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The length of the humerus in human embryos at developmental stages 18–23

W. Woźniak, M. Bruska

Department of Anatomy, University School of Medical Sciences, Poznań, Poland

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The length of the humerus was measured in 69 staged human embryos at developmental stages 18–23 (7 and 8 weeks). It was found that the length of the humerus increases from 2.0 mm at stage 18 to 5.4 mm at stage 23. There were slight variations at certain of the stages investigated. Asymmetry between limb lengths was not found during the embryonic period under examination.

Key words: human embryonic period, upper limb, humerus, metric features

INTRODUCTION

The first morphological evidence of the developing limb is the swelling of the lateral body wall at pectoral and lumbar levels [9]. This swelling forms a limb bud, which is considered a morphogenetic field and develops via a series of interactions of specialised regions of the somatopleuric mesoderm with the overlying ectoderm. As the limb buds grow, four segments are progressively specified: 1) the girdle (zonoskeleton), which includes the clavicle and scapula or hip bone, 2) the proximal segment (stylopodium), which forms a single skeletal element (the humerus/femur), 3) the medial segment (zygopodium), which has two skeletal elements (the radius-ulna/tibia-fibula) and 4) the distal segment (autopodium), containing the skeletal elements of the hand or foot.

The growth and patterning of the limb bud is under the control of limb organisers. The type of limb bud and its patterning is specified before formation [23]. In each limb bud three signalling centres have been identified, each operating mainly in one of the three orthogonal axes [3, 16]. These centres are the apical ectodermal ridge (AER), the zone of polarising activity (ZPA) and the non-AER ectoderm [9].

Apical ectodermal ridge is the ectoderm-forming longitudinal ridge of pseudostratified columnar cells. This ridge is an essential inductor in limb development and is required for elongation in the proximodistal axis. The function of the AER is mediated by several members of the fibroblast growth factor family, which are synthesised by AER cells [22].

In the formation of the variety of tissues that comprise the limb, such as the skeleton, musculature, and vasculature, the Notch family signalling pathway is involved and is required for proper functioning of AER [12].

Zone of polarising activity is a small group of mesenchymal cells located at the posterior border of the bud. These cells control the patterning in the anteroposterior (rostrocaudal) axis through their production of *sonic hedgehog (Shh)*. This region also specifies the positional identity of the digits. Patterning in the dorsoventral axis depends on the non-AER ectoderm. The dorsal ectoderm expresses *Wnt7a*, which induces expression of the homeobox gene *Lmx1*, which is responsible for the dorsalisation of mesoderm. The ventral ectoderm expresses *Engrailed-1 (En-1)*, which controls ventral patterning by restricting *Wnt7a* expression to the dorsal ectoderm.

Address for correspondence: Prof. W. Woźniak, Department of Anatomy, University School of Medical Sciences, Święcickiego 6, 60–781 Poznań, Poland, tel: +48 61 854 65 64, fax: + 48 61 854 65 68, e-mail: wwozniak@amp.edu.pl

The upper limb buds are present at stage 12 (30 days) at the somitic levels s7-12. During stage 15 (36 days) the hand plate appears. The bone of the upper limb develops at the end of embryonic period (stages 22–23).

The prenatal development of the skeleton of the limbs has been extensively studied [1, 14, 18, 19, 21, 27–30, 32]. Most of these studies were concerned with assessment of foetal maturity and have concentrated on foetuses *in utero* near term [2, 10, 11, 15, 17, 24, 25, 33]. The length of the developing human humerus has been studied in the foetal period and was related to age rather than to crown-rump length [24, 25, 31].

The present study was performed on staged human embryos during the last two weeks of the embryonic period.

MATERIAL AND METHODS

A study was made of 69 embryos from the Collection of the Department of Anatomy of the University School of Medical Sciences, Poznań and from the Collection of the Human Developmental Anatomy Center in Washington D.C. Embryos were staged according to 23 developmental stages and age was expressed in postovulatory days. Investigated embryos were at developmental stages 18 to 23, and crown-rump lengths ranged from 13 to 30.5 mm. Approximate ages of the embryos ranged from 44 to 57 days.

The majority of specimens were fixed in formalin and paraffin or paraplast were used for embedding. All embryos in which the humerus was measured were sectioned serially in the coronal or sagittal planes. Sections were stained with various techniques.

Measurements were made under a low magnification light microscope with the aid of a micrometer ocular and with the Leica Image Processing and Analysis system.

RESULTS

In embryos at the 7th week, stages 18, 19, and 20, the skeletal elements of the arm, forearm and hand are in cartilage. The rounded head and thickened distal part of the humerus are discernible and the longitudinal axes of the limbs are parallel. During the 8th week (stages 21–23) the ossification point appears in the shaft of the humerus [18, 26, 28–30].

It was found that the humerus showed continuous growth during the last two weeks of the embryonic period. The length of the humerus increased from 2.0 mm in stage 18 to 5.4 mm in stage 23 (Tables 1–6).

Table 1. C-R length, developmental stage, age, and length of humerus in embryos at stage 18

No.	C-R length [mm]	Developmental stage	Age [days]	Length of humerus [mm]
1	13.0	18	44	2.0
2	13.1	18	44	2.0
3	13.3	18	44	2.0
4	13.5	18	44	2.0
5	13.7	18	44	2.1
6	14.0	18	44	2.1
7	14.2	18	44	2.1
8	14.3	18	44	2.1
9	14.5	18	44	2.2
10	14.6	18	44	2.2
11	14.8	18	44	2.2
12	15.0	18	44	2.2

Table 2. C-R length, developmental stage, age, and length of humerus in embryos at stage 19

No.	C-R length [mm]	Developmental stage	Age [days]	Length of humerus [mm]
1	16.0	19	46	2.5
2	16.1	19	46	2.5
3	16.3	19	46	2.5
4	16.5	19	46	2.5
5	17.0	19	46	2.56
6	17.2	19	46	2.56
7	17.3	19	46	2.6
8	17.5	19	47	2.6
9	17.5	19	47	2.7
10	17.6	19	47	2.8
11	17.6	19	47	2.7

At stage 18 the length of the humerus varied from 2.0 mm to 2.2 mm (Table 1). In stage 19 the length of the humerus was between 2.5 mm and 2.7 mm (Table 2). Embryos at stage 20 have a length of the humerus between 2.9 mm and 3.2 mm (Table 3). In stage 21 the length of the humerus varied from 3.4 mm to 3.5 mm (Table 4). At stage 22 the length of the humerus was from 3.7 mm to 4.3 mm (Table 5) and at stage 23 this length varied from 4.3 mm to 5.4 mm (Table 6). It is evident that the humerus elongates markedly during developmental stages 22 and 23 (the end of the embryonic period).

Table 3. C-R length, developmental stage, age, and length of humerus in embryos at stage 20

No.	C-R length [mm]	Developmental stage	Age [days]	Length of humerus [mm]
1	18.0	20	49	2.9
2	18.1	20	49	2.0
3	18.3	20	49	2.9
4	18.3	20	49	2.9
5	18.6	20	49	3.0
6	18.9	20	49	3.0
7	19.2	20	49	3.0
8	19.5	20	49	3.0
9	19.5	20	49	3.1
10	20.1	20	50	3.1
11	20.4	20	50	3.1
12	20.6	20	50	3.2
13	21.2	20	50	3.2

Table 4. C-R length, developmental stage, age, and length of humerus in embryos at stage 21

No.	C-R length [mm]	Developmental stage	Age [days]	Length of humerus [mm]
1	22.0	21	51	3.4
2	22.1	21	51	3.4
3	22.3	21	51	3.3
4	22.6	21	51	3.33
5	23.0	21	51	3.4
6	23.0	21	52	3.44
7	23.0	21	52	3.45
8	23.1	21	52	3.5
9	23.1	21	52	3.5
10	23.2	21	52	3.5
11	23.4	21	52	3.5

Table 5. C-R length, developmental stage, age, and length of humerus in embryos at stage 22

No.	C-R length [mm]	Developmental stage	Age [days]	Length of humerus [mm]
1	23.9	22	53	3.7
2	24.0	22	53	3.9
3	24.7	22	53	4.0
4	25.0	22	54	4.0
5	25.5	22	54	4.1
6	25.9	22	55	4.1
7	26.0	22	55	4.1
8	26.3	22	55	4.3
9	26.7	22	55	4.3

Table 6. C-R length, developmental stage, age, and length of humerus in embryos at stage 23

No.	C-R length [mm]	Developmental stage	Age [days]	Length of humerus [mm]
1	27.9	23	56	4.3
2	28.1	23	56	4.5
3	28.3	23	56	4.5
4	28.5	23	56	4.6
5	28.7	23	56	4.6
6	28.7	23	56	4.7
7	29.1	23	56	4.9
8	29.5	23	56	5.0
9	29.9	23	57	5.0
10	30.0	23	57	5.1
11	30.2	23	57	5.2
12	30.3	23	57	5.1
13	30.5	23	57	5.4

The mean values for the investigated stages were as follows: stage 18 — 2.1 mm, stage 19 — 2.6 mm, stage 20 — 3.0 mm, stage 21 — 3.4 mm, stage 22 — 4.0 mm, stage 23 — 4.8 mm.

DISCUSSION

The skeleton tendons, and the sheaths of the muscles of the limbs are derived from the lateral plate mesenchyme, whereas the muscles of the limbs are of somitic (myotomic) origin [18, 27–30].

Differential proximodistal growth of parts of the limb bud result in two main changes: 1) the dorsal aspect of the limb grows faster than the ventral, and later 2) the preaxial (rostral) border grows faster than the postaxial (caudal). These events cause the limb to curve around the body wall and form particular parts of the limb (shoulder, arm, forearm).

Skeletal elements of the upper limb appear in the mesenchyme during stages 13–16. Mesenchymal cell condensation is a critical event in the initiation of chondrogenesis and subsequent cartilage formation.

However, the molecular mechanisms underlying the condensation process itself remain largely unknown [20]. One of the cell adhesion molecules implicated in this process is N-cadherin. Displays a diffuse expression pattern in the central core region in the precartilaginous stage. In the development of particular parts of the limb an important role play *Hox* genes [4–8, 13, 34].

The humerus begins to chondrify before the radius and the radius before the bones of the hand.

Kelemen et al. [17] measured the long bones with a calliper and their results are not exact.

The present study was made in staged embryos with precisely determined ages. The humerus was measured along its long axis from exact proximal and distal points. There was no difference between male and female embryos in the length of the humerus. Such differences appear later in foetal life [1]. Asymmetry between limb lengths was not found in the examined embryos.

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