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PAMIDRONATE ATTENUATES MUSCLE LOSS FOLLOWING PEDIATRIC BURN INJURY†

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Abstract

Children who are burned > 40% total body surface area lose significant quantities of both bone and muscle mass due to acute bone resorption, inflammation and endogenous glucocorticoid production, which result in negative nitrogen balance. Because administration of the bisphosphonate pamidronate within 10d of the burn injury completely prevents the bone loss we asked whether muscle protein balance was altered by the preservation of bone. We reviewed the results from 17 burned pediatric subjects previously enrolled in a double-blind randomized controlled study of pamidronate in the prevention of post-burn bone loss and who were concurrently evaluated for muscle protein synthesis and breakdown by stable isotope infusion studies during the acute hospitalization. We found a significantly lower fractional protein synthesis rate (FSR) in the pamidronate group and a correspondingly lower rate of appearance of the amino acid tracer in venous blood suggesting lower muscle protein turnover. Moreover, net protein balance (synthesis minus breakdown) was positive in the subjects receiving pamidronate and negative in those receiving placebo. Muscle fiber diameter was significantly greater in the pamidronate subjects and leg strength at 9 months post-burn was not different between subjects who received pamidronate and normal physically fit age-matched children studied in our lab. Leg strength in burned subjects who served as controls tended to be weaker, although not quite significantly so. If substantiated by a larger study, these results suggest that bone may have a paracrine mechanism to preserve muscle and this finding may have implications for the treatment of sarcopenia in the elderly.

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Keywords

Corticosteroids < CELL/TISSUE SIGNALING - Endocrine Pathways; Sarcopenia < SKELETAL MUSCLE; Bone-muscle interactions < SYSTEMS BIOLOGY; BONE INTERACTORS; Antiresorptives < THERAPEUTICS; EXERCISE

INTRODUCTION

Burn injury in children is associated with resorptive bone loss (1) likely due to the acute systemic inflammatory response (2) and the stress-associated production of endogenous glucocorticoids (2,3). We have previously shown that a single dose of the bisphosphonate pamidronate within the first ten days of burn injury followed by a second dose a week later completely blocks the resorptive bone loss (4) and that the effect is maintained for at least two years post-burn (5).

Muscle wasting also occurs post-burn along with negative nitrogen balance and this effect may be attributable to either the endogenous glucocorticoid production or the systemic inflammatory response (2,3).

Because the mechanism of bisphosphonate action involves interference with cholesterol biosynthesis in the osteoclast (6), it is possible that these drugs could act to interfere with steroid production during the stress response and attenuate muscle breakdown.

The aim of our study was to review data previously obtained from patients enrolled in the completed randomized controlled double blinded trial of pamidronate who were concurrently enrolled in stable isotope infusion studies of muscle protein synthesis and breakdown to determine whether the parameters of muscle protein kinetics, synthesis, breakdown and net balance between synthesis and breakdown, differed between subjects receiving pamidronate and those receiving placebo.

METHODS

We reviewed the database of burned children who enrolled in the randomized controlled double-blinded study of pamidronate and placebo between 2000 and 2004 (4) who were simultaneously enrolled in infusion studies of stable isotopes in order to evaluate muscle protein synthesis, breakdown, and net balance in the first 30-60d post-burn. The infusion protocol is well-established (7,8) and consisted of an 8 hr primed (2 μ mol/kg) continuous (0.08 μ mol/kg/min intravenous infusion of L[-ring-²H₅] phenylalanine with an additional infusion of unlabelled amino acids during the last 3h. Arterial and venous blood samples were drawn at baseline, 2h, and every 15 min between hours 4-5 and 7-8. Vastus lateralis muscle biopsies were obtained at 2,5, and 8h. Leg blood flow was measured by the indocyanine green dilution technique (7) between hours 3-4 and 6-7. Muscle protein fractional synthesis rate (FSR) was determined by the direct incorporation method. FSR 1 is the synthesis rate during the basal period (incorporation of tracer into the muscle in the period between the first two muscle biopsies. FSR 2 is the synthesis rate during the infusion of unlabeled amino acids (incorporation of tracer into the muscle in the period between the

second and third muscle biopsies). Changes in muscle phenylalanine enrichment over time was divided by plasma enrichment and expressed as %/hr. A two-compartment model was used in addition to calculate muscle protein synthesis and breakdown rates. Because phenylalanine is neither synthesized nor oxidized in the muscle, its rate of appearance (Ra) in venous blood is taken as the rate of muscle protein breakdown provided there are no changes in intracellular enrichment. The net balance (NB) of phenylalanine is calculated from the arterio-venous difference in phenylalanine multiplied by the leg blood flow (7) and represents the difference between muscle protein synthesis and breakdown as calculated from the initial 5 hr infusion period.

Additional muscle biopsy tissue was frozen and later thawed in warm saline, embedded in paraffin, sectioned, and examined by light microscopy for determination of muscle fiber diameter).

In addition, a functional cognate of these studies, muscle strength, was estimated by leg strength as peak torque corrected for body weight at 9 m post-burn utilizing isokinetic dynamometry (9) Briefly, assessment of isokinetic strength was made on the patient's dominant leg extensors at an angular velocity of 150°/second using the Biodex System 3 dynamometer (Biodex Medical System, Shirley NY). All patients performed a warm-up session of three sub-maximal repetitions without load. Following this warm-up, the patient was asked to perform 10 maximal full leg extensions and full leg flexions. The Biodex system calculated and provided the peak torque (PKT) measurement corrected for gravitational movements of the lower leg and the lever arm. The highest measurement of the 10 repetitions was selected.

Finally, this PKT was corrected for each subject's body weight.

Urine for determination of free cortisol excretion expressed as micrograms (μg)/24h was analyzed by previously published methods (2,3).

Statistical analyses were carried out by use of one-way ANOVA, unpaired t tests, or Welch t tests in cases of unequal variances between groups.

RESULTS

Post hoc analysis of our patient database identified 17 subjects who had participated in the pamidronate study, 10 receiving pamidronate, 7 receiving placebo (4,5), who underwent concurrent stable isotope infusion studies at our institution. There were no differences in age between pamidronate and placebo groups, 13.4 + 4.0 (SD) vs 12.5 + 3.6 yr respectively. Total body surface area burns 65.7 + 18.1% for the pamidronate group and 58.4 + 17.8% for the placebo subjects. The differences in age and burn size between the two groups were not significant. In the control group FSR2 was significantly higher than FSR1, reflecting the increased supply of building blocks for muscle protein synthesis. In the pamidronate group, however, FSR2 was not different from FSR1 (Figure 1).

Similarly, Ra, or muscle breakdown rate, was significantly higher in the control group than in the group receiving pamidronate (Figure 2). Moreover, NB was negative in the control

group, a usual finding in the burned patient with muscle protein breakdown exceeding the synthesis, and negative nitrogen balance. However, in the subjects receiving pamidronate NB was positive, and there was a significant difference between the pamidronate and placebo groups (Figure 3).

With regard to the additional muscle biopsies, 12 were found for the group receiving pamidronate and 8 were found for patients in the control group. A mean of $30 + 8$ (SD) muscle fibers were counted per biopsy of the subjects receiving pamidronate and $28 + 8$ fibers were counted in biopsies for the control subjects. As shown in Figure 4, mean muscle fiber diameter in the pamidronate group was significantly greater than that in the controls, substantiating the stable isotope findings by different means.

Furthermore, subjects receiving pamidronate had a peak torque of $149.5 + 62\%$ ($n=5$) of their body weight on the Biodex machine at 9 months post-burn. This compared to $153.4 + 29.9\%$ for normal, age-matched, physically fit children ($n=22$), and contrasts to $112.2 + 51.7\%$ ($n=6$) for the control subjects, $p=0.052$ by ANOVA. There were no significant differences in burn size between groups of children undergoing the exercise program.

Urine free cortisol excretion in subjects receiving pamidronate was $319.9 + 292.6 \mu\text{g}/24\text{h}$ ($n=12$) compared to $399 + 183.5 \mu\text{g}/24\text{h}$ in the controls ($n=9$) during the corresponding time period. There was no significant difference between the groups, $p=0.486$. The upper limit of normal for pediatric cortisol values ranges from 50–125 $\mu\text{g}/24\text{h}$.

DISCUSSION

This post-hoc analysis of a completed clinical trial demonstrates that in the burned patients administration of pamidronate within the first 10d following burn injury attenuated muscle loss as indicated by decreased muscle synthesis and breakdown, a positive net balance of muscle protein, and larger muscle fiber diameter. Muscle strength exhibited a tendency to be greater than in controls at 9 m post-burn but the difference was not quite significant between controls and unburned physically fit children of similar age. The question raised by these data is how did this happen?

There are at least three possible explanations. The first is that pamidronate acts by inhibiting farnesyl pyrophosphate synthase, an enzyme active in cholesterol biosynthesis (6) and therefore might interfere with the biosynthesis of glucocorticoid precursors or other intracellular signaling proteins. For pamidronate to protect muscle by reduction of steroid biosynthesis reduction of free cortisol excretion should be seen. Inasmuch as urine free cortisol excretion is not significantly reduced by pamidronate, this possibility is remote.

A second scenario is that pamidronate attenuates the post-burn inflammatory response, which also can contribute to muscle wasting (10). We think this is unlikely. The inflammatory response results in high levels of circulating cytokines, especially interleukins (IL)- 1β and IL-6 (2). These cytokines are known to cause up-regulation of the parathyroid calcium-sensing receptor (CaSR) (11–13), a condition documented in a sheep model of burn injury (14) that may be an adaptive response to the acute resorption of bone (1). Following pamidronate administration there was no amelioration of the hypocalcemic

hypoparathyroidism resulting from CaSR up-regulation (4) suggesting that the inflammatory response had not been significantly altered.

A third possibility, and one that would appear to be most likely, is that bone produces myogenic factors that reach muscle by means of an as yet unidentified paracrine mechanism or mechanisms. It is known that during development bone produces Indian hedgehog (15) and vascular endothelial growth factor (16) that can both aid in muscle development. Other osteocyte products may also affect muscle. It has previously been shown that ketoconazole, an inhibitor of glucocorticoid production, can reduce endogenous steroid synthesis to normal in burned patients but fails to prevent muscle breakdown following burn injury (17). Thus reducing glucocorticoid production does not halt the catabolic response. Moreover, pamidronate administration appears to overcome the combined effects of inflammatory and glucocorticoid-mediated muscle breakdown. This speaks to the possibility that the anti-apoptotic properties of bisphosphonates (18) permit the development of healthy bone following the burn injury and that healthy bone is capable of producing substances that either protect the muscle from breakdown or support rapid regeneration of muscle tissue.

However, there also remains the possibility that these results represent an artifact of the retrospective nature of the study and the attendant possibility of inadvertent selection bias along with admittedly low numbers of subjects. A prospective study is needed to substantiate these findings.

If, however, these data are confirmed the necessity to treat osteoporosis would become greater as treatment may prevent or delay the onset of frailty.

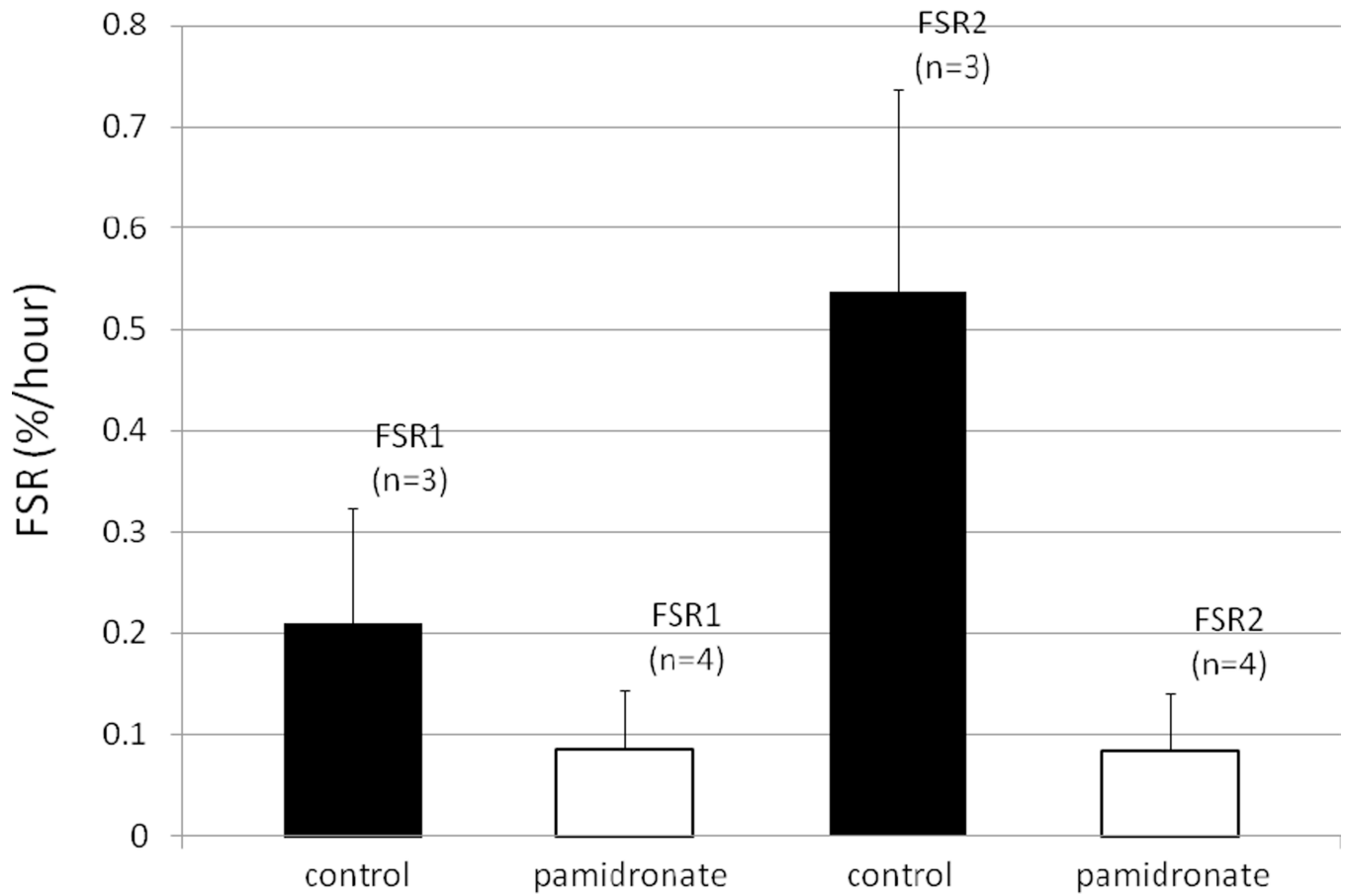
Acknowledgments

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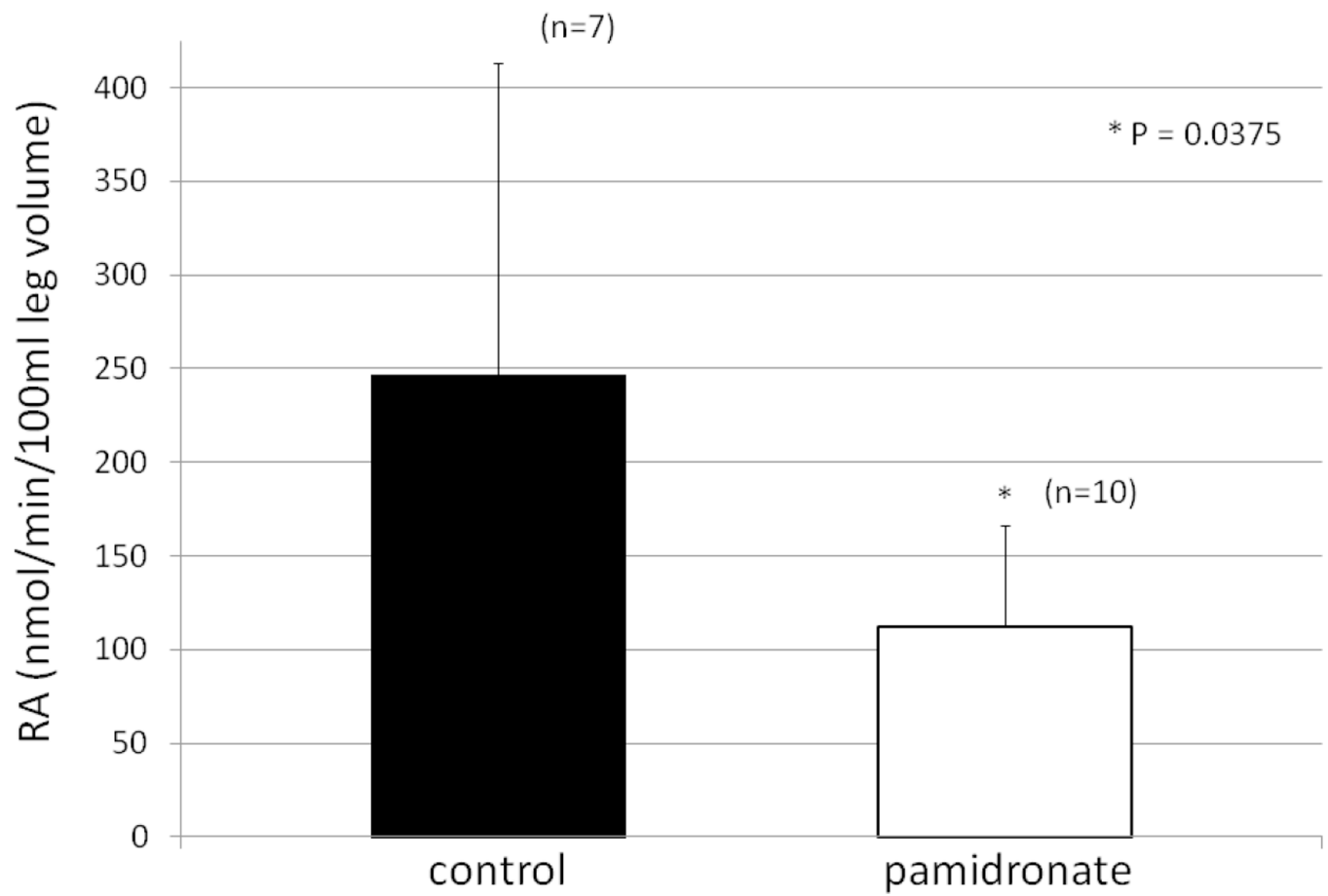
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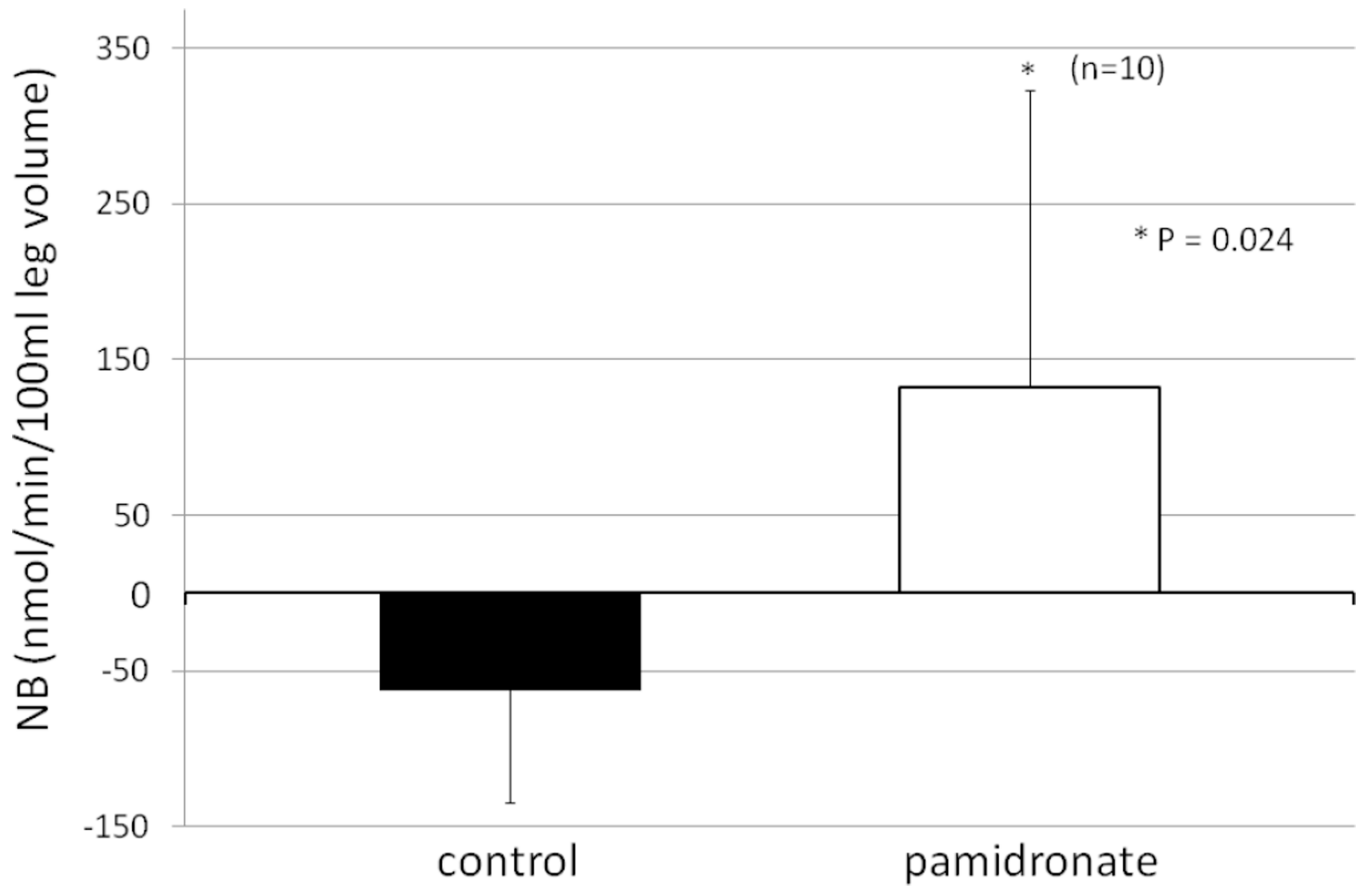
**1.**

Fractional synthetic rate (FSR) of muscle protein during a basal period (FSR 1) and during an unlabelled amino acid infusion period (FSR2) in pamidronate and control subjects. Results are expressed as percent per hour.

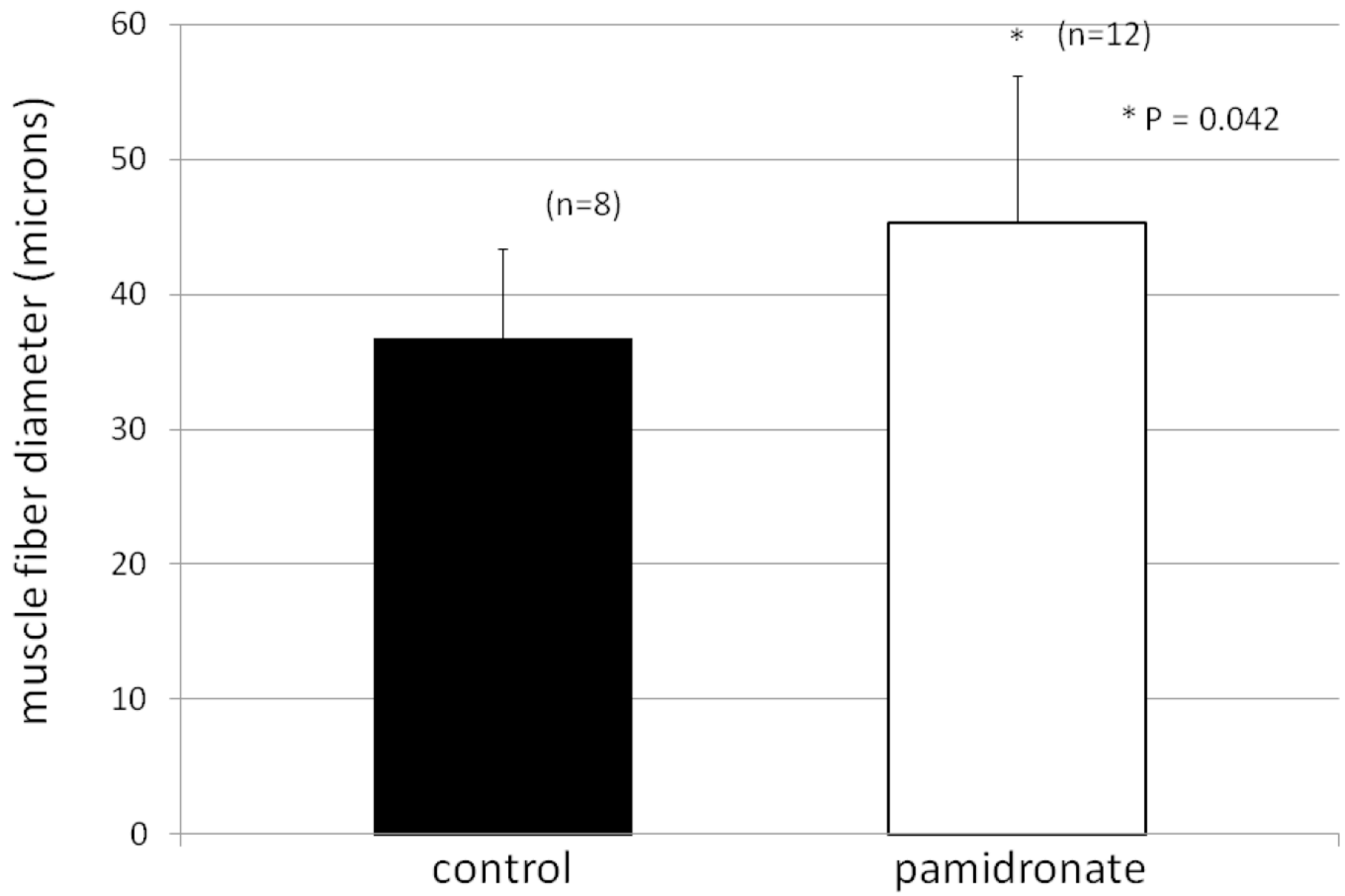


2.

Rate of appearance (Ra) of phenylalanine in venous blood in pamidronate and control subjects expressed as nmol/min/100 ml leg volume.

**3.**

Net balance (NB, synthesis minus breakdown) of phenylalanine as calculated from the arterio-venous difference in phenylalanine concentration multiplied by the blood flow and expressed as nmol/min/100 ml leg volume.

**4.**

Muscle fiber diameter from m. vastus lateralis biopsies of subjects receiving either pamidronate or placebo and expressed in microns.