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Case report

## Three Patient Kindred with a Novel Phenotype of Osteogenesis Imperfecta due to a *COL1A1* Variant

*Short title: Novel phenotype of OI*

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### What is already known on this topic?

Osteogenesis imperfecta (OI) is a heterogeneous connective tissue disorder characterized by reduced bone mass and increased bone fragility. Peak fracture rates in OI occur during the toddler and adolescent years, decline during adulthood, and increase again after age 55 years.

### What this study adds?

We describe a kindred of 3 members who presented with a unique phenotype of OI, presumably due to a proline-to-leucine missense variant in the *COL1A1* gene. All 3 members had a pattern of prenatal bone deformities followed by multiple nontraumatic long bone fractures within the first two years of life and then an absence of nontraumatic fractures thereafter. To our knowledge, a clinical phenotype of OI characterized by cessation of nontraumatic fractures after the first two years of life has not been described previously.

### Abstract

Osteogenesis Imperfecta (OI) is characterized by fractures and progressive bone deformities. Fracture rates peak during the toddler and adolescent years and decline during adulthood but do not stop entirely. We describe a 3-person kindred (mother and 2 sons) who presented with a unique phenotype of OI. Our patients demonstrated a pattern of prenatal bone deformities followed by multiple nontraumatic long bone fractures within the first two years of life and then an absence of nontraumatic fractures thereafter. No extraskelatal manifestations have been noted to date. The mother did not receive bisphosphonate therapy but had no nontraumatic fractures after age 5 months. Intravenous bisphosphonate therapy was started for both sons within 2 months of birth with the most recent infusions at age 18 months and 28 months in patient 2 and 3 respectively. Two patients harbored a variant of uncertain significance in the *COL1A1* gene. This heterozygous variant, c.3548C>T; p.(Pro1183Leu), is listed in the OI Variant Database as affecting only 1 other individual with osteopenia. We describe 3 family members with a unique presenting phenotype of OI, characterized by cessation of nontraumatic fractures after the first two years of life.

**Key words:** fragility fractures, collagen, child, bisphosphonates, bone density

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## Introduction

Osteogenesis imperfecta (OI) is a heterogeneous connective tissue disorder characterized by reduced bone mass and increased bone fragility [1, 2]. The disorder is primarily caused by variants in the genes involved in the synthesis or post-transcription modification of type 1 collagen [3]. Type 1 procollagen is composed of two  $\alpha 1(I)$  and one  $\alpha 2(I)$  chains that are encoded by the *COL1A1* (OMIM #120150) and *COL1A2* (OMIM #120160) genes, respectively [2]. The triple-stranded procollagen molecule is assembled and then secreted into the extracellular space, where the propeptides are enzymatically cleaved to form mature collagen [4]. Although there are numerous other OI-related genes, most cases of OI (85-90%) are caused by variants in the *COL1A1* and *COL1A2* genes.

Typically, point variants affecting glycine residues in the triple-helix structure of type 1 collagen have been predicted to disrupt protein folding, resulting in OI [5]. The substitution of leucine for proline in exon 49 of the *COL1A1* gene is listed in the OI Variant Database but to date, it has been associated with only one individual affected by osteopenia and it has been designated as a variant of unknown pathogenicity [6].

The severity of OI varies from mild to progressively deforming to perinatally lethal [7]. Peak fracture rates in OI occur during the toddler and adolescent years, decline during adulthood, and increase again after age 55 years [8]. However, to our knowledge, a clinical phenotype of OI characterized by cessation of nontraumatic fractures after the first two years of life has not been described previously.

We describe a kindred of 3 members who presented with a unique phenotype of OI, presumably due to a proline-to-leucine missense variant in the *COL1A1* gene. All 3 members had a pattern of prenatal bone deformities followed by multiple nontraumatic long bone fractures within the first two years of life and then an absence of nontraumatic fractures thereafter.

## Case Reports

We describe a nonconsanguineous family of European descent, in which 3 members were affected by OI (**Table 1, Figure 1**). Informed verbal consent was obtained from the mother.

### Patient 1: Mother

The mother received the diagnosis of OI when she was 23 years old, after the birth of her second child (patient 2), whose prenatal ultrasound suggested OI. The mother had 9 nontraumatic long bone fractures, primarily femoral, before age 5 months. Her prenatal ultrasound findings were unknown. She did not receive bisphosphonate treatment but did not have any other nontraumatic fractures after age 5 months. She had normal sclerae, dentition, hearing, echocardiogram and stature (159.8 cm, 25<sup>th</sup> percentile) [9]. She had no spinal or cranial deformities, no joint hypermobility and did not bruise easily. We were unable to obtain data regarding her bone turnover markers and bone mineral density (BMD). She had an unaffected 17-year-old daughter. As of manuscript writing, patient 1 was 39 years old.

### Patient 2: Older sibling

Patient 2 was the second child of patient 1 and an unaffected father. The father's height was 188 cm (94<sup>th</sup> percentile) [9]. At 5 months' gestation, a prenatal ultrasound showed evidence of bowing of both femurs. Patient 2 was born at 38 weeks' gestation via caesarean delivery without respiratory distress at birth. His birth weight was 4.28 kg (90<sup>th</sup> percentile), length was 52 cm (50<sup>th</sup> percentile) and head circumference was 38 cm (90<sup>th</sup> percentile) [9]. At age 3 days, during circumcision, he had a right femur fracture (**Figure 1**). Subsequently, he had fractures of bilateral humeri and femurs at 6 days of life and sustained a fracture of the left femur at age 4 weeks. There was minimal or no trauma associated with any of these fractures.

A diagnosis of OI was considered and pamidronate infusions were initiated (0.75 mg/kg per day on 2 consecutive days, every 2 months, total of 8 infusions) from age 6 weeks through 18 months at another center. He tolerated the infusions well. Age-related developmental milestones were normal. At age 3 months, he was at the 95<sup>th</sup> percentile for length and 97<sup>th</sup> percentile for weight. His sclerae, hearing and dentition were normal. He had no spinal or cranial deformities, no joint hypermobility, and did not bruise easily. At age 14 months, his lumbar spine BMD was 0.51 g/cm<sup>2</sup> and left hip BMD was 0.57 g/cm<sup>2</sup> (reference range not available for this age group) [10]. He had normal serum calcium (10.6 mg/dL; reference range 9-11 mg/dL) and total alkaline phosphatase (274 U/L; reference range 150-420 U/L).

Given the paucity of fractures (no fractures since age 4 weeks, despite being very active), pamidronate therapy was discontinued at age 18 months. As of manuscript writing, he was 17 years old, had normal ambulation and no deformities. A follow-up BMD was not available for him. His height was at the 75<sup>th</sup> percentile and weight was at the 95<sup>th</sup> percentile. His corrected midparental height was at the 65<sup>th</sup> percentile.

### Patient 3: Younger sibling

Patient 3 was born at 39 weeks' gestation to patient 1 and a different father. The father's height was 170 cm (17<sup>th</sup> percentile) [9]. At 26 weeks' gestation, an ultrasound showed bowing of both femurs. Patient 3 was born via caesarean delivery, without respiratory distress at birth. His birth weight was 3.8 kg (75<sup>th</sup> percentile), length was 48 cm (25<sup>th</sup> percentile) and head circumference was 36.5 cm (65<sup>th</sup> percentile). Radiographic studies on the first day of life confirmed left posterior ninth rib fracture and femoral bowing bilaterally (**Figure 2**). Wormian bones were noted along the lambdoid sutures. He had nontraumatic

fractures of the eighth through tenth ribs on the right side at 2 weeks, right tibia and bilateral femur fractures at 5 weeks, bilateral femur fractures at 6 weeks (distinct locations each time) and left tibia fracture at 15 weeks.

Physical examination showed that he had white sclerae and no dysmorphic features. His hearing screen was normal. Serum ionized calcium (5.89 mg/dL; reference range 3.9-6.0 mg/dL), phosphorus (6.3 mg/dL; reference range 4.5-9.0 mg/dL), total alkaline phosphatase (250 U/L; reference range 150-420 U/L) and 25-hydroxy-vitamin D (37 ng/mL; reference range  $\geq 20$  ng/mL) concentrations were normal. Zoledronic acid infusions were initiated every 3 months from age 3 weeks to 9 months (0.0125 mg/kg for the first dose, 0.025 mg/kg for the next 3 doses, each infused over 60 minutes). The fracture at age 15 weeks occurred after he had received 2 infusions of zoledronic acid. Given the paucity of further symptoms of OI and the fact that his mother and older sibling had no fractures after the first few months of life, bisphosphonate therapy was discontinued when he was 9 months old.

At age 21 months, he was noted to have a new mild compression of the anterior-superior endplate of the 5<sup>th</sup> lumbar vertebral body (L5). He also had a mildly displaced oblique fracture of left distal tibial metaphysis due to moderate trauma (his leg was caught on the edge of a slide while sitting on his father's lap) at age 25 months. He was treated with 2 additional infusions of zoledronic acid at 21 months and 28 months (total 6 infusions). He has not sustained additional low-trauma fractures since. The mild L5 vertebral body deformity was less apparent on subsequent imaging 7 months later.

He achieved normal developmental milestones, including appropriate dentition for age. Baseline BMD was obtained at 12 months (lumbar spine 0.44 g/cm<sup>2</sup>; total body excluding head 0.34 g/cm<sup>2</sup>; reference range not available for this age group) [10]. A follow-up BMD at age 28 months after 6 zoledronic acid infusions revealed an improvement of 23.7% in spinal bone density (0.54 g/cm<sup>2</sup>) and 36.5% in total body excluding head bone density (0.46 g/cm<sup>2</sup>), compared to baseline. As of manuscript writing, he was 36 months old and at 27<sup>th</sup> percentile for length and 81<sup>st</sup> percentile for weight. His corrected midparental height was at the 20<sup>th</sup> percentile.

#### DNA Sequencing and *In silico* Analysis

Clinical information was collected from the patients and abstracted from the medical records. Conformation-sensitive gel electrophoresis (CSGE) was performed by Matrix DNA Diagnosis (New Orleans, LA). The variant was described using the Human Genome Variation Society nomenclature [11]. The pathogenicity of identified variant was analyzed using the *in silico* prediction software Alamut Visual (missense predictors: Align GVGD v.2007, SIFT v.6.20 and Polyphen-2 HumVar; splicing algorithms: SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer and HSF) [12].

#### Results

##### Identification of the *COL1A1* variant

Given the concern for OI in Patient 2, CSGE was performed for the *COL1A1*- and *COL1A2*- coding exons. The CSGE analysis identified a heterozygous missense alteration in exon 49 of the *COL1A1* gene resulting in a C to T nucleotide substitution that converted a proline (CCT) to a leucine (CTT), [NM\_000088.3:c.3548C>T; p.(Pro1183Leu), (RefSeqGene NG\_007400.1:g.19734C>T)].

The patient's mother was heterozygous for the same variant and no variations were present in the father. The variant was classified as 'variant of uncertain significance'. *In silico* analysis was equivocal for potential pathogenicity of this variant (Align GVGD - Class C65 deleterious; SIFT - deleterious; PolyPhen-2 - benign). No significant changes were noted in canonical splice sites.

#### Discussion

We describe a kindred with a unique presenting phenotype of OI. The OI was presumably attributable to a missense variant c.3548C>T; p.(Pro1183Leu) that replaced proline with leucine in the Y position of the glycine-Xaa-Yaa sequence of the pro $\alpha$ 1(I) chain (exon 49 of *COL1A1*). The same variant has been reported previously in only 1 individual [c.3548C>T; p.(Pro1183Leu)] [6]. The referral reason for testing that individual was reported as symptoms of OI and a strong family history of recurrent femoral fractures and osteopenia. The variant was designated to be of unknown pathogenicity, and further family studies to clarify pathogenicity were requested. As of manuscript writing, that patient was 49 years old, and updates on the patient and results from family studies could not be obtained (personal communication, Dr. Meena Balasubramanian, 2018). As more individuals with this specific variant are identified, the clinical phenotype of this form of OI will become clearer.

Lim *et al.* [13] described a patient with Ser40Trp variant in *IFITM5* gene, who presented with multiple fractures in the prenatal period. She remained fracture free after birth with normal BMD. She had blue sclerae, progressive lower limb deformities during childhood and severe short stature (SD -3.5). Her mother, who did not have a history of fractures, was noted to have somatogonadal mosaicism for this variant and became pregnant with a second child with multiple prenatal fractures, found to have the same variant. This is in contrast with our patients who had normal sclerae, absence of postnatal limb deformities, normal stature and a variant in the *COL1A1* gene, although with similar phenotypic bone deformity pattern.

Type 1 procollagen helical domains contain a repetitive glycine-Xaa-Yaa sequence, where Xaa and Yaa are often the amino acids proline (Pro) or hydroxyproline (Hyp) [14, 15]. Some evidence suggests that proline and hydroxyproline are responsible for the thermal stability of the triple-helix structure [16]. Bryan *et al.* [17] hypothesized that a missense variant in the highly stable region of the collagen molecule such as Gly-Pro-Hyp will lead to a greater disruption and more severe clinical consequences than the same missense variant in a less stable region. Collagen chains with these abnormal molecules are overmodified and secreted inefficiently, which disrupts helix stability [18].

Various factors have been proposed to explain how the substitution of one amino acid for another affects collagen processing and the phenotype of OI, including the coordinates of the variant and its position relative to the C-terminus of the propeptide [5, 14, 19]. A variant's proximity to the C-terminus of the molecule is important because assembly of the collagen triple-helix chain begins at the C-terminal end and propagates toward the N-terminal end (Dagleish 1997). Our kindred harbored a variant close to the C-terminus, at amino acid position 1183 on exon 49 of the *COL1A1* gene. Exon 49 is 283 base pairs long (cDNA base numbers 3532-3814) and encodes the last 15 amino acids of the triple-helix region (amino acids 1178-1192), the entire C-telopeptide (amino acids 1193-1218) and part of the C-propeptide domain (amino acids 1219-1271) [20]. A small number of point variants in the C-terminal propeptide have been identified as causing varying clinical phenotypes of OI, ranging from mild to lethal and high bone-mass OI [18, 19, 21]. Although missense variants in the C-propeptide are known to impair or prevent pro $\alpha$ 1(I) chain assembly in the endoplasmic reticulum [19], the outcome of each substitution may differ, depending on the specific gene transcript.

For patients with milder forms of OI, the incidence of fracture declines with age but does not stop entirely [8]. Interestingly, cessation of OI associated fractures after the first two years of life, as seen in our patients, has not been described in the literature in association with *COL1A1* variants. The pathophysiology resulting in this phenotype remains uncertain. There is evidence that posttranslational modification of procollagen is temporally regulated, and this regulation may be crucial for its folding, secretion, and extracellular matrix assembly [22]. We speculate that a sequential improvement in these processes during the first two years of life in our patients resulted in paucity of fractures thereafter.

Finally, the long-term benefit of intravenous bisphosphonate therapy in our patients remains unclear, particularly because patient 1 did not receive this therapy and did not sustain any nontraumatic fractures after age 5 months. Patient 2 received his last infusion of pamidronate at age 18 months. At age 17 years, he was of normal adult height and remained fracture free despite an unrestricted, active lifestyle. We speculate that prolonged bisphosphonate therapy may not provide additional benefit to those with this form of OI, although our sample size and duration of follow-up is too small to draw a definitive conclusion.

Our case series has certain other limitations including lack of genetic testing for variants in other OI-related genes such as *IFITM5*, which have been associated with a phenotype similar to that seen in our patients. Patient 1 and patient 2 underwent genetic testing almost 17 years ago. To our knowledge, at that point in time, typical OI genetic testing included primarily *COL1A1* and *COL1A2* genes. Currently, we do not have the funding available to update genetic testing for patient 1 and patient 2, or send genetic testing for patient 3 for research purposes. It would have entailed a huge economic burden on the family and the genetic testing results were unlikely to influence clinical decision making.

#### **Conclusion**

We describe a 3-patient kindred with a unique phenotype of OI, presumably due to a variant in the *COL1A1* gene. The OI phenotype of affected individuals included a pattern of mild-moderate bone deformities prenatally and multiple nontraumatic fractures limited to the first two years of life. We speculate that the proline-to-leucine substitution in close proximity to the C-propeptide domain might have influenced folding of the triple helix or helix stability. Given the small number of patients in our kindred, the full range of phenotypes associated with this variant remains to be established. Whether antiresorptive therapy is beneficial later in life also remains unclear.

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#### **Authorship Contribution**

NG, SWG, DRD and PJT contributed to study design, data collection, data analyses and manuscript preparation.

#### **Disclosure Statement**

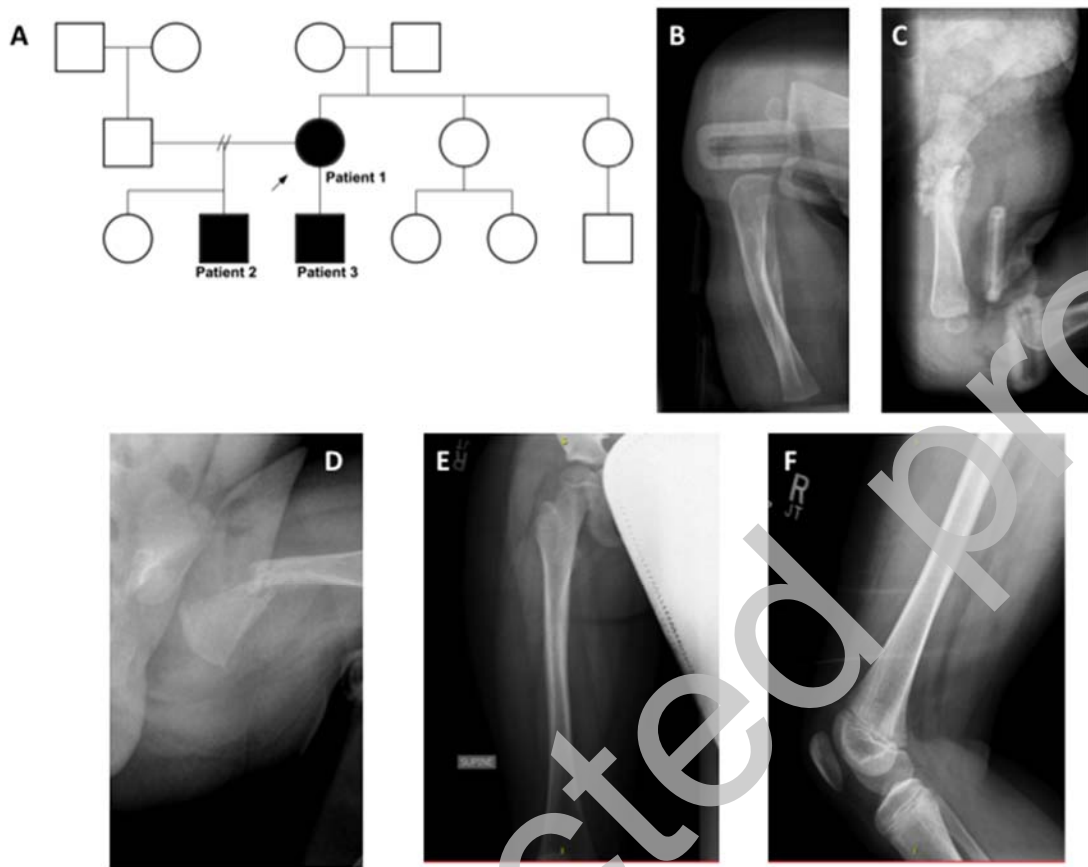
The authors have no conflicts of interest to declare.

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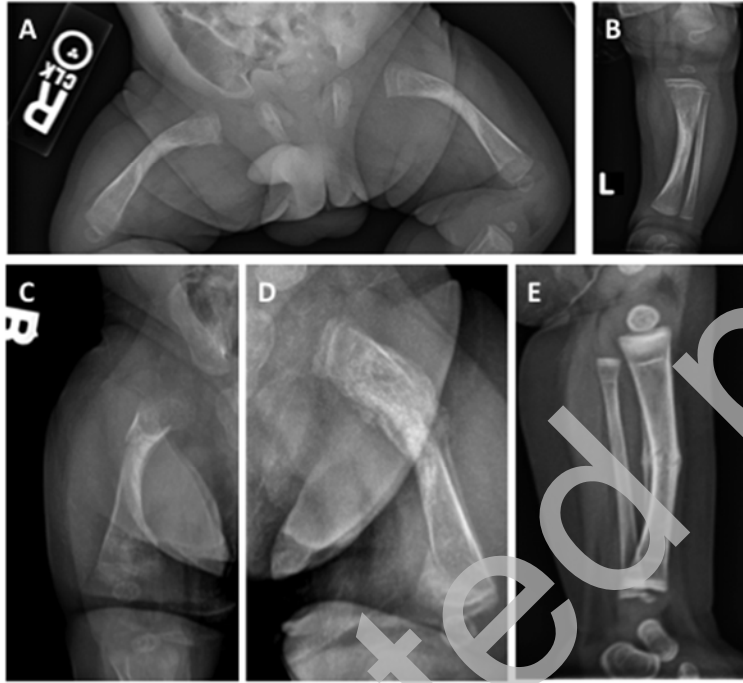
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**Figure 1.** [A] Pedigree chart. The index case is indicated by the arrow. [B-F] Radiographs of patient 2. [B] Bowing of midright tibial shaft at age 3 weeks, lateral view. [C] Abundant callus formation around a healing midshaft right femoral fracture at age 3 weeks, lateral view. [D] Acute proximal-shaft left femoral fracture at age 4 weeks, anteroposterior view. [E] Right femur anteroposterior view at age 10 years. [F] Right femur lateral view at age 10 years.



**Figure 2.** Radiographs of patient 3. [A] Proximal femoral shaft bowing, right greater than the left, at age 2 weeks, anteroposterior view. Note the mild diffuse osteopenia. [B] Left tibial midshaft bowing at age 2 weeks, anteroposterior view. [C] Proximal right femoral fracture at age 6 weeks, lateral view. [D] Periosteal reaction and callus formation around a healing proximal left femoral fracture sustained at age 6 weeks, lateral view. [E] Transverse midshaft fracture of left tibia at age 15 weeks, lateral view.



**Table 1: Clinical characteristics of kindred cases**

	<b>Patient 1 Mother</b>	<b>Patient 2 Older sibling</b>	<b>Patient 3 Younger sibling</b>
<b>Current age</b>	39 y	17 y	36 m
<b>Current height (percentile)</b>	25	75	27
<b>Fractures, age</b>	R femur, < 5 m L femur, < 5 m	R femur, 3 d R humerus, 6 d L humerus, 6 d R femur, 6 d L femur, 6 d L femur, 4 w	L 9 <sup>th</sup> rib, 1 d R 8-10 <sup>th</sup> rib, 2 w R tibia, 5 w R femur, 5 w L femur, 5 w R femur, 6 w L femur, 6 w L tibia, 15 w L5 compression, 21 m
<b>Total fractures, n</b>	9	6	11
<b>BMD (g/cm<sup>2</sup>), age</b>	N/A	14 m Lumbar spine: 0.51 L hip: 0.57	<b>12 m</b> Lumbar spine: 0.44 TBLH: 0.34 <b>28 m</b> Lumbar spine: 0.54 TBLH: 0.46
<b>Bisphosphonate treatment</b>	No	Yes Pamidronate	Yes Zoledronic acid
<b>Total infusions, n</b>	-	8	6
<b>Age at last infusion</b>	-	18 m	28 m
<b>Sclerae</b>	Normal	Normal	Normal
<b>Dentition</b>	Normal	Normal	Normal
<b>Hearing</b>	Normal	Normal	Normal
<b>Echocardiogram</b>	Normal	N/A	N/A
<b>Spinal deformity</b>	None	None	None
<b>Extremity deformity</b>	None	None	None
<b>Joint mobility</b>	Normal	Normal	Normal
<b>Easy bruising</b>	None	None	None
<b>Genotype</b>	c.3548 C>T; p.(Pro1183Leu) Heterozygous	c.3548 C>T; p.(Pro1183Leu) Heterozygous	N/A

y, years; m, months; d, days; w, weeks; R, right; L, left; L5, 5<sup>th</sup> lumbar vertebrae; BMD, bone mineral density; N/A, not available; TBLH, total body less head