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(71) Demandeur/Applicant:
ALEXION PHARMACEUTICALS, INC., US

(72) Inventeur/Inventor:
ODRLJIN, TATJANA, US

(74) Agent: GOUDREAU GAGE DUBUC

(54) Titre : METHODES DE TRAITEMENT D'UN SUJET ATTEINT D'UNE DEFICIENCE EN PHOSPHATASE ALCALINE
(54) Title: METHODS OF TREATING A SUBJECT WITH AN ALKALINE PHOSPHATASE DEFICIENCY

(57) Abrégé/Abstract:
Disclosed herein are methods for treating a subject with an alkaline phosphatase deficiency, further comprising monitoring additional anaiytes, e.g., calcium, parathyroid hormone and/or vitamin D, with treatment modifications as indicated by the levels, e.g., serum levels, of the additional anaiytes.

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(71) Applicant: ALEXION PHARMACEUTICALS, INC. [US/US]; 100 College St., New Haven, CT 06510 (US).

(72) Inventor: ODRLJIN, Tatjana; 1070 Beacon St. #5A, Brookline, MA 02446 (US).

(74) Agents: BIEKER-BRADY, Kristina et al.; Clark & Elbing LLP, 101 Federal Street, 15th Floor, Boston, MA 02110 (US).

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(54) Title: METHODS OF TREATING A SUBJECT WITH AN ALKALINE PHOSPHATASE DEFICIENCY

(57) Abstract: Disclosed herein are methods for treating a subject with an alkaline phosphatase deficiency, further comprising monitoring additional anaiytes, e.g., calcium, parathyroid hormone and/or vitamin D, with treatment modifications as indicated by the levels, e.g., serum levels, of the additional anaiytes.

METHODS OF TREATING A SUBJECT WITH AN ALKALINE PHOSPHATASE DEFICIENCY

This application claims priority to U.S. provisional patent application No. 62/108,689, filed January 28, 2015, the contents of which are hereby incorporated by reference in their entirety.

BACKGROUND

Enzyme replacement therapy (ERT) has been successfully implemented to treat subjects with deficiencies in alkaline phosphatase (AP) activity. In particular, such therapies are useful for treating bone mineralization defects associated with deficient AP activity. Several factors regulate bone formation and resorption, including, for example, serum calcium and phosphate concentrations, and circulating parathyroid hormone (PTH). FGF23, for example, is a hormone that contributes to the regulation of calcium and phosphate homeostasis- promoting renal phosphate excretion and reducing circulating levels of active vitamin D (diminishing intestinal absorption of calcium). ERT treatment that leads to normalized bone formation can potentially have an effect on the production of modulators (*e.g.*, hormones such as, for example, parathyroid hormone (PTH), or vitamin D) that regulate or are regulated by bone mineralization factors (*e.g.*, serum calcium and phosphate).

PTH, also referred to as "parathormone" or "parathyrin," is secreted by the parathyroid gland as an 84-amino acid polypeptide (9.4 kDa). PTH acts to increase the concentration of calcium (Ca^{2+}) in the blood by acting upon the parathyroid hormone 1 receptor (high levels of the parathyroid hormone 1 receptor are present in bone and kidney) and the parathyroid hormone 2 receptor (high levels of the parathyroid hormone 2 receptor are present in the central nervous system, pancreas, testes, and placenta).

PTH enhances the release of calcium from the large reservoir contained in the bones by affecting bone resorption by modulation of expression of key genes that regulate bone resorption and formation. Bone resorption is the normal degradation of bone by osteoclasts, which are indirectly stimulated by PTH. Since osteoclasts do not have a receptor for PTH, PTH's effect is indirect, through stimulation of osteoblasts, the cells responsible for creating bone. PTH increases osteoblast expression of the receptor activator of nuclear factor kappa-B ligand (RANKL) and inhibits the expression of osteoprotegerin (OPG). OPG binds to RANKL and blocks it from interacting with RANK, a receptor for RANKL. The binding of RANKL to RANK (facilitated by the decreased amount of OPG available for binding the excess RANKL) stimulates fusion of osteoclasts into multinucleated osteoclasts, ultimately leading to bone resorption. The downregulation of OPG expression thus promotes bone resorption by osteoclasts.

PTH production (synthesis of PTH) is stimulated with high serum levels of phosphates (often present in late stages of chronic kidney disease) by direct effect of serum phosphates on PTH synthesis in the parathyroid gland by promoting the stability of PTH. PTH negatively impacts retention of phosphates in kidneys (promoting loss through urine) affecting homeostasis of phosphates and calcium. The importance of this signaling pathway in the renal response to PTH is highlighted by the renal resistance to PTH associated with deficiency of PTH receptor G protein subunit (Gsalpha) deficiency in patients with pseudohypoparathyroidism. PTH also enhances the uptake of phosphate from the intestine and bones into the blood. In the bone, slightly more calcium than phosphate is released from the breakdown of bone. In the intestines, absorption of both calcium and phosphate is mediated by an increase in activated vitamin D. The absorption of phosphate is not as dependent on vitamin D as is that of calcium. The end result of PTH release from the parathyroid gland is a small net drop in the serum concentration of phosphate.

Secretion of PTH is controlled chiefly by serum Ca^{2+} through negative feedback. Increased levels of calcium reduce PTH secretion, while diminished levels increase PTH secretion. Calcium-sensing receptors located on parathyroid cells are activated when Ca^{2+} is elevated. G-protein coupled calcium receptors bind extracellular calcium and are found on the surface of a wide variety of cells distributed in the brain, heart, skin, stomach, parafollicular cells (“C cells”), and other tissues. In the parathyroid gland, high concentrations of extracellular calcium result in activation of the Gq G-protein coupled cascade through the action of phospholipase C. This hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to liberate intracellular messengers IP3 and diacylglycerol (DAG). Ultimately, these two messengers result in a release of calcium from intracellular stores and a subsequent flux of extracellular calcium into the cytoplasmic space. The effect of this signaling of high extracellular calcium results in an intracellular calcium concentration that inhibits the secretion of preformed PTH from storage granules in the parathyroid gland. In contrast to the mechanism that most secretory cells use, calcium inhibits vesicle fusion and release of PTH.

Additional mechanisms that affect the amount of PTH available for secretion involve, for example, calcium-sensitive proteases in the storage granules. Upon activation increase the cleavage of PTH (1-84) into carboxyl-terminal fragment, further reducing the amount of intact PTH in storage granules.

PTH also increases the activity of 1-α-hydroxylase enzyme, which converts 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol, the active form of vitamin D in kidneys. Vitamin D decreases transcription of the PTH gene. Vitamin D deficiency (often seen in chronic renal disorders) thus causes increases in PTH production. FGF23 is another regulator of parathyroid function, it is secreted by osteocytes or osteoblasts in response to increased oral phosphate intake and other factors. It acts on kidney to reduce expression transporters of phosphates in kidney reducing phosphate retention. In early stages of chronic renal disease, levels of FGF23 are increased to help promote the urinary excretion of phosphates. Elevated FGF23 in chronic renal disorders reduces activity of the Vitamin D 1-α-hydroxylase enzyme and results low production of the active form of vitamin D. In the intestines, absorption of calcium is mediated by an increase in activated vitamin D. Diminished intestinal calcium absorption, which leads to serum hypocalcemia, does not provide strong negative feedback to production/release of PTH from parathyroid gland, causing increased release of PTH from the parathyroid gland. FGF23 appears to directly inhibit PTH secretion as well.

As AP replacement therapy replaces part of a complex pathway, for example, for proper bone formation, there is a need to further characterize the pathway, and to identify analytes that are indicative of therapeutic effects. Such tracking may indicate therapeutic efficacy and/or may identify additional therapies that may become necessary as a result of AP replacement therapy.

SUMMARY

Described herein are methods for treating a subject with an alkaline phosphatase deficiency that comprise monitoring one or more analytes to determine additional therapeutic treatments and procedures.

One aspect of the disclosure is directed to a method of treating a subject with an alkaline phosphatase deficiency, comprising: administering a therapeutically effective amount of an alkaline phosphatase; and monitoring the concentration of one or more bone mineralization analytes, wherein the monitoring the concentration of one or more bone mineralization analytes is indicative for at least one additional treatment regimen for the subject. A non-limiting example for all methods described herein provides that the one or more bone mineralization analytes is at least one analyte selected from the group consisting of: vitamin D, Ca^{2+}, and parathyroid hormone. A non-limiting example for all methods described herein provides that the alkaline phosphatase deficiency is hypophosphatasia. A non-limiting example for all methods described herein provides that the alkaline phosphatase is a tissue non-specific alkaline phosphatase, a placental alkaline phosphatase, an intestinal alkaline phosphatase, an engineered alkaline phosphatase, a fusion protein comprising an alkaline phosphatase moiety, or a chimeric alkaline phosphatase. A non-limiting example for all methods described herein provides that the alkaline phosphatase is asfotase alfa (STRENSIQ®) (see, *e.g.*, U.S. Patent No. 7,763,712; International Pub. No. WO 2005/103263, both herein incorporated by reference in their entirety). A non-limiting example for all methods described herein provides that the bone mineralization analyte is Ca^{2+}. A non-limiting example for all methods described herein provides that the subject is determined to be hypocalcemic, the method further comprising treating the subject with a therapeutically effective amount of calcium gluconate, calcium chloride, calcium arginate, vitamin D or a vitamin D analog or parathyroid hormone or a fragment or analog thereof. A non-limiting example for all methods described herein provides that the subject is determined to be hypercalcemic, the method further comprising treating the subject with a therapeutically effective amount of a calcimimetic, a bisphosphonate, prednisone, intravenous fluids, or a diuretic. A non-limiting example for all methods described herein provides that the calcimimetic is cinacalcet. A non-limiting example for all methods described herein provides that the bone mineralization analyte is parathyroid hormone. A non-limiting example for all methods described herein provides that the subject has a statistically significantly low serum concentration of parathyroid hormone, the method further comprising administering a therapeutically effective amount of calcium or vitamin D. A non-limiting example for all methods described herein provides that the subject has a statistically significantly high serum concentration of parathyroid hormone, the method further comprising treating the subject with surgery or by administering a therapeutically effective amount of a calcimimetic, parathyroid hormone or an analog thereof, or a bisphosphonate. A non-limiting example for all methods described herein provides that the calcimimetic is cinacalcet. A non-limiting example for all methods described herein provides that the bone mineralization analyte is vitamin D. A non-limiting example for all methods described herein provides that the subject has a statistically significantly low serum concentration of vitamin D, the method further comprising administering a therapeutically effective amount of vitamin D or an analog thereof.

BRIEF DESCRIPTION OF THE DRAWING(S)

FIG. 1 shows the mean results for serum PTH (Intact pmol/L) over time by disease onset (HPP phenotype) and pooled safety set for all clinical trials (N=71). The time axis shows length of treatment with asfotase alfa in weeks. "Intact" indicates full-length PTH (not the PTH fragment). Bars at each timepoint represent 95% confidence intervals.

FIG. 2 shows mean laboratory test results over time for phosphate (mmol/L). The time axis refers to length of treatment with asfotase alfa in weeks. Bars at each timepoint represent 95% confidence intervals.

FIG. 3 shows mean laboratory test results over time for 25-hydroxyvitamin D (mmol/L). The time axis refers to length of treatment with asfotase alfa in weeks. Bars at each time point represent 95% confidence intervals.

FIG. 4 shows mean results for calcium (mmol/L) over time by disease onset and overall safety set. The time axis refers to length of treatment with asfotase alfa in weeks. Bars at each timepoint represent 95% confidence intervals.

FIG. 5 shows calcium (top panel) and PTH levels (lower panel) with reference ranges for a single patient during treatment with asfotase.

FIG. 6 shows the mean results for serum PTH (Intact, pmol/L) over time through week 312 by disease onset (HPP phenotype) and overall safety set. The time axis shows length of treatment with asfotase alfa in weeks. "Intact" indicates full length PTH (not the PTH fragment). Bars at each timepoint represent 95% confidence intervals.

FIG. 7 shows mean laboratory test results over time through week 312 for phosphate (mmol/L). The time axis refers to length of treatment with asfotase alfa in weeks. Bars at each timepoint represent 95% confidence intervals.

FIG. 8 shows mean laboratory test results over time through week 312 for 25 hydroxyvitamin D (mmol/L). The time axis refers to length of treatment with asfotase alfa in weeks. Bars at each time point represent 95% confidence intervals.

FIG. 9 shows mean results for calcium (mmol/L) over time through week 312 by disease onset and overall safety set. The time axis refers to length of treatment with asfotase alfa in weeks. Bars at each timepoint represent 95% confidence intervals.

FIG. 10 shows the patient values for calcium (mmol/L) and PTH (pmol/L) as a function of treatment week for the patient of FIG. 5. Vertical lines mark the start and end of 3 mg/kg/week dosing and the start of 6 mg/kg/week dosing.

FIG. 11 shows the amino acid sequence of asfotase alfa monomer (SEQ ID NO: 1). Asfotase alfa exists as a dimer with inter-subunit disulfide bonds.

DETAILED DESCRIPTION

Described herein are materials and methods for monitoring and further treating subjects who are in need of treatment with an alkaline phosphatase or who are being treated with an alkaline phosphatase. The unexpected findings that additional analytes can be monitored to indicate additional treatment regimens led to the materials and methods described herein. Particular analytes can lead to additional treatments, for example, for hypocalcemia, hypercalcemia, osteoporosis, hyperparathyroidism, and vitamin D deficiency.

Various definitions are used throughout this document. Most words have the meaning that would be attributed to those words by one skilled in the art. Words specifically defined either below or elsewhere in this document have the meaning provided in the context of the present disclosure as a whole and as are typically understood by those skilled in the art. For example, as used herein, the singular forms “a,” “an,” and “the” include plural references unless the content clearly dictates otherwise. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Methods and materials are described herein for use in the present disclosure; other suitable methods and materials known in the art can also be used. In case of conflict, the present specification, including definitions, will control.

The materials and methods described herein relate to monitoring and further treating subjects who are in need of alkaline phosphatase (AP) replacement therapy or who are undergoing AP replacement therapy. The terms “individual,” “subject,” “host,” and “patient” are used interchangeably and refer to any subject for whom diagnosis, treatment, or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses and the like. APs are responsible for dephosphorylating a variety of enzymes, and at least one isoform is substantially involved in bone mineralization and formation.

There are at least three APs in humans- intestinal (ALPI), placental (ALPP) and tissue non-specific (TNAP; sometimes referred to as liver/bone/kidney AP or ALPL), in addition to germline AP. TNAP is a membrane-anchored AP that is active extracellularly. Defects in TNAP result in, for example, elevated blood and/or urine levels of three phosphocompound substrates: inorganic pyrophosphate (PPi), phosphoethanolamine (PEA) and pyridoxal-5’-phosphate (PLP) (Whyte, M., *Endocr. Rev.*, 15:439-61, 1994). TNAP is primarily responsible for regulating serum PPi levels (major inhibitor of hydroxyapatite crystal deposition in the bone matrix), and, therefore, is important for bone formation and mineralization. Genetic defects in TNAP, for example, lead to diseases, conditions, or disorders associated with low or decreased bone mineralization symptoms, *e.g.*, hypophosphatasia (HPP).

Defects in TNAP activity can lead to a variety of diseases, disorders, and symptoms. Hypophosphatasia (HPP), for example, is a rare, heritable form of rickets or osteomalacia (Whyte, M. “Hypophosphatasia,” In *The Metabolic and Molecular Bases of Disease*, 8th ed., 5313-29, Eds C. Scriver, A. Beaudet, W. Sly, D. Valle & B. Vogelstein. New York: McGraw-Hill Book Company, 2001). HPP is caused by loss-of-function mutation(s) in the gene (ALPL) that encodes TNAP (Weiss, M. *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:7666-9, 1988; Henthorn, P. *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:9924-8, 1992; Henthorn, P. & Whyte, M., *Clin. Chem.*, 38:2501-5, 1992; Zurutuza, L. *et al.*, *Hum. Mol. Genet.*, 8:1039-46, 1999). The biochemical hallmark is subnormal AP activity in serum (hypophosphatasemia).

HPP is an ultra-rare genetic disorder whereby TNAP activity is either absent or barely detectable in affected patients. While differences in patterns of inheritance and mutations cause variability in age at symptom onset and disease severity, all HPP patients share the same primary pathophysiological defect, the failure to mineralize bone matrix (resulting in rickets or osteomalacia) due to lack of TNAP. This primary defect in infants and children, alone or in combination with associated metabolic disturbances, can lead to deformity of bones, impaired growth, and decreased motor performance. This primary pathophysiological mechanism can rapidly lead to progressive damage to multiple vital organs, seizures due to a CNS deficiency in functional vitamin B6, and developmental delays. Subjects with HPP, left untreated, can develop, for example, hypercalcemia, and hyperphosphatemia.

All forms of HPP share the same underlying genetic and biochemical defect; however, the diagnosis of HPP actually encompasses a spectrum of disease. Published classifications of HPP have historically taken into account the age at which clinical manifestation(s) first appear, dividing the disease into the following categories: perinatal (onset *in utero* and at birth), infantile (onset post-natal to 6 months of age), juvenile (also described as childhood, onset from 6 months to 18 years), and adult (onset after 18 years of age). Other milder forms of the disease, including benign perinatal HPP and odontohypophosphatasia, have also been described.

HPP manifest *in utero* and may cause stillbirth. At the time of delivery, limbs may be shortened and deformed from profound skeletal hypomineralization, and radiographic examination often reveals an almost total absence of bony structures. Most patients with perinatal HPP have life-threatening disease, and death generally results from respiratory insufficiency due to pulmonary hypoplasia and poor functioning due to a rachitic chest. Patients with infantile-onset HPP often appear normal at birth but typically present with skeletal abnormalities and failure to thrive within the first six months life. These patients can have a flail chest from rachitic deformity of the thorax; and, together with rib fractures, this may predispose them to pneumonia and respiratory compromise. Mortality, usually due to pulmonary complications, has been reported to be as high as 50% (Whyte M. Hypophosphatasia. In: Glorieux FH, Pettifor JM, Juppner H, editors. Pediatric Bone: Biology and Diseases. London, UK, Academic Press; 2012: pp. 771-94; Caswell A. *et al.*, *Crit. Rev. Clin. Lab. Sci.*, 28:175-232, 1991). Other clinical features may include, for example, functional craniosynostosis with resultant increased intracranial pressure and papilledema, and non-traumatic fractures. Hypercalcemia and hypercalciuria are also common, and nephrocalcinosis with renal compromise may occur. Weakness and delayed motor development are also common complications of infantile-onset HPP and seizures may occur secondary to vitamin B6 deficiency in the central nervous system.

In juvenile-onset patients, radiographs of the long bones often reveal focal bony defects that project from the growth plates into the metaphyses, sometimes described as "tongues" of radiolucency. Physeal widening, irregularities of the provisional zones of calcification, and metaphyseal flaring with areas of radiolucency adjacent to areas of osteosclerosis may also be present. Premature bony fusion of cranial sutures has also been observed in some patients, leading to potential increased intracranial pressure, proptosis, and cerebral damage. Rachitic deformities, including, for example, beading of the costochondral junctions, either bowed legs or knock-knees, and enlargement of the wrists, knees, and ankles from flared metaphyses, are common, and often result in short stature. Walking is frequently delayed, and a nonprogressive myopathy characterized by limb weakness, especially of the proximal muscles of the lower extremities, has also been described (Seshia, S. *et al.*, *Arch. Dis. Child.*, 65:130-1, 1990). Skeletal pain and stiffness may also be present and non-traumatic fractures are common. Nephrocalcinosis may develop in juvenile-onset HPP as well.

First signs of HPP may also present later in life (as in the adult form of HPP); however, upon questioning, many adult patients report a history of early tooth loss or rickets during childhood. In adult HPP, hypomineralization manifests as osteomalacia. Adult HPP patients are subject to recurrent, poorly healing fractures, often in the metatarsals and/or femur. Complaints of pain in the thighs and hips from subtrochanteric femoral pseudofractures are also common. Radiographs often reveal the presence of osteopenia and chondrocalcinosis. In some patients, deposition of calcium pyrophosphate dehydrate occurs, leading to PPi arthropathy. Although adult HPP has been described as 'mild', manifestations of the disease in adults can be severe and debilitating, often requiring multiple surgeries and the use of supportive devices to perform activities of daily living.

Subjects with a defect in an endogenous AP, *e.g.*, TNAP, are in need if AP enzyme replacement therapy (ERT). AP-ERT has been successful, for example, in treating HPP. ERT replaces an enzyme in subjects in whom that particular enzyme is deficient or absent. ERT does not affect the underlying genetic defect, but increases the concentration of enzyme in which the patient is deficient. The copy of the enzyme to be replaced, for example, can be a copy of the endogenous enzyme, an isoform of the enzyme, an ortholog of the enzyme, a chimeric version of the enzyme, a fusion protein with the relevant active site of the enzyme or an otherwise engineered version of the enzyme. ERT can be accomplished, for example, by providing the enzyme itself or by causing the enzyme to be expressed in particular tissues or cells of the subject (*e.g.*, through gene therapy methods, mRNA methods, transcriptional or translational activation methods, etc.).

Asfotase alfa or STRENSIQ®, for example, is a dimeric fusion protein that comprises two monomers with a TNAP phosphatase domain fused to an Fc chain and a bone tag to target the molecule to bone. The APs described herein can be, for example, intact native proteins, modified proteins or fusion proteins. Fusion proteins can comprise, for example, sequences to stabilize the protein, increase residence time in a patient, and/or target the fusion protein to a particular tissue, *e.g.*, bone. Fusion proteins, for example, can comprise Fc domains or albumin moieties. Bone tags are typically negatively charged regions, *e.g.*, poly-aspartate or poly-glutamate sequences, *e.g.*, between about 5 to about 50, between about 10 to about 25, between about 67 to about 30, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50 or more aspartates, glutamates, or other negatively charged amino acids (natural or non-naturally occurring).

As described herein, treatment of a subject with AP-ERT can result in, for example, hypocalcemia, hyperparathyroidism, hypophosphatemia, vitamin D deficiency, and/or symptoms or side effects thereof. Such situations can occur, for example, in cases where the mineral defect is profound and availability of calcium and phosphorous for formation of hydroxyapatite is not adequate (*e.g.*, not enough supplementation in food, not enough utilization of the minerals available in the food or profound loss through urine). Treatment of one or more of these effects can lead to "overcorrection" of the effect, and, therefore, require additional treatment to reverse the overcorrection. As described herein, monitoring of calcium, PTH, phosphate and vitamin D, therefore, can improve the treatment of a subject in need of or being treated with AP-ERT.

Described herein are also materials and methods for identifying subjects who, prior to or at the time of treatment, for example, AP-ERT, need to undergo treatment to normalize one or more metabolites associated with bone mineralization (*e.g.*, PTH, Ca^{2+}, vitamin D, and/or phosphate). A subject in need of AP-ERT, for example, who is hypocalcemic prior to AP-ERT treatment, would benefit from having normalized calcium levels prior to AP-ERT treatment.

Routine urinalysis and serum hematology and chemistries, for example, can be obtained before, during and after treatment using AP-ERT (*e.g.*, treatment with asfotase alfa). Calcium and phosphate metabolism should be monitored periodically with measurements of serum calcium, phosphate and PTH levels and urinary calcium excretion. Dietary intake of calcium should be adjusted according to PTH levels and urinary calcium levels (ionized and adjusted for other markers, *e.g.*, creatinine or albumin). When using asfotase alfa in patients with hypomineralization, *e.g.*, with HPP rickets or osteomalacia, it is useful to monitor calcium concentration closely, as rapid intake of calcium into the bone matrix can result in episodes of hypocalcemia. In certain examples, this is particularly relevant during the initial month or months of treatment. To prevent sequelae of hypocalcemia, including potential hypocalcemia-induced seizures, supplementation of calcium, or treatment with calcimimetics, for example, can be useful for those patients whose calcium levels are statistically significantly low or high.

As used herein, an “engineered” molecule is one that can be isolated from natural sources, synthesized and/or modified chemically. If the engineered molecule is a biological molecule, an engineered molecule can be one that is mutagenized, fused to a second molecule, *e.g.*, forming a fusion protein, attached to a specific functional moiety, *e.g.*, a targeting domain, purification domain, active site, etc., humanized, or made into a chimeric protein by switching particular domains with other proteins or isoforms. The engineering is the process of modifying the molecule in a particular manner to achieve a desirable result.

As used herein, “fusion protein” refers to an engineered protein that comprises residues of moieties from two or more different proteins. Fusion genes, which can be used to generate fusion proteins, are created through the joining of two or more coding sequences that code for separate proteins. Translation of a fusion gene results in a single or multiple polypeptides with functional properties derived from each of the original proteins. Recombinant fusion proteins are created by recombinant DNA technology.

As used herein, “chimeric proteins” are proteins that comprise moieties from at least two distinct proteins. The term refers to hybrid proteins made of polypeptides having different functions or physicochemical patterns.

The subjects described herein have an AP activity defect. Such a defect can arise, for example, due to a genetic anomaly (*e.g.*, a mutation) that causes the AP enzyme to not be produced or to be produced in an inactive form. Although such a defect can occur in any of the AP isoforms, of particular interest for the present disclosure are AP defects that lead to bone mineralization defects, *e.g.*, HPP.

Described herein are findings indicating that patients who are in need of or who are undergoing treatment, *e.g.*, ERT-AP treatment, for a bone mineralization disease, disorder, condition or symptoms thereof, *e.g.*, HPP, can be monitored for one or more analytes that are indicative of the need for additional treatments or a need to alter the current treatment regimen, *e.g.*, alter the dosage and/or frequency of dosage.

"Treatment" refers to the administration of a therapeutic agent or the performance of medical procedures with respect to a patient or subject, for any of prophylaxis (prevention), cure, or reduction of the symptoms of the disease, disorder, condition, or symptoms from which the subject suffers.

The treatments (therapies) described herein can also be part of "combination therapies." Combination therapy can be achieved by administering two or more agents, each of which is formulated and administered separately, or by administering two or more agents in a single formulation. One active ingredient can be, for example, useful for treating, for example, a disease, disorder, condition or symptoms associated with a TNAP defect, *e.g.*, hypophosphatasemia, or symptoms associated with treatment by the active agent ("side effects"). Other combinations are also encompassed by combination therapy. For example, two or more agents can be formulated together and administered in conjunction with a separate formulation containing a third agent. While the two or more agents in the combination therapy can be administered simultaneously, they need not be. For example, administration of a first agent (or combination of agents) can precede administration of a second agent (or combination of agents) by minutes, hours, days or weeks. Thus, the two or more agents can be administered within minutes of each other or within any number of hours of each other or within any number or days or weeks of each other.

As used herein, a "therapeutically effective dosage" or "therapeutically effective amount" results in a decrease in severity of disease, disorder, condition or symptoms thereof (*e.g.*, associated with aberrant AP activity, *e.g.*, HPP), an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction.

As described herein, treatment with AP replacement therapy is more effective or leads to overall improved health or quality of life, when the treated subject is further monitored for one or more additional analytes. PTH, calcium (Ca^{2+}), phosphate and vitamin D concentrations can each be monitored or individually monitored during AP-ERT, and the specific concentrations are indicative of, for example, efficacy of treatment and/or the need for one or more additional therapeutic regimen(s).

PTH acts on osteoblasts in bone and tubular cells within the kidney via G-protein-linked receptors that stimulate adenylate cyclase production of cyclic AMP. In bone, within one or two hours, PTH stimulates a process known as osteolysis in which calcium in the minute fluid-filled channels (canaliculi/lacunae) is taken up by syncytial processes of osteocytes and transferred to the external surface of the bone and, hence, into the extracellular fluid. Some hours later, it also stimulates resorption of mineralized bone; a process that releases both Ca^{2+} and phosphate into the extracellular fluid.

Monitoring PTH concentration in a sample obtained from a subject, for example, is of interest for better treating the subject, as AP-ERT can have an effect on PTH concentration. A determination that the treated subject's serum PTH concentration is statistically significantly lower or higher than normal, for example, can lead to revised treatment plans (*e.g.*, combining the AP-ERT plan with one or more therapeutic agents for treating, for example, hyperparathyroidism, *e.g.*, with cinacalcet). In cases where PTH concentration is determined to be statistically significantly high, *e.g.*, hyperparathyroidism, the subject can be treated with higher levels of the AP-ERT, *e.g.*, asfotase alfa, to reduce PTH levels in the case where a patient does not show good response in term of bone mineralization to initial dose.

As used herein, the term "sample" refers to biological material from a subject. Although serum concentration is of interest, samples can be derived from many biological sources, including, for example, single cells, multiple cells, tissues, tumors, biological fluids, brain extracellular fluid, biological molecules or supernatants or extracts of any of the foregoing. Examples include tissue removed for biopsy, tissue removed during resection, blood, urine, lymph tissue, lymph fluid, cerebrospinal fluid, amniotic fluid, mucous and stool samples. The sample used will vary based on the assay format, the detection method and the nature of the tumors, tissues, fluids, cells or extracts to be assayed. Methods for preparing samples are known in the art and can be readily adapted to obtain a sample that is compatible with the method utilized.

As used herein, "statistical significance" is a statistical term that informs as to the certainty that a difference or relationship exists, *e.g.*, that a sample value id statistically significantly different from a normal or baseline value. It is conferred by finding a low probability of obtaining at least as extreme results given that the null hypothesis is true. It is an integral part of statistical hypothesis testing where it determines whether a null hypothesis can be rejected. In any experiment or observation that involves drawing a sample from a population, there is the possibility that an observed effect would have occurred due to sampling error alone. But if the probability of obtaining at least as extreme result (large difference between two or more sample means), given the null hypothesis is true, is less than a pre-determined threshold (*e.g.*, 5% chance), then an investigator can conclude that the observed effect actually reflects the characteristics of the population rather than just sampling error. A test for statistical significance involves comparing a test value to some critical value for the statistic. The procedure to test for significance is the same- decide on the critical alpha level (*i.e.*, the acceptable error rate), calculate the statistic and compare the statistic to a critical value obtained from a table. P-values, the probability of obtaining the observed sample results (or a more extreme result) when the null hypothesis is actually true, are often coupled to a significance or alpha (α) level, which is also set ahead of time, usually at about 0.05 (5%). Thus, if a p-value was found to be less than about 0.05, then the result would be considered statistically significant and the null hypothesis would be rejected. Other significance levels, such as about 0.1, about 0.075, about 0.025 or about 0.01 can also be used. As used herein, a "statistically significantly low" concentration of an analyte is one that is lower than the normal or baseline situation for a control, *e.g.*, healthy, subject. Conversely, a "statistically significantly high" concentration of an analyte is one that is higher than the normal or baseline concentration of the analyte for a control subject.

PTH promotes osteoclast function and leads to bone resorption, thereby increasing serum Ca^{2+} and phosphate concentrations. Low levels of serum Ca^{2+} fail to exhibit negative feedback effect on the release or production of PTH from the thyroid, whereas high concentrations of serum Ca^{2+}, by exhibiting a negative feedback effect on release and/or production of PTH, lead to decreased PTH levels in blood. In a related pathway, vitamin D increases adsorption of Ca^{2+} and phosphate in the intestine, leading to elevated levels of serum Ca^{2+} and, therefore, lower bone resorption. Effects of active vitamin D (1, 25 $(OH)_2D$ on bone, however, are diverse and can affect formation or resorption.

Although, hypoparathyroidism (hypoPT) is one of the few major hormone deficiency diseases that is often not treated with the missing hormone, hormone replacement therapies are available. Bovine PTH has been purified and used as experimental treatment, however utility as a treatment was diminished, mainly because of antibody formation and costs. Approval of fully humanized truncated PTH (Teriparatide, PTH (1-34)) and intact parathyroid hormone (Preotact, PTH(1-84)) for treatment of osteoporosis, has made the PTH drugs more accessible and thereby made clinical trials with PTH treatment of hypoPT feasible. Patients with hypoPT experience an improved quality of life when treated with PTH compared with conventional treatment with 1α-hydroxylated vitamin D metabolites and calcium supplements, although hypoPT is still treated, for example, by supplementing calcium and/or vitamin D.

Primary hyperparathyroidism results from a hyperfunction of the parathyroid glands. Over secretion of PTH can be due, for example, to a parathyroid adenoma, parathyroid hyperplasia or a parathyroid carcinoma. This disease is often characterized by the presence of kidney stones, hypercalcemia, constipation, peptic ulcers and depression.

Secondary hyperparathyroidism is due to physiological secretion of PTH by the parathyroid glands in response to hypocalcemia (low blood calcium levels). The most common causes are vitamin D deficiency and chronic renal failure. Lack of vitamin D leads to reduced calcium absorption by the intestine leading to hypocalcemia and increased PTH secretion. This increases bone resorption. In chronic renal failure the problem is more specifically failure to convert vitamin D to its active form in the kidney. The bone disease in secondary hyperparathyroidism caused by renal failure is termed renal osteodystrophy.

Tertiary hyperparathyroidism is seen in patients with long-term secondary hyperparathyroidism, which eventually leads to hyperplasia of the parathyroid glands and a loss of response to serum calcium levels.

Quaternary and quintary hyperparathyroidism are rare conditions that may be observed after surgical removal of primary hyperparathyroidism, when it has led to renal damage that now again causes a form of secondary (quaternary) hyperparathyroidism that may itself result in autonomy (quintary) hyperparathyroidism. Additionally, quaternary hyperparathyroidism may ensue from hungry bone syndrome after parathyroidectomy.

Primary hyperparathyroidism can be treated, for example, by surgery (parathyroidectomy) if treatment, for example, with calcimimetics is unsuccessful. Secondary hyperparathyroidism can be treated, for example, by vitamin D supplementation and/or by the use of calcimimetics (*e.g.*, cinacalcet). Other forms of hyperparathyroidism are variations of secondary hyperparathyroidism, and treatments involve approaches similar to those used for primary and secondary hyperparathyroidism.

Low plasma calcium stimulates PTH release (by negating the inhibition of PTH release), and PTH acts to resorb Ca^{2+} from the pool in bone and to enhance renal re-absorption of Ca^{2+}. High plasma calcium stimulates calcitonin secretion, which lowers plasma calcium by inhibiting bone resorption.

Normal blood calcium level is between about 8.5 to about 10.5 mg/dL (2.12 to 2.62 mmol/L; some reports use the values of between about 8.0 to about 10.0 mg/dL) and that of ionized calcium is 4.65 to 5.25 mg/dL (1.16 to 1.31 mmol/L). Hypocalcemia or hypercalcemia is characterized by a statistically significantly low or high serum calcium concentration. Hypocalcemic subjects, for example, typically display a serum calcium concentration of about 2.5 mg/dL or lower (Sorell, M. & Rosen, J., *J. Pediatr.*, 87:67-70, 1975). A hypocalcemic subject, for example, can have serum calcium concentration of about 7.0 mg/dL or lower, about 5.0 mg/dL or lower, about 1.0 mg/dL or lower, or about 0.5 mg/dL or lower.

Common causes of hypocalcemia include hypoparathyroidism, vitamin D deficiency and chronic kidney disease. Symptoms of hypocalcemia include, for example, neuromuscular irritability (including tetany as manifested by Chvostek's sign or Trousseau's sign, bronchospasm), electrocardiographic changes and seizures. Treatment options include, for example, supplementation of calcium and some form of vitamin D or its analogues, alone or in combination. Intravenous calcium gluconate 10% can be administered, or if the hypocalcemia is severe, calcium chloride can be given. Other treatments involve multivitamin supplementation, in oral, chewable, or liquid forms.

Hypercalcemia is an elevated Ca^{2+} level in the blood, which is often indicative of other disease(s). It can be due to excessive skeletal calcium release, increased intestinal calcium absorption or decreased renal calcium excretion. The neuromuscular symptoms of hypercalcemia are caused by a negative bathmotropic effect due to the increased interaction of calcium with sodium channels. Since calcium blocks sodium channels and inhibits depolarization of nerve and muscle fibers, increased calcium raises the threshold for depolarization. Symptoms of hypercalcemia include, for example, renal or biliary stones, bone mineralization defects and bone pain, abdominal pain, nausea, vomiting, polyuria depression, anxiety, cognitive dysfunction, insomnia, coma, fatigue, anorexia and pancreatitis. Hypercalcemia is defined as a serum calcium level greater than about 10.5 mg/dL (>2.5 mmol/L). Hypercalcemia can also be classified based on total serum and ionized calcium levels, as follows: Mild: total calcium 10.5-11.9 mg/dL (2.5-3 mmol/L) or ionized calcium 5.6-8 mg/dL (1.4-2 mmol/L); Moderate: total calcium 12-13.9 mg/dL (3-3.5 mmol/L) or ionized calcium 5.6-8 mg/dL (2-2.5 mmol/L); Hypercalcemic crisis: total calcium: >14-16 mg/dL (3.5-4 mmol/L) or ionized calcium 10-12 mg/dL (2.5-3 mmol/L).

Hypercalcemia is treated a number of ways, including, for example, using fluids and diuretics for an initial therapy (hydration, increasing salt intake, and forced diuresis). Diuretic treatments include, for example, furosemide, and they can be given to permit continued large volume intravenous salt and water replacement while minimizing the risk of blood volume overload and pulmonary edema. In addition, loop diuretics tend to depress renal calcium reabsorption thereby helping to lower blood calcium levels. Caution must be taken to prevent potassium or magnesium depletion. Additional therapies include, for example, bisphosphonates, plicamycin, gallium nitrate, glucocorticoids and calcitonin. Bisphosphonates are pyrophosphate analogues with high affinity for bone, especially areas of high bone turnover.

They are taken up by osteoclasts and inhibit osteoclastic bone resorption. Available drugs include, for example, etidronate, tiludronate, IV pamidronate, alendronate, zoledronate, and risedronate. Calcitonin blocks bone resorption and also increases urinary calcium excretion by inhibiting renal calcium reabsorption. Phosphate therapy can correct the hypophosphatemia in the face of hypercalcemia and lower serum calcium. Calcium mimetics, *e.g.*, cinacalcet, are also used to lower serum calcium concentrations.

Hypovitaminosis D is a deficiency of vitamin D. It can result from inadequate nutritional intake of vitamin D coupled with inadequate sunlight exposure (in particular sunlight with adequate ultraviolet B rays), disorders that limit vitamin D absorption, and conditions that impair the conversion of vitamin D into active metabolites including certain liver, kidney, and hereditary disorders. Deficiency results in impaired bone mineralization and leads to bone softening diseases including rickets in children and osteomalacia and osteoporosis in adults. Maintenance doses of both calcium and vitamin D are often necessary to prevent further decline.

EXEMPLIFICATION

The following examples do not limit the scope of the invention as disclosed and described in the claims.

EXAMPLE 1. PTH and Calcium

When evaluating results by age at disease onset, mean and median PTH levels were notably higher in patients with infantile- and juvenile-onset HPP during the first 12 weeks of treatment compared with later time points, and were likely associated with the bone mineralization process and the monitoring thereof. In some cases, multivitamins, calcium, vitamin D, vitamin A, vitamin K, cinacalcet, pyridoxal phosphate calcium, and/or calcitonin were administered to patients in order to normalize PTH, calcium, and phosphate levels concomitantly with the monitoring process.

Mean PTH levels in patients with adult-onset HPP tended to be lower than those observed in the infantile- and juvenile-onset HPP patients through approximately Week 72. These comparisons, however, involved only two patients with adult-onset HPP. FIG. 1 provides the change in serum PTH over time in the clinical studies.

Patients were subdivided by Baseline PTH level, and the details of the changes in the initial period after the start of asfotase alfa treatment are as follows:

For the nine patients with low PTH at Baseline:

Seven patients with normal calcium levels at Baseline and lower calcium levels post-treatment had a rise in PTH. All except one patient showed radiological improvements, as determined by the RSS score (scoring of rickets).

One patient, who had high Baseline serum calcium, showed no change in serum calcium levels and no change in PTH by Week 6, at which time the patient was discontinued from the study. Note that this patient received only two doses of asfotase alfa and was subsequently withdrawn; therefore, no change in calcium or PTH was expected.

One patient with a high Baseline serum calcium level had normalization of serum calcium by Week 24. PTH data beyond Week 6 is not available for this patient (at Week 6, PTH was unchanged). This patient showed no improvements in rickets at Week 12 (7 weeks after asfotase alfa dose was increased), however showed a decrease in RSS score by Week 24.

For the13 patients with normal PTH at Baseline:

12 patients with normal PTH and normal calcium levels at Baseline responded with small or no change in serum calcium levels and no or slight change in PTH levels. All except 1 patient showed radiographic improvement.

One patient with calcium levels at the upper limit of normal at Baseline responded with normalization of calcium levels and large increase in PTH. This patient showed radiographic improvement.

Only one patient had high PTH at Baseline with normal calcium levels. The patient responded with lowering of serum calcium levels and large rise in PTH levels. This patient did not show radiological improvement during the periods of rise in PTH.

One patient had no available Baseline PTH results, but the earliest result at Week 6 showed values in the normal range. Calcium remained within normal ranges with some oscillation until Week 60. The patient showed worsening of rickets (RGI-C score at Week 12 was negative; -1.67) and growth scores on the initial dose of asfotase alfa of 6 mg/kg/week. After the dose was increased to 9 mg/kg/week due to continued worsening of growth delays, the patient showed improvement in radiographic signs (at Week 36, RSS score improved 4.5 points since week 24 assessment) and PTH increased above the normal range. PTH further increased more steeply until it peaked at Week 72. Note that calcium levels showed a drop at Week 48 (although were still within the normal limits) and then fell below normal at Week 60, however rebounded to normal at Week 72. In this patient, low vitamin D levels and low urine calcium/creatinine ratios were found coincident with the observed elevation of PTH level.

EXAMPLE 2. Phosphate

Mean serum phosphate values tended to be variable through Week 24 in all HPP onset categories, and then appeared to normalize and stabilize with continued treatment. Some decreases in serum phosphate levels appeared to coincide with decreases in serum calcium levels during the first several weeks of treatment, which likely was due to the intense bone mineralization processes that occurred early in treatment.

Three patients (infantile-onset) had a shift from normal values for phosphate at Baseline to low values during treatment, and 21 patients (15 infantile-onset; 5 juvenile-onset; 1 adult-onset) with low or normal values at Baseline had a shift to high values during treatment; at the last visit, no patients had shifted from normal or high values to low values, and 5 patients (infantile-onset) had shifted from normal values to high values; see FIG. 2.

EXAMPLE 3. Vitamin D

Mean 25-OH vitamin D values were consistently higher in patients with adult-onset HPP than in patients in the other HPP onset categories; however, these values did decrease slightly over time, and there were only 2 patients with adult-onset HPP included in the study. Mean and median 25-OH vitamin D values in the other HPP onset categories were relatively consistent over time; see FIG. 3. In patients where vitamin D values were monitored and identified as deficient or lower than desired by the clinician (i.e., less than than 20 ng/ml), vitamin D was supplemented with vitamin D in the form of an oral medication, i.e., as children's vitamins, adult multivitamins, or vitamin D capsules. Vitamin D in some cases was administered as an intramuscular injection or in combination dosages with calcium, vitamin A, and/or vitamin K. Calcitriol, cholecalciferol, and/or ergocalciferol were also administered as needed.

EXAMPLE 4.

Systematic analyses of pre- and post-treatment serum calcium, parathyroid hormone (PTH), phosphate and vitamin D were performed.

Calcium: serum calcium levels were variable at Baseline, ranging from 1.92 to 4.03 mmol/L. Although the changes in mean and median calcium levels over the course of treatment with asfotase alfa were not remarkable, levels tended to stabilize and become less variable; calcium levels ranged from 1.82 to 2.80 mmol/L at Week 24, and from 2.12 to 3.67 mmol/L at the last visit, basically eliminating the episodes of hypocalcemia and the range of hypercalcemia was lowered to nearly normal (*i.e.*, upper range is 3.5 mmol/L). Increases in calcium above the normal range were generally small and, when present, were most notable at Baseline and tended to normalize over the course of asfotase alfa treatment with the increased calcium deposition in the bone as noted on X-ray. When evaluating results by age at disease onset, the small increases in calcium were generally seen in patients with infantile-onset HPP, and calcium levels in these patients tended to normalize during treatment (Table 1 and FIG. 4).

Parathyroid Hormone: mean and median PTH levels increased with treatment, most notably during the first 12 weeks of treatment with asfotase alfa. This increase was likely due to a physiologic response secondary to increases in the bone mineralization process associated with asfotase alfa treatment. The variability in PTH levels noted at Baseline and throughout treatment may be due to factors that affect PTH levels, including, but not limited to: age, body mass index (BMI), serum creatinine levels, serum calcium levels and vitamin D levels. When evaluating results by age at disease onset, mean and median PTH levels were notably higher in patients with infantile- and juvenile-onset HPP during the first 12 weeks of treatment compared with later time points, and were likely associated with the bone mineralization process. Mean PTH levels in patients with adult-onset HPP tended to be lower than those observed in the infantile- and juvenile-onset HPP patients through approximately Week 72; there are several variables that can affect PTH levels (Table 1 and FIG. 1).

Phosphate: mean serum phosphate values were variable through Week 24 in patients with infantile-, juvenile- and adult-onset HPP, and then appeared to normalize and stabilize with continued treatment with asfotase alfa. Some decreases in serum phosphate levels appeared to coincide with decreases in serum calcium levels during the first several weeks of treatment, which were likely due to the intense bone mineralization processes occurring early in treatment (Table 1 and FIG. 2).

Vitamin D: changes over time for vitamin D were not clinically meaningful; some of the variability seen in vitamin D results may be reflective of concomitant vitamin D supplements taken by some patients. When evaluating results by age at disease onset, mean vitamin D values were consistently higher in patients with adult-onset HPP than in patients with infantile- or juvenile-onset HPP; however, higher values did decrease slightly over time. Mean and median vitamin D values in patients with infantile- and juvenile-onset HPP were relatively consistent over time (Table 1 and FIG. 3).

Table 1. Changes from Baseline to Week 24 and last visit for serum Ca^{2+}, PTH, phosphate and Vitamin D; pooled safety set overall.

Parameter Statistic	Baseline	Week 24	Change from Baseline to Week 24	Last Visit	Change from Baseline to Last Visit
Calcium (mmol/L)					
n	71	64	64	70	70
Mean (SD)	2.540 (0.2727)	2.487 (0.1580)	-0.055 (0.2459)	2.504 (0.2109)	-0.039 (0.2693)
Median	2.500	2.485	-0.025	2.470	-0.045
Range	1.92, 4.03	1.82, 2.80	-1.33, 0.38	2.12, 3.67	-1.33, 0.67
Parathyroid Hormone (pmol/L)					
n	57	61	51	69	56
Mean (SD)	2.68 (1.813)	3.41 (3.848)	0.86 (4.230)	3.66 (4.988)	0.73 (2.406)
Median	2.40	2.50	0.50	2.40	0.45
Range	0.6, 8.0	0.6, 27.9	-6.2, 26.7	0.6, 38.7	-4.4, 7.8
Phosphate (mmol/L)					
n	70	63	62	70	69
Mean (SD)	1.814 (0.3922)	1.900 (0.3520)	0.090 (0.3962)	1.771 (0.2967)	-0.042 (0.3960)
Median	1.870	1.970	0.025	1.760	-0.090
Range	0.42, 2.74	0.90, 2.50	-0.51, 1.49	1.00, 2.50	-0.71, 1.23
25-Hydroxy Vitamin D (pmol/mL)					
n	68	65	63	67	64
Mean (SD)	76.5 (28.99)	86.2 (33.58)	9.8 (35.56)	78.3 (25.08)	3.5 (35.32)
Median	77.0	80.7	4.0	75.0	-1.0
Range	17, 169	23, 212	-54, 135	18, 179	-83, 91

SD = standard deviation.

EXAMPLE 5.

One patient had a low serum calcium level at Baseline, but upon initiation of asfotase alfa treatment it further decreased, and was responsive to changes in asfotase alfa dosage changes. At Week 24 the dose was reduced from 6mg/kg/week to 3mg/kg/week and at Week 48 it was raised to 6mg/kg/week. During this period PTH levels initially rose but stayed within the normal range up to Week 72, when levels were temporarily elevated above the normal range. Simultaneously, calcium was raised and entered to the normal range. The patient showed signs of radiographic improvement starting at Week 12, although the RGI-C score did not reach 2 or above (meaning substantial improvement in radiographic signs of rickets). At Week 168, serum calcium fell below the normal range and PTH increased above the normal range, at which time six-minute walk test (6MWT) results showed a large drop compared with the previous result at Week 120. See FIG. 5 and FIG. 10.

EXAMPLE 6.

An additional year of data was subsequently collected for the patients contained in Examples1-5 which demonstrated continuation of the previously described trends, see FIGs. 6-9.

OTHER EMBODIMENTS

It is to be understood that while the present disclosure has been described in conjunction with the detailed description herein; the foregoing description is intended to illustrate and not limit the scope as defined by the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims. References cited in the Specification are herein incorporated by reference in their entireties.

CLAIMS

What is claimed is:

1. A method of treating a subject with an alkaline phosphatase deficiency, comprising: administering a therapeutically effective amount of an alkaline phosphatase; and monitoring the concentration of one or more bone mineralization analytes, wherein the monitoring the concentration of one or more bone mineralization analytes is indicative for at least one additional treatment regimen for the subject.

2. The method of Claim 1, wherein the one or more bone mineralization analytes is selected from the group consisting of: vitamin D, Ca^{2+}, and parathyroid hormone.

3. The method of any of the preceding claims, wherein the alkaline phosphatase deficiency is hypophosphatasia.

4. The method of any of the preceding claims, wherein the alkaline phosphatase is a tissue non-specific alkaline phosphatase, a placental alkaline phosphatase, an intestinal alkaline phosphatase, an engineered alkaline phosphatase, a fusion protein comprising an alkaline phosphatase moiety, or a chimeric alkaline phosphatase.

5. The method of any of the preceding claims, wherein the alkaline phosphatase is asfotase alfa (FIG. 1, SEQ ID NO: 1).

6. The method of any of the preceding claims, wherein the bone mineralization analyte is Ca^{2+}.

7. The method of any of the preceding claims, wherein the subject is determined to be hypocalcemic, the method further comprising treating the subject with a therapeutically effective amount of calcium gluconate, calcium chloride, calcium arginate, vitamin D, a vitamin D analog, or parathyroid hormone, or a fragment or analog thereof.

8. The method of any of the preceding claims, wherein the subject is determined to be hypercalcemic, the method further comprising treating the subject with a therapeutically effective amount of a calcimimetic, a bisphosphonate, prednisone, intravenous fluids, or a diuretic.

9. The method of Claim 8, wherein the calcimimetic is cinacalcet.

10. The method of any of the preceding claims, wherein the bone mineralization analyte is parathyroid hormone.

11. The method of any of the preceding claims, wherein the subject has a statistically significantly low serum concentration of parathyroid hormone, the method further comprising administering a therapeutically effective amount of calcium or vitamin D.

12. The method of any of the preceding claims, wherein the subject has a statistically significantly high serum concentration of parathyroid hormone, the method further comprising treating the subject with surgery or by administering a therapeutically effective amount of a calcimimetic, parathyroid hormone or an analog thereof, or a bisphosphonate.

13. The method of any of the preceding claims, wherein the calcimimetic is cinacalcet.

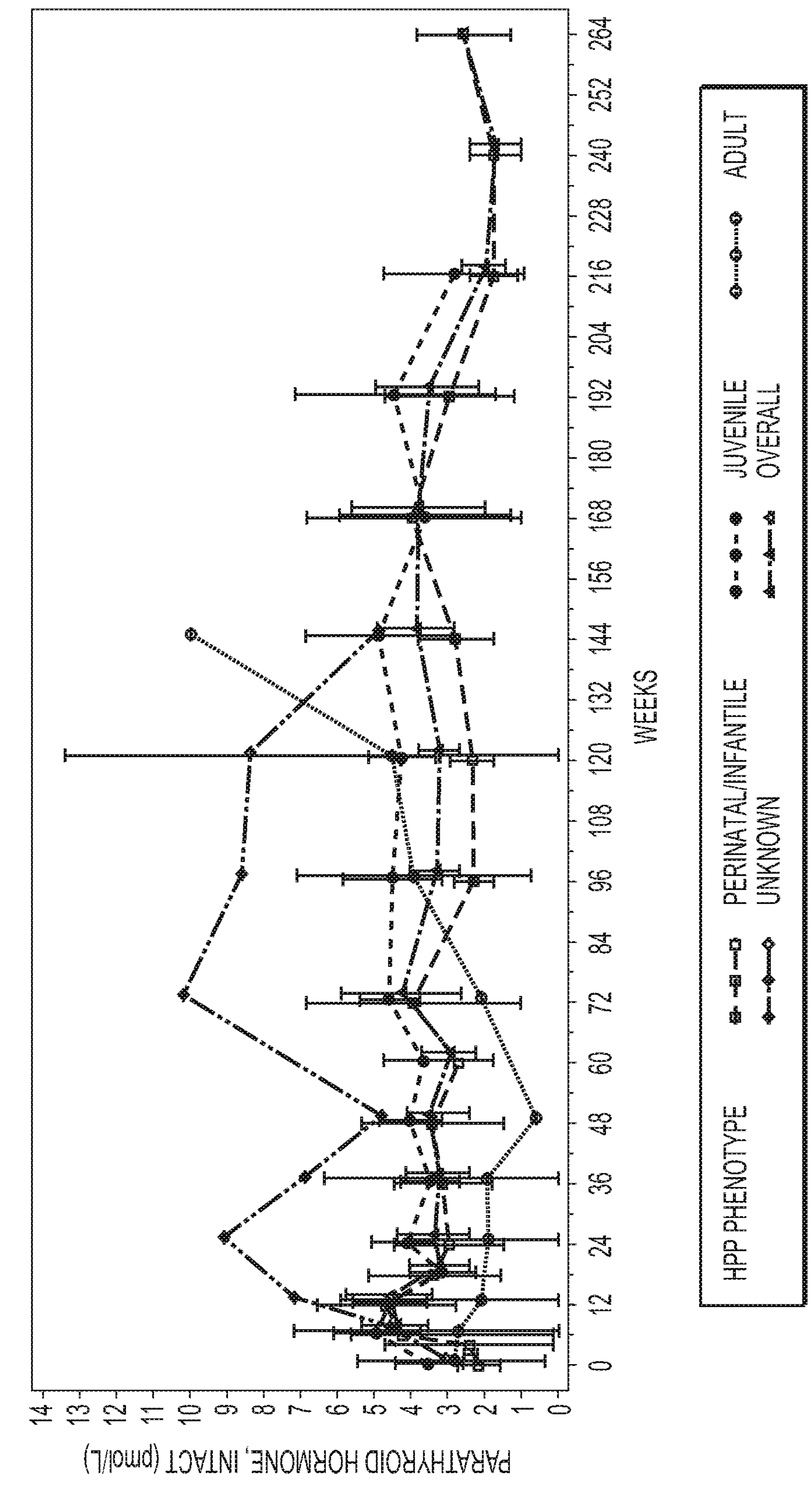
14. The method of any of the preceding claims, wherein the bone mineralization analyte is vitamin D.

15. The method of any of the preceding claims, wherein the subject has a statistically significantly low serum concentration of vitamin D, the method further comprising administering a therapeutically effective amount of vitamin D or an analog thereof.

16. The method of claim 3, further comprising administering a therapeutically effective amount of at least one of vitamin D, vitamin K, vitamin B, calcium, and a multivitamin.

17. The method of claim 3, further comprising administering an effective amount of at least one therapeutic treatment which decreases the amount of at least one of vitamin D, calcium, and PTH.

18. The method of claim 16 or claim 17, wherein the therapeutically effective amount is administered orally, intramuscularly, or intravenously.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4
5
6
7
8
9
10
11
12
13
14
WEEKS
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
192
204
216
228
240
252
264
HPP PHENOTYPE
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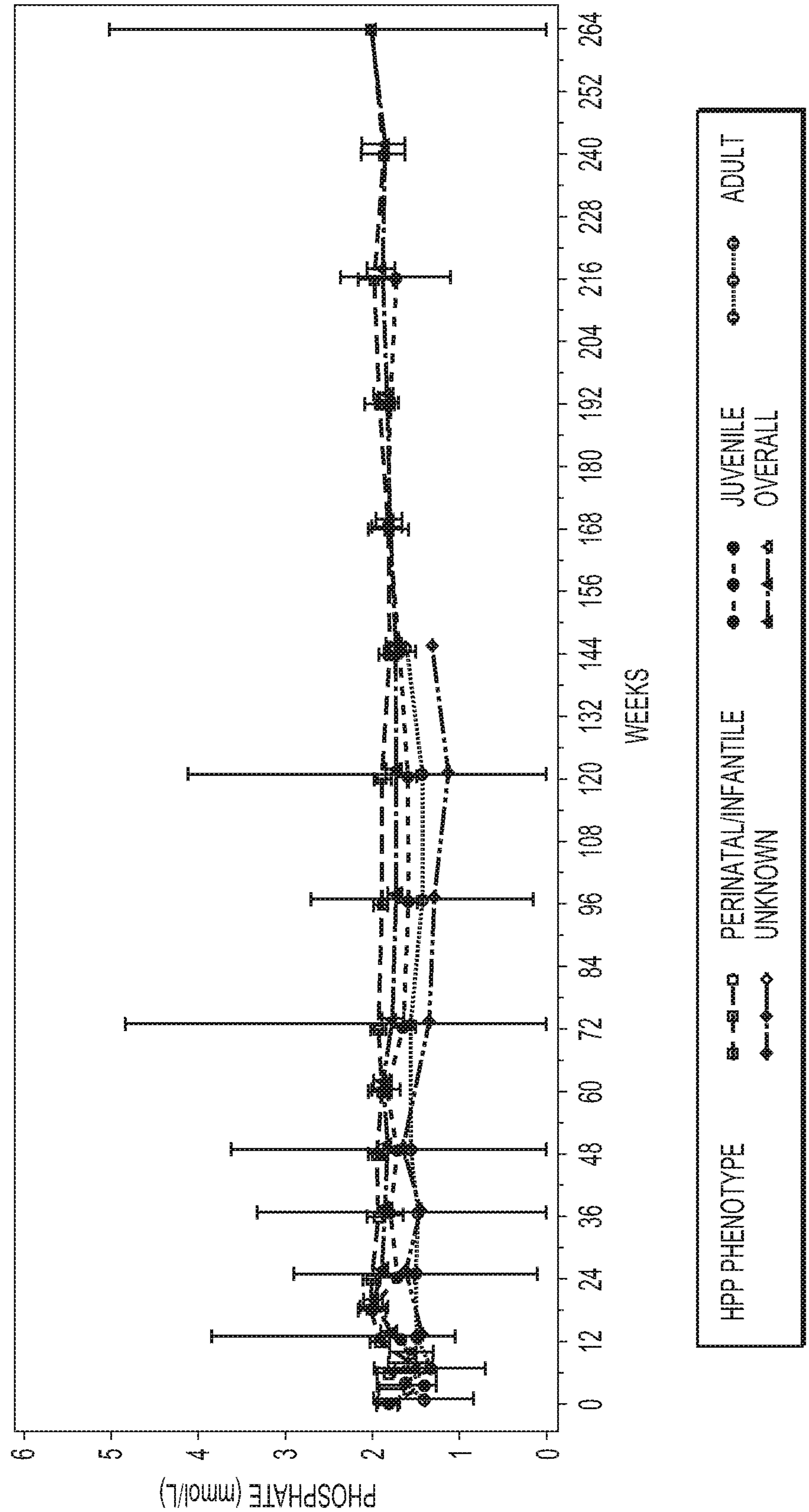
UNKNOWN
JUVENILE
OVERALL
ADULT

FIG. 1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1
2
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5
6
WEEKS
0
12
24
36
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108
120
132
144
156
168
180
192
204
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228
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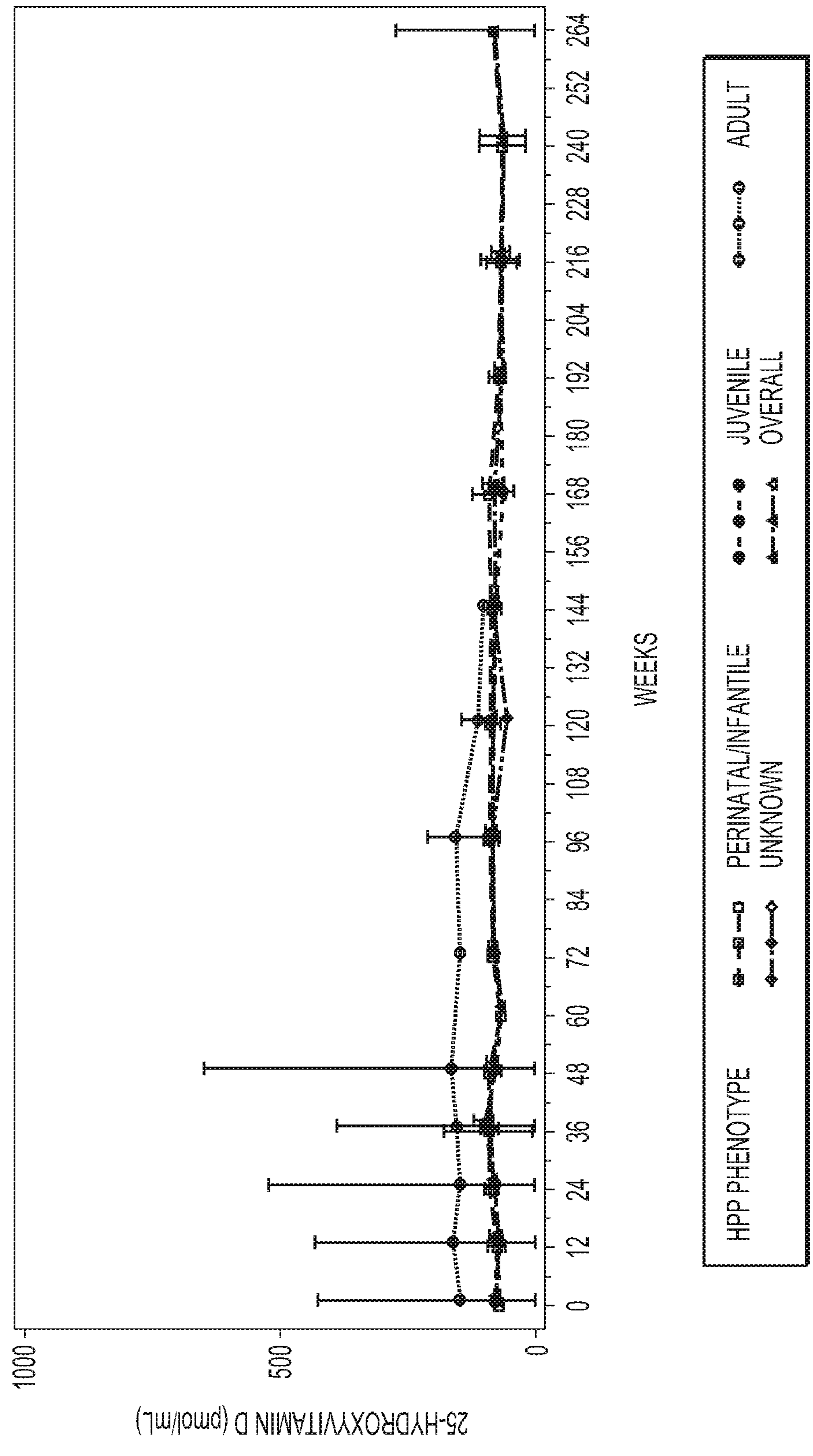
HPP PHENOTYPE
PERINATAL/INFANTILE
UNKNOWN
JUVENILE
OVERALL
ADULT

FIG. 2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1000
500
0
WEEKS
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
192
204
216
228
240
252
264
HPP PHENOTYPE
PERINATAL/INFANTILE
UNKNOWN
JUVENILE
OVERALL
ADULT

FIG. 3

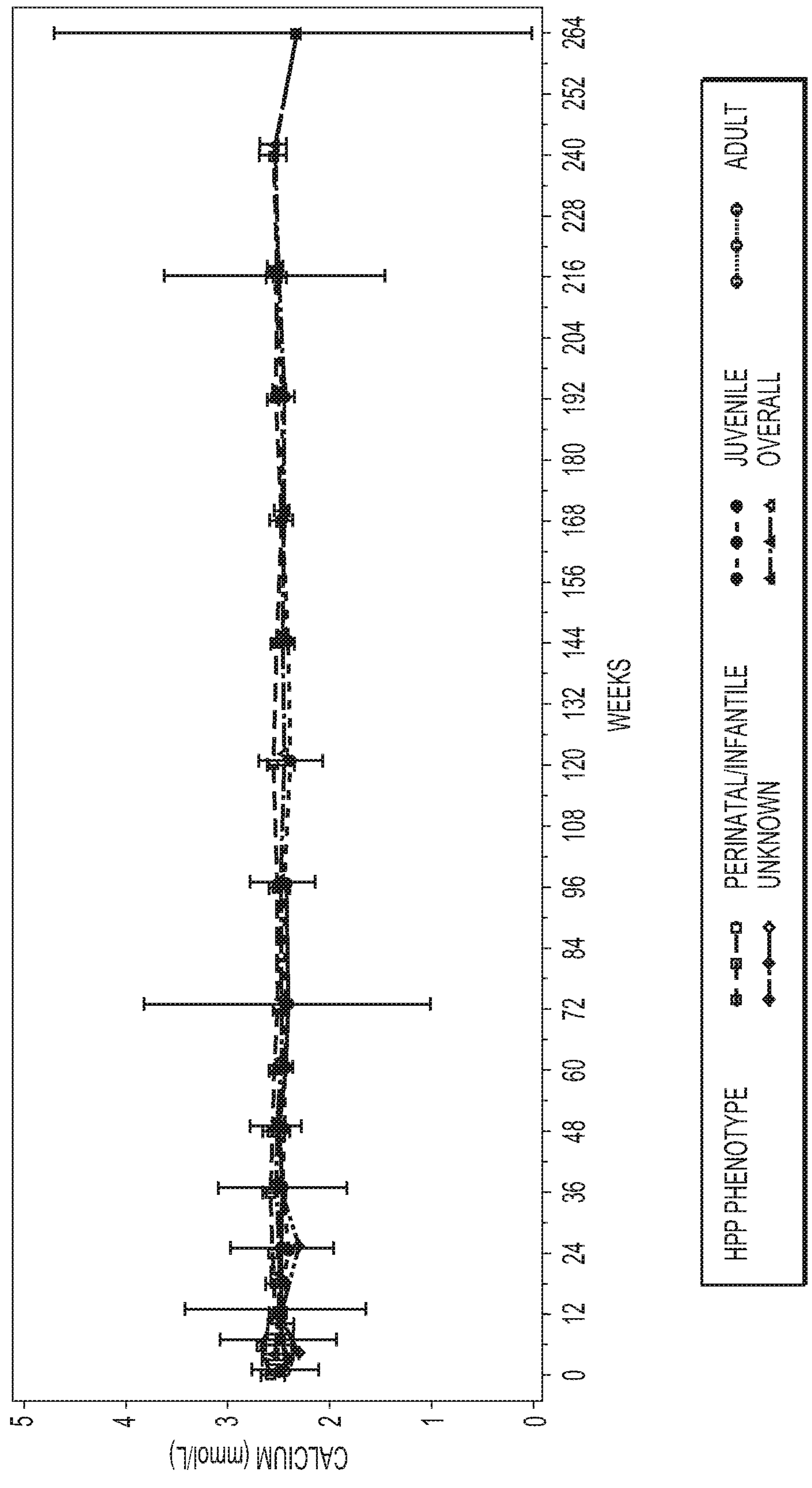

CALCIUM (mmol/L)
0
1
2
3
4
5
WEEKS
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
192
204
216
228
240
252
264
HPP PHENOTYPE
PERINATAL/INFANTILE
UNKNOWN
JUVENILE
OVERALL
ADULT

FIG. 4

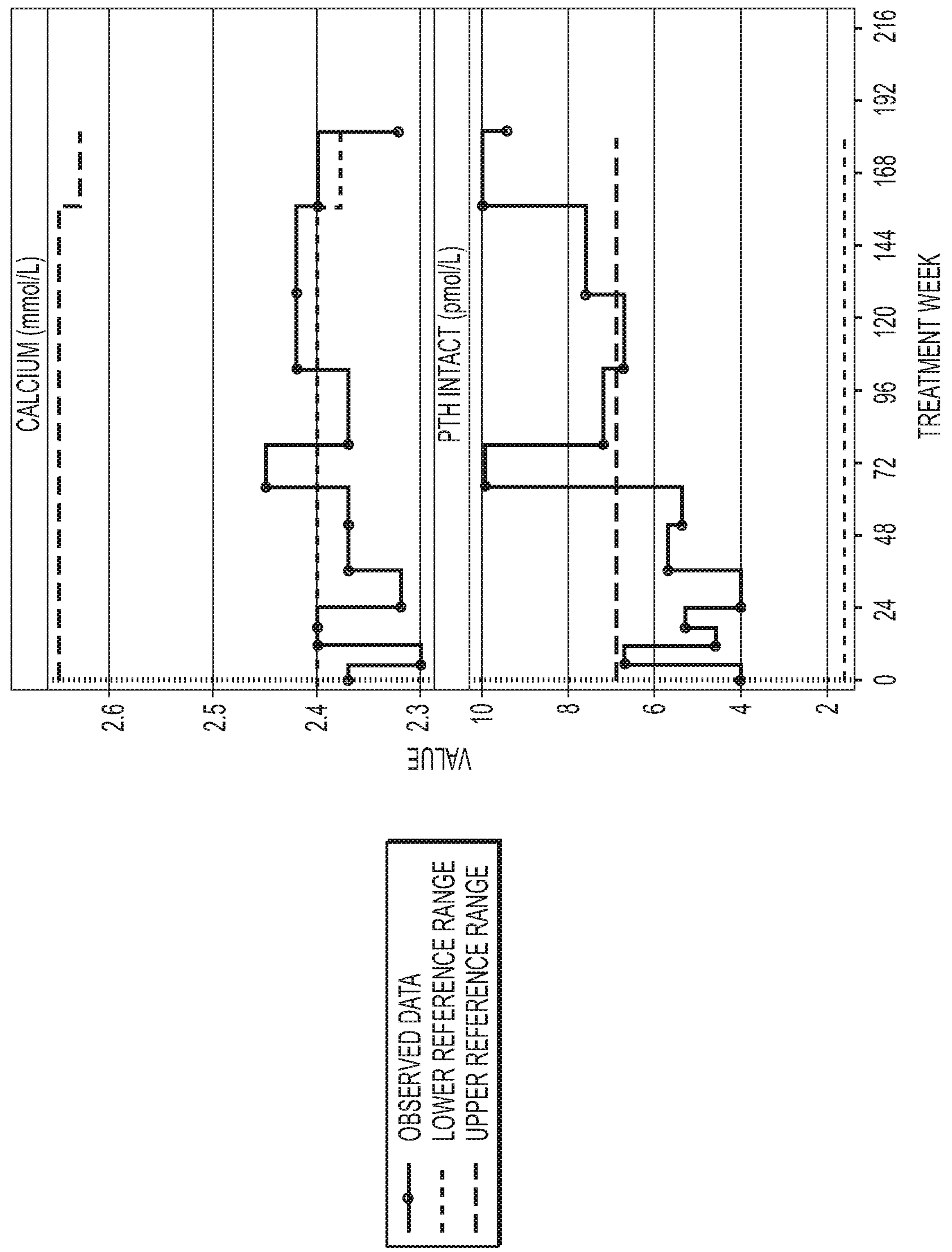

CALCIUM (mmol/L)
PTH INTACT (pmol/L)
VALUE
2.6
2.5
2.4
2.3
10
8
6
4
2
0
24
48
72
96
120
144
168
192
216
TREATMENT WEEK
OBSERVED DATA
LOWER REFERENCE RANGE
UPPER REFERENCE RANGE

FIG. 5

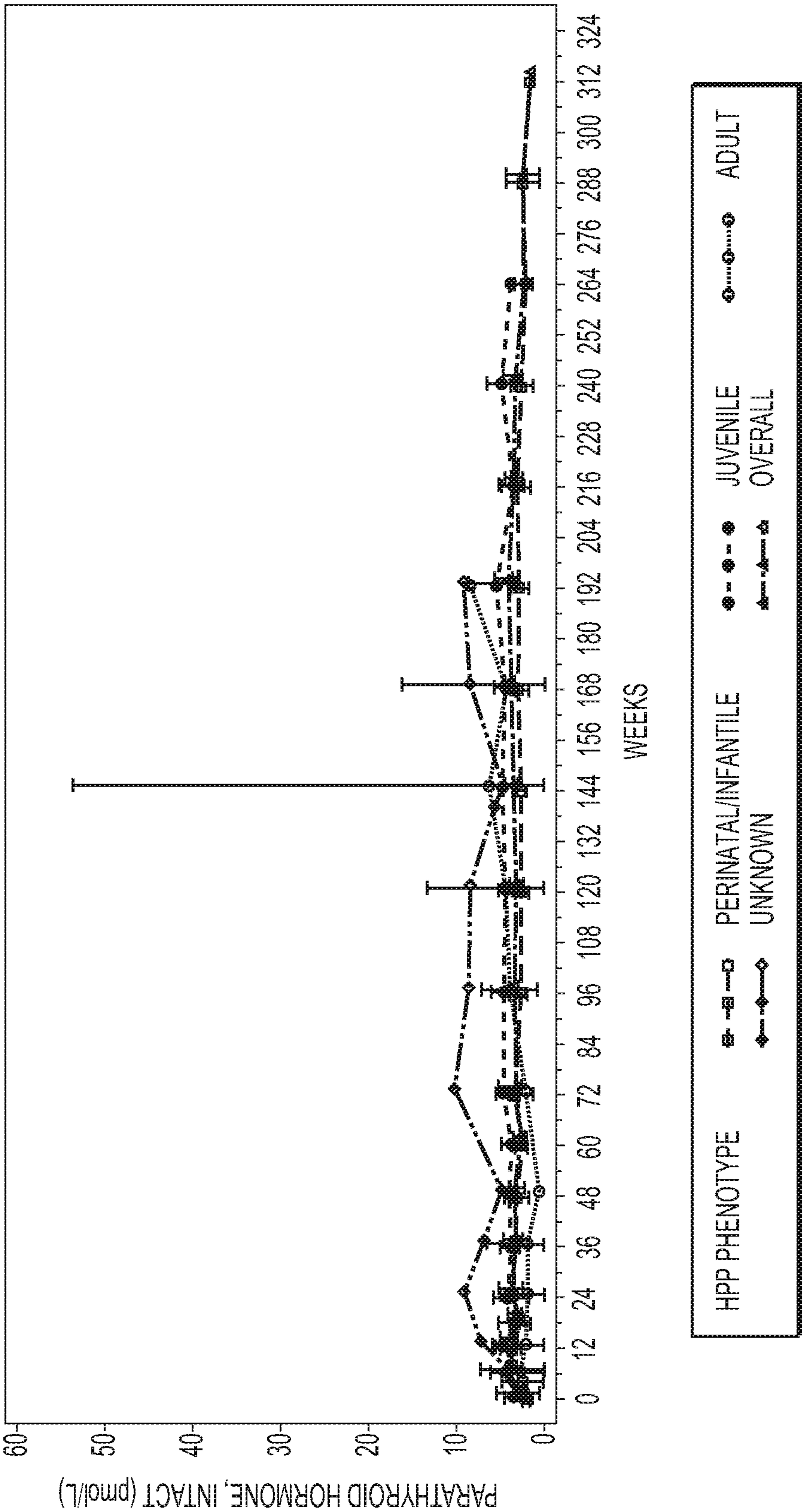

PARATHYROID HORMONE, INTACT (pmol/L)
WEEKS
HPP PHENOTYPE
PERINATAL/INFANTILE
UNKNOWN
JUVENILE
OVERALL
ADULT

FIG. 6

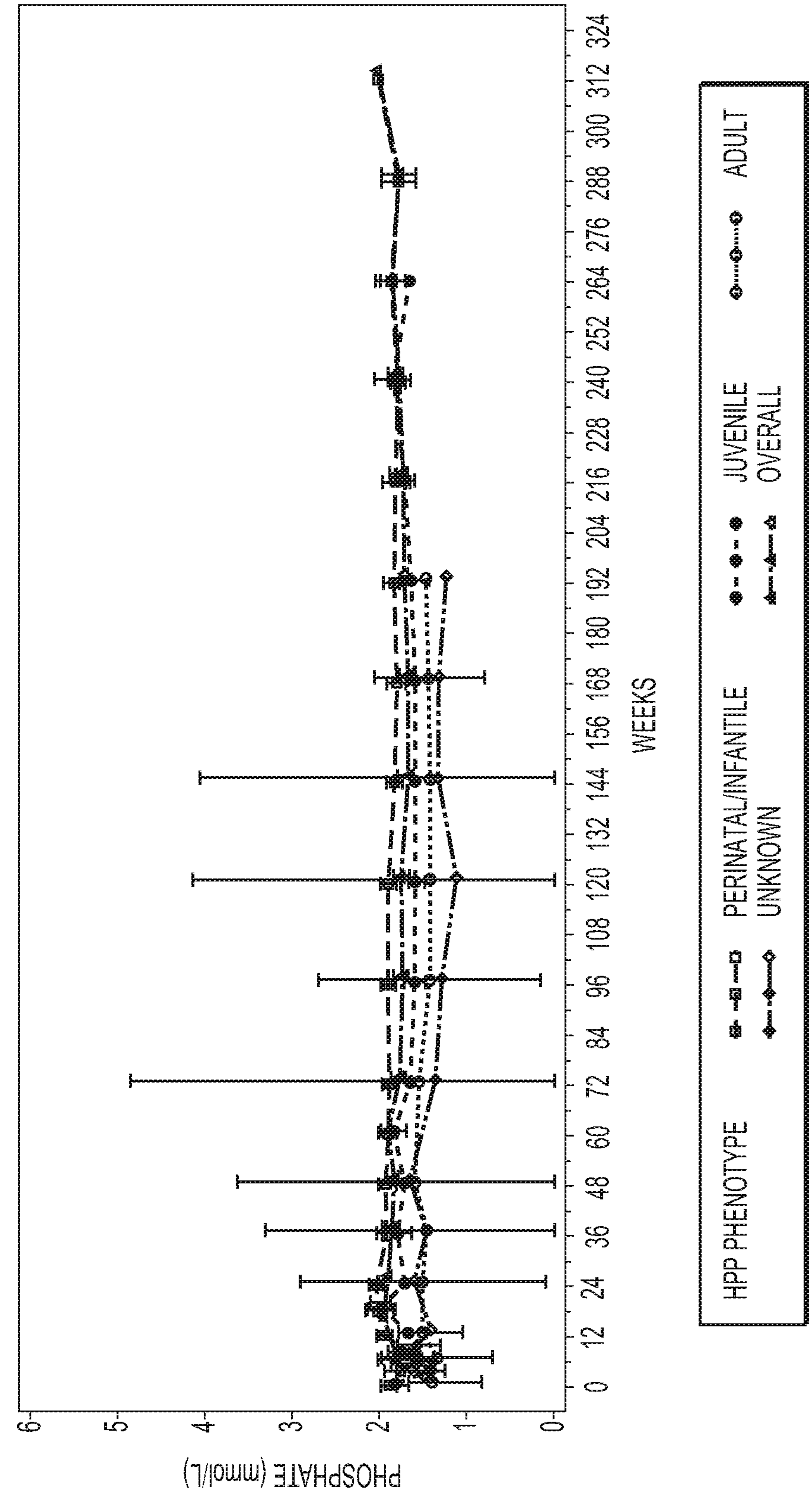

PHOSPHATE (mmol/L)
WEEKS
HPP PHENOTYPE
PERINATAL/INFANTILE
UNKNOWN
JUVENILE
OVERALL
ADULT

FIG. 7

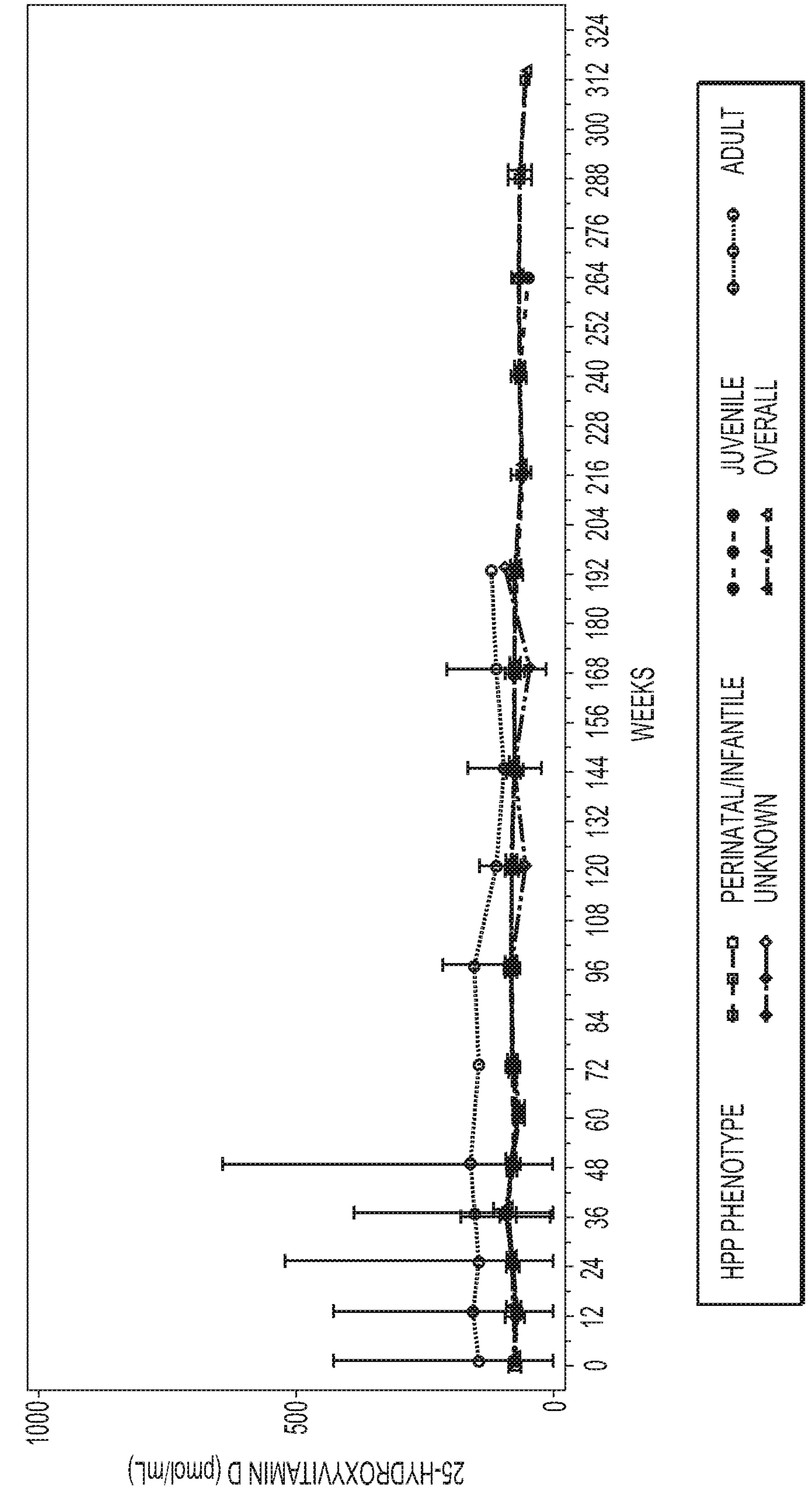

25-HYDROXYVITAMIN D (pmol/mL)
1000
500
0
WEEKS
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216 228 240 252 264 276 288 300 312 324
HPP PHENOTYPE
PERINATAL/INFANTILE
UNKNOWN
JUVENILE
OVERALL
ADULT

FIG. 8

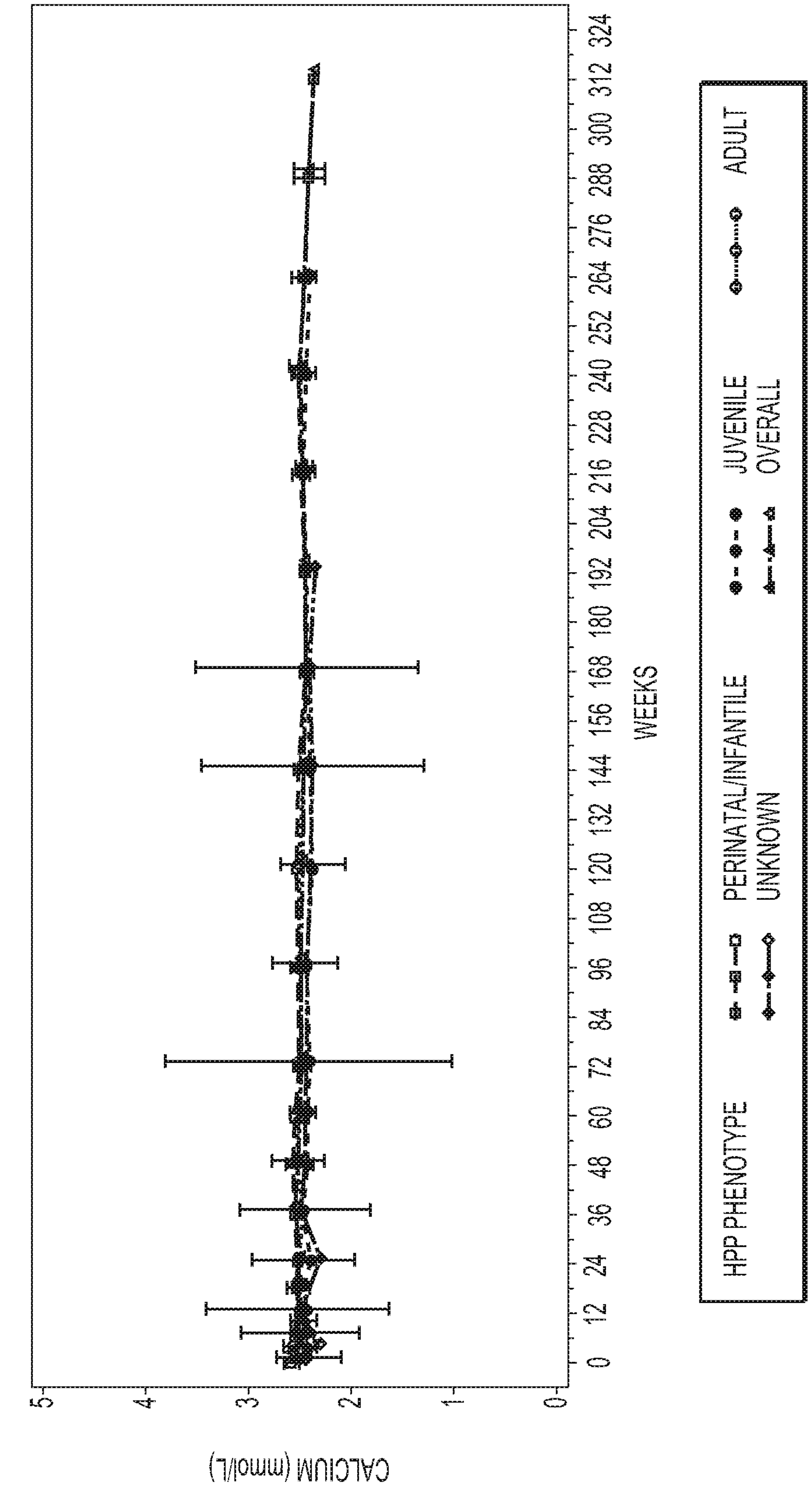

CALCIUM (mmol/L)
WEEKS
HPP PHENOTYPE
PERINATAL/INFANTILE
UNKNOWN
JUVENILE
OVERALL
ADULT

FIG. 9

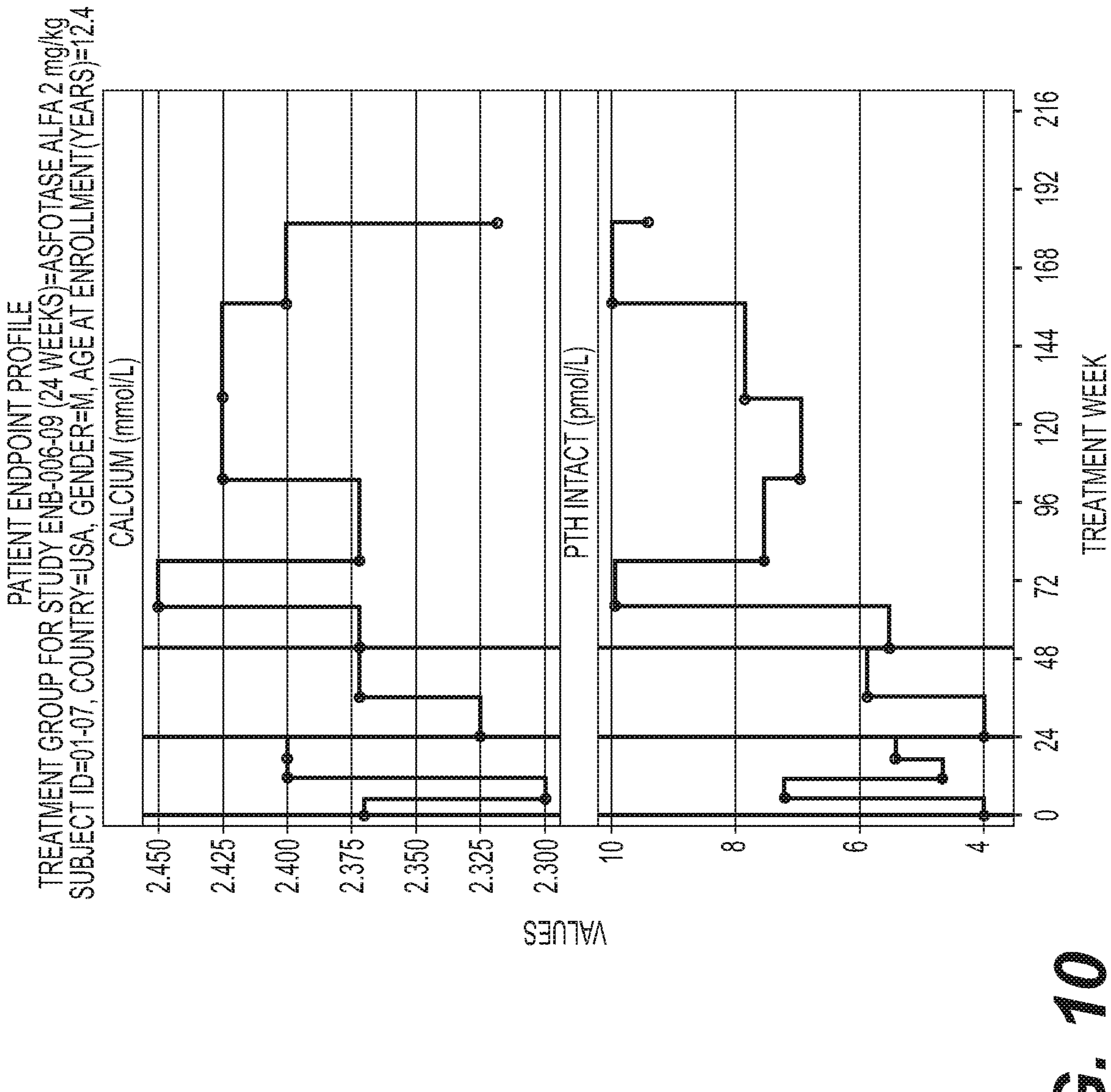
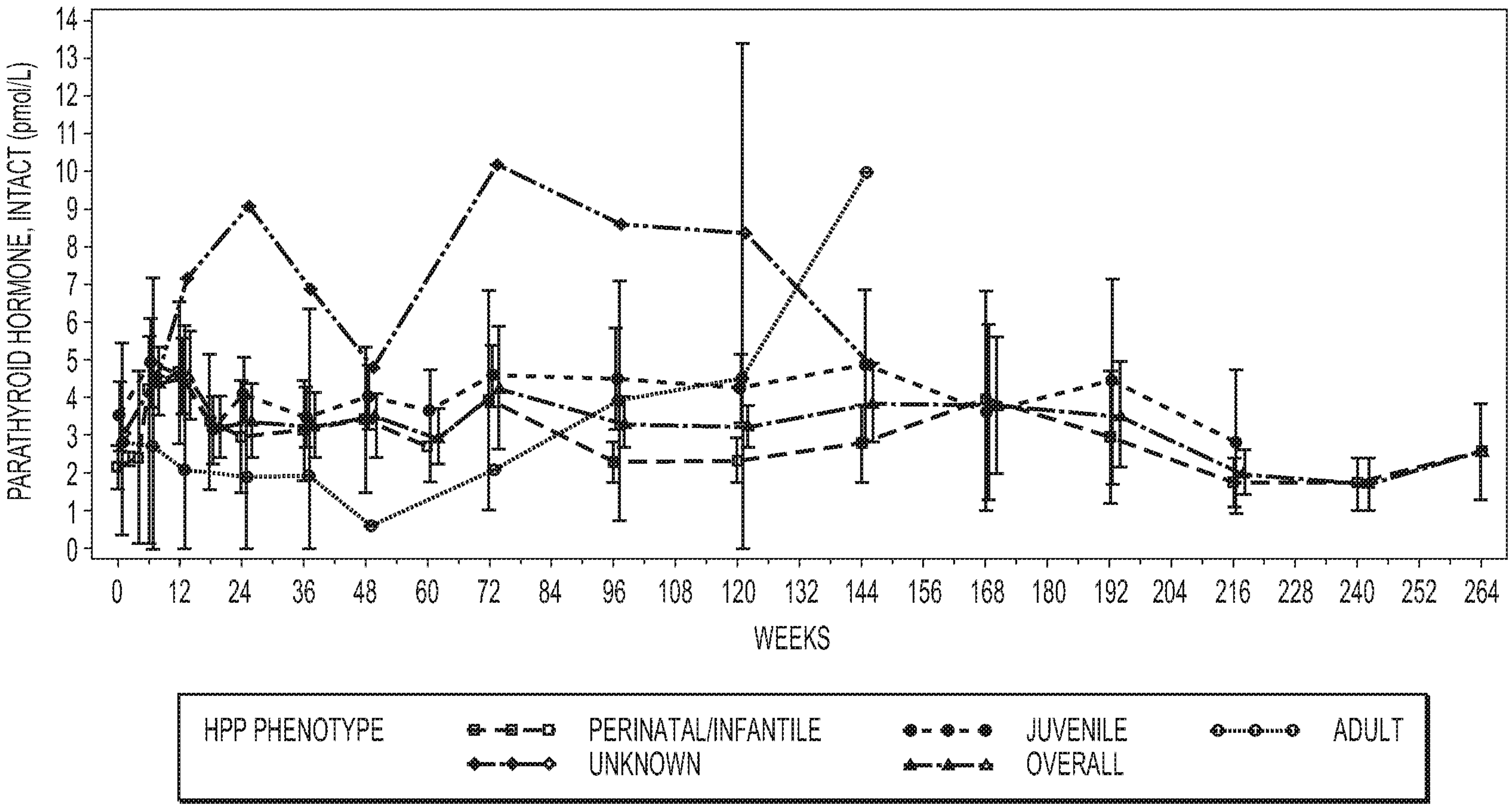

PATIENT ENDPOINT PROFILE
TREATMENT GROUP FOR STUDY ENB-006-09 (24 WEEKS)=ASFOTASE ALFA 2 mg/kg
SUBJECT ID=01-07, COUNTRY=USA, GENDER=M, AGE AT ENROLLMENT(YEARS)=12.4
CALCIUM (mmol/L)
2.450
2.425
2.400
2.375
2.350
2.325
2.300
PTH INTACT (pmol/L)
10
8
6
4
VALUES
0
24
48
72
96
120
144
168
192
216
TREATMENT WEEK

FIG. 10

ASFOTASE ALFA SEQ ID NO:1

```
  1 LVPEKEKDPK YWRDQAQETL KYALELQKLN TNVAKNVIMF LGDGMGVSTV
 51 TAARILKGQL HHNPGEETRL EMDKFPFVAL SKTYNTNAQV PDSAGTATAY
101 LCGVKANEGT VGVSAATERS RCNTTQGNEV TSILRWAKDA GKSVGIVTTT
151 RVNHATPSM YAHSADRDWY SDNEMPPEAL SQGCKDIAYQ LMHNIRDIDV
201 IMGGGRKYMY PKNKTDVEYE SDEKARGTRL DGLDLVDTWK SFKPRYKHSH
251 FIWNRTELLT LDPHNVDYLL GLFEPGDMQY ELNRNNVTDP SLSEMVVVAI
301 QILRKNPKGF FLLVEGGRID HGHHEGKAKQ ALHEAVEMDR AIGQAGSLTS
351 SEDTLTVVTA DHSHVFTFGG YTPRGNSIFG LAPMLSDTDK KPFTAILYGN
401 GPGYKVVGGE RENVSMVDYA HNNYQAQSAV PLRHETHGGE DVAVFSKGPM
451 AHLLHGVHEQ NYVPHVMAYA ACIGANLGHC APASSLKDKT HTCPPCPAPE
501 LLGGPSVFLF PPKPKDTLMI SRTPEVTCW VDVSHEDPEV KFNWYVDGVE
551 VHNAKTKPRE EQYNSTYRW SVLTVLHQDW LNGKEYKCKV SNKALPAPIE
601 KTISKAKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES
651 NGQPENNYKT TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH
701 NHYTQKSLSL SPGKDIDDDD DDDDDD
```

FIG. 11



PARATHYROID HORMONE, INTACT (pmol/L)
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
192
204
216
228
240
252
264
WEEKS
HPP PHENOTYPE
PERINATAL/INFANTILE
JUVENILE
ADULT
UNKNOWN
OVERALL

FIG. 1