that the dynamics of growth can be described by an incremental continuous function (Tanner, 1962; Coleman, 1969). The most appropriate statistical model was then selected on the basis of three factors: (1) the strength of the correlation coefficient (R2); (2) the significance of the function expressed by the F value; and (3) the significance of the coefficients of the function obtained by the ANOVA test.

Finally, in order to permit age prediction, inverse regression analysis was performed for age and each metrical variable of the femur using age as dependent variable. In other words, each metrical variable of the femur (x) was regressed on age (y). This method was selected because generally when the individual under study comes from the same distribution as the referenced sample (same or similar population from a nearby geographical area), inverse regression is the correct methodology to use (Konigsberg et al., 1998).

Polynomial regression was calculated for both sexual series separately. However, in series that displayed no sexual differences, calculus was applied to the data as a whole (males and females combined). This last function is employed in situations where sex is unknown. All these data analyses were carried out with the statistical programs SPSS 20.0 and R.

RESULTS

Table 1 shows the results for the metrical variables. Table 2 shows the functions obtained for subadult age estimation. For the sake of clarity, the results for each variable will be presented separately.

Maximum length of the tibia

Results in Table 1 show that the maximum length of the tibia for boys ranges from 92.73 mm at birth to 371.40 mm at 18 years of age. For girls, the values range from 90.79 mm at birth to 342.96 mm at 18 years of age. In all ages boys have higher values than girls, but these differences are statistically significant only form 14 years onwards.

Variable	Age																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Maximum length of the tibia																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	92,73	114,15	144,35	163,88	187,85	201,87	226,53	237,54	250,96	275,87	286,65	301,04	315,43	325,28	355,00	370,50	368,07	369,45	371,40
DS	4,01	6,44	8,68	8,69	9,28	10,71	11,40	12,84	20,43	15,16	23,15	20,41	28,12	22,51	24,41	31,91	26,43	24,22	19,00
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	90,79	115,73	142,73	169,06	191,37	214,04	231,50	248,27	261,36	278,63	295,63	311,34	335,20	344,77	346,79	344,76	343,99	344,66	342,96
DS	4,59	6,10	7,57	8,11	11,64	15,11	13,10	16,30	13,67	18,33	20,60	23,21	25,79	28,20	21,33	24,32	19,56	17,91	17,27
t	1,28	0,78	0,78	0,97	0,67	-0,36	-1,34	-1,61	-1,14	1,77	-4,15	-4,15	-1,801	1,26	1,88	1,69	5,15	5,96	8,84
p	0,087	0,145	0,143	0,345	0,555	0,675	0,132	0,232	0,343	0,324	0,067	0,123	0,077	0,086	0,032*	0,000*	0,000*	0,000*	0.000*
Diaphyseal Length of the tibia																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	-	-	-	-	-
Mean	84,33	109,53	141,48	163,34	183,71	202,35	221,80	236,54	258,70	271,39	284,16	299,22	312,73	325,55	-	-	-	-	-
DS	4,06	5,24	6,65	7,35	9,70	9,84	10,62	11,21	11,75	15,10	11,02	16,43	15,86	15,30	-	-	-	-	-
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	-	-	-	-	-
Mean	86,63	107,56	140,14	162,35	184,98	203,77	220,57	232,48	250,24	266,56	285,35	300,72	310,14	333,88	-	-	-	-	-
DS	4,10	5,33	6,24	7,08	9,00	7,11	12,73	12,60	15,16	12,36	17,57	21,22	19,16	26,29	-	-	-	-	-
t	-2,19	0,78	0,78	0,97	0,67	-0,36	-1,34	-1,61	-1,14	1,77	-4,15	-4,15	-1,801	1,26	-	-	-	-	-
p	0,063	0,122	0,122	0,338	0,567	0,723	0,238	0,112	0,259	0,281	0,183	0,232	0,077	0,086	-	-	-	-	-
Proximal Epiphysis breadth																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	21,64	24,58	27,88	30,15	34,24	39,17	44,17	49,17	53,41	58,07	63,40	68,35	70,02	72,98	76,65	77,96	78,14	78,59	78,76
DS	1,21	0,95	1,89	1,59	2,23	2,37	2,23	1,95	1,89	2,91	2,54	3,02	3,79	3,59	3,86	3,48	4,33	2,75	3,00
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	23,95	26,76	29,77	34,88	41,66	47,04	49,92	55,25	61,57	63,92	67,81	69,85	73,03	74,22	74,88	75,26	75,15	75,02	75,58
DS	1,31	1,51	2,15	1,60	2,67	2,27	2,23	2,22	2,64	1,74	2,16	2,46	2,89	3,41	2,04	2,83	3,51	3,16	3,15
t	-2,65	-5,43	-4,67	1,51	-0,43	-5,43	4,01	-1,59	4,16	8,18	9,08	12,18	10,45	15,01	13,55	10,92	12,55	12,94	13,12
p	0,000	0,545	0,435	0,138	0,099	0,543	0,073	0,118	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	0.000*
Distal Epiphysis breadth																			
Male n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	12,21	14,08	16,88	18,91	22,41	24,97	27,78	33,25	38,08	40,01	41,47	44,99	47,01	47,77	50,45	51,12	52,47	53,88	53,25
DS	0,94	1,29	1,56	1,15	1,36	1,87	2,01	1,45	1,09	1,47	1,55	0,95	0,85	1,21	2,26	1,86	1,54	2,23	1,45
Female n	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Mean	13,03	15,25	15,96	18,56	20,76	24,79	28,46	32,51	37,90	40,70	42,20	45,59	47,06	47,87	50,58	51,04	52,20	53,05	52,88
DS	0,77	1,56	1,19	1,00	2,01	1,94	1,83	2,08	1,32	1,44	1,14	1,95	1,86	1,76	2,08	2,15	1,66	1,77	1,83
t	4,54	6,54	5,43	7,65	4,54	9,78	9,76	2,45	7,87	4,69	7,30	9,94	17,73	17,83	21,58	16,31	23,70	21,13	26,19
p	0,143	0,435	0,564	0,765	0,443	0,232	0,237	0,156	0,059	0,071	0,087	0,123	0,098	0,215	0,135	0,132	0,232	0,065	0,075

Table 1: Descriptive statistics of the four variables classified according to each age category and sex. Sexual differences by t-Student test. The significance is indicated by asterisk (*).

The best growth model for the maximum length of the tibia for both boys and girls (Figure 2) is a second degree polynomial. The explained variability for the boy model of growth is 95.2%, and for girls it is 95.0%. Because of their being second degree polynomial curves, it is not possible to see any growth restraint, making it impossible to observe the beginning of the growth spurt in males and females. However, it is possible to see that the sexual differences between boys and girls are due to the greater growth rate displayed for boys (Figure 2), which seems to increase at around 14 years of age (Table 1). Male and Female values become constant, approximately around 15 and 14 years of age respectively (Figure 2, Table 1).

Figure 2: Polynomial regression line of the maximum length of the tibia for both sexes (continuous line for boys, discontinuous for girls), from 0 to 18 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

In order to assess age at death, the inverse regression between maximum length of the tibia and age was calculated (Table 2). A first-degree polynomial for male, female and combined sexual series (unisex series) was selected with a 94.0%, 90.1% and 94.0% explained variability respectively. The unisex series function was calculated taking into account only individuals under 14 years of age, because sexual differences begin at this point.

	R2	Age limit
Tibial maximal length		
Male		
Age = 0.0583 x Max Length - 5.744	0,940	Up to 18 years
Female		
Age = 0.060 x Max Length - 6.239	0,901	Up to 18 years
Combined		
Age = 0.051 x Max Length - 4.536	0,940	Up to 13 years
Tibial diaphyseal length		
Male		
Age = 0.053 x Diaphysis length - 4.696	0,972	Up to 18 years
Female		
Age = 0.052 x Diaphysis length - 4.501	0,950	Up to 18 years
Combined		
Age = 0.052 x Diaphysis Length - 4.597	0,953	Up to 13 years
Tibial Proximal Epiphysis breadth		
Male		
Age = 0,001 x Prox ep breadth^2 +1.161 x Prox ep. breadth - 2.722	0,951	Up to 18 years
Female		
Age = 0,003 x Prox ep breadth^2 + (-0.031) x Prox ep. breadth + 0.425	0,978	Up to 18 years
Combined		
Age = -0,003 x Dist ep breadth^2 + 0.445 x Dist ep breadth - 7.658	0,901	Up to 7 years
Tibial Distal epiphysis breadth		
Male		
Age = 0,004 x Dist ep breadth^2 +0.101 x Dist ep breadth - 0.356	0,983	Up to 18 years
Female		
Age = 0,004 x Dist ep breadth^2 +0.089 x Dist ep breadth - 0.141	0,981	Up to 18 years
Combined		
Age = 0,004 x Dist ep breadth^2 + 0.095 x Dist ep breadth - 0.252	0,960	Up to 18 years

Table 2: Inverse polynomial functions for the studied variables

Diaphyseal length of the tibia

In boys, the length of the shaft of the tibia ranges from 84.33 mm at birth to 325.55 mm at the age of 13 (Table 1). In girls this variable ranges from 86.63 mm at birth to 333.88 mm at 13 years of age (Table 1). The length of this variable could no longer be measured once the union of the distal epiphyses had begun, impeding the analyses of this variable in posterior ages. No sexual differences were observed.

The best growth model for the diaphyseal length of the tibia was a second degree polynomial for both sexes with an explained variability 97.2% and 96.1% for boys and girls respectively (Figure 3).

The lack of sexual differences in all the age groups indicates that the diaphyseal length of the tibia is not a useful variable for sex determination, but it can be of use for age estimation before the fusion of the proximal epiphysis in individuals of both known and unknown sex takes place. In order to estimate subadult age, the inverse regression was calculated. A first-degree polynomial for both sex series and the unisex series (boys and girls combined) was selected, with an explained variability (Table 2) of 97.2% in boys, 95.0% in girls, and 95.3% in unisex series.

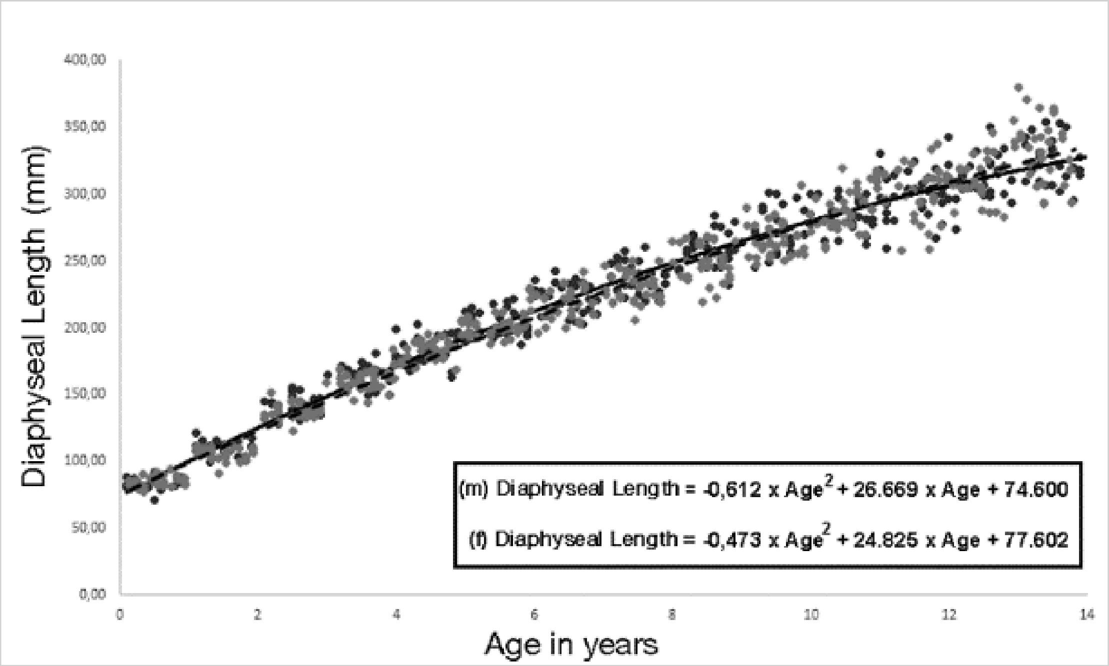

Figure 3: Polynomial regression line of the diaphyseal length of the tibia for both sexes (continuous line for boys, discontinuous for girls), from 0 to 13 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

Proximal epiphysis breadth of the tibia

The values of the proximal epiphysis in boys ranged from 21.64 mm at birth to 78.76 mm at 18 years of age (Table 1). In girls, the values of this variable ranged from 23.95 mm at birth till 75.58 mm at 18 years of age. Girls have greater values for the breadth of the proximal epiphysis than boys until 14 years old, but from this age onwards boys have greater values than girls. These sexual differences are significant from 8 years of age (Table 1). The change in pattern of the sexual differences in this variable at 14 years of age is probably due to the female cessation of growth at 13 (from then on values start to be constant) and the fact that boys continue growing until they become 15 (from 15 years on male values start to be constant). The early evidence of sexual differences in the proximal epiphysis breadth of the tibia (at 8 years of age) is in accordance with the well-defined sexual dimorphism on the tibial proximal epiphysis in sub-adults (Yscan, 1984; Holland, 1991).

The best growth model for the proximal epiphysis breadth of the tibia was a third degree polynomial for both the male and female series (Figure 4), with an explained variability of 97.8% in boys and 97.8% in girls.

The proximal epiphysis of the tibia is useful for sex determination of individuals over the age of 8 (Table 1). Regarding age estimation, this variable can be useful for osteological remains of known sex and also for remains of unknown sex between 0-8 years of age. The absence of sexual differences until the age of 7 made it possible to use the combined series to calculate a unisex model. To assess age, the inverse regression was calculated (Table 2). A second-degree polynomial model was selected for the male, female and unisex series, with a 95.1%, 97.8% and 90.1% explained variability, respectively.

Figure 4: Polynomial regression line of the proximal epiphysis breadth of the tibia for both sexes (continuous line for boys, discontinuous for girls), from 0 to 18 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

Distal epiphysis breadth of the tibia

Distal epiphysis breadth of the tibia in boys ranges from 12.21 mm at birth to 53.25 mm at 18 years of age (Table 1). In girls, it ranges from 13.03 mm at birth to 52.88 mm at 18 of age. The values for both sexes remain fairly equal during all the ages, displaying no sexual differences.

The best growth model for the maximum breadth of the distal epiphysis was a third-degree polynomial in boys and girls, with an explained variability of 98.3% and 98.1, respectively (Figure 5). In both cases, the maximum growth comes during childhood and finishes at 16-17 years of age.

Distal epiphysis breadth of the tibia seems not be an adequate variable for sex diagnosis due to the lack of significant sexual differences in any of the analysed ages. However, it can be useful for subadult age estimation, especially for cases in which the sex is unknown. In order to estimate age, the inverse regression between the distal epiphysis breadth and age was calculated. A second-degree polynomial for male, female and unisex series was selected with an explained variability of 98.3%, 98.1% and 96.0%, respectively (Table 2).

Figure 5: Polynomial regression line of the distal epiphysis breadth of the tibia for both sexes (continuous line for boys, discontinuous for girls), from 0 to 18 years of age. Polynomial functions shown in the box below (m, masculine; f, feminine).

DISCUSSION

The growth pattern of the five variables indicates a different growth behaviour between length (maximum length and diaphyseal length) and breadth measures (both epiphysis). The longitudinal variables showed a more linear growth and the breadth variables revealed a pattern with a growth restraint before the growth spurt; these results are in accordance with current literature (Rissech and Malgosa, 2003, Rissech and Malgosa, 2007, Twiesselmann, 1969). Moreover, the growth of both epiphysis seems to start with a moderate rhythm till the individual reaches 5 years of age, and then the rhythm accelerates till the end of puberty.

In general, the curves have a good fit and there is not a great deal of scatter, as evidenced by the consistently high correlation and the significance of the functions and coefficients achieved in the models. The category of the polynomial obtained for the variables of the tibia in this work coincides with those of López-Costas et al. (2012), which are based on a documented skeletal sample from Iberian Peninsula. However, it must be said that the results on metrical measurements of their study are not comparable with those obtained in our study due to secular differences between both samples. Their study is based on a documented sample from the Iberian Peninsula dated from the late 18th century to the mid-20th century, whereas our sample is constituted by living Spaniards from the 21st century. That means that the individuals of our study are in general higher and larger than those found in López-Costas et al. (2012). For example, in their study the diaphyseal length of males and females individuals between 15-19 years old is 315.67 mm and 293 mm respectively, whereas in our study this variable is 325.55 mm for males and 333.88 mm for females at 13 years of age. These results indicate that the functions and values obtained by these authors are not useful for current samples of the 21st century, and should be used exclusively to examine individuals who lived during the period of time in which the individuals of their study belong (late 18th to mid-20th century) and archaeological samples. These differences can be attributed to the noticeable height increase (10 cm) registered in Spain in the second half of the 20th century as a result of improved living conditions (Spijker et al., 2012). Age estimation is essential in any osteological study and biological identification, since it is characteristic of Forensic Anthropology. The success in determining the identity of a deceased individual is not only a requisite in order to declare it officially dead, but also the basis to investigate interpersonal and war crimes and mass disasters. Therefore, an increase in the accuracy and reliability of the ageing methods is imperative in the field of Forensic Anthropology.

Sexual dimorphism

Our results show that the sexual differences observed in this study, which were in the maximum and diaphyseal length of the tibia, are related to the cessation of female growth rather than to the spurt itself, as it was observed in the longitudinal variables (Tanner et al., 1976). The maximum length and the proximal epiphysis are useful for sexual diagnosis from 14 years and 8 years of age respectively. The statistical significance of both variables are in agreement with the importance of these variables in adult sexual diagnosis (Olivier, 1960).

Growth pattern – age estimation

During our study we observed that the fusion of the proximal epiphysis begins at 13 years of age and finishes at 16 in girls, while in boys it begins at 15 and ends at 18 years of age approximately. As for the fusion of distal epiphysis, it begins at 14 and finishes at 16 years of age in girls, whereas in boys it starts at 15 and finishes at 18 years of age. These data are in accord with current information on the maturation process in living individuals (Scheuer & Black, 2000). The values obtained in the epiphyseal fusion of the proximal epiphysis of the tibia are related to the age at which boys and girls finish growing. These values are approximately 18 years of age in boys and 16 in girls, in agreement with those in the bibliography (19), since the end of the longitudinal growth of the tibia comes from the fusion of both epiphysis (Scheuer & Black, 2000).

In the analyzed sample, the age of proximal epiphysis fusion takes place at 13 years in most girls. Current standards based on radiographic atlas of the knee joint zone (Pyle and Hoerr, 1955; Scheuer and Black, 2000) indicate that girls' fusion of proximal epiphysis occurs at age 13 and is completed 1.5 years later. In the analyzed sample the fusion occurs one year ahead compared to standard values. The same happens with the male sample, showing fusion marks at 14 years, when in radiographic atlas (Pyle and Hoerr, 1955) fusion starts at 15 years. This difference in the age of fusion of proximal epiphysis may be due to secular effect. The sample of this study comes from a Spanish population born in the ending of the 20th century and beginning of the 21st. On the contrary, Pyle and Hoerr's (1955) sample contains Caucasian children from North America of the early twentieth century. The total fusion of the epiphysis varies, beginning at 16 years in girls and 17-18 in boys, and these values are inside the parameters of others studies (Hansman, 1962; Scheuer and Black, 2001).

Inverse polynomial functions of tibia variables are useful for age estimation in subadult individuals. The functions obtained from the four variables analyzed in this study are useful for forensic medicine and anthropology, for age estimation of current living individuals. The functions of both epiphysis and the functions of the diaphysis of the tibia are interesting due to the possibility they bring to determine the age of subadult victims of mass disasters, for example in cases when few parts are recovered.

It is important to create specific growth patterns for each population because the bones react to external factors as well as to the physical activity patterns of each population (Rauch et al, 2004), and therefore it is better to have functions that can be applied to populations with similar habits and environments than to apply functions from geographically distant series. The functions obtained in this study, taken from a current living Spanish sample, allow us to estimate the age of modern subadult skeletal remains in a reliable way for individuals from Spain and the Mediterranean area.

The creation of an accurate and reliable biological profile of subadult population from the Iberian Peninsula and Mediterranean area for the correct age estimation of forensic cases is required, because most of the available subadult skeletal ageing and stature standards were developed from US reference samples. The magnitude of error involved in applying these methods to Iberian and Mediterranean individuals is unknown. For example, the method to calculate adult stature based on US reference samples fails when applied to the estimation of living height in Spain and Italy. Therefore, methodology should not be applied to skeletal material without considering the secular and regional origin of the reference collection(s) used for its creation. For these reasons, the data presented in this study is of great importance to forensic and medicine anthropology of the region of Western Europe, and particularly of the Iberian Peninsula, due to the time proximity of the sample to the living population.

CONCLUSIONS

This cross-sectional study based on a living subadult population of the 21st century of the Iberian Peninsula provides valuable information related to the tibial growth pattern. The big size of the sample allowed for the creation of reliable functions to determine age at death in forensic cases. At same time, the study brings information about sexual differences in different elements of the tibia, such as diaphysis and proximal and distal epiphysis, which could be useful for sex determination. The study provides useful tools for age and sex determination in cases related to populations from the Mediterranean area, and more specifically the Iberian Peninsula.

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5. Discusión

5. DISCUSIÓN

Los resultados obtenidos en nuestros diferentes estudios han permitido observar los cambios que se producen en las extremidades inferiores a lo largo del desarrollo, desde el nacimiento hasta el final de la pubertad de una muestra de niños y niñas de Barcelona, nacidos todos ellos prácticamente en el siglo XXI. A través de la morfometría geométrica se observó que el máximo incremento en las dimensiones femorales tenía lugar alrededor de los 13 años en las niñas y alrededor de los 15 años en los niños. Estos resultados coinciden con la edad dada para el final del brote puberal en las niñas (12,8 años según Fernández-Méndez y Seara-Aguilar, 1994) y niños (10–15 años según Ferrández et al., 2009) españoles y es anterior los 17 años, edad en la que se considera que la mayoría de niños españoles adquieren el tamaño adulto, (Fernández-Méndez y Seara-Aguilar, 1994; Carrascosa et al., 2008; Ferrández et al., 2009; García Cuartero et al., 2010). Tales resultados están dentro del rango de edad normal de variación para el brote puberal femenino (9,5–14,5 años) y masculino (12,5-17,5 años), respectivamente, de la población actual viva (Tanner, 1962; Marshall y Tanner 1970; Gasser et al., 1991).

A continuación se detallan y discuten los hechos más destacados que se han observado en este estudio:

PATRÓN DE CRECIMIENTO Y ESTIMACIÓN DE LA EDAD

Las técnicas de morfometría geométrica y sus análisis estadísticos asociados han demostrado ser de gran utilidad para el estudio del desarrollo del fémur durante la pubertad, tanto en el caso de las chicas (Pujol et al., 2014) como en el de los chicos, y entre ambos sexos (Pujol et al., 2015).

Estudiando el desarrollo del fémur en su totalidad se han podido observar los cambios tanto en forma como en tamaño. El cambio en tamaño es notable durante todo el proceso de desarrollo del individuo, en especial durante el brote puberal, momento en que el crecimiento se acelera. Se inicia con el crecimiento del hueso en longitud y, después, en robustez. Los resultados concuerdan con estudios previos, que demuestran que el crecimiento longitudinal precede al transversal (Bass et al., 1999; Bradney et al., 2000; Rauch et al., 2001). Tanto la masa muscular como la fuerza que esta ejerce en el hueso es de crucial importancia para el correcto desarrollo de la robustez del fémur, y de cualquier hueso (Van der Meulen et al., 1993, 1996, 2002; Moro et al., 1996; Schoenau, 1998, 2005; Schoenau et al., 2000). Al aumentar la carga muscular, las fuerzas biomecánicas generan mayor robustez del hueso a su vez (Schiessl et al., 1998; Schoenau et al., 2000; Ruff, 2003). Suele ser habitual que la masa muscular sea mayor en chicos que en chicas, debido principalmente al incremento de testosterona que tiene lugar en el sexo masculino durante el brote puberal (Round et al., 1999).

Por otro lado, en el análisis de las variables métricas ha permitido observar como el crecimiento de fémur y tibia difieren. En el caso del fémur, algunas de las variables muestran patrones de crecimiento en los que se observa un estancamiento del crecimiento justo antes del brote puberal (Longitud máxima del fémur, ángulos cuello diafisario y bicondilar), que es normal en el patrón de crecimiento estándar en los humanos (Tanner y Whitehouse, 1976; Hernánndez, 2000 Ferrandez et al., 2005, Vizmanos et al., 2001, Kalberg et al., 2003). Las fórmulas obtenidas para la determinación de la edad muestran que son los valores de las longitudes, y en segundo lugar los de las epífisis, los que tienen un mayor peso en el ritmo de desarrollo de los individuos. En cambio, la tibia presenta un desarrollo mucho más lineal durante la niñez y la pubertad, lo que corrobora que el crecimiento de la tibia en general responde a un patrón lineal mientras que el fémur crece más o menos según etapas, con crecimiento acelerado en la niñez, ligera desaceleración antes de la pubertad y aceleración del crecimiento en la pubertad. Estos resultados concuerdan con los de otros autores (Scheuer y Black, 2000).

Existen pequeñas variaciones en el patrón de crecimiento de las diferentes poblaciones debido a factores genéticos, culturales, y ambientales, por lo que es de vital importancia generar estándares específicos de cada región para su uso tanto en antropología física como forense (Cameriere y Ferrante, 2008; Boccome et al., 2010; Charisi et al., 2011; Rissech et al., 2013). Actualmente la mayoría de los estándares se basan en poblaciones Norte Americanas de muestras esqueléticas o radiográficas de los años 50, y la desviación que se obtiene al aplicarlas en muestras europeas es desconocida. Por ejemplo, el uso de funciones basadas en población blanca Norte Americana para el cálculo de la estatura de la población española o italiana sobreestima la estatura en vida de los individuos analizados de manera sistemática (Formicola, 1993; Formicola y Franceschi, 1996). Contrariamente, en estas poblaciones se obtienen mejores resultados al aplicar las funciones desarrolladas por Pearson (1899), las cuales están basadas en población francesa del siglo XIX debido a la entrelazada historia biológica de estas poblaciones y porque provenían de muestras de mediana estatura (Formicola, 1993; Formicola y Franceschi, 1996). De manera similar, el método de Gindhart (1973) y el de Hoffman (1979), basados en norteamericanos blancos de origen europeo, infraestiman la edad de los individuos subadultos de la Península Ibérica aunque tanto la colección en la que se basa la metodología como aquella en la que se aplicó pertenecen a la mitad del siglo XX (Rissech et al., 2013). Una razón que explica estas diferencias entre la estatura y la edad estimada es que la población norteamericana es más alta que la mayoría de las poblaciones europeas (Komlos, 2001; Komlos y Baur, 2004; Smith y Norris, 2004), y aunque la estatura de los europeos ha aumentado recientemente debido a las mejoras en las condiciones de vida todavía existen algunas diferencias (Pebles y Norris, 2011).

La estimación de la edad es un elemento importantísimo en cualquier estudio osteológico y en los casos de identificación biológica, siendo una característica indispensable en Antropología Forense. El éxito en determinar la identidad de un cadáver o de restos esqueléticos no es sólo el requisito para decretar la muerte oficial del individuo, sino para la investigación de crímenes interpersonales, de guerra, y de desastres masivos. Por ello hay un progresivo aumento en la investigación científica para la generación de fórmulas lo más similares posible a las que son objeto de estudio. Nuestros resultados cooperan a este fin respecto a la población actual del siglo XXI de la Península Ibérica y en la región Mediterránea.

Fusión epifisaria

En los fémures de nuestra muestra, la fusión de la epífisis proximal se inicia a los 12.9 en chicas y a los 13.9 años en chicos, mientras que en la población portuguesa se inicia un año más tarde (Cardoso, 2008), a los 14 años en las chicas y a los 15 en los chicos, aunque ciertamente la muestra portuguesa corresponde a población portuguesa de principios-mediados del siglo XX con una condición socio-económica medio-baja. El final de la fusión empieza a darse, en nuestra muestra, a partir de los 14 años en las chicas y a los 15 años de edad en los chicos, mientras que en la portuguesa (Cardoso, 2008) se produce a los 15 años en chicas y a los 16 en chicos, esto es existiendo un adelanto del crecimiento en nuestro estudio, debido seguramente a un mejor status nutricional de la serie española.

En lo que respecta a la epífisis distal del fémur, en las chicas del presente estudio empieza la fusión a los 12.9 años, mientras que en los chicos se inicia a los 15.6 años. Tales resultados concuerdan con los obtenidos en niños Irlandeses, quienes muestran un inicio de fusión de la epífisis distal a los 13.3 años en chicas y a los 16 en chicos (O'Connor, 2007). No obstante, los valores obtenidos en nuestra muestra se adelantan cuando se comparan con poblaciones norteamericanas y bosnias de población de principios del siglo XX en ambos casos. (McKern y Stewart, 1957; Schaefer y Black, 2005). En estas últimas poblaciones la fusión de la epífisis distal del fémur tiene lugar a los 18 años en chicos. En cuanto a la edad de fusión completa de la epífisis distal de la muestra estudiada coincide con las observaciones realizadas en la población irlandesa en las que la fusión completa tiene lugar en las chicas alrededor de los 15.6 y en los chicos a los 18. No obstante, los valores observados en la población analizada se muestra adelantada respecto a las muestras norteamericanas y bosnias en la muestra masculina, que van más allá de los 18 años.

En la muestra de la tibia, la mayoría de chicas presentan el inicio de la fusión de la epífisis proximal a los 12 años y 14 años en los chicos. Los estándares actuales de la región de la rodilla (Pyle y Hoerr, 1955; Scheuer y Black, 2000) indican que la fusión de dicha epífisis suele inciarse en las niñas a los 13 años y en los niños a los 15 años, completándose 1.5 años después de su inicio (Pyle y Hoerr, 1955; Scheuer y Black, 2000). En la muestra española analizada la fusión de esta epífisis parece darse aproximadamente un año antes, en ambos sexos. Por otro lado la fusión completa de ambas epífisis, distal y proximal de la tibia, finaliza alrededor de los 16 años en chicas, y de los 17-18 años en chicos, valores que coinciden con los datos hallados en la bibliografía actual (Hansman, 1962; Scheuer y Black, 2000).

Las diferencias observadas, tanto en fémur como en tibia, respecto al tiempo de inicio de la fusión epifisaria parecen mostrar que en la muestra española aquí se inicia antes el crecimiento, quizá este factor puede venir por efecto secular. Nuestra muestra está formada por individuos de finales del siglo XX, principios del XXI, contrariamente a la muestra utilizada por los autores antes citados (Pyle y Hoerr, 1955; Cardoso, 2008; McKern y Stewart, 1957; Schaefer y Black, 2005) cuyas muestras provienen de norteamericanos de origen europeo y de europeos de inicios del siglo XX, de condición socio-económicas bajas. Nuestra muestra tiene unas condiciones socio-económicas buenas, pues todos los individuos muestran un ritmo de crecimiento y unas estaturas adecuadas para la población europea actual (Pujol et al., 2015). Además las muestras comparadas utilizan restos óseos y no material de tipo radiográfico como el del presente estudio, aunque la metodología utilizada para la determinación del grado de fusión ha sido muy similar tanto en los estudios de referencia como en el estudio de la muestra de este trabajo. Cuando comparamos con muestras más modernas observamos que las etapas de fusión se aproximan más a las aquí observadas, auqnue en el caso de la rodilla parece ser haber un ligero adelantamiento aun comparando con estudios que utilizan material radiográfico para su estudio (O'Connor 2007). Ello reflejaría el efecto secular comentado anteriormente.

DIMORFISMO SEXUAL

En el caso del fémur, las diferencias sexuales se observan a partir de los 9-10 años en función de las variables observadas. Para la longitud máxima, la longitud de la diáfisis, así como los ángulos cuello-diafisario y bicondilar, las diferencias sexuales empiezan a ser significativas a partir de los 10 años en adelante. En cambio, en el caso de la cabeza del fémur y de la epífisis distal estas diferencias se adelantan un año presentándose a partir de los 9 años hacia adelante. En cuanto a la longitud del fémur, el diámetro de la cabeza femoral y la anchura de la epífisis distal, se observa que las diferencias entre ambos sexos vienen dadas principalmente por el cese del crecimiento de estas variables en las chicas, lo cual se produce en ellas antes que en los chicos (Rissech et al., 2008). Estos prolongan su crecimiento hasta edades más tardías, aproximadamente unos 2 años después (Paterson, 1929; Flecker, 1932, 1942; Narayan y Bajaj, 1957; Hansman, 1962; Saksena y Vyas, 1969). Los valores de la longitud del fémur parecen no mostrar unos valores muy separados entre ambos sexos, aunque hay diferencias significativas entre ambos sexos. No obstante, en los valores de la cabeza femoral y de la epífisis distal, las diferencias entre ambos sexos son más visibles a partir de la pubertad. Tales resultados concuerdan con la opinión de que las anchuras de las epífisis son buenos indicadores del dimorfismo sexual humano (Olivier, 1960; Mall et al., 2000).

En el caso de la tibia, las diferencias sexuales se observan algo más tarde que en el caso del fémur. En la longitud máxima estas diferencias son significativas a partir de los 14 años, mientras que para la epífisis proximal las diferencias lo son desde los 9 años, al igual que la de la epífisis distal del fémur. Las diferencias sexuales observadas en las variables estudiadas también responden al cese del crecimiento femenino más que al brote puberal por sí mismo (Tanner et al., 1976), pues el crecimiento en chicos se alarga en el tiempo, aproximadamente unos 2 años (Paterson, 1929; Flecker, 1932, 1942; Narayan Bajaj, 1957; Hansman, 1962; Saksena y Vyas, 1969). No obstante, según los resultados obtenidos en este trabajo sólo la longitud máxima de la tibia y la epífisis proximal de la misma son útiles para la diferenciación sexual a través de este elemento esquelético. Hecho que reafirma la idea de que la región de la articulación de la rodilla (epífisis distal del fémur y proximal de la tibia) es una región con alto poder discriminante sexual (Gill, 2001; Holliday y Ruff, 2001).

Ángulo cuello-diafisario

En los valores de los ángulos cuello-diafisario y bicondilar es donde se dan las mayores diferencias entre ambos sexos de todas las medidas analizadas. Los valores observados entran dentro del rango normal de variabilidad para cada edad (Billing, 1954; Tardieu y Damsin, 1997; Muñoz-Gutiérrez, 2001). Los individuos al nacer muestran valores de 140.86° en el caso de los chicos y 141.86° en las chicas, estos valores descienden durante el desarrollo de la locomoción y durante la pubertad, sobre todo en las mujeres, alcanzando valores de 128.88° en chicos y 123.68° en chicas. Tales resultados entran dentro de la normalidad (121.7°-136-5°, para muestra europea) (Anderson y Trinkaus, 1998) en las que a partir de los 16 años el ángulo cuello-diafisario se estabiliza, en concordancia con estudios previos (Humphry, 1889; Yamaguchi, 1993).

El desarrollo del ángulo cuello-diafisario observado durante la infancia viene dado por la adquisición de la locomoción bípeda, con una posición inicial del cuello mucho más vertical y pasando a una posición más horizontal, debido probablemente a las fuerzas ejercidas por la musculatura, concretamente el músculo abductor durante la locomoción (Lovejoy, 2005).

El ángulo cuello-diafisario (al igual que el bicondilar) no se diferencian entre ambos sexos hasta el brote puberal. Tales ángulos, asociados al proceso de remodelación angular (Pujol et al., 2014; Pujol et al., 2015), son muy sensibles a diferentes factores. Por ejemplo el ángulo cuello-diafisario se ve claramente modificado por la actividad física ejercida por la población, lo que se comprueba al comparar poblaciones sedentarias e industrializadas con poblaciones menos desarrolladas económicamente y mayores patrones de actividad física (Anderson y Trinkaus 1998, Igbigbi, 2003) donde el ángulo cuello-diafisario es más cerrado que en las poblaciones más industrializadas (con menor actividad física asociada a la economía). También influyen en esta angulación la estatura de los individuos (Lofgren, 1956; Lusted y Keats, 1966; Singh y Singh, 1975; Igbigbi, 2003), pues los individuos de mayor estatura, y por consiguiente de mayor peso, presentan un ligero cierre del ángulo cuello-diafisario respecto a los de menor estatura. El ensanchamiento de la pelvis es otro de los factores que más influye en la variación de estos dos ángulos, reduciendo el cuello-diafisario y aumentando el bicondilar, Este factor es muy importante en la mujer (Tague, 2005; Birkenmaier et al., 2010), a la vez que el ensanchamiento, por ser el fémur femenino más corto (por tener las piernas más cortas que los chicos) (Singh & Singh, 1975; Gulan et al., 2000; Igbigbi, 2003). Para mantener el equilibrio en posición bípeda durante la marcha es necesario, en este último caso, cerrar más el ángulo cuello-diafisario para llevar las rodillas lo más próximas al centro de simetría del cuerpo (Aiello y Dean, 1990).

Ángulo bicondilar

Los valores obtenidos para este ángulo en la muestra aquí analizada van desde los 3.07° al nacer hasta los 8.04° a los 18 años en el caso de los niños, y de los 3.18° hasta los 10.70° en el caso de las niñas. Estos resultados concuerdan con los valores normales obtenidos por otros estudios (Pearson y Bell, 1919; Keats et al., 1966; Singh y Singh, 1975; Pandya et al., 2008).

A pesar de la importancia paleoantropológica del ángulo bicondilar, muy pocos estudios se han centrado en el estudio de su desarrollo (Salenius y Vankka, 1975; Tardieu y Damsin, 1997). Los resultados del presente estudio coinciden con las observaciones realizadas por Tardieu y Damsin (1997) basados en chicas y chicos de 0 a 12 años. Estos autores propusieron dos funciones para calcular el ángulo bicondilar de las niñas y para calcular el ángulo bicondilar de los niños según la edad de estos. Según tales autores y su función, el ángulo bicondilar en las niñas es de 6° a los 10 meses de vida postnatal, de 8° a los 9 años y de 10,8° a los 14 años. Este mismo estudio en varones, sugiere un ángulo bicondilar de 0° en el recién nacido, de 6° a los 9 años y 7,18° a los 12 años. En nuestra muestra, el ángulo bicondilar a los 9 años fue de 6,92° en las niñas y de 6,93° en los niños, a los 12 años fue de 10,48° en las niñas y de 7,52° en los niños, aumentando a 10,8° en las niñas de 14 años y a 8,12° en los niños de 16. Tales resultados contrastan con aquellos estudios, donde se apunta que el ángulo bicondilar sólo varía hasta los 7 años de edad, edad en la cual el ángulo bicondilar adquiere un valor adulto (Tardieu et al., 2006). En el presente estudio, el desarrollo de este ángulo se da hasta el cese del brote puberal (13 años en chicas, 15 en chicos), lo cual concuerda con estudios previos (Tardieu y Damsin, 1997). Los valores en chicas son mayores a los de los chicos, lo que coincide con observaciones previas (Tardieu y Damsin, 1997; Scheuer y Black, 2000, Igbigbi y Sharrif, 2005; Pandya et al., 2008). Las diferencias sexuales empiezan a ser significativas a partir de los 10 años, edad en que el brote puberal del pubis femenino tiene lugar (Rissech y Malgosa, 2007). El ángulo bicondilar muestra una mayor variabilidad en los chicos respecto a las chicas, esto muestra una canalización del crecimiento mayor en chicas. La canalización, descrita por Tanner (1962), considera este hecho una evidencia del poder de control que ejercen los genes frente al desarrollo del organismo. La canalización significa que los niños crecen con fidelidad respecto a la curva de crecimiento normal. Si esta curva se ve alterada por alguna enfermedad que modifica el correcto desarrollo de peso y estatura, se da el fenómeno de 'recuperación', denominado en inglés "Catch up", por el que el sujeto recupera aceleradamente su ritmo ordinario, cuando las condiciones son favorables, y vuelve a su curva de crecimiento correspondiente (Boersma y Wit, 1997). Este fenómeno de canalización también afectaría previsiblemente al de la pelvis, asociada inevitablemente al canal del parto, elemento de vital importancia para la reproducción y que junto con la locomoción parecen ser los dos de los factores selectivos que alteran el ángulo bicondilar (Parsons, 1914; Pearson y Bell, 1919; Walmsley, 1933; Heiple y Lovejoy, 1971; Tardieu, 1981, 1983; Berge, 1993; Tardieu y Trinkaus, 1994).

Las constantes diferencias sexuales de tamaño observadas en ambos ángulos femorales así como los valores constreñidos de estos ángulos en las niñas, en especial el bicondilar, **es un hallazgo nuevo que sugiere que el dimorfismo sexual de estos dos ángulos podría estar de forma parcial genéticamente predeterminado en las niñas**. Igualmente, nuestros resultados en cuanto al dimorfismo sexual del fémur contribuyen al debate de los factores subyacentes en relación con el desarrollo de la morfología de la pelvis. Ambos ángulos están relacionados con desarrollo de la amplitud pélvica, en especial con el desarrollo del diámetro biacetabular y el diámetro transverso del plano medio del canal del parto. Diferentes autores han encontrado sistemáticamente diferencias sexuales significativas en esta última variable del canal del parto (Kurki, 2013; Crespo et al., 2015), señalando la importancia de la misma en la estructura del canal del parto humano y en el proceso del parto (Kurki, 2013; Crespo et al., 2015). El patrón de crecimiento del fémur femenino podría significar que las constricciones observadas en estos dos ángulos femorales son debidas a las necesidades funcionales del canal del parto femenino en *Homo sapiens*. De hecho algunos autores han sugerido que la angulación del fémur parece estar sometida, al igual que la pelvis, por dos grandes presiones. Por un lado las exigencias locomotoras de la bipedestación y por otro a las dimensiones del canal del parto en los individuos femeninos (Parsons, 1914; Pearson y Bell, 1919; Walmsley, 1933; Heiple y Lovejoy, 1971; Tardieu, 1981, 1983; Berge, 1993; Tardieu y Trinkaus, 1994). No obstante, aún es necesario confirmar este extremo con más estudios sobre la existencia de patrones del desarrollo del dimorfismo sexual del fémur compartidos por primates humanos y no humanos.

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6. Conclusiones

6. CONCLUSIONES

1. La base de datos creada con un total de 1140 individuos subadultos sanos representa una importante fuente de información sobre el desarrollo en individuos de la Península Ibérica de principios del siglo XXI, con unas condiciones socio-económicas favorables para su perfecto crecimiento.

2. Los 22 landmarks utilizados en sendos estudios de la pubertad, tanto en chicos como en chicas, han demostrado ser de gran utilidad para la observación del patrón de cambios que se da durante esta fase del desarrollo subadulto.

3. Los estudios de morfometría geométrica han permitido observar el desarrollo del fémur y de sus centros de osificación así como el desarrollo de la forma general, tanto en tamaño como en forma.

4. Los estudios de morfometría geométrica así mismo han permitido observar las diferencias sexuales y el patrón de desarrollo de las mismas, las cuales se dan durante la pubertad entre chicos y chicas.

5. El desarrollo del fémur durante todo su crecimiento está en concordancia con los valores mostrados por otros estudios.

6. El desarrollo de la tibia durante todo su crecimiento está en concordancia con los valores mostrados por otros estudios.

7. Los resultados obtenidos así como las fórmulas desarrolladas, tanto para el fémur como para la tibia, son de gran utilidad como herramientas de diagnóstico para edad y sexo en el campo forense.

8. La fusión de las epífisis proximal y distal del fémur muestran un adelantamiento de 1 año aproximadamente comparado con poblaciones de la Península de mediados del siglo XX, mostrando un posible efecto secular en la población actual.

9. La remodelación angular, que implica el ángulo cuello-diafisario y el ángulo bicondilar, presentan un desarrollo que dura hasta el final de la pubertad en ambos sexos. Los valores obtenidos muestran una gran variabilidad poblacional en ambos valores lo cual concuerda con estudios previos.

10. La variación en la remodelación angular del fémur es mucho más marcada en chicas que no en chicos. A su vez durante la pubertad se ve una canalización en el desarrollo de esta variable en las chicas, lo que podría suponer una posible determinación genética prenatal a este elemento según el sexo.

11. La remodelación angular viene dada tanto por las cargas ejercidas por el fenómeno de la locomoción como por el ensanchamiento pélvico asociado al canal del parto.

12. La estrecha relación de la remodelación angular con el bipedismo y el desarrollo pélvico pueden ser de gran utilidad para el estudio de la adaptación del bipedismo en individuos subadultos en el registro fósil, teniendo en cuenta la variabilidad entre hombres y mujeres.

7. Artículos Publicados

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Ontogeny of the female femur: geometric morphometric analysis applied on current living individuals of a Spanish population

Aniol Pujol,[1] Carme Rissech,[1] Jacint Ventura,[2] Joaquim Badosa[3] and Daniel Turbón[1]

[1]*Unitat d'Antropologia Física, Departament de Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain*
[2]*Unitat de Zoologia, Departament Biologia Animal, de Biologia Vegetal i d'Ecologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Barcelona, Spain*
[3]*Unitat de Radiogiagnòstic de l'Hospital Sant Joan de Déu de Barcelona, Barcelona, Spain*

Abstract

In this study we describe the development of the female femur based on the analysis of high-resolution radiographic images by means of geometric morphometrics, while assessing the usefulness of this method in these kinds of studies. The material analysed consisted of digital images in DICOM format (telemetries), corresponding to 184 left femora in anterior view, obtained from the database of the Hospital Sant Joan de Déu of Barcelona (Spain). Bones analysed corresponded to individuals from 9 to 14 years old. Size and shape variation of the entire femur was quantified by 22 two-dimensional landmarks. Landmark digitisation errors were assessed using Procrustes ANOVA test. Centroid size (CS) variation with age was evaluated by an ANOVA test. Shape variation was assessed by principal component analysis. A MANCOVA test between the first five principal components and age, using the CS as covariable, was applied. Results indicated that both size and shape vary significantly with age. Several age-related shape changes remained significant after removing the allometric effect. In general, an increase in the robustness of the bone and noticeable phenotypic changes in certain areas of the femur were observed. During growth in the proximal region of the femur, the collo-diaphyseal angle decreases, the neck of the femur widens and the fovea moves to a lower position, standing more in line with the plane of the neck. Likewise, the size of the greater and lesser trochanters increase. In the distal region, a significant increase of epiphyseal dimensions was recorded, mainly in the medial condyle. The angular remodelling of the neck and the bicondylar region of the femur in females continues until 13 years old. The information provided in the present study increases our knowledge on the timing and morphology of the femur during development, and in particular the morphology of the different femoral ossification centres during development.

Key words: development; female femur; geometric morphometric; lower limb; ontogeny; sub-adult individuals.

Introduction

Developmental studies on the human skeleton are very important for: (i) reconstructing demographic profiles in past populations; (ii) estimating sub-adult age in skeletal remains; (iii) the biological identification of living individuals in legal proceedings where a minor is involved; and (iv) the determination and interpretation of many factors, including indicators of health status and living conditions. Methods of age estimation in infant and juvenile individuals rely on the degree of skeletal and dental development. Based on the skeletal age of the individual, these methods provide a specific age range based on the degree of bone growth and development. Therefore, to estimate the biological age of living sub-adult individuals or in a sub-adult skeletal sample, it is essential to understand the normal pattern of growth and maturation of every skeletal element, and to develop the corresponding growth model. These growth models must be devised from two fully documented sources: (i) skeletal collections provided with information on age, sex, ancestry, pathology and cause of death; or (ii) on radiographic material of high resolution, which does

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Correspondence
Carme Rissech, School of Archeology and Ancient History, University of Leicester, University Road, Leicester LH1 7RH, UK.
T: +34 66 1142408; E: carme.rissech@gmail.com

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not distort the actual measurements. It is even better if the demographic, socioeconomic and temporal contexts in which the individuals lived are also known (López-Costas et al. 2012; Rissech et al. 2013a). It is essential that the origin of the reference sample used in the creation of the method is biologically comparable to the population under study, because the accuracy of the estimations depends on the application of appropriate data relating to the growth and maturation of the skeletal elements with regard to genetic, environmental and cultural factors (Biewener & Bertram, 1993; Slemenda et al. 1994; Arden & Spector, 1997; McGuigan et al. 2002).

However, despite the importance of having a register of growth and developmental data for different skeletal elements and populations, there is still a gap in the studies based on direct or radiographic material from which actual measurements can be taken. In fact, these studies are scarce because of the lack of sub-adult skeletal collections (Rissech, 2008), and because until recently there has not been appropriate radiographic material of high resolution, such as multislice computerised tomographies and telemetries (García et al. 2010).

Although there is a relatively large number of studies on human skeletal growth (Greulich & Thoms, 1938; Reynolds, 1945, 1947; Ghantus, 1951; Maresh, 1955; Greulich, 1960; Garn, 1962; Tanner, 1962; Coleman, 1969; Pyle et al. 1971; Gindhart, 1973; Tanner et al. 1976; Hoffman, 1979; Alduc-le Bagousse, 1988; Gasser et al. 1985, 1991; Hoppa, 1992; Miles & Bulman, 1994, 1995; Majó, 2000; Rissech et al. 2001, 2003, 2008, 2013a,b; Rissech & Malgosa, 2005, 2007; Rissech & Black, 2007; Schillaci et al. 2011, 2012; López-Costas et al. 2012 among others), very few works have been based on the study of material from Western European children (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles & Bulman, 1994, 1995; Majó, 2000; Rissech et al. 2001, 2003, 2008, 2013a,b; Rissech & Malgosa, 2005, 2007; Rissech & Black, 2007; López-Costas et al. 2012), and particularly on children of Spanish populations (Rissech et al. 2013b). Most of these latter studies have been based on archaeological material, in which age and sex were estimated in the laboratory (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles & Bulman, 1994, 1995; Majó, 2000). Analyses on documented skeletal collections are scarce and restricted to specific skeletal elements, such as the pelvic bone (Rissech et al. 2001, 2003; Rissech & Malgosa, 2005, 2007), scapula (Rissech & Black, 2007), tibia (López-Costas et al. 2012) and humerus (Rissech et al. 2013a). The femur is a lower limb skeletal element that is anthropologically important for its locomotor function (Scheuer & Black, 2000), and because of its robustness and post-depositional resistance. Despite the anthropological and forensic interest of the femur and the amount of research pertaining to this bone (Scheuer & Black, 2000), only a study of its developmental osteology has been conducted on documented skeletal individuals originating from Western Europe (Rissech et al. 2008). We have also encountered no femoral growth studies based on telemetries and geometric morphometric analysis, and even less femoral growth studies based on the current Spanish living population. For all this, it is important to complete and detail the postnatal development of the skeleton in Western European populations, and specifically in those from the Iberian Peninsula. Additionally, this information would be useful in palaeoanthropology and forensic anthropology.

Landmark-based geometric morphometrics (Bookstein et al. 1985; Bookstein, 1991; Reyment, 1991; Rohlf, 1999, 2000a,b) is a powerful tool because it permits the discrimination of anatomical differences through detailed shape analysis. Statistical analyses applied to landmark-based data allow testing hypotheses about the factors that affect the phenotype. Although geometric morphometrics is an appropriate procedure to analyse shape differences in skeletal structures (Lockwood et al. 2002; Pretorius et al. 2006; Scholtz et al. 2010; Toro-Ibacache et al. 2010), so far there have been no studies applying their method to track the development of human postcranial skeletal elements.

Our aim is to provide information on the postnatal development of the femur in the current living Spanish population. However, because of the extent of the subject we divided the study into two sub-studies, one for female and one for male femurs. For this reason, this first study will focus exclusively on the female femur, and we evaluate the morphological changes of the femur in females between 9 and 14 years old by applying landmark-based geometric morphometric techniques.

Materials and methods

High-resolution digital radiological images (telemetries) in Digital Imaging and Communication in Medicine (DICOM) format from the archives of Hospital Sant Joan de Déu de Barcelona were used for this analysis. All personal data were omitted with the exception of the demographic information (sex and age) following the Spanish Organic Law of Data Protection (Ley Orgánica de Protección de Datos; for details see García et al. 2010). This study has the approval of the Bioethical Committee of the Hospital Sant Joan de Déu de Barcelona (Ref. number 2938). The telemetries were chosen for this study due to the absence of a significant image deformation involved. This improvement of the image quality is the result of both the distance at which telemetries are taken (more than 180 cm from the centre of the X-ray focus and the subject) and the contact that the subject has with the radiographic film (it is in the bucky mural in telemetries). The bucky is a grid used in conventional radiology that selectively filters the radiation produced by the X-ray machine and eliminates non-perpendicular rays to the chassis that produce radiographic opacities that corrupt the image. For these reasons, these distant types of high-resolution digital radiographies are routinely used for medical purposes of evaluation and accurate measurement of the limbs (telemetries) and rachis (vertebral column radiographies, also known as scoliograms).

All the telemetries were taken in the Hospital Sant Joan de Déu following the standardised procedure of the hospital. The subject was made to undress from the waist down and to stand upright with the back of his body on the bucky mural for the purpose of telemetry, with arms extended along the body, knees and feet together, and hips and knees in full extension, the patellae facing forward with tibiae vertical and femora with slight internal rotation as is the natural anatomical position in this vertical position. There was equal weight bearing on both limbs. The tube was focused perpendicularly at the inferior angle of the patellae in the knee; the film-focus distance was 200 cm. Exposure was 120 Kv at 50–60 mA s^{-1}.

The left femora in anterior view of living girls aged from 9 to 14 years were analysed. This age range was selected because the female growth spurt takes place in this age interval (Tanner et al. 1981; Tanner, 1986). Those individuals displaying any type of pathology or anatomical deformation that could affect the analysis were excluded. Femora of 184 girls were analysed (girls of 9 years old: $n = 30$; 10 years: $n = 30$; 11 years: $n = 30$; 12 years: $n = 32$; 14 years: $n = 30$).

Twenty-two bidimensional landmarks were digitised using the Thin Plate Spine program series (Rohlf, 2006). Landmarks were placed in the femoral regions that show greater changes during growth, such as the head, the fovea, the neck, the trochanter, and the distal diaphysis and epiphysis. These landmarks (Fig. 1) were the following: (1) the most medial point of the growth plate between the head and neck; (2) the most angulated point of the growth plate between the head and neck; (3) the most lateral point of the growth plate between the head and neck; (4) the lowest point of the fovea; (5) the most upper point of the fovea; (6) the lowest point of the narrowest region of the neck; (7) the most upper point of the narrowest region of the neck; (8) the most proximal point of the growth plate between the greater trochanter and the shaft; (9) the midpoint of the growth plate between the greater trochanter and the shaft; (10) the most distal point of the growth plate between the greater trochanter and the shaft; (11) the tip of the greater trochanter; (12) the most proximal point of the growth plate between the lesser trochanter and the shaft; (13) the tip point of the lesser trochanter; (14) the most distal point of the growth plate between the lesser trochanter and the shaft; (15) the midpoint of the medial aspect of the shaft; (16) the midpoint of the lateral aspect of the shaft; (17) the most medial point of the growth plate between the diaphysis and distal epiphysis; (18) the midpoint of the growth plate between the diaphysis and distal epiphysis; (19) the most lateral point of the growth plate between the diaphysis and distal epiphysis; (20) the most distal point on the medial condyle; (21) the intercondylar superior angle of distal epiphysis in the articular margin; and (22) the most distal point of the lateral condyle.

Measurement error is an important source of variation affecting morphometric data that can increase the likelihood of type II errors and lead to biased results (Arnqvist & Martensson, 1998; Bailey & Byrnes, 1990) . In order to evaluate the impact of error on the current set of landmarks, each landmark was located on each radiograph 10 times over two separate days. A Procrustes ANOVA comparing variation in landmark location both among and within femora was performed (Klingenberg & McIntyre, 1998; Klingenberg et al. 2002). Because the variation between femora clearly exceeded that of the measurement error, we considered the effect of the measurement error in the landmark location process negligible (see Results).

To test size-related differences during growth, an analysis of variance (ANOVA test) on centroid size (CS) was applied. In order to determine changes in femur shape related to age, a principal component analysis (PCA) of the covariance matrix of the partial warps scores (tpsRelw program, version 1.46; see also Rohlf, 1999) was performed. Allometry, assumed as size-dependent shape variation, was evaluated through a multivariate analysis of covariance (MANCOVA) on the first five principal components, using CS as covariate. Statistical analyses were performed using SPSS 16.

Fig. 1 Landmark locations on the anterior aspect of the left femur in telemetry. For the definition of each landmark, see the text.

Results

The Procrustes ANOVA showed high significant differences between femora, both in size and shape ($P < 0.0001$). The mean squares for femur size variation (MS femur = 115603.84) exceeded the mean squares for the replicates (MS error = 12.49) by 9254.66-fold. For shape, mean squares for femur variation (MS femur = 0.000022) exceeded the mean squares for the replicates (MS error = 0.000011) by 1.92-fold. These results indicate low measurement error and consequently strong repeatability of the landmark location of the femur.

The ANOVA test showed that CS increased significantly with age ($F = 4.496$, $*P = 0.032$). This size variation was related to the growth spurt that takes place between 9 and 13 years old in the girls studied (Fig. 2). MANCOVA test revealed that, after removing the allometric effect, shape variation related to age was statistically significantly (Lambda de Wilks = 0.093, $F = 2.652$, $*P = 0.003$).

In the PCA, the first (PC1) and second (PC2) principal components explained 63.3% and 9.2% of the shape variation, respectively. Distribution of the different individuals in the shape space defined by these components (Fig. 3) showed that femur shape varied progressively according to age. The traits that accounted for this variation along PC1 were the robustness of the femur (Fig. 4), the verticality of the head with respect to the femur (Fig. 5), the angle of the neck of the femur with respect to its diaphysis (Fig. 5), the size of the distal epiphysis (Fig. 6), and the position of the greater trochanter (Fig. 5). The second principal component allowed the separation of the different age groups by the increase in size of the lesser and greater trochanter (Fig. 5), the increase of the diaphysis width (Fig. 4), and the increase of width of the femoral neck (Figs 4 and 5).

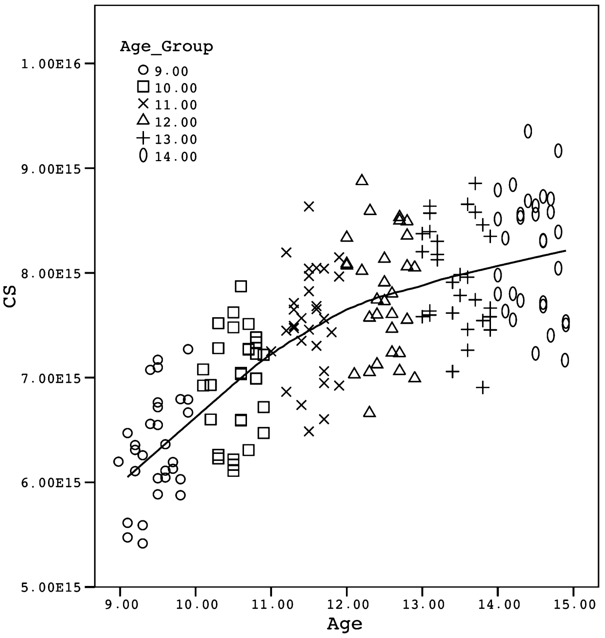


Fig. 2 Scatter plot of age and CS, including the Lowess regression line.

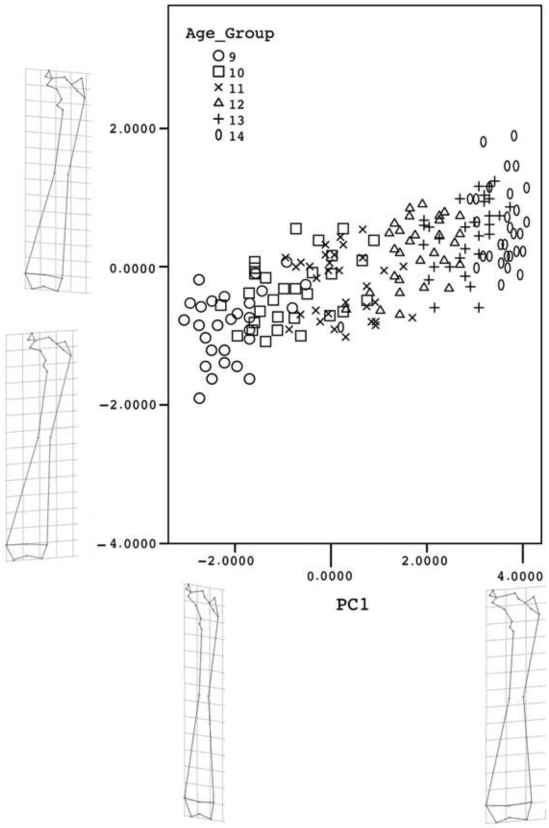


Fig. 3 Ordination of the individuals in the space of the two first principal components (PC1, PC2) based on the weight matrix for the anterior aspect of the femur.

In general terms, in younger individuals the head of the femur tends to be in a more vertical position in relation to the diaphysis (Fig. 4), the lesser trochanter is unfused and the greater trochanter is relatively large; meanwhile, the distal and proximal epiphysis are well developed but have not reached the final size. In older individuals, the whole femur gains robustness. The diaphysis and the femoral neck increase their diameter, and the femoral head assumes a more horizontal position relative to the shaft. The lesser and the greater trochanter enlarge considerably and, in turn, the greater trochanter holds a superior position in relation to the juvenile femur. The distal epiphysis also grows in size, but mainly focuses on increasing the medial condyle, giving rise to a greater femoral angle in relation to the central axis of the body, and the angle between the femoral neck and the diaphysis becomes smaller. All these changes were related to the angular femoral remodelling that occurs during growth, and according to our results they finish at the end of puberty (Figs 4–6).

In order to quantify the changes in the angulation of both femoral epiphyses, the collo-diaphyseal and bicondylar

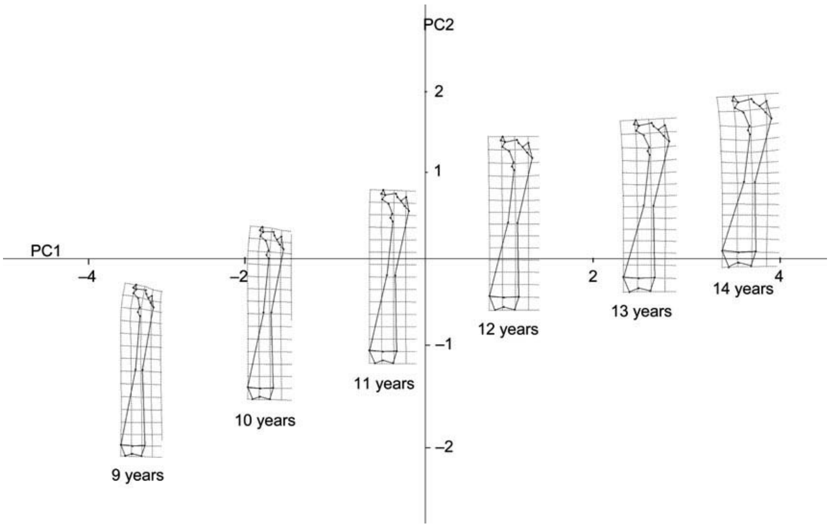


Fig. 4 Graphic representation of the consensus form in each age of the analysed interval of age (from 9 to 14 years) based on the space of the two first principal components.

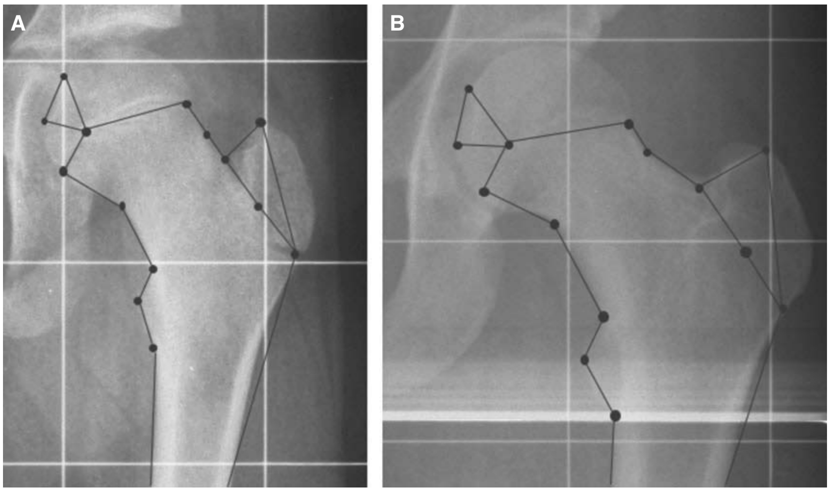


Fig. 5 Shape changes in the proximal femur between the individuals who were (A) 9 years old and (B) 14 years old.

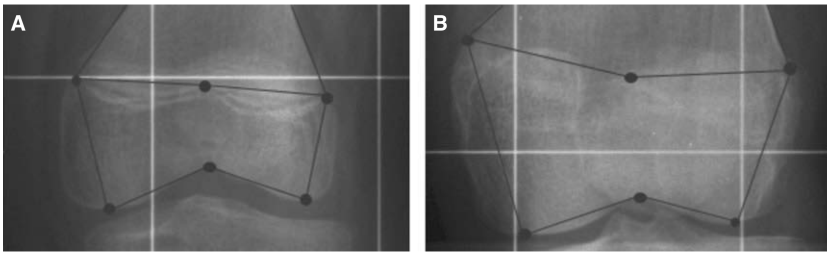
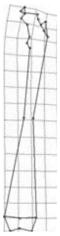
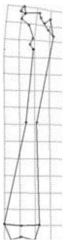
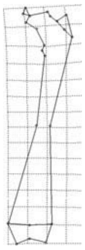
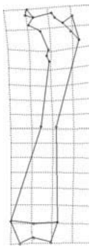


Fig. 6 Shape changes in the distal epiphysis between individuals who were (A) 9 years old and (B) 14 years old.

angles were measured and graphically represented in relation to the individual age (Table 1; Figs 7 and 8). The former is the angle formed by the intersection between the longitudinal axis of the femoral diaphysis and the longitudinal axis of the neck (Tardieu & Damsin, 1997). The bicondylar angle is that between the longitudinal axis of the diaphysis and a perpendicular line to the infracondylar plane of the femur (Shefelbine et al. 2002). The longitudinal axis of the femoral diaphysis is the midline along the diaphysis, which links the middle of the infra-condylar segment and the middle of the proximal segment of the femur, located 2 cm below the great trochanter. The longitudinal axis of the neck is the midline between the distal and proximal borders of the neck. Results reveal that both angles clearly change until 13 years old, when they tend to stabilise, and show divergent trends. Thus, during this period, whereas the collo-diaphyseal angle decreases, the bicondylar angle shows a substantial increase.

Table 1 Descriptive statistics of the bicondylar and collo-diaphysal angles by age with the corresponding consensus form in each age.

Age		Bicondylar angle	Collo-diaphyseal angle	Consensus form in each specific age
9	Mean	6.92	137.00	
	n	30	30	
	DS	0.53	3.52	
10	Mean	8.72	133.36	
	n	30	30	
	DS	0.50	3.24	
11	Mean	9.51	130.35	
	n	30	30	
	DS	0.33	4.68	
12	Mean	10.48	127.39	
	n	30	30	
	DS	0.57	3.51	
13	Mean	11.09	125.92	
	n	30	30	
	DS	0.42	3.70	
14	Mean	10.80	123.95	
	n	31	31	
	DS	0.45	2.77	

Discussion

Geometric morphometrics and associated statistical analyses allowed us to describe the morphological changes in the female femur between 9 and 14 years old. Results revealed that both the size and the shape of the femur vary significantly during this period. Due to the presence of the pubertal growth spurt within this age interval, the size increase of the femur is one of the most significant changes observed in this bone, increasing first in length and later in robustness. These results are in accordance with those by other authors who suggest that the elongation of the femur precedes its diameter (robustness) and mass increases (Bass et al. 1999; Bradney et al. 2000; Rauch et al. 2001). In fact, mass and muscle strength are important for bone development in robustness (Van der Meulen et al. 1993, 1996, 2002; Moro et al. 1996; Schöenau, 1998, 2005; Schöenau et al. 2000). By raising the muscular load, biomechanical forces increase, leading to the development of higher robustness (Schiessl et al. 1998; Schöenau et al. 2000; Ruff, 2003). The gain in muscle mass is generally higher in boys than in girls due to the increase of testosterone during male growth (Round et al. 1999). Consequently, the ratio of bone to muscle is higher in females than in males (Högler et al. 2008), and hence the femur of the girls is less robust (Ruff, 2003).

The shape changes associated with growth were significant even after removing the allometric effect. Among

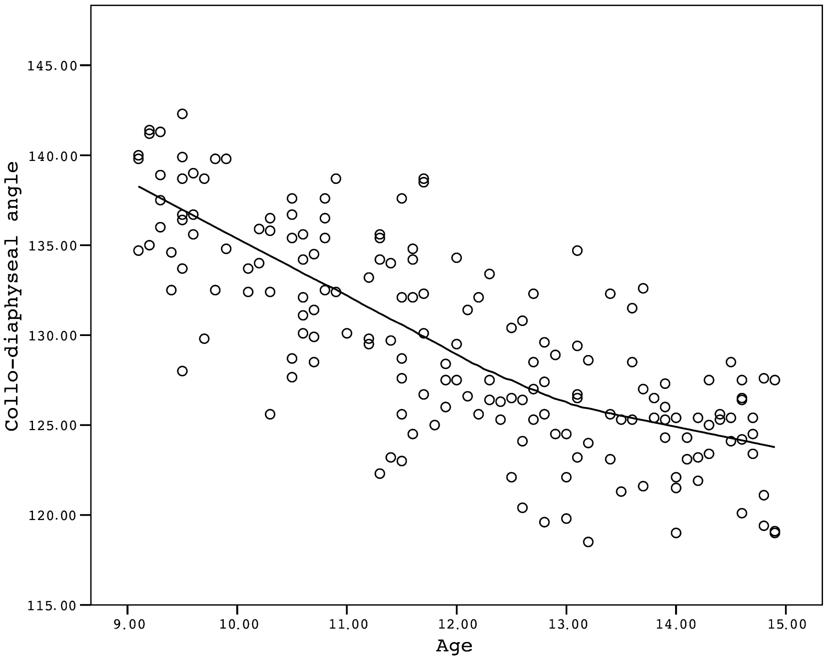


Fig. 7 Collo-diaphyseal angle variation regarding age. The curve was calculated using the Lowess method.

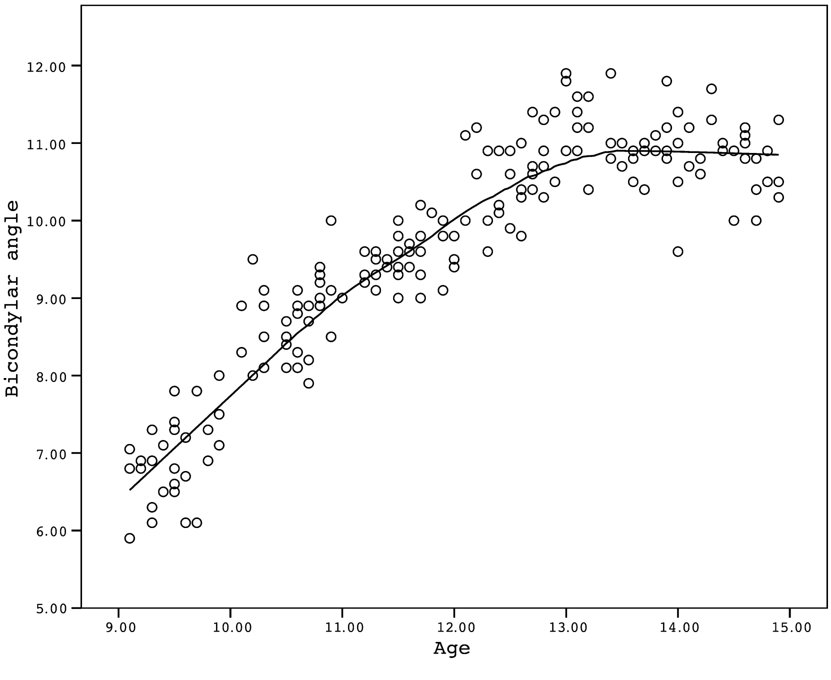


Fig. 8 Bicondylar angle variation regarding age. The curve was calculated using the Lowess method.

these changes, those in both proximal and distal epiphyses are outstanding, due to the angular remodelling of the femur determined by a more horizontal position of the femoral neck (angular decrease of the femoral neck) and the size increase of the medial condyle of the distal epiphysis (bicondylar angular increase).

Results reported here indicate that the angular remodelling of the femur (collo-diaphyseal and bicondylar angles) takes place until approximately 13 years old in females, which corresponds to the mean age of the end of Spanish female puberty (12.8 years old according to Fernández-Méndez & Seara-Aguilar, 1994). This age falls in the middle of the normal age range (9.5–14.5 years) of growth spurt in girls in the current living population (Tanner, 1962; Gasser et al. 1991). The obtained average for collo-diaphyseal angle in each age group of our study is inside the normal range of variability given in current literature on the growth process of this variable (Billing, 1954;

Muñoz-Gutiérrez, 2001). Our sample starts with a value of 137 ° at 9 years old, and reaches 123.95 ° at 14 years old, which falls within the normal range of variation (122.3–135.9 °) for adult European women (Anderson & Trinkaus, 1998). Beyond 13 years old, the collo-diaphyseal angle stabilises in our sample, which is congruent with the results obtained in other studies that have shown that the values of this angle are very stable from mid-adolescence throughout most adulthood (Humphry, 1889; Yamaguchi, 1993; Anderson & Trinkaus, 1998). The morphology of the femoral diaphysis changes considerably throughout growth (Scheuer & Black, 2000). The proximal epiphysis, the neck, the head and both trochanters increase in size. During the early stages of life, the angle of the femoral neck with the shaft is more obtuse, namely the neck has a more vertical position, as the abductor muscle develops in response to the start of locomotion. The collo-diaphyseal angle decreases from an average of 150 ° at birth to approximately 127 ° at the end of growth after the end of the pubertal growth spurt (Humphry, 1889; Keats et al. 1966; Hefti, 2000; Igbigbi, 2003). Previous studies have identified considerable populational variability in the collo-diaphyseal angle (Anderson & Trinkaus, 1998; Igbigbi, 2003). This variability is related to both individual stature (Lofgren, 1956; Lusted & Keats, 1966; Singh & Singh, 1975; Igbigbi, 2003) and differential economic activity (Anderson & Trinkaus, 1998). This variable increases significantly across populations with an increasingly sedentary existence and with mechanisation (Anderson & Trinkaus, 1998). High angles commonly encountered in modern industrial societies cluster at the high end of normal recent human ranges of variation. Lower values, even below 120 °, are by no means unusual in medically normal individuals in non-industrial societies (Anderson & Trinkaus, 1998). More recently, some authors have pointed out the correlation of this angle with climate (Gilligan et al. 2013).

Furthermore, females generally have smaller collo-diaphyseal angles than males because of their wider pelvis and shorter femur (Singh & Singh, 1975; Gulan et al. 2000; Igbigbi, 2003). In fact, the change in angulation of the femoral neck is not only associated with an increased mechanical load generated by the muscles and ligaments, but it is also depends on the widening of the pelvis, which is important in females at this age (Tague, 2005; Birkenmaier et al. 2010). With regard to the trochanters, size and shape are also conditioned by the increase of the mass of the gluteus maximus and minimus (greater trochanter), and pectineus (lesser trochanter) muscles.

The bicondylar angle is the skeletal feature that permits the adduction of the lower limb in humans. This is different from the tibio-femoral angle, which is the angle between the tibial and femoral diaphyseal axes in the coronal plane of the leg. Conversely, the tibio-femoral angle measures the evolution of a physiological phenomenon and not an osteological angular remodelling (Tardieu & Damsin, 1997). The bicondylar angle allows the knee and ankle joints to be placed almost directly under the centre of gravity of the body, making the human displacements more economical, as the load transmitted by the lower limbs is close to the vertical axis of gravity of the body during the phases of walking and running. Due to its implication of bipedal walking, the bicondylar angle was one of the most important characteristics that permitted the attribution of some fossils to human lineage (LeGros Clark, 1947; Johanson & Coppens, 1976; Tardieu & Trinkaus, 1994), as it indicates a clear adaptation to bipedal locomotion (Tardieu & Damsin, 1997). Although this has palaeoanthropological significance and there is great angular remodelling that implies femur growth in humans (changing from a varus position in newborns to a valgus position in adults), only an extremely few studies have focused on the development of the bicondylar angle (Salenius & Vankka, 1975; Tardieu & Damsin, 1997). Our results are in agreement with those obtained previously in girls from 0 to 14 years old (Tardieu & Damsin, 1997), which propose a regression function to calculate the female bicondylar angle by age with 97% expressed variability (bicondylar angle = 4.1244 + 0.03958 × age in months). This function indicates a bicondylar angle of 6 ° at 10 months of post-natal life, an angle of 8 ° at 9 years old, and an angle of 10.8 ° at 14 years old.

The distal epiphysis reacts to the mechanical loads caused by the change of angulation of the femur. This shape modification results in pressures generated in the bicondylar region. The stress exerted by the different muscle groups as well as the patella generates an asymmetrical load in the condyles, focusing the force mainly on the medial condyle. This leads to a reaction in the endochondral cartilage of the medial condyle, causing a greater development of that condyle and consequently the angulation of the distal femur (Shefelbine et al. 2002). The bicondylar angle undergoes a major transformation from the beginning of locomotion to 8 years old and, according to our results, it continue after the femoral growth spurt before the completion of epiphyseal closure, which is in accordance with the observations of Tardieu & Damsin (1997). The age of stabilisation of this variable has remained an open question (Tardieu & Damsin, 1997) because of the scarcity of studies on this subject, and because the few studies that exist (Tardieu & Trinkaus, 1994; Tardieu & Damsin, 1997) do not have enough information to show it clearly. Most of the studies are based on the tibio-femoral angle (Salenius & Vankka, 1975; Cahuzak et al. 1995; Mehmet-Arazi et al. 2001) and they do not agree in relation to the stabilisation age of this variable. Selenius and Vankka stated that the tibio-femoral angle would stabilise at about 6 or 7 years old, and from this age the femur would grow in length maintaining a constant angle. Other authors affirm that this would take place at about 10 years old (Cahuzak et al. 1995), and others at about 12 years (Mehmet-Arazi et al. 2001). But the fact is that these studies are based on the development of the tibio-femoral angle not the bicondylar angle. In relation to the development of the bicondylar angle, Tardieu & Damsin (1997) observed that in females it could stabilise at 13 years old. Our study clearly shows that the age of stabilisation in the Spanish female bicondylar angle is about 13 years old, after the female growth spurt and coinciding with the observations of Tardieu & Damsin (1997). In the present study, the average bicondylar angle in older individuals is 10.8 °, which falls into to the current normal range of variation, between 8 and 15 ° (Pearson & Bell, 1919; Keats et al. 1966; Singh & Singh, 1975; Pandya et al. 2008), around the given value from the London population and higher than that given from the Paris population (London = 10.2 °, Paris = 7.9 °; Tardieu & Trinkaus, 1994). Like the collo-diaphyseal angle, the bicondylar angle varies between populations (Pandya et al. 2008) as a consequence of mechanical loading experienced by the femur due to diverse physical activity coupled with several genetic, cultural and environmental processes that take place in different individuals and populations (Tardieu & Trinkaus, 1994).

In summary, the angular remodelling of the femoral neck causes a displacement of the axis load, which results in it not coinciding with the central axis of the femoral diaphysis but intercepting it in the distal epiphysis. This change causes the knee to come nearer to the midline of the pelvis. This phenomenon is associated with an increase of the bicondylar angle and coincides with the development of a more efficient locomotion, which is given by the knee, lower leg and foot being nearer to the medial axis body (Aiello & Dean, 1990). Consequently, the centre of gravity of the body needs to move only a short lateral distance to stay on the supporting leg during walking (Aiello & Dean, 1990).

It is true that all human populations grow and develop similarly under healthy conditions. However, it is also true that the existence of specific standards for individuals of specific regions is necessary and important in both anthropological and forensic fields (Cameriere & Ferrante, 2008; Boccome et al. 2010; Charisi et al. 2011; Rissech et al. 2013b). Most of the current standards are based primarily on North American (USA) skeletal or radiographic samples, and the magnitude of the error in its application to European populations is unknown. For example, the method for calculating adult stature based on USA reference samples fails in the estimation of living height in Spain and Italy (Formicola, 1993; Formicola & Franceschi, 1996; Lalueza-Fox, 1998). In Spain and Italy, the use of the formulae proposed by Pearson (1899) at the end of the 19th century, based on a French sample, performs better (Formicola, 1993; Formicola & Franceschi, 1996; Lalueza-Fox, 1998) than the Trotter and Gleser formulae for Whites, because of the intertwined biological population history of French, Spanish and Italian populations (Formicola, 1993; Formicola & Franceschi, 1996; Lalueza-Fox, 1998), and because they are populations of medium stature (Formicola, 1993; Formicola & Franceschi, 1996). In contrast, the equations of Trotter and Gleser for Whites systematically overestimate stature in both female and male skeletons of Spanish and Italian origin (Formicola & Franceschi, 1996; Lalueza-Fox, 1998). In sub-adult age estimation, for example, according to the data given by Gindhart (1973) and Hoffman (1979) studies based on a White American sample belonging to the middle of the 20th century of North Western European descent, a tibial diaphyseal length of 203 mm from a Portuguese individual aged 7 years from the same historical period is situated in the lower limit of the expected normal variation range of growth (200–260 mm) given by these authors for individuals of 7 years old (Rissech et al. 2013). For this reason, the estimated age (which is an average age of the normal range interval) for this individual is 4.5 years (Rissech et al. 2013b). To assume that all populations grow like those from the USA would not be exactly correct. It is well known that the USA population is taller than most European populations (Komolos, 2001; Komlos & Baur, 2004; Smith & Norris, 2004). Although in recent years stature has increased in Europeans due to improvements in living conditions, some differences still exist (Pebles & Norris, 2011). Forensic age estimation of unidentified corpses and skeletons for the purpose of identification has been a traditional feature of forensic science. Successfully determining the identity of a decedent is of considerable significance from the ethical, legal and criminal perspective; not only is it the prerequisite for officially declaring an individual dead, but it is also the basis for investigating crimes, mass disasters or war crimes. There is a pressing need for accuracy and reliability of the methods in the field of Forensic Anthropology. In this way, the data presented in this study are appropriate for current living populations from the 21st Century, especially for those belonging to Western Europe and more specifically from the Iberian Peninsula.

The information provided in the present study increases our knowledge of the timing and morphology of the femur during development, and in particular the morphology of the different femoral ossification centres during development. Improvement of our knowledge of bone development helps in age estimation, which will be determined with greater accuracy in both the clinical context, and in archaeological and forensic assemblages. In a clinical context it can help to increase the accuracy in assessing maturity. In archaeological and forensic assemblages, the data of the present study may be useful in age estimation of juveniles. Furthermore, it can be of utility in some palaeonthological cases, as for example the given values for the bicondylar angle by age, which can be extremely useful in determining bipedal locomotion in young individual fossils. However, further research on the development of the femur is necessary to obtain better information for skeletal diagnosis. In particular, it is necessary to study male femur development in order to compare it with the data presented here.

Conclusions

The findings in this study constitute the first approach to elucidate the size and shape changes in the female human femur during puberty by using geometric morphometrics of landmark-based data. Furthermore, it is also the first skeletal growth study based on the Spanish population, and more specifically on the current living Spanish population from the 21st Century. The results obtained agree with the development patterns observed in previous studies on the development of the femur during puberty. Furthermore, they bring new information on the timing of the angular remodelling of the neck and bicondylar region during postnatal growth of the female femur based on a considerable sample (184 females). Results indicate continuous remodelling until 13 years old for both variables (collo-diaphyseal and bicondylar angle), and agree with the high variability demonstrated by different studies among different regions. The angular remodelling of the neck and the bicondylar region of the femur is determined by the increase in the mechanical load generated by muscles and ligaments, and by the widening of the pelvis, which is important in females (Tague, 2005; Birkenmaier et al. 2010). Widening of the pelvis does not finish until the end of the female juvenile stage (Rissech et al. 2003; Rissech & Malgosa, 2005, 2007). These results reveal that geometric morphometric analyses can be very useful for the understanding of skeletal development. Furthermore, it could be useful as a method for detecting morphological criteria of differentiation between the different ages in sub-adult individuals, which could be useful for sub-adult age estimation. Results reported here can also be an interesting resource for palaeoanthropological studies on immature individuals from the fossil record, especially those in which it is important to detect bipedalism.

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Ontogeny of the Male Femur: Geometric Morphometric Analysis Applied to a Contemporary Spanish Population

Aniol Pujol,[1] Carme Rissech,[1]* Jacint Ventura,[2] and Daniel Turbón[1]

[1]*Departament de Biologia Animal, Facultat de Biologia, Unitat d'Antropologia Física, Universitat de Barcelona, Barcelona, Spain*
[2]*Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Unitat de Zoologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Barcelona, Spain*

KEY WORDS development; male femur; geometric morphometric; lower limb; ontogeny; subadult individuals

ABSTRACT

Objective: To describe the morphological changes of the male femur during the adolescent growth spurt and to compare the pattern obtained with that reported previously for females.
Material and Methods: Two hundred and forty males from a Spanish population aged between 9 and 16 years were analysed, based on telemetries. Size and shape variation of the femur was quantified by 22 2D-landmarks and analysed using geometric morphometric methods. Likewise, the variation of neck–shaft and bicondylar angles were also determined and evaluated by Student's *t*-test. Sexual differences were analysed by comparing results here obtained on boys with those corresponding to girls reported in a previous study.
Results: In males, both size and shape varied significantly with age, with males having larger dimensions than females. In general terms, these changes are generally characterised by an increase in robustness of the femur and shape modifications in the epiphyses. During growth, the neck–shaft angle decreases and the size of the greater and lesser trochanters increase. A significant increase of distal epiphyseal dimensions was recorded, mainly in the medial condyle. The angular remodeling of both the neck and the bicondylar regions of the male femur continues until 16 and 15 years, respectively. Female and male femur each followed divergent growth trajectories. Males showed a greater variability in neck–shaft and bicondylar angles than females.
Discussion: The timing, morphology and growth trajectories provided on the femur during development can be very helpful in anthropological, paleoanthropological and evolution studies. Am J Phys Anthropol 000:000–000, 2015. © 2015 Wiley Periodicals, Inc.

Research on human development has a long tradition in the field of physical anthropology, dating the first longitudinal growth study from 17th century (Bogin, 1999). At present, growth and maturation processes, specifically developmental studies on the human skeleton, are considered essential for resolving different medical and anthropological questions, such as: i) establishing the degree of growth and maturation of subadult individuals in cases of orthopaedic surgery or growth hormone treatment; ii) establishing the health status and living conditions of children; iii) reconstructing demographic profiles in past populations; iv) estimating the age of skeletal remains of subadult individuals; v) the biological identification of living individuals in legal proceedings where a minor is involved; vi) understanding the current human variability and vii) understanding the evolution of human ontogeny which will ultimately provide a framework for reliable interpreting the evolution in fossil hominins (Neubauer et al., 2009).

Basically, these estimations rely on the relationship observed between chronological age and the degree of bone growth and maturation for infant and juvenile individuals. Taken into account the skeletal age of one individual, the methods for subadult age estimation provide a specific age range based on the degree of growth and maturation. Each ageing method is based on a model of the bone development process based on a specific reference sample. These models can be based on documented skeletal collections (with known sex, age, biological origin and cause of death) or high-resolution digital radiographies, ranging from telemetries (accurate radiographies of the limbs) and scoliograms (vertebral column radiographies; also known as rachis) to multislice computerised tomographies, which do not distort the actual measurements. Furthermore, it is essential that the reference population used to create the model be biologically comparable to the analysed sample, since the accuracy of the estimations depends on the application of appropriate data relating to the development of the skeletal elements with regard to genetic,

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*Correspondence to: Carme Rissech, School of Archaeology and Ancient History, University of Leicester, University Road, Leicester LE1 7RH, UK. E-mail: carme.rissech@gmail.com

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environmental and cultural factors (Biewener and Bertram, 1993; McGuigan et al., 2002; Rissech et al., 2008). However, in spite of the importance of having a register of growth and maturation values from different skeletal elements and populations, and the large amount of studies on human skeletal development (Greulich and Thoms, 1938; Greulich, 1960; Garn, 1962; Tanner, 1962, 1986; Coleman, 1969; Pyle et al., 1971; Gindhart, 1973; Tanner et al., 1976, 1981; Hoppa, 1992; Miles and Bulman, 1994, 1995; Rissech et al., 2013a,b; Frelat and Mitteroecker, 2011; Schillaci et al., 2011, 2012; López-Costas et al., 2012), there is still a serious lack of information regarding the development of many of the elements of the human skeleton in different populations (Rissech et al., 2013a,b).

Of the growth standards currently available for osteological studies of Western European individuals, many are based on radiological images and skeletal assemblages from white North American children from the 1950s and beforehand (Greulich and Thoms, 1938; Reynolds, 1945, 1947; Ghantus, 1951; Maresh, 1955; Coleman, 1969; Pyle et al., 1971; Gindhart, 1973; Hoffman, 1979; Gasser et al., 1985, 1991). The few studies which focus on Western European children (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles and Bulman, 1994, 1995; Majó, 2000; Rissech et al., 2001, 2003, 2008, 2013a, 2013b; Rissech and Malgosa, 2005, 2007; Rissech and Black, 2007; Frelat and Mitteroecker, 2011; Lopez-Costas et al., 2012) and particularly on Spanish children are mostly based on: i) archaeological material with age and sex estimated in the lab (Alduc-le Bagousse, 1988; Hoppa, 1992; Miles and Bulman, 1994, 1995; Majó, 2000); and ii) documented skeletal remains from the late 19th and first half of the 20th century (Rissech et al., 2001, 2003, 2013a, 2013b; Rissech and Malgosa, 2005, 2007; Rissech and Black, 2007; Frelat and Mitteroecker, 2011; López-Costas et al., 2012; Cardoso et al., 2014). Furthermore, these last studies are restricted to specific skeletal elements such as the pelvic bone (Rissech et al., 2001, 2003; Rissech and Malgosa, 2005, 2007), scapula (Rissech and Black, 2007), tibia (Frelat and Mitteroecker, 2011; López-Costas et al., 2012; Cardoso et al., 2014) and humerus (Rissech et al., 2013a; Cardoso et al., 2014).

For identification and age estimation purposes, the existence of developmental models for individuals of specific populations is necessary and important. However, because most of the current standards are based primarily on North American (USA) samples, the magnitude of error when applied to European populations is unknown. For example, the method for calculating adult stature (it is necessary to remember that the stature results from the growing process) based on White American reference samples systematically overestimates stature in both female and male skeletons of Spanish and Italian origin (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998). Because of the intertwined biological population history of French, Spanish and Italian populations, and because they are populations of medium stature, the use of the formulae proposed by Pearson (1899) at the end of 19th century, based on a French sample performs better than the Trotter and Gleser (1952) formulae for "Whites" (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998). Similarly, the data given by Gindhart (1973) and Hoffman (1979), based on a White Americans of North Western European descent, also underestimate ages in subadult individuals from the Iberian Peninsula, even though both the reference sample and children derive from the mid-20th century (Rissech et al., 2013b). One reason of these errors in stature calculation and subadult age estimation is that the current North American population is taller than most European populations (Komlos, 2001; Komlos and Baur, 2004; Smith and Norris, 2004), and even though the stature has increased in Europeans recently, due to the improvements in health and living conditions, some differences still exist (Pebles and Norris, 2011).

Age estimation is basic in any osteological study and biological identification, being a characteristic of forensic anthropology. The success in determining the identity of a deceased is not only a requisite for officially declaring an individual dead but it is also the basis for investigating interpersonal and war crimes and mass disasters. Therefore, an increase in the accuracy and reliability of the ageing methods is urgent in the field of Forensic Anthropology. This becomes even more evident considering the noticeable height increase (10 cm) seen in Spain in the second half of the 20th century as a result of improved living conditions (Spijker et al., 2012). Moreover, the need for studies on skeletal development based on different populations becomes more important when we take into account their broad relevance in biological anthropology and human evolution. The study of changes in size increase, shape and form of different skeletal elements in different populations during growth can help to elucidate, for example, how patterns of bone growth have changed in recent generations of children and how selective forces influence the development of sexual dimorphism in the different anatomical regions of the skeleton (Bulygina et al., 2006; Coquerelle et al., 2011; Crespo et al., 2015).

In light of this background, there is an obvious need for studies regarding the developmental osteology of individuals from contemporary populations, as has been recommended by different authors (Martrille et al., 2007; Cameriere and Ferrante, 2008; Boccone et al., 2010; Charisi et al., 2011; López-Costas et al., 2012; Rissech et al., 2012; San Millán et al., 2013) and demonstrated in several studies (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998; Bulygina et al., 2006; Rissech et al., 2013b; Crespo et al., 2015).

Given the anthropological importance of the femur as a result of its locomotory function (Aiello and Dean, 1990; Anderson and Trinkaus, 1998; Andriacchi and Alexander 2000) and the postdepositional resistance exhibited by this element due to its structural density, we analysed femoral development during puberty in a contemporary Spanish population. This research was divided into two studies, one for the female and another for the male femur. Results from the study of the female femur (Pujol et al., 2014) revealed an overall increase in the robustness and length of the female femur, together with marked morphological changes in specific regions of this bone with age. In general, an increase in the robustness of the bone and noticeable phenotypic changes in certain areas of the femur were observed. The aims of this study are twofold: i) to determine the morphological changes during the adolescent growth spurt of the femur in boys and youths between 9 and 16 years of age in the current living Spanish population and ii) to evaluate the development of the sexual dimorphism in femoral morphology by the comparison of the results here obtained in boys with our previous findings for girls. For these purposes, we applied landmark-based geometric

TABLE 1. Height (in cm) of a sample of the analysed individuals

	Males		Females	
Age (years)	*n*	Height Mean (SD)	*n*	Height Mean (SD)
9	10	135.6 (15.3)	11	134.3 (14.3)
10	12	138.9 (16.4)	9	139.4 (14.9)
11	15	144.2 (17.5)	10	146.7 (15.9)
12	12	148.3 (17.3)	14	153.9 (15.3)
13	17	156.6 (18.1)	18	157.1 (16.9)
14	19	163.7 (17.4)	20	160.3 (17.5)
15	22	172.4 (17.5)	20	163.4 (15.8)
16	22	174.9 (16.1)	21	164.1 (13.6)

The data of the female series were obtained from the study by Pujol et al. (2014). SD, standard deviation.

morphometric techniques (GMM) to high-resolution radiographic images.

MATERIALS AND METHODS

High-resolution radiographic images of the lower half of the body (telemetries), in DICOM (Digital Imaging and Communication in Medicine) format, were obtained from the database at the Hospital Infantil Sant Joan de Déu de Barcelona (Spain). In accordance with current Spanish data protection legislation (García et al., 2010), all personally identifying information other than age and sex was deleted from these images prior to use. This study was approved by the Bioethics Committee at the Hospital Sant Joan de Déu, Barcelona (Ref.: 2938).

The suitability of using telemetries (distant type of digital radiography of the limbs which lacks of significant image deformation) and the radiography protocols followed by the Hospital Sant Joan de Déu are explained in detail by Pujol et al. (2014). However, it is worth clarifying that in the radiographic procedure of the Hospital, the knee joints were always aligned to the center of the body gravity to permit the comparison of both femoral angles between individuals (Pujol et al., 2014).

In brief, the left femur of male children from 9 to 16 years old born in the 21st century was analysed in anterior view. This age range was selected to include the onset of the male pubertal growth spurt (Tanner et al., 1981; Tanner, 1986). Individuals presenting disease or anatomical variation that could affect the ability to determine "normal" development osteology were discarded. A total of 240 male femora were analysed and distributed into groups of 30 individuals per year. We did not have access to the entire clinical information of each individual, since the radiographic images were separated from the clinical histories due to the law of individual data protection. However, some of the radiographs included the height of the individual. To offer information on the general body size variation related to age in the samples analysed, in Table 1 we summarised the mean values of the height obtained for the boys here considered and for the girls analysed in our previous study (Pujol et al., 2014). All the individuals come from the subadults' radiological collection of the Universitat de Barcelona, recently created by one of the authors of this study (A.P.) for his doctoral thesis under the supervision of D.T. and C.R. This collection comprises radiological images obtained from 1080 individuals (540 boys and 540 girls) of Spanish origin, born between 1991 and 2010, ranging from birth to 18 years of age. All of these individuals come from middle social-economic class, showing the boys of 18 years of age a mean height of 177.5 cm and the girls a mean height of 164.3 cm. These values correspond to the expected male and female adult mean height in current living Spanish population: 174.20 cm (SD: 7.08) for males and 162.09 cm (SD: 6.37) for females (Spijker et al., 2012).

Fig. 1. Landmark locations on the anterior aspect of the left femur. For definitions of the landmarks see Table 2.

GMM: landmarks selection and location

The landmark-based GMM procedure is an effective method for capturing information on the shape of an individual and useful for analysing statistically shape differences between individuals or groups (Bookstein et al., 1985; Rohlf 2000a,b; Scholtz et al., 2010). Furthermore, it is a potent and intuitive tool for visualising shape, and shape differences between the analysed individuals and groups because data are recorded to capture the geometry of the analysed structure (Bookstein et al., 1985; Rohlf 2000a,b; Zelditch et al., 2004; Toro-Ibacache et al., 2010). Landmarks can be bidimensional or three-dimensional (2D or 3D) coordinates of morphological points fitted by superimposition methods, which pair homologous landmarks as closely as possible by

TABLE 2. Description of the 22 bidimensional landmarks used in this study

Anatomical area	Landmark	Description
Head of the femur	1	The most medial point of the growth plate between the head and neck
	2	The most angulated point of the growth plate between the head and neck
	3	The most lateral point of the growth plate between the head and neck
	4	The lowest point of the fovea
	5	The most upper point of the fovea
Neck of the femur	6	The lowest point of the narrowest region of the neck
	7	The most upper point of the narrowest region of the neck
Trochanters	8	The most proximal point of the growth plate between the greater trochanter and the shaft
	9	The midpoint of the growth plate between the greater trochanter and the shaft
	10	The most distal point of the growth plate between the greater trochanter and the shaft
	11	The tip of the greater trochanter
	12	The most proximal point of the growth plate between the lesser trochanter and the shaft
	13	The tip point of the lesser trochanter
	14	The most distal point of the growth plate between the lesser trochanter and the shaft
Femoral diaphysis	15	The midpoint of the medial aspect of the shaft
	16	The midpoint of the lateral aspect of the shaft
Distal epiphysis	17	The most medial point of the growth plate between the diaphysis and distal epiphysis
	18	The midpoint of the growth plate between the diaphysis and distal epiphysis
	19	The most lateral point of the growth plate between the diaphysis and distal epiphysis
	20	The most distal point on the medial condyle
	21	The intercondylar superior angle of distal epiphysis in the articular margin
	22	The most distal point of the lateral condyle

minimizing the summed squared distances of the Procrustes between corresponding landmarks. According to this, shape changes may be defined as the residuals of the superimposition given as transformation vectors (Rohlf and Marcus, 1993).

A total of 22 bidimensional landmarks (Fig. 1) were digitised using the thin plate spline (TPS) program series (Rohlf, 2006). These landmarks were the same as those we defined and used in the study by Pujol et al. (2014) to ensure that the data obtained in both series were fully compatible and comparable. These 22 landmarks were defined on the femoral regions which displayed greater changes during growth, specifically (see Table 2 and Fig. 1): the head, the fovea, the neck, the trochanter and the distal diaphysis and epiphysis. Taking into account the results obtained in females (Pujol et al., 2014) and the fact that analysis was undertaken by the same person (A.P.), the measurement error in the landmark location was considered negligible.

Data analyses: femur development in boys

In males, after digitising the 240 configurations of 22 landmarks, we used Procrustes-based geometric morphometrics (Rohlf and Slice, 1990; Bookstein, 1991) to obtain shape variables for statistical analyses. For this purpose, we first performed a generalised procrustes analysis (GPA; Rohlf and Slice, 1990) to compute shape coordinates. This implies translation to a common centroid, scaling to unit centroid size (CS: square root of the summed squared Euclidean distances from all landmarks to their centroid), and rotation of the configurations to minimize the overall sum of squared distances between pairs of corresponding landmarks. Then, the coordinates of superimposed configurations were orthogonally projected to the tangent space to obtain shape variables for statistical analyses. This resulted in a vector of 44 shape variables and a CS for each individual. Procrustes superimposition and projection to the tangent space were performed using the TPS programs series (Rohlf, 2006).

First of all and to test femoral size-related differences during growth, an analysis of variance (ANOVA) was performed on the CS and age. Secondly, to evaluate age-related changes in the femoral shape of the boys sample a principal components analysis (PCA in shape space or relative warp analysis) was applied (tpsRelw program, version 1.46; see also Rohlf, 1999). This analysis reduces the complex multidimensional data into a few eigenvectors that were linear combinations of the landmark displacements (Zelditch et al., 2004). A multivariate analysis of covariance (MANCOVA) was performed with the first five components, using the CS as covariable, to evaluate the effect of the allometric factor on shape changes. Furthermore, plots of the corresponding PC scores of this analysis were complemented by the results of applying a multivariate regression of shape on lnCS to evaluate allometry (Mitteroecker et al., 2013). Additionally, and to better evaluate the allometric relation between shape and size of the femur during growth, we applied a PCA in size–shape space (Mitteroecker et al., 2004a,b). This is a composite approach which consists of a relative warp analysis of the usual matrix of Procrustes shape coordinates (Bookstein, 1991; Rohlf, 1993) augmented by one single additional column for the natural logarithm of CS (Mitteroecker et al., 2004a,b). The resulting low-dimensional eigenspace allows comparisons of size and shape in one analysis (the form), whereas the classical PCA of the Procrustes coordinates allows the analysis of the shape only. In addition, plots of the corresponding size–shape PC scores of this analysis were complemented by the results of applying a multivariate regression of size and shape coordinates on

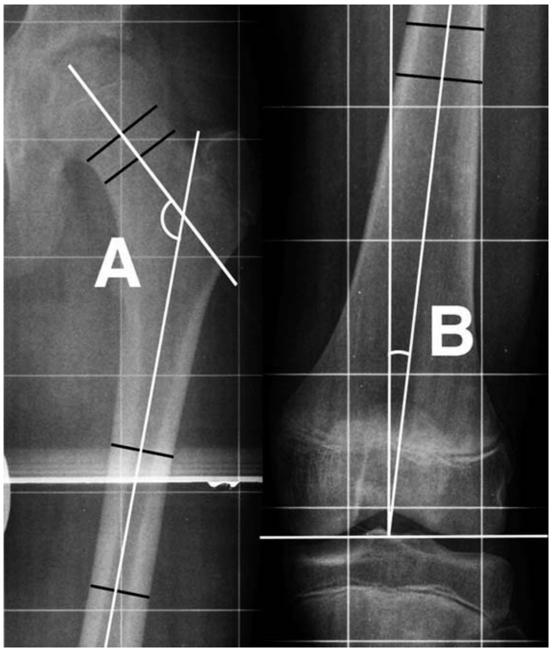

Fig. 2. Measurement process of the neck–shaft angle (**A**) and bicondylar angle (**B**). White lines indicate the diaphyseal and neck axes. Black lines are the lines drawn to locate the neck and shaft axes, see the text.

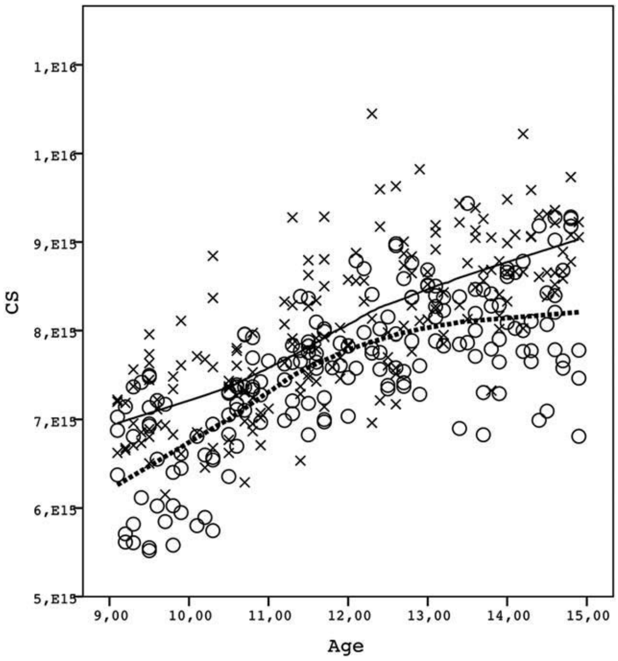

Fig. 3. Centroid size (CS) variation regarding age and sex. Males (X); females (O). The data of the female series were obtained from Pujol et al. (2014).

lnCS to evaluate allometry (Mitteroecker et al., 2013). The study and comparison of both generated spaces (shape space and size–shape space) allow a better comprehension of differences and dissociations of the femur size–shape relationship between ages during the individual development.

Changes in femur shape were visualised in all cases using TPS deformation grids (Bookstein, 1991). All the statistical analyses of this study were performed using SPSS 20 and R v2.15.3.

Data analyses: boys–girls femur variation during development

To determine possible sexual differences of the femur during growth we first performed a single GPA, using our boys sample together with the girls sample analysed in the study by Pujol et al. (2014). The resulting GPA displacement vectors were ordinated by PCA of shape coordinates to explain shape variation among samples. Taking into account the lower age range considered for girls (Pujol et al., 2014), only individuals between 9 and 14 years of age were analysed. To evaluate the size effect on shape changes, a MANCOVA was performed with the three first components, using CS as co-variable. We considered only the three first PCs because of the low percentage of variation explained by the remaining PCs. As in boys, to better assess the differences and dissociations of the size–shape relationship in the femur, a size–shape PCA was performed.

To compare sexual differences in femoral shape (shape space) and form (size–shape space) at the different ages, we calculated sex-specific mean shapes for 9, 10, 11, 12, 13 and 14 years of age by a moving average algorithm (Bulygina et al., 2006; Coquerelle et al., 2011). This method calculates the mean shape and form change in the analysed period of time by a quadratic regression of all the shape PC, in the case of shape space, and size–shape PC, in the case of size–shape space.

To visualize the sexual dimorphism in shape during femoral development, TPS deformation grids (Bookstein, 1991) were obtained in each age group to its next older stage for males and females separately.

Metrical analysis

As in the study of the female femur (Pujol et al., 2014), development of the male neck–shaft and bicondylar angles was quantified and graphically represented in relation to individual chronological age. The neck–shaft angle (Fig. 2) is the angle between the longitudinal axis of the femoral diaphysis and the longitudinal axis of the neck (Tardieu and Damsin, 1997). The bicondylar angle (Fig. 2) is located between the longitudinal axis of the diaphysis and a line perpendicular to the infracondylar plane of the femur (Shefelbine et al., 2002). The longitudinal axis of the femoral shaft is the midline along the shaft which connects the middle of the infra-condylar and proximal segments of the femur. The longitudinal axis of the neck is the midline along the neck which connects the distal and proximal limits of this anatomical region. To graphically locate these axes on the radiological images, two perpendicular lines to the shaft and neck directions were plotted on the middle of these anatomical regions (Fig. 2). The line which crosses perpendicularly the middle of these two lines was considered the shaft and neck axes, respectively (Fig. 2). To evaluate the sexual differences in femoral size (CS) and in the neck–shaft and bicondylar angles, Student's *t*-test was applied.

TABLE 3. Sexual differences in the collo-diaphysal and bicondylar angles according to age

	Age (years)							
Variables	9	10	11	12	13	14	15	16
CS								
Males *n*	30	30	30	30	30	30	30	30
Mean	7.100×10^{15}	7.300×10^{15}	7.940×10^{15}	8.380×10^{15}	8.600×10^{15}	8.940×10^{15}	9.520×10^{15}	9.260×10^{15}
SD	4.586×10^{14}	5.572×10^{14}	6.340×10^{14}	8.315×10^{14}	5.118×10^{14}	4.689×10^{14}	5.944×10^{14}	7.851×10^{14}
Females *n*	30	30	30	30	30	30	30	30
Mean	6.490×10^{15}	7.040×10^{15}	7.610×10^{15}	7.940×10^{15}	8.080×10^{15}	8.230×10^{15}	–	–
SD	6.693×10^{14}	5.808×10^{14}	4.008×10^{14}	5.461×10^{14}	5.386×10^{14}	6.946×10^{14}	–	–
P	0.081	0.087	0.021*	0.019*	0.000*	0.000*	–	–
Neck–shaft angle								
Males *n*	30	30	30	30	30	30	30	30
Mean	138.65	137.11	137.41	137.63	135.13	132.56	129.68	129.49
SD	4.94	3.25	4.25	5.23	6.51	5.72	5.33	5.19
CV	3.56	2.37	3.09	3.80	4.82	4.32	4.11	4.01
Females *n*	30	30	30	30	30	31	–	–
Mean	137.00	133.36	130.35	127.39	125.92	123.95	–	–
SD	3.52	3.24	4.68	3.51	3.70	2.77		
CV	2.57	2.43	3.59	2.76	2.94	2.23		
P	0.140	0.000	0.000	0.000	0.000	0.000		
Bicondylar angle								
Males *n*	30	30	30	30	30	31	31	31
Mean	6.93	7.21	7.40	7.51	7.99	8.03	8.20	8.11
SD	1.32	1.25	1.33	1.07	0.89	1.40	1.32	1.23
CV	19.05	17.34	17.97	14.25	11.14	17.44	16.10	15.17
Females *n*	30	30	30	30	30	31	–	–
Mean	6.92	8.72	9.51	10.48	11.09	10.80	–	–
SD	0.53	0.50	0.33	0.57	0.42	0.45	–	–
CV	7.66	5.73	3.47	5.44	3.79	4.17		
P	0.977	0.000	0.000	0.000	0.000	0.000		

The data of the female series were obtained from the study of Pujol et al (2014).

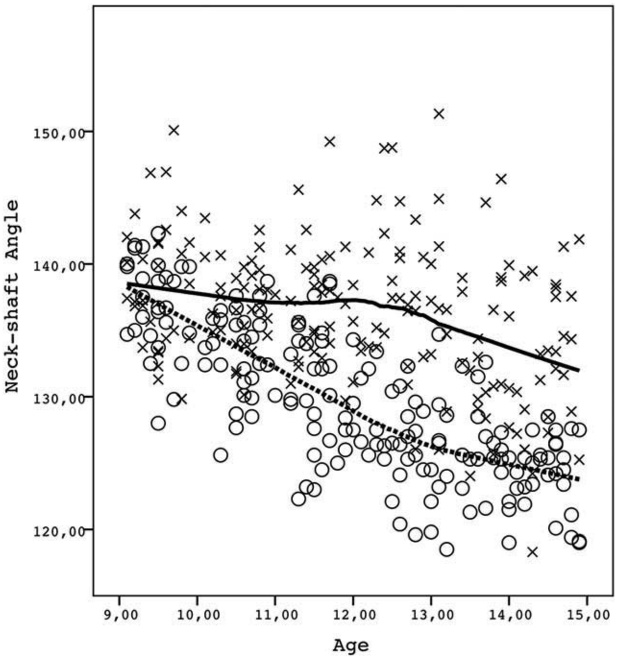

Fig. 4. Neck–shaft angle variation in males (X) and females (O) according age. The data of the female series were obtained from Pujol et al. (2014).

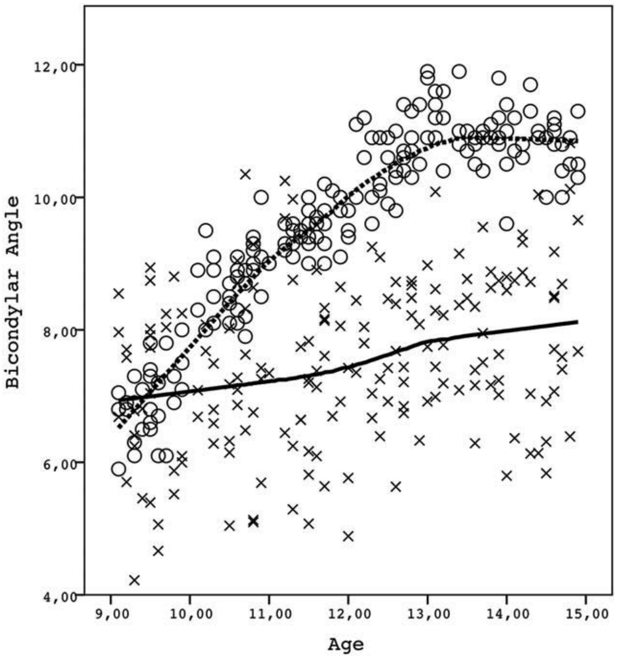

Fig. 5. Bicondylar angle variation between males (X) and females (O) according age. The data of the female series were obtained from Pujol et al. (2014).

RESULTS

Size variation

The ANOVA test showed that male CS increased significantly with age ($F = 6.689$, $*P = 0.0000$). The MANCOVA test performed on the male sample indicated that the effect of size on shape was significant (Wilks' Lambda = 0.925, $F = 3{,}777$, $*P = 0.012$) and that after removing the effect of allometry, age-related shape changes were significant (Wilks' Lambda = 0.767, $F = 4.655$, $*P = 0.0000$). Likewise, the MANCOVA test performed on both sexual series together also showed a significant effect of size on shape (Wilks' Lambda = 0.933, $F = 3.411$, $*P = 0.031$) and showed that after removing the allometry effect, age-related (Wilks' Lambda = 0.096, $F = 10.266$, $*P = 0.0000$) and sex-related shape changes (Wilks' Lambda = 0.632, $F = 72.641$, $*P = 0.0000$) were significant.

A comparison between both sexual series (Fig. 3) showed that the mean CS was higher for males at all ages (9–14 years; see Table 3). These differences were significant from 11 years upwards (Table 3). The mean CS value in males at 15 years of age was similar to that obtained at 16 years (Table 3), which indicates a possible stabilisation of this variable from 15 years of age. This happens before in females, at around 13 years of age (Pujol et al., 2014).

In males, the neck–shaft angle undergoes major changes between 9 and 16 years of age (Fig. 4, Table 3). Although a constant decrease of this angle occurs during this period, the most dramatic reduction occurs from approximately 12 until around 16 years of age (Table 3). The decrease in neck–shaft angle averaged 10° between 9 and 16 years of age (Table 3, Fig. 4). A comparison of the results obtained in this study with those corresponding to the female series (Pujol et al., 2014) showed that males had larger neck–shaft angles (Table 3, Fig. 4) and that these differences were significant from 10 years of age upward (Table 3). Males exhibited a greater variability in neck–shaft angles than females, particularly from 12 years of age upward (Table 3, Fig. 4). The variability in the neck–shaft angle increased more with age in males than in females (Table 3).

As expected, the bicondylar angle in the male series increased with age (Fig. 5), although the most marked increase was detected at around 11 years of age, with values tending to stabilize at around 15 years of age (Table 3). These ages are slightly earlier (by around 1 year) than those observed for the neck–shaft angle. The bicondylar angle increased by around 2–3° on average over the period considered, from 10 years old (Table 3, Fig. 5). Males consistently exhibited smaller bicondylar angles than females (Table 3, Fig. 5). These differences were statistically significant from 10 years of age upward (Table 3). Likewise, males showed greater variability in bicondylar angle than females at all ages (Fig. 5, Table 3). These sex-related differences in bicondylar angle are much more marked than those observed for the neck–shaft angle. The female bicondylar angle increases within a very narrow range over the period considered, with the variability remaining unchanged. In contrast, this range was greater in males (standard deviation range for males: 0.87–1.4; for females: 0.33–0.57; see Fig. 5).

Change of shape and form in boys

The first two components (PC1 and PC2) in the PCA in shape space (Fig. 6) explained 74.3% of the male femur shape variation (PC1: 65.3%; PC2: 9%). The most dominant factor of shape variation was the shape difference between young and old individuals, which varied progressively with age (Fig. 6). The arrow represents the coefficient vector of the regression of shape on LnCS ($r = 0.81$), indicating the actual ontogenetic allometry.

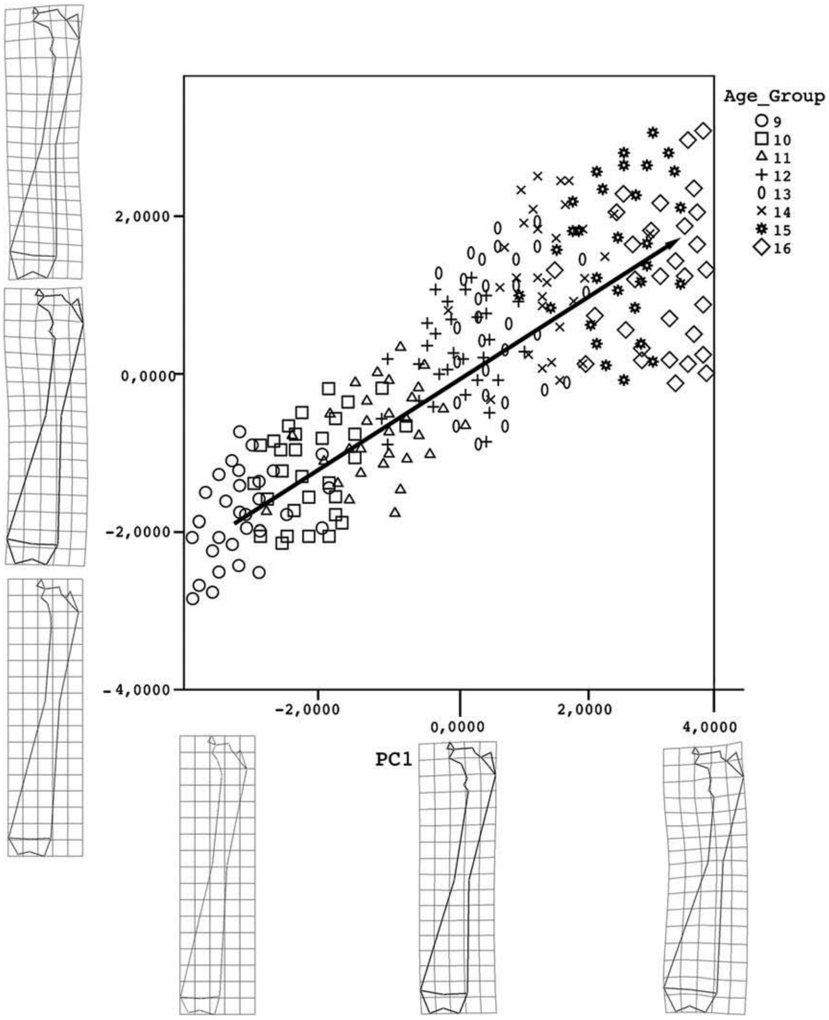

Fig. 6. Ordination of the male individuals in the space of the two-first principal components (PC1, PC2) based on the weight matrix for the anterior aspect of the femur. The arrow represents ontogenetic allometry (allometric shape and size)—the regression of shape on ln CS. Grids show the shape changes throw the negatives values (untransformed shape) to positive values of the axis.

This arrow is positively correlated with PC1 and PC2 and indicates similar significant correlation of both PC to the CS during growth (Fig. 6). As in girls (Pujol et al., 2014), the shape changes associated with the increase in PC1 score were the following: i) increase of the robustness of the femur in both the diaphyseal and neck regions; ii) vertical decrease of the femoral head in relation to the overall femur, revealed by the decrease of the neck–shaft angle (Fig. 6); and iii) increase of the proportions of the distal epiphysis in relation to the overall femur (Fig. 6). An increase in PC2 scores was related to a change in the proportions between the upper (greater and lesser trochanters) and the lower epiphyses in relation to the diaphysis (Figs. 6 and 7).

In general, the femoral head showed a much more vertical position in the youngest male individuals (9 years of age; Fig. 8), with both trochanters already present, albeit small, and the distal epiphysis well-developed (Fig. 9). Femora from the older males (15 and 16 years) mainly presented changes in the femoral head region (Fig. 8), which takes a much more horizontal position, resulting in a reduction in the neck–shaft angle (Fig. 8). The greater trochanter increased in size with respect to the lesser trochanter and the femur as a whole. In addition, the angle of the greater trochanter was located in a relatively higher position than in the femora of subjects aged 9 years (Fig. 8). The distal epiphysis reached approximately its final shape at 15–16 years, which was indicated by fusion of this epiphysis to the shaft at 17 years of age in the great majority of the individuals, which in turn indicated the ending of male growth of this anatomical area. This observation is consistent with the given standards of union of the knee epiphyses for the current male population (Scheuer and Black, 2000), and more specifically for the current male population of the Iberian Peninsula (Rissech et al., 2008; López-Costas et al., 2012). At 15 and 16 years, the distal epiphysis presented proportionally larger condyles (especially the medial condyle) in relation to the femur as a whole. This modification is associated with the adjustment of the femur to the change in neck angle (Fig. 9). These changes in the bicondylar region and in the femoral neck are related to the angular remodeling that occurs during male femoral development.

The first two components (PC1 and PC2) of the PCA in size–shape space (Fig. 10) explained 87% male femur

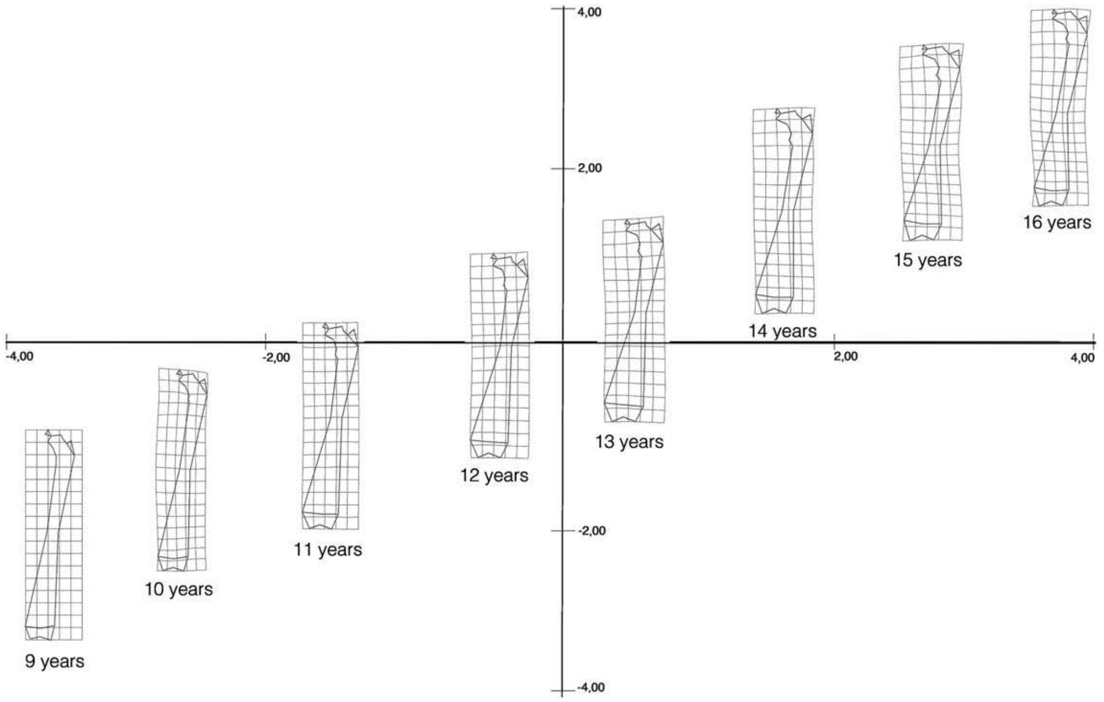

Fig. 7. Distribution of the consensus form of each age [from 9 (untransformed shape) to 16 years] in the space defined by the two first PCs in males.

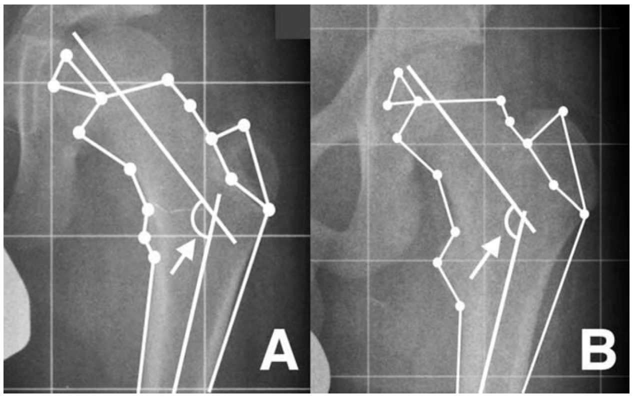

Fig. 8. Consensus forms of the proximal male femur for individuals aged 9 (A) and 16 years (B). The arrow shows the neck–shaft angle.

form variation (PC1: 82%; PC2: 5%). Femoral growth includes changes in both shape and size, because of this femoral form (size–shape space) closely reflects an individual's age femoral than femoral shape. The arrow represents the coefficient vector of the regression of size–shape on LnCS ($r = 0.802$). Consequently PC1 represents the axis of allometric development of these individuals. Shape differences corresponding to PC1 are similar to the PC1 in shape space (Fig. 6), but PC1 in form space additionally comprises differences in size, and because of this the regression vector (the arrow) was more stretched along PC1. It indicates the significance of the increasing in size of the male femur during development. The increase in PC1 score was associated with: the increased robustness of the diaphysis and distal epiphysis; the decreased verticality of the femoral head in relation to the overall femur (decreasing in neck–shaft angle); and the gained size in distal epiphysis in relation to the overall femur (Fig. 10). The increase in PC2 score is associated with the change in proportions between the upper and lower epiphyses in relation to the diaphysis.

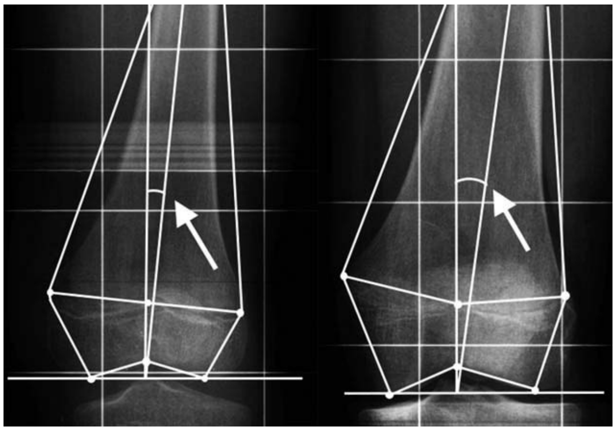

Fig. 9. Consensus forms of the distal male femur for individuals aged 9 (A) and 16 (B). The arrow shows the bicondylar angle.

Change of shape and form between boys and girls

Figure 11 shows the distribution of the male and female individuals on shape space. The first two components accounted for 72% of shape variation (PC1 = 65%, PC2 = 7%). Female and male lines, computed via local linear regression of the PC scores on age, show the ontogenetic trajectories of the femur in each sex on the

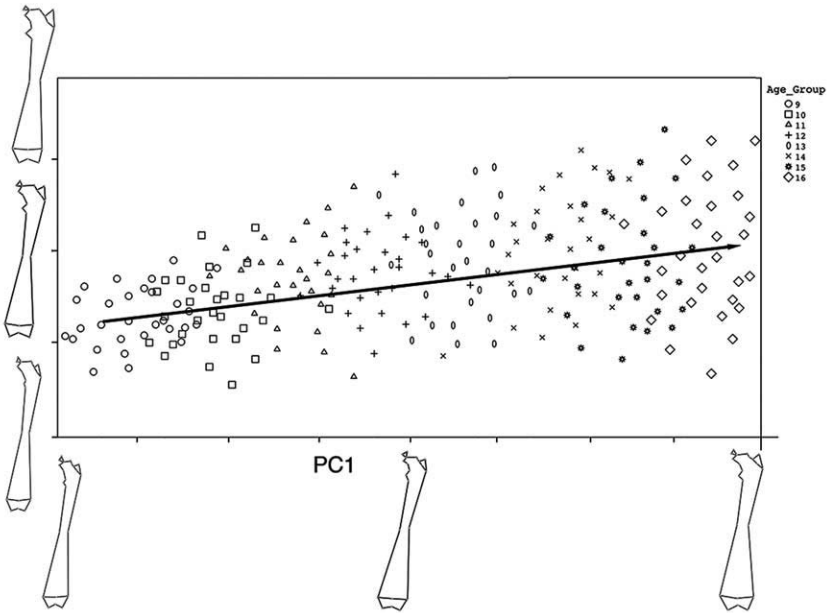

Fig. 10. Scatter plot of the first two PCs of male femur form (Procrustes shape coordinates augmented by ln CS). The arrow represents ontogenetic allometry (allometric shape and size)—the regression of form on ln CS. Grids show the shape–size changes throw the negatives values (untransformed shape) to positive values of the axis.

shape space (Fig. 11). Results indicated that the male and female ontogenetic trajectories diverged progressively from 9 to 14 years of age, with boys having higher values on PC2 than girls. Specifically, sexual shape differences in the average scores estimated at each age were significant from 11 to 14 years ($P < 0.001$). In particular, these differences increase much more after approximately 12 years of age, around the time of the female pubertal growth spurt (12.5 years). After this age the female ontogenetic trajectory tends towards a more horizontal slope, indicating that in this period the most evident age-related changes in girls are associated exclusively with the angular remodeling of the femur, which corresponds to the main changes along PC1. Contrarily, in boys, from 9 to 14 years of age, the most evident age-related modification correspond to the increase in diaphyseal length and size of both trochanters, although angular remodeling of the femur is still evident. These changes correspond to the main variation along PC2.

Figure 12 shows sexual dimorphism in femur shape between 9 and 14 years. It is visualised as a series of six TPS deformation grids displaying the different consensus shape age stages for males and females separately. Results indicate that, in early stages, 9 and 10 years of age, girls and boys have similar femur shapes; however, at those ages, the girls' neck–shaft angle is reduced in relation to those of boys. Clear differences between the shapes of both femora appear from 11 years of age upwards, probably because of the proximity of the female growth spurt. In general, during the pubertal period, the female femur "seems" to increase its diaphyseal thickness in relation to the total femur, although in absolute values the male femora are somewhat broader and more robust than female femora at the same age (Fig. 12). This is because of the great increase in length of the male femur in relation to the female one. Furthermore, the female greater and lesser trochanters have smaller dimensions than those of boys; the femoral neck becomes more horizontal and the medial condyle of the distal epiphysis increases in size due to the angular remodeling of the femur, which is higher in girls than in boys.

Figure 13 shows the ontogenetic trajectories (Fig. 13) of females and males in the size–shape space (Fig. 13). The first two components of the PCA in this space account for 88% of the size–shape variation ($PC1 = 83\%$, $PC2 = 5\%$). The ontogenetic trajectories are slightly different from those obtained in shape space. In this case the ontogenetic trajectories of both sexes run closely parallel (with boys having higher values in PC2) until approximately 12 years of age, from which they start to diverge significantly ($P < 0.001$). These differences between trajectories in shape space and size–shape space indicate that the amount of shape change per size change differs between sexes depending on age. From 9 to 11 years of age the size–shape relationship is more or less similar between males and females, however from the age of 12 years significant differences in shape occur (Fig. 12). In addition, in all ages from 10 years of age, male size–shape average grows faster than that of females (Fig. 13), due to the significant size increase of the male femur related to female femur during development. However, at 9 years this situation is reversed.

DISCUSSION

The results obtained in this study allowed us to describe the variation in the size and shape of the male femur between the ages of 9 and 16 years. The maximum rate of increase in femoral dimensions is around 15 years, coinciding with the age range of the Spanish male pubertal growth spurt (10–15 years of age according to Ferrández et al., 2009) and before the age at which most Spanish males reach adult size which is 17 years (Fernández-Méndez and Seara-Aguilar, 1994; Carrascosa et al., 2008; Ferrández et al., 2009; García Cuartero et al., 2010). This is concordant with our

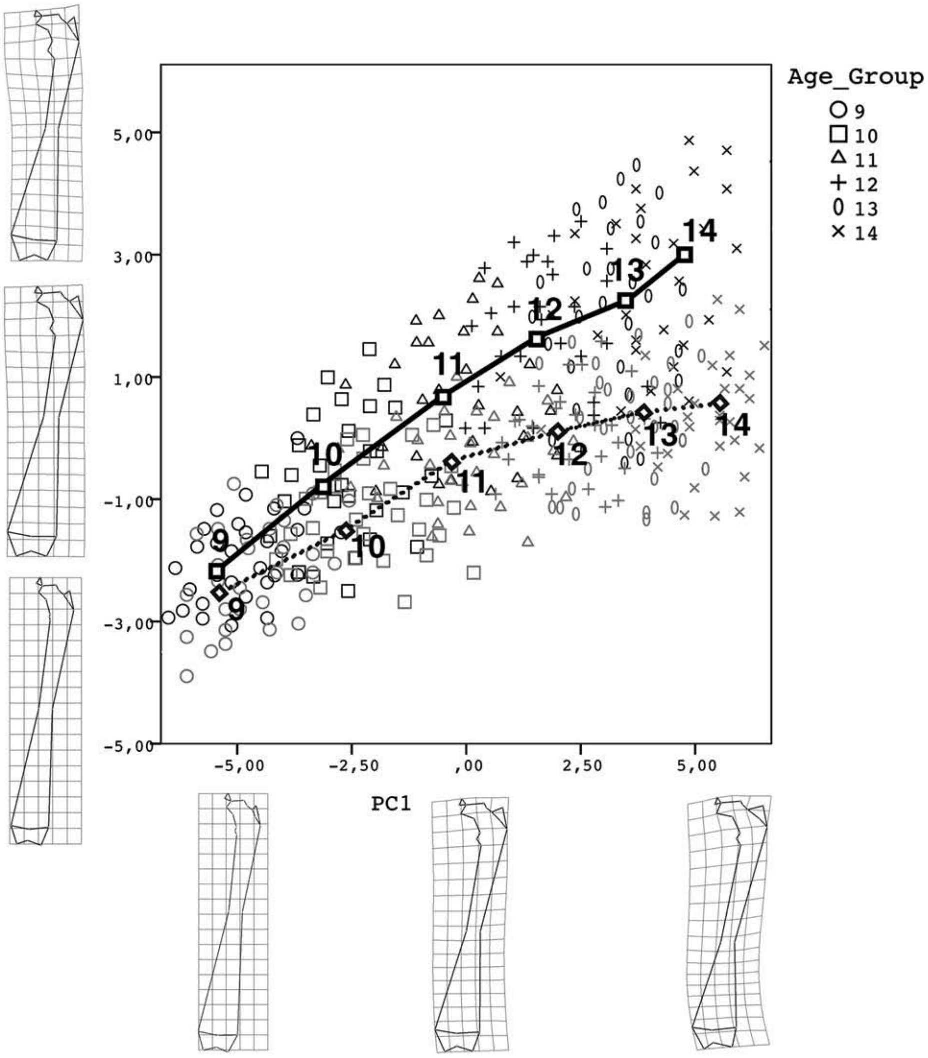

Fig. 11. Scatter plot of males and females in shape space PCA and ontogeny trajectories established from the average PC scores at different ages in both sexes. Continuous line for boys and discontinuous line for girls. Grids show the shape changes throw the negatives values (untransformed shape) to positive values of the axis.

observation on the fusion of the distal epiphysis in boys. These values are within the normal age range for the male pubertal growth spurt (12.5–17.5 years) in the current living male population (Tanner, 1962; Marshall and Tanner 1970; Gasser et al., 1991). Our findings indicate that the size differences between male and female femora can be detected from 11 years of age, when most Spanish females are entering or have already entered the pubertal growth spurt. The girls growth spurt starts about 2 years earlier (8–3 years according to Ferrández et al., 2009) than in males. As is well known, the male femur can grow for longer, thereby leading to longer femora (Bachrach and Smith, 1996; Scheuer and Black, 2000; Seeman, 2003). These femora and the fact that the elongation of the bone precedes the thickening (Bass et al., 1999; Bradney et al., 2000; Rauch et al., 2001; Pujol et al., 2014) probably make the male femur appear in our study less robust (although it is not) than that of females of the same age (Pujol et al., 2014). In fact, mass and muscle strength are important for the development of bone robustness (Van der Meulen et al., 1993, 1996, 2002; Moro et al., 1996; Schöenau, 1998, 2005; Schöenau et al., 2000). By raising the muscular load, biomechanical forces increase, leading to the development of higher robustness (Schiessl et al., 1998; Rauch and Schöenau, 2001; Ferretti et al., 2003; Ruff, 2003). Males generally show a greater increase in muscle mass than females due to the increase in testosterone levels during growth (Round, 1999). Consequently, the muscle/bone ratio is higher in females than in males (Högler et al., 2008) and the female femur is less robust (Ruff, 2003).

The shape variation in the male femur varied significantly between the ages of 9 and 16 years. Among these changes, those in both proximal and distal epiphysis are particularly striking (see below).

Proximal epiphysis: Neck–shaft angle and trochanters

The obtained average for neck–shaft angle in each age group falls within the normal range of variability for each of these ages (Billing, 1954; Tardieu and Damsin, 1997; Muñoz-Gutiérrez, 2001). The youngest individuals

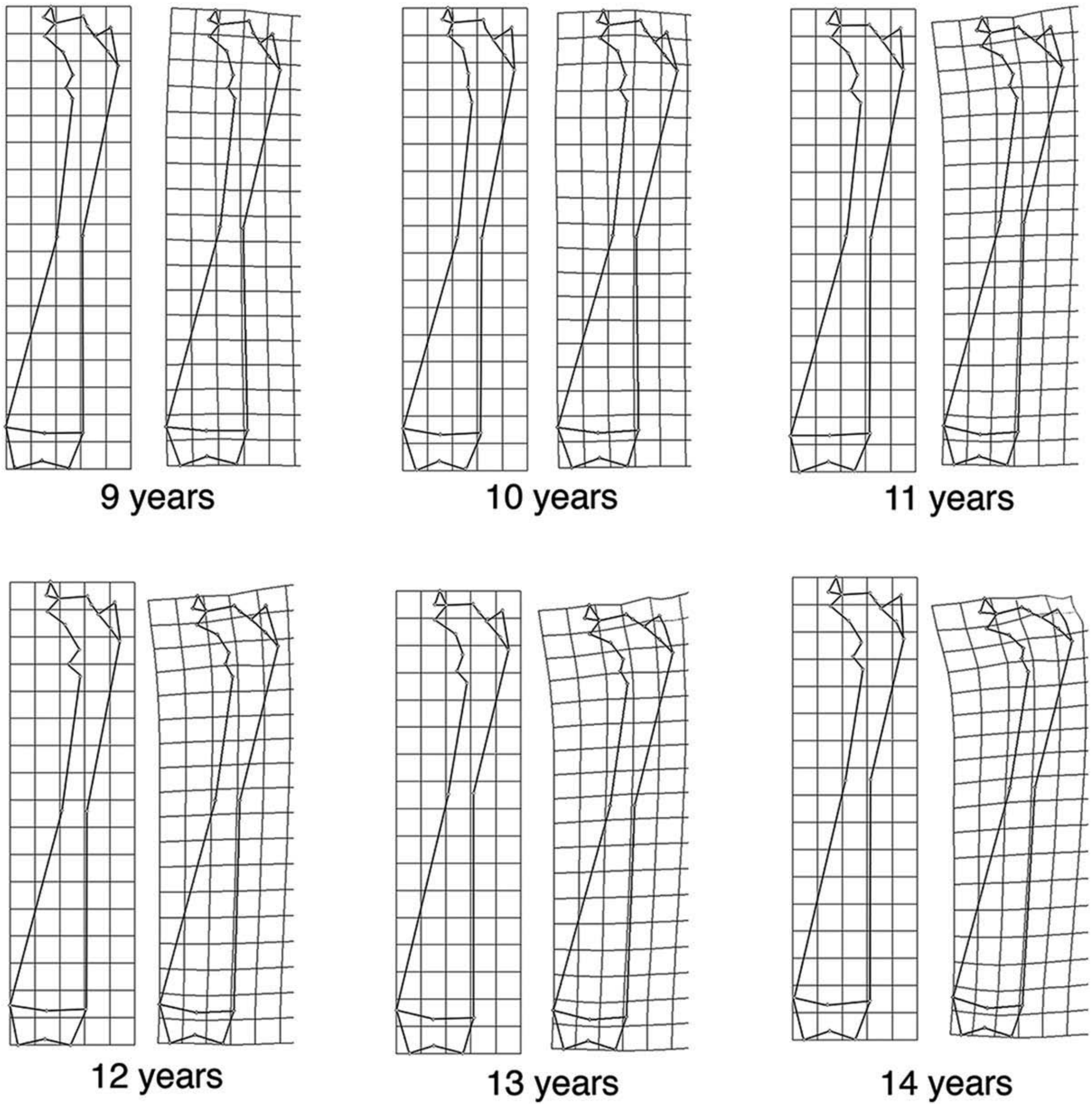

Fig. 12. TPS deformation grids in shape for the age stages of 9, 10, 11, 12, 13 and 14 years for males and females separately.

of our sample (9 years of age) showed a mean value of 138°, and the oldest (16 years of age) an average of 129°. This last value is inside the normal range of variation (121.7°–136.5°) reported for adult European males (Anderson and Trinkaus, 1998). After 16 years of age, the neck–shaft angle became stable, agreeing with observations of previous authors who indicated that the values of this angle are stable from mid-adolescence throughout most adult life (Humphry, 1889; Yamaguchi, 1993), and with previous study on female femur (Pujol et al., 2014). During growth, there is a great increase in size in the proximal epiphysis, neck, head and both trochanters. The neck–shaft angle is more obtuse during the first years of individual life. Namely, the neck has a more vertical position, because the abductor muscle only develops as response to the onset of locomotion (Lovejoy, 2005). The neck–shaft angle decreases during growth, starting from an average of 150° at birth to approximately 127° at the end of the pubertal growth spurt, (Humphry, 1889; Keats et al., 1966; Hefti, 2000; Igbigbi, 2003).

Considerable population-based variability in the neck–shaft angle has been highlighted in the literature (Anderson and Trinkaus, 1998; Igbigbi, 2003). This variability seems to be related to physical activity (Anderson and Trinkaus, 1998), individual high (Lofgren, 1956; Lusted and Keats, 1966; Singh and Singh, 1975; Igbigbi, 2003) and climate (Gilligan et al., 2013). In mechanised societies with sedentary habits, the neck–shaft angle acquires greater values (Anderson and Trinkaus, 1998), which are located in the upper end of normal recent human ranges of variation. Lower values, which sometimes can be smaller than 120°, are not rare in clinically normal individuals of nonindustrialised societies (Anderson and Trinkaus, 1998).

According to our results, sex-based differences in the neck–shaft angle are not significant during the early years of life and do not exceed 2° at 9 years of age.

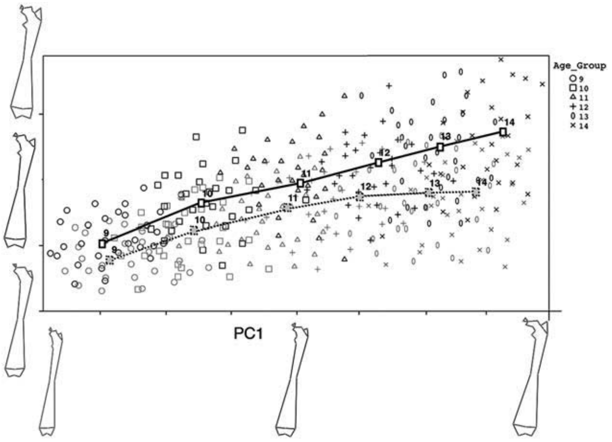

Fig. 13. Distribution of males and females in size–shape space PC with ontogeny trajectories established from the average PC scores at different ages in both sexes. Continuous line for boys and discontinuous line for girls. Grids show the shape–size changes throw the negatives values (untransformed shape) to positive values of the axis.

However, sexual differences increase markedly with the onset of puberty, reaching an average difference between sexes of 10° at 14 years of age and statistically significant from 10 years of age onwards, age of the onset of the pubertal growth spurt in the female pubis (Rissech and Malgosa, 2007). The presence of sexual differences in the neck–shaft angle in the older age groups was concordant with the observations on adult femur by Walmsley (1933), Igbigbi (2003), and Bernard et al., (2012), and disagrees with the results by Otsianyi et al., (2011) and Gilligan et al., (2013). However, it must be taken into account that these two last studies mixed together individuals of different populations and ethnicities, with different levels and types of physical activities that could mask sexual differences. Likewise, the study of Otsianyi does not show a strong lack of sexual differences. Probably, the reason of these discordant results between authors could also be related to the possible different growth pattern of the neck–shaft angle in girls and boys. As can be seen from Figure 4, while girl values of neck–shaft angle are more or less constrained to a specific region of the graph, boys have a larger variability that in some cases overlaps with female values. Actually, the decreasing angulation of the femoral neck depends on both mechanical load generated by the muscles and ligaments (Serafimov, 1974; Tompkins et al., 1988) and the widening of the pelvis, which is important in females at this age (Tague, 2005; Birkenmaier et al., 2010). The broadening of the pelvis in females plays a key role in decreasing the neck–shaft angle (Singh and Singh, 1975; Gulan et al., 2000; Igbigbi, 2003; Tague, 2005; Birkenmaier et al., 2010) and could explain the lower variability observed for this angle in the female series. The lower values of some boys could be explained by their higher physical activity.

The size and shape of the trochanters are also related to the increase in mass of the gluteus maximus, gluteus minimus (greater trochanter) and pectineus (lesser trochanter) muscles, which actively participate in locomotion and in the stabilization of the femoro-pelvic and femoro-tibial joints. As expected, the comparison of the results of this study with those obtained in the female series (Pujol et al., 2014) shows that the increase in size and robustness of this region is greater in males (Fig. 13).

Distal epiphysis: bicondylar angle

The bicondylar angle is associated with the abduction movement of the lower limb. It is important to note that the bicondylar and the tibio-femoral angles must not be confused; the latter is the angle between the tibial and femoral diaphyseal axes in the coronal plane of the leg. The tibio-femoral angle measures a physiological phenomenon and the bicondylar angle measures an osteological one (Tardieu and Damsin, 1997). The bicondylar angle permits the location of the knee and ankle joints almost directly under the body's center of gravity to generate biomechanically efficient bipedal locomotion (Aiello and Dean, 1990). This makes the load transmitted through the lower limbs to be close to the vertical axis of gravity of the body during the phases of walking and running. Consequently, the center of gravity of the body needs to move only a short distance laterally to lie above the standing leg during walking (Aiello and Dean, 1990). The bicondylar angle has been key of the assignation of fossils to human lineage (LeGros Clark, 1947; Johanson and Coppens, 1976; Tardieu and Trinkaus, 1994) because its implication in bipedal locomotion (Tardieu and Damsin, 1997).

Despite the paleoanthroplogical significance of the bicondylar angle, few studies have focused on its development (Salenius and Vankka, 1975; Tardieu and Damsin, 1997). Our results are consistent with those obtained by Tardieu and Damsin (1997) in boys from 0 to 12 years of age. These authors proposed a function for calculating the male bicondylar angle taking in relation to age. Accroding to this study, at birth the bicondylar angle is 0°; at 9 years of age 6°; and at 12 years 7.1°. In our sample, the angulation was 6.93° at 9 years of age, 7.51° at 12 years, increasing to 8.11° at 16 years. These results contrast with those found in other studies, which show that the male bicondylar angle only varies up to 7 years of age, reaching its adult value at that age (Tardieu et al., 2006). The values of the bicondylar angle in our sample stabilise at around 15 years of age, the mean age at which the male pubertal growth spurt finishes in the population of Barcelona (Ferrández et al., 2009).

The change of angulation of the femur produces specific mechanical loads that in turn pressure in the femoral bicondylar region which makes to react the distal epiphysis. The force produced to the action of the different muscle groups and the patella causes an asymmetrical load in the condyles, which is greater on the medial condyle. This generates a reaction in the endochondral cartilage of the medial condyle, which lead to a greater size increase of this condyle which in turn produces the angulation of the distal femur (Shefelbine et al., 2002). The greatest changes of the bicondylar angle take place from the beginning of locomotion to 8 years of age and, according to our results, keep on until the femoral growth spurt (15 years), before the epiphyseal closure. These findings are in agreement with the variation observed during puberty in the female Spanish series analysed by Pujol et al. (2014). The growth cessation of the bicondylar angle is an open question (Tardieu and Damsin, 1997) due to the paucity of studies on this subject and the scarcity of available studies (Tardieu and Trinkaus, 1994; Tardieu and Damsin, 1997) do not furnish enough information to clarify the question. Most of these studies are based on the tibio-femoral angle (Salenius and Vankka, 1975; Cahuzak et al., 1995; Mehmet-Arazi et al., 2001), and they do not offer concordant results on the age of growth cessation for the bicondylar angle. Salenius and Vankka (1975) claim that the tibio-femoral angle would stabilise at 6 or 7 years of age, and thus the femur would grow in length keeping this angle constant from this age. In contrast, other authors have suggested that the values for this angle stabilise at around 10 (Cahuzak et al., 1995) or 12 years of age (Mehmet-Arazi et al., 2001). But the point is, that all this studies and given ages of growth cessation are built on the development of the tibio-femoral angle and not on the bicondylar angle. In relation to the increase of the bicondylar angle, Tardieu and Damsin (1997) and Pujol et al. (2014) observed that in females it finishes at around 13 years of age, which is around the feminine growth spurt in height. This study suggests that the age of growth cessation of the bicondylar angle in males is about 15 years, around the male growth spurt and concordant with the observations of Tardieu and Damsin (1997) and Pujol et al. (2014). In this study, the mean bicondylar angle at 16 years of age is 8.11°, value that falls within the normal range of variation for the current population (8°–15°; Pearson and Bell, 1919; Keats et al., 1966; Singh and Singh, 1975; Pandya et al., 2008). This value is also close to that reported for a sample of Parisians (7.9°) but slightly lower than that obtained for a sample of Londoners (10.2°; Tardieu and Trinkaus, 1994). As the neck–shaft angle, the bicondylar angle shows great variation between different populations (Pandya et al., 2008). This variability is the result of different mechanical loads supported by the femur, due to the different physical activities performed by the individuals and the influence of different genetic, cultural and environmental factors (Tardieu and Trinkaus, 1994).

In females, the bicondylar angle (Pujol et al., 2014) is higher than in males. This result is concordant with previous observations (Tardieu and Damsin, 1997; Scheuer and Black, 2000, Igbigbi and Sharrif, 2005; Pandya et al., 2008). These sexual differences are statistically significant from 10 years of age, when the pubertal growth spurt in the female pubis occurs (Rissech and Malgosa, 2007) and agree with the significant sexual differences found in adults (Igbigbi and Sharrif, 2005; Pandya et al., 2008). This finding further supports the idea that the distal region of the femur has a higher sexual discrimination power for adult individuals (Gill, 2001; Holliday and Ruff, 2001). The bicondylar angle in males is more variable than in females, which exhibit a greater canalised growth. Canalization in human growth is a widely understood phenomenon and extended concept in pediatrics, indicating that children stay in one or two adjacent growth channels following a parallel-to-centile pattern in growth (Hermanussen et al., 2001). This idea of growth canalization was described by Tanner (1962) and it is considered an evidence of the robust control that genes exert over body size—See Nelson, Text book of Pediatrics pp. 58 (Behrman et al., 1999). Canalisation means that children normally grow with remarkable fidelity relative to the normal growth curve, and physicians are trained to note abnormal height velocity since it always warrants further evaluation (Wilson et al., 1998). Even when illness or starvation temporally interrupts the process of normal height acquisition, catch up growth usually seems to compensate for these losses (Boersma and Wit, 1997), leading stature back to the original centile. Catch up growth has been considered one of the strongest examples of developmental canalization in humans (Prader et al., 1963).

These findings of different growth patterns of both femoral angles between boys and girls, specifically the bicondylar, could indicate the importance of pelvic breadth development in females. Given the anatomical and functional relationship femur–pelvis, the former is subjected to two selective pressures, namely locomotory needs and the interacetabular distance in females, a variable that is related to the width of the birth canal (Parsons, 1914; Pearson and Bell, 1919; Walmsley, 1933; Heiple and Lovejoy, 1971; Tardieu, 1981, 1983; Berge, 1993; Tardieu and Trinkaus, 1994). It is not possible to compare exhaustively the findings on the canalised growth in the bicondylar angle in girls. Most studies on this variable are based on adults and, as we stated before, there is a lack of information on its changes during growth. Furthermore, the few published studies on this subject based on subadults used small samples which may bias findings. For example, Tardieu and Damsin (1997) used a sample of 77 postnatal individuals between 5 months and 17 years old, and Tardieu et al. (2006) used a sample of 48 individuals between 6 fetal months and 18 years of age. In addition, Tardieu et al. (2006) applied logarithms in their analysis of correlation between this variable and age, which could be another possible reason which obscures the differences in the bicondylar angle pattern of variation between boys and girls detected in our study.

The bicondylar angle arises due to the asymmetric growth of the femoral condyles. The greater size of the medial condyle is related to asymmetric loads due to the decrease in the neck–shaft angle and the action of muscles from the lower limb, as well as the load from the patella. All these forces determine that the metaphyseal cartilage generates more bone tissue in the medial condyle (Shefelbine et al., 2002). In turn, the decrease in the neck–shaft angle is related to the increase in mechanical loads generated by muscles and ligaments, structures that also depend on the increase in pelvic breadth, which is particularly important in young females. Indeed, the appearance of sex-based differences in these two angles occurs at around 10 years of age, when the pubertal growth spurt in the female pubis generally starts (Rissech and Malgosa, 2007).

According to our results, the angular remodeling of the femur is extremely significant in girls, specifically from 12 years of age upward. Conversely in boys, both the increase in size and the angular remodeling of the femur are equal in importance during the age period analysed. These different patterns of femoral development are expressed by divergent trajectories, especially from 12 years of age. The postnatal divergent trajectories during development is an aspect of the process to acquire adult shape differences (Mitteroecker et al., 2004a; Frelat and Mitteroecker, 2011), in this case, sexual differences. The extension or truncation of growth trajectories to generate sex-specific and species-specific differences is currently a subject of discussion. Older and univariate studies presented sex-specific and species-specific differences as extension or truncation of identical developmental trajectories (see Bulygina et al., 2006). However, current studies indicate that this vision is too simplistic (Bulygina et al., 2006). Contrarily, several studies on humans (Bulygina et al., 2006;

Coquerelle et al., 2011) and other vertebrates (Hingst-Zaher et al., 2000) demonstrated that developmental trajectories are not frequently the same between species or between sexes of the same species.

The constant size difference between boys and girls in both femoral angles and the constrained values acquired in girls, especially in the bicondylar angle, are new findings, which suggests that the sexual dimorphism in these two angles could be partially prenatally established in girls. Likewise, our results on sexual dimorphism of the femur contribute to the discussion about the underlying factors that affect pelvic morphology. Both angles, neck–shaft and bicondylar, are related with the development of the pelvic breath, specifically with the biacetabular and transverse diameter of the mid plane of the pelvis. The significant sexual differences in this last variable of the birth canal have been found by different authors and types of studies (Kurki, 2013; Crespo et al., 2015), pointing out its importance in the structure of the birth canal and the human parturition process (Kurki, 2013; Crespo et al., 2015). The growth pattern of the girl femur could indicate that the observed constrictions of these two femoral angles are due to the functional need of the female birth canal in *Homo sapiens*. Quantitative explorations on the development patterns of the femoral sexual dimorphism in other humans and primates will be helpful to elucidate some of these questions and to determine the existence of shared developmental patterns among primates.

CONCLUSIONS

The information provided by this study increases the understanding of femoral development in 21st-century children, especially those from Spain. Specifically, it provides information concerning the period and morphology of the different secondary ossification centers in the male femur during development, which is in agreement with the development patterns observed in previous studies on the development of the femur during puberty. Furthermore, our results provide new information on the patterns of femoral development, the possible factors that contribute to femoral sexual differences and the timing of the angular remodeling of the postnatal male femur. Our analyses also indicate a continuous remodeling until 16 years old for male neck–shaft angle and 15 years old for bicondylar angle and agree with the high variability shown by different studies. Sexual dimorphism analysis showed a divergent pattern of development between boys and girls, especially from 12 years of age. From that age, the angular remodeling of the femur predominates almost exclusively in girls. In boys, the angular remodeling and the increase in size have equal importance during the ages considered. In the female femur, the observation of a canalised growth in both angles, specifically in the bicondylar, is a new finding, which suggests that the sexual dimorphism observed in these angles could be partially established prenatally. Furthermore, our results on sexual dimorphism may contribute to the discussion on the underlying factors that affect pelvic morphology because the close relationship with the pelvic morphology and bipedal locomotion. Our findings may be also useful for helping to determine bipedal locomotion in immature fossil individuals, especially considering the differences in variability of these angles between males and females, and the effect of pelvic breadth on the formation of these angles, especially the bicondylar angle. Sex-related differences here detected should be taken into account in future analyses on these angles (e.g., in studies of human evolution in which the sex of the fossil individuals is unknown). In general, results of this study can be meaningful to improve our understanding of the onset of bipedalism in the human lineage and to refine sub-adult age-estimation methods, both of which are essential to progress in osteoarchaeology, forensic sciences and paleoanthropology.

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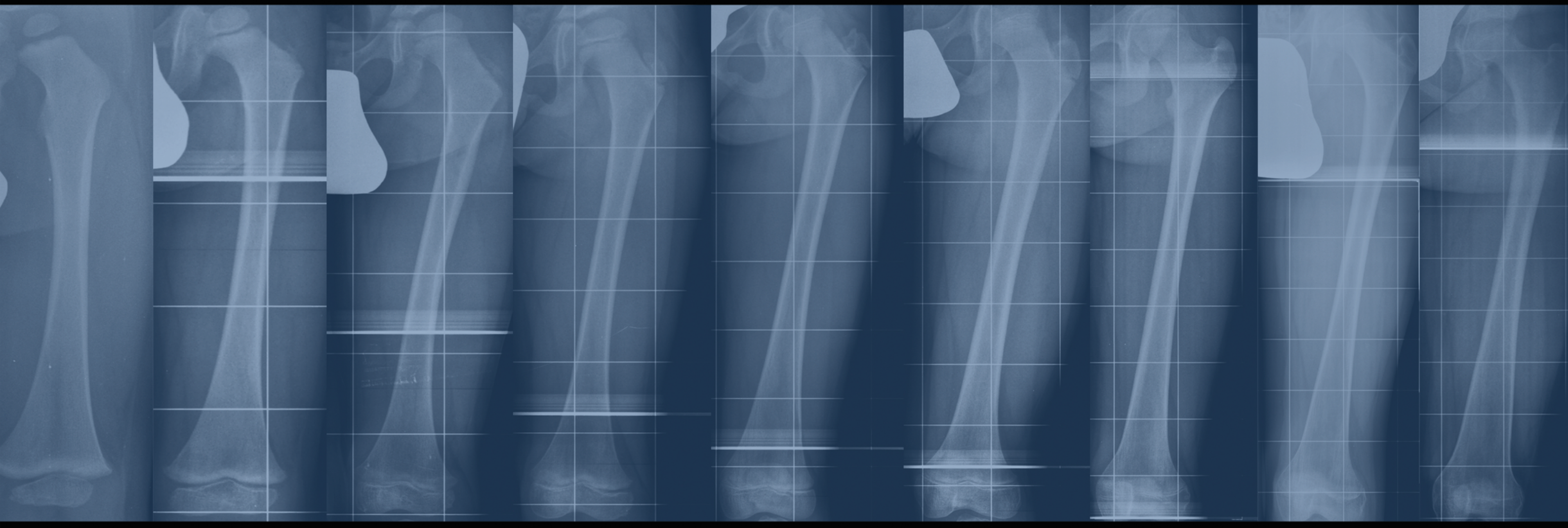

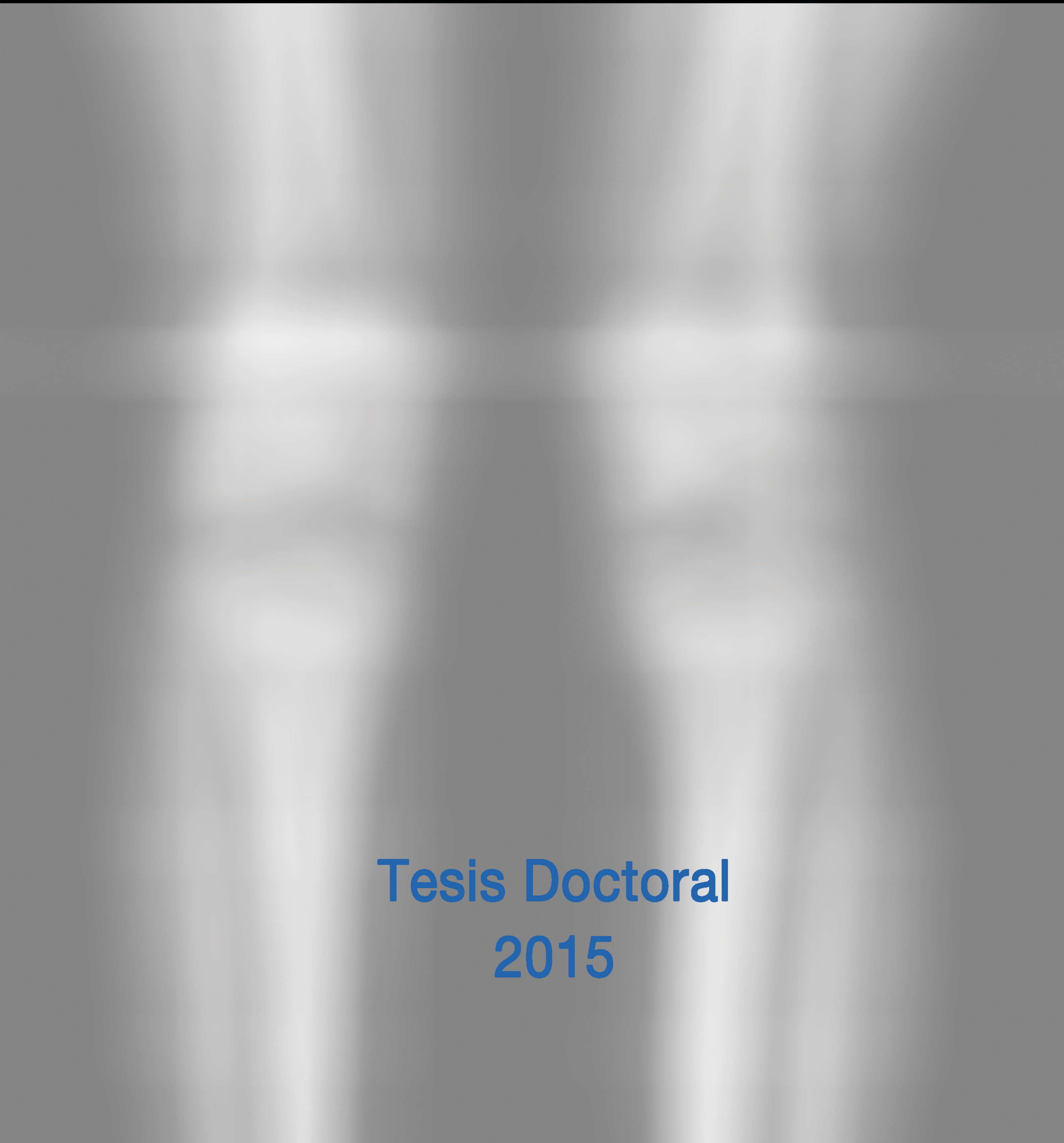

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