J Musculoskel Neuron Interact 2002; 2(4):327-334

Original Article

Hylonome

Nerve growth factor and norepinephrine concentrations in weight-bearing and non-weight-bearing bones of euthyroid and hyperthyroid rats

M. Yao, P.C. Dooley, J.A. Schuijers, B.L. Grills

Department of Human Physiology and Anatomy, School of Human Biosciences, La Trobe University, Victoria, Australia

Abstract

Thyroid hormone, nerve growth factor (NGF) and norepinephrine (NE) and weight-bearing affect bone metabolism, yet interactions between these factors and osseous tissue have not been investigated. Therefore, the aims of the study were to measure NGF and NE concentrations in weight-bearing and non-weight-bearing bones from euthyroid (control) and hyperthyroid (HT) rats. Hyperthyroidism was induced by oral intake of triiodothyronine (90 mg/kg/day) for 21 days. Histomorphometry on distal femurs verified significant trabecular bone loss in HT rats compared to euthyroid animals. NGF concentrations were assayed via ELISA, whilst NE concentrations were measured via HPLC and ECD. In euthyroid rats: (i) the concentration of NGF in ribs (914 ng/g) was almost 3-fold greater than in femurs (326 ng/g wet weight of tissue) (ii) the concentrations of NE in ribs (74.7 ng/g) and calvaria (87.4 ng/g) were 2.5–3.5-fold greater than either femurs (24.0 ng/g) or tibiae (30.5 ng/g) and (iii) NE concentrations were comparable between ribs (74.7 ng/g) and calvaria (87.4 ng/g) and similar between tibiae (30.5 ng/g) and femurs (24.0 ng/g). In HT rats: (i) the concentration of NGF in ribs (1802 ng/g) was 4-fold greater than in femurs (402 ng/g) (ii) NE concentrations in ribs (23.3 ng/g) and calvaria (13.6 ng/g) were 4.5-fold and 2.6-fold greater respectively than in tibiae (5.2 ng/g), while ribs had almost a 2-fold higher concentration of NE than calvaria. In HT rats compared to euthyroid animals: (i) NGF concentrations almost doubled in ribs but there was little change in the NGF concentration in femurs (ii) there was a reduction in NE concentrations in calvaria by 84%, in ribs by 69% in tibiae by 83% and 55% in femur (NS). Conclusions: (i) Non-weight-bearing is associated with higher concentrations of NGF and NE than weight-bearing in bones in euthyroid and HT rats; (ii) Hyperthyroidism exerts opposite effects on NGF and NE in bone and (iii) Hyperthyroidism interacts with weight-bearing to determine NGF and NE concentrations in bone. Therefore, the influence of thyroid hormone on NGF and NE in bone may need to be taken into account when considering the action of thyroid hormone on bone in either euthyroid or hyperthyroid states.

Keywords: Bone, Hyperthyroidism, Nerve Growth Factor, Norepinephrine, Weight-Bearing

Introduction

Normal thyroid hormone plasma concentrations are essential for typical bone remodeling. Hyperthyroidism stimulates both bone formation and bone resorption, however there is a net loss of cortical and trabecular bone due to a higher activity of osteoclasts compared to that of osteoblasts[1-4]. These skeletal effects are primarily attributable to the direct stimulation of thyroid hormone receptors in osteoblasts[5].

There is also the possibility that thyroid hormone may affect bone indirectly through an effect on peripheral nerves. Peripheral sensory and sympathetic nerves that supply bone influence skeletal remodeling[6]. Thyroid hormone has a critical role in the maintenance and regeneration of these nerves partly through its regulation of nerve growth factor (NGF) synthesis in non-neuronal tissues[7]. NGF is implicated in bone metabolism as it has been identified along with receptors in various cells of unfractured and fractured bone[8-10] as well as in osteoblastic cell lines[11]. The trophic action of NGF on bone is indicated by the improved rate of both repair and

Corresponding author: Dr. Brian L. Grills, School of Human Biosciences, La Trobe University, Victoria 3086, Australia.
Email: brian.grills@latrobe.edu.au

Accepted 6 January 2002

biomechanical strength of calluses in fractured rat ribs when NGF was applied topically[12]. NGF also stimulated the formation of osteoid tissue around nerves during sensory nerve regeneration in mandibles of rabbits[13]. A relationship between hyperthyroidism and NGF in bone has not been previously investigated, but may be anticipated, as high thyroid hormone plasma levels affect the concentration of NGF or NGF gene expression in non-osseous tissues of experimental animals[14,15].

Thyroid hormone also modulates norepinephrine (NE) concentrations in various tissues. NE affects bone remodeling and its major source in osseous tissue comes from adrenergic nerves that supply osseous blood vessels and regions of high osteogenic activity such as the periosteum[16]. Actions of NE on bone are not completely understood, however one of its main effects is to increase osteoclastic bone resorption, possibly acting indirectly by stimulating beta-2-adrenergic receptors in osteoblasts[17].

Hyperthyroidism reduces NE concentrations in both human plasma[18] and rat brain[19] but increases NE concentrations in rat cardiac muscle[19]. Therefore, the action of thyroid hormone in modulating NE tissue concentrations appears to be organ specific. It is not known how hyperthyroidism influences NE concentrations in bone.

Given that thyroid hormone influences both NGF and NE concentrations in various tissues and that these neural factors are implicated in bone metabolism, the aim of this study was to investigate whether thyroid status alters the concentrations of NGF and NE bones. We predict that concentrations of NGF and NE in bones of rats are likely to be different between euthyroid and hyperthyroid (HT) states.

It is well established that the load placed on bone influences its remodeling[20]. Non-weight-bearing bones (eg. calvaria, mandible) have higher concentrations of IGF-II and TGF-β than weight–bearing bones (eg. vertebra, iliac crest) and these growth factors may protect non-weight-bearing bones from bone loss[21]. It is not known whether NGF and NE concentrations are different between these two types of bone. Furthermore, if there is a difference in the levels of these neural substances between weight-bearing and non-weight-bearing bones, does hyperthyroidism affect their concentrations?

Therefore, it is of interest to investigate whether osseous NE and NGF concentrations differ between non-weight-bearing and weight-bearing bones of euthyroid and HT animals.

Materials and methods

Animals

Ethics approval for this experiment was granted by the La Trobe University Animal Ethics Committee. Sixteen male Sprague Dawley rats aged 12 to 13 weeks (300 to 400 g) were used and randomly allocated to either euthyroid (control; n = 8) or HT groups (n = 8) and were held at 21°C under controlled 12 h light-dark cycles. Daily weight and water consumption was recorded in order to estimate the daily drug intake by each animal and to observe the effects on weight gain. Animals were allowed unlimited access to food and drinking water.

Treatment

Hyperthyroidism was induced by oral administration of triiodo-L-thyronine (T3) in drinking water. T3 (20 mg) was dissolved in 400 μl of 4 M NH_4OH in methanol and added to 100 ml of water containing 1% bovine serum albumin. Aliquots of this solution were added to drinking water on alternate days, such that a final T3 concentration was 1 μg/ml[22]. The intended dosage of T3 administered to each rat was 90-100 μg/kg of body weight/day[23]. Euthyroid animals received a vehicle solution (above mixture minus the added T3). Three HT rats died during the experimental period; one at 16 days and two at 17 days into the experiment. It is envisaged that thyrotoxicosis contributed to their deaths.

After 21 days of treatment the animals were killed via an overdose of carbon dioxide. At autopsy blood samples were collected and bone samples were excised, cleaned, weighed wet and recorded.

Bone samples

The left femur was bisected cross-sectionally through the mid-shaft using bone cutters and the distal end processed for histology. The proximal part of this femur and the left sixth rib from each rat were analysed for NGF concentrations using an ELISA. The calvarium, the right sixth rib, the right proximal femur and the tibia from each rat were analysed for NE concentrations using HPLC and ECD.

Measurement of T3 and thyroxine (T4) concentrations

Concentrations of free T3 (FT3) and free T4 (FT4) from both euthyroid and HT groups were measured in blood plasma samples obtained after 21 days of treatment using a Microparticle Enzyme Immunoassay (MEIA; Abbott Axsym System Kit, Abbott Laboratories, Abbott Park, Il, USA).

Histological and histomorphometric analyses

To verify bone loss associated with hyperthyroidism, distal femoral samples were immersion fixed for two days at 4°C in paraformaldehyde-glutaraldehyde (4% paraformaldehyde; 0.25% glutaraldehyde; 7% sucrose in 0.1 M cacodylate buffer; pH 7.4) immediately after excision. Bones were subsequently processed to LR Gold resin (London Resin Company, England)[24]. Upon resin polymerization, samples were sectioned (4 μm) mid-line longitudinally using a Leica RM 2155 microtome (Leica Instruments, Nussloch, Germany) with a tungsten-carbide knife. Sections were stained with Goldner trichrome[25] for histomorphometric analysis.

Sections were viewed using a Leica DMRBE microscope (Leica Mikroskopie und Systemie GmbH, Wetzlar, Germany) with an associated Sony PowerHAD 3CCD color video camera (Sony Corporation, Tokyo, Japan). Images were downloaded onto a desktop computer and qualitatively assessed. Histomorphometric measurements of distal femoral metaphyseal trabecular bone were made from downloaded images using Leica Qwin® software (Leica Imaging Systems Ltd., Cambridge, England) at 25 times magnification. Trabecular bone area measurements were obtained from a 4 mm^2 field, positioned 1 mm anterior and distal to the lowest point of the epiphyseal growth plate in the metaphysis. Captured images were blindly measured and assessed before comparisons were made between euthyroid and HT groups.

Nerve growth factor analysis

NGF Extraction

Femurs and ribs were crushed with a mortar and pestle. One ml cold extraction buffer (20 mM Tris-HCl buffer; pH 8; 137 mM NaCl; 1% NP40 detergent; 10% glycerol; 1 mM phenylmethylsulfonyl fluoride; 10 µg/ml aprotinin; 1 mg/ml leupeptin; 0.5 mM sodium vanadate) was added to all samples before homogenization (30 s) and sonication (30 s). The homogenates were centrifuged for 30 min (6000 rpm at 4˚C), after which the supernatant was removed and stored at –80˚C.

Enzyme linked immunosorbent assay (ELISA)

The NGF ELISA was performed according to the ELISA kit manufacturer's protocol (Promega Corporation, Madison, WI, USA), with modifications as described by Bennett and co-workers[26].

Five 96-well ELISA plates were coated with anti-NGF polyclonal antibody, allowed to incubate (16 h at 4˚C) and washed with Tris buffered saline containing Tween 20 (TBST). Block and Sample Buffer (BSB) was added to wells and incubated (1 h) at room temperature, followed by washing with TBST. Known concentrations of NGF (standards) was added to each 96-well plate (7.8 – 500 pg/ml) in conjunction with unknown concentrations of extracted NGF from tissue samples. The extracted NGF samples were diluted with cold extraction buffer (1:5; 1:25) and added sequentially to assigned wells. All plates were sealed and incubated with shaking (6 h) at room temperature. Plates were washed five times with TBST prior to incubation with anti-NGF monoclonal antibody at 4˚C overnight. On the following day, plates were washed five times with TBST prior to a shaking incubation (2.5 h at room temperature) with anti-rat IgG horseradish peroxidase conjugate solution, after which the plates were washed another five times with TBST. Color development was achieved during a shaking incubation (7 min) with TMB solution containing peroxidase substrate and halted immediately with 1 M phosphoric acid. Absorbance was read with a Labsystems Multiskan MS spectrophotometer (Labsystems Multiskan MS, Finland) at wavelength of 450 nm. A standard curve was constructed for each plate using GraphPad Prism™ software (GraphPad Software, Inc., San Diego, CA, USA).

NE analysis

The procedures used in the analysis of NE concentrations in bone were adapted from Schuijers and co-workers[27] as follows:

NE extraction

Bone samples were stored in 1 ml solution of 0.1 M perchloric acid (PA) containing 0.1 mM sodium metabisulfide ($Na_2S_2O_5$) and 20 ng 3,4-dihydroxybenzylamine (DHBA) (Sigma-Aldrich, Pty Ltd, NSW, Australia) as an internal standard. All samples were kept on ice with minimal light exposure throughout the extraction procedure. Samples were homogenized (3 min), transferred into 13 x 100 mm culture tubes and centrifuged for 20 min (3600 rpm at 4°C). The supernatant was transferred into small test tubes containing 25 mg acid-washed alumina and 30 µl of 10 mM $Na_2S_2O_5$, with a subsequent addition of 1 ml of 1 M Trizma Base containing 2% EDTA. The samples were vortexed and centrifuged and the supernatant was discarded. The alumina was washed three times with deionized, distilled water before the NE was desorbed from the alumina with the addition of 100 ml of 0.1 M PA containing 0.1 mM $Na_2S_2O_5$. Samples were stored at –80°C until analysis.

High performance liquid chromatography (HPLC) with electrochemical detection (ECD)

The ECD mobile phase contained 0.07 M di-sodium hydrogen orthophosphate, 0.2 mM EDTA, 3 mM heptanosulphonate in 940 ml degassed, deionized, and distilled water (pH 4.8), with the addition of 60 ml of filtered methanol (0.4 µm FH type Millipore organic filter). A 15 cm ion exchange column (NovaPak C18; Waters Corporation, Milford, MA, USA) with an in-line pressure filter was used. The HPLC-ECD flow rate was 0.7 ml/min, with the electrochemical detector sensitivity at 20 nA and an applied potential between 0.65 V - 0.72 V. Fifty µl of either standard or sample were injected using an automatic injector (WISP; Waters Corporation, Milford, MA, USA), at a filter mode of 5 s/reduction potential.

Statistical analysis

The results obtained from the measurement of FT3 and FT4, NE and NGF were analysed using a two-tailed t-test. Bone histomorphometric measurements were analysed via a one-tailed t-test. Differences between euthyroid and HT groups were accepted when $p < 0.05$. Results are presented as mean ± SEM.

Results

Thyroid hormone concentrations in plasma

The mean daily T3 intake by HT animals was approximately 100 μg/day/kg body weight. After 21 days, the mean concentration of FT3 (Table 1) was 46.7 ± 3.2 pmol/l in these animals, which was approximately 10-fold higher than in euthyroid animals (4.3 ± 0.4 pmol/l; $p < 0.001$). The concentration of FT4 fell to 8.1 ± 1.2 pmol/l which was approximately 2-fold smaller than euthyroid animals (20.2 ± 0.8 pmol/l; $p < 0.001$). Plasma concentrations of both FT3 and FT4 in euthyroid animals were similar to other reported values[28].

Changes in body weight associated with altered thyroid status

Euthyroid rats gained weight over the 21 day period, such that at Day 21 the mean body weight (466 ± 8 g) was approximately 15% greater than that at Day 1 (406 ± 8 g). HT rats exhibited a significant reduction in body weight compared to euthyroid controls ($p < 0.001$). During the first 8 days of treatment, body weight was relatively constant at 372 ± 9 g. Thereafter, body weight decreased, such that at Day 21, HT animals had lost approximately 10% of their original weight (336 ± 14 g). This weight loss was similar to that reported in other HT rat models over similar experimental periods[29,30].

Effect of hyperthyroidism on distal femoral metaphyseal trabecular bone volume

Histomorphometric measurements showed there was approximately 36% less trabecular bone volume in femurs of HT animals (19.7 ± 1.7%) compared to that in euthyroid animals (30.7 ± 1.2%; $p < 0.001$). These data are similar to previous reports in HT rats[31,32].

NGF concentrations in femurs and ribs

In both euthyroid and HT groups, mean NGF concentrations were greater in ribs than in femurs (Figure 1). In euthyroid rats, NGF concentrations were almost 3-fold greater in ribs than in femurs (914 ± 128 ng/g and 326 ± 63 ng/g respectively; $p<0.01$). While in HT rats, NGF concen-trations were over 4-fold greater in ribs than in femurs (1802 ± 371 ng/g and 402 ± 55 ng/g respectively; $p < 0.01$; Figure 1).

Hyperthyroidism had different effects in ribs and femurs. The concentrations of NGF in ribs of HT animals were almost twice that of ribs from the euthyroid group ($p < 0.05$). However, hyperthyroidism failed to increase femoral NGF concentrations (Figure 1).

NE concentrations in femurs, tibiae, ribs and calvaria

In the euthyroid group of rats, the NE concentrations were similar in ribs (74.7 ± 14.4 ng/g) and calvaria (87.4 ± 15.8 ng/g; Figure 2). Further, the concentrations of NE were similar to tibiae (30.5 ± 4.8 ng/g) and femur (24.0 ± 4.7 ng/g; Figure 2). However, the NE concentrations were 3- to 4-fold greater in calvaria and rib compared to femurs ($p < 0.01$) and tibiae ($p < 0.05$).

In HT rats, NE concentrations in calvaria, ribs and tibiae were smaller than in the euthyroid rats (Figure 2), but the decrease was not significant in the femur. As for the euthyroid rats, non-weight-bearing bones had higher concentrations of NE than weight-bearing bones. Thus, calvaria (13.6 ± 1.4 ng/g) and ribs (23.3 ± 2.7 ng/g) had significantly higher concentrations of NE than tibiae (5.2 ± 2.2 ng/g; $p<0.05$), but NE concentrations in femurs (13.0 ± 6.6 ng/g) were not significantly different from the values from other bones.

Discussion

Three significant findings from the present study on euthyroid rats were that: (i) ribs contained greater amounts of NGF than femurs, (ii) ribs and calvaria each had higher concentrations of NE than either femurs or tibiae and (iii) NE concentrations were the same in ribs and calvaria and the same in tibiae and femurs.

There are at least two possible explanations for these results. Firstly, calvaria and ribs may be more neurotrophic

	HT rats n = 5	Euthyroid rats n = 8
[FT3] (pmol/l)	46.7 ± 3.2***	4.3 ± 0.4
[FT4] (pmol/l)	8.1 ± 1.2***	20.2 ± 0.8

Table 1. Plasma concentrations of triiodothyronine (T3) and thyroxine (T4) in HT and euthyroid animals. Data presented as mean ± SEM; ***$p < 0.001$.

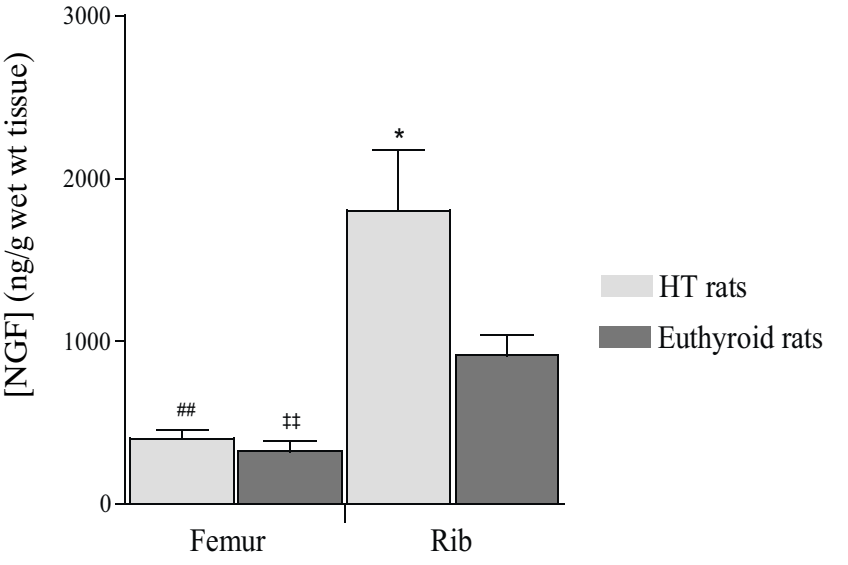

Figure 1. Nerve growth factor (NGF) concentrations in femurs and ribs of HT (n = 5) and euthyroid (n = 8) groups.
Data presented as mean ± SEM.
* $p < 0.05$ (HT ribs vs. euthyroid ribs), ## $p < 0.01$ (HT ribs vs. HT femurs) and $p < 0.01$ (euthyroid ribs vs. euthyroid femurs).

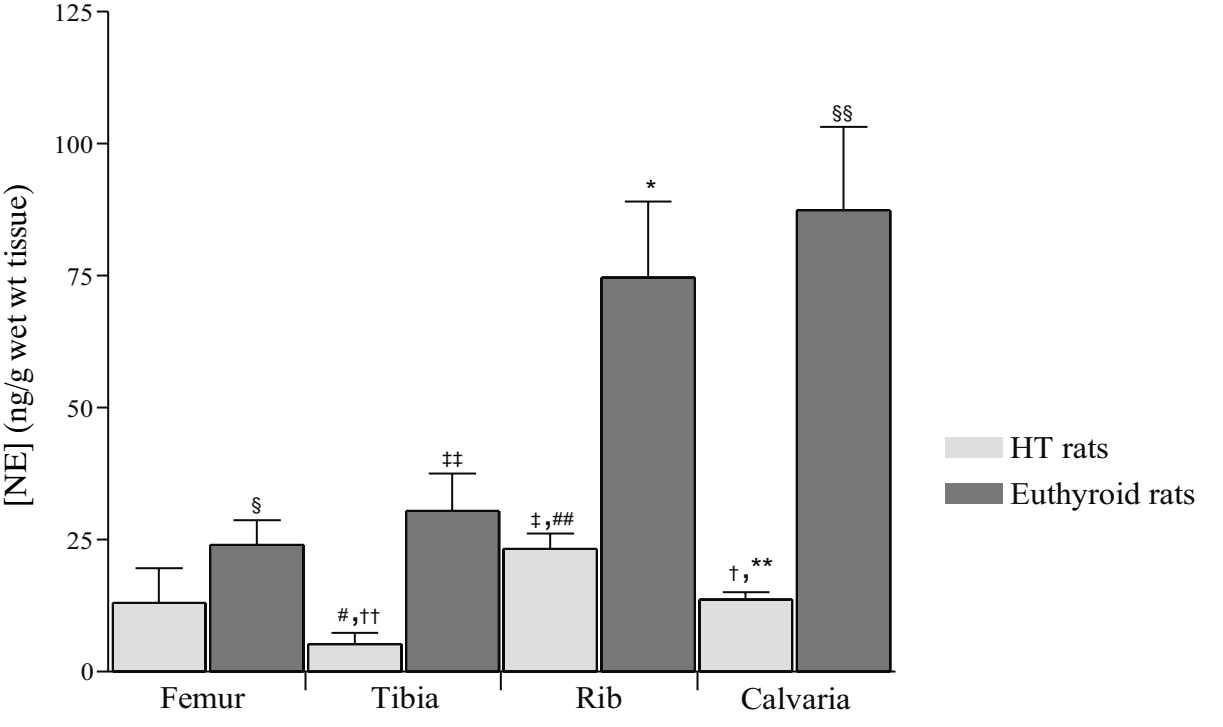

Figure 2. Norepinephrine (NE) concentrations in femurs, tibiae, ribs and calvaria of HT (n = 5) and euthyroid (n = 8) groups.
Data presented as mean ± SEM.

#	$p < 0.05$ (HT tibiae vs. euthyroid tibiae),	##	$p < 0.01$ (HT ribs vs. HT tibiae),
‡	$p < 0.05$ (HT ribs vs. euthyroid ribs),	‡‡	$p < 0.01$ (euthyroid tibiae vs. euthyroid calvaria),
*	$p < 0.05$ (euthyroid ribs vs. euthyroid tibiae)	**	$p < 0.01$ (HT calvaria vs. euthyroid calvaria),
†	$p < 0.05$ (HT calvaria vs. HT ribs)	††	$p < 0.05$ (HT tibiae vs. HT calvaria),
§	$p < 0.05$ (euthyroid femurs vs. euthyroid ribs)	§§	$p < 0.01$ (euthyroid calvaria vs. euthyroid femurs).

and more widely innervated by adrenergic nerves than femurs and tibiae. There are no published reports that indicate that non-weight-bearing bones are possibly more neurotrophic and adrenergically innervated than weight-bearing bones. Adrenergic nerves, however, extensively innervate newborn rat calvarial bone, with the majority of fibres being in close relationship to or within developing bone[33]. This nerve supply to rat calvaria, however, is evidently different to that of the adrenergic innervation of adult rat long bone, where nerve fibres are almost exclusively found close to or within blood vessel walls rather than juxtaposing bone cells or matrix[34]. Secondly, weight-bearing on bones could reduce the osseous formation of either NGF or NE. There have been a number of reports showing that weight-bearing bones and non-weight-bearing bones are physiologically different in relation to the way they react to load. Bone ash was reduced in weight-bearing vertebrae (L5) but not in non-weight-bearing calvaria or mandibles of rats subjected to microgravity for 12 days[35]. In astronauts (n=2), the bone mineral density (BMD) of non-weight-bearing skulls increased, whilst the BMD decreased in their weight-bearing vertebrae after space flight[36]. Furthermore, calvarial bone appears to be resistant to osteoporosis compared to other bones[21]. Whether increased NE concentrations reduce bone loss in skulls in situations such as weightlessness or osteoporotic conditions is presently unknown. However, increased concentrations of IGF-II and TGF-β in human calvaria compared to iliac crest or vertebral bodies have been suggested to contribute to the osteoporotic resistance of the skull[21]. Further work will be needed to identify the roles of endogenous NGF and NE in these separate bone types.

Significant findings from the present study on HT rats were that concentrations of NGF increased in ribs but not in femurs. These observations give rise to the possibility that bone type (possibly weight-bearing or non-weight-bearing) influences the response of osseous NGF levels to hyperthyroidism. High plasma thyroid hormone concentrations increase NGF concentrations in submaxillary gland, liver[14] and hippocampus[37] of rats but not cardiac muscle and kidney[14], which suggests the effect of thyroid hormone on NGF synthesis, may be tissue dependent. The present results indicate that the effect of thyroid hormone on osseous NGF synthesis may also be bone-type dependent. This finding is consistent with the observations that in femurs of thyrotoxic rats, bone densities were decreased and osteoblastic and osteoclastic gene expression markers were raised, whilst vertebral bone density was unchanged[38].

Presently, the role of NGF in bone metabolism remains obscure. It is well known that NGF is important for the appropriate development and maintenance of both peripheral sensory and post-ganglionic sympathetic nerves[39] and such nerves are known to influence bone remodeling[6]. Therefore, NGF may have an indirect influence on bone remodeling through its action on peripheral nerves. Recent studies, however, have located NGF receptors and the expression of NGF mRNA in various osteoblastic cell lines[40,41], implicating a direct effect of NGF on bone. NGF appears to elicit trophic actions on bone, as this growth factor improved the rate of repair and the biomechanical strength of calluses in fractured rat ribs[12] and stimulated the formation of osteoid tissue around nerves during sensory nerve regeneration in mandibles of rabbits[13]. These observations suggest that NGF promotes bone formation. Both bone formation and bone resorption increase in the HT state, however, resorption is greater than formation and this results in a net bone loss[1-4]. Therefore, it is possible that the raised osseous NGF concentrations reported in the current experiment could be one of the contributing factors to an increased bone formation which occurs in hyperthyroidism.

The final significant finding from the present study was that hyperthyroidism depressed NE levels in calvaria, ribs and tibiae (but not femurs, where the decrease was not significant). Previous studies have shown that hyperthyroidism reduces plasma NE concentrations in both humans[42] and animals[43] and this reduction is possibly due to an increase in the activity of the enzyme, monoamine oxidase, which degrades NE in various tissues[44,45]. The reduction of osseous NE concentrations in bones of HT animals in the current study indicates that thyroid hormone may modulate the adrenergic influence on bone. Adrenergic nerves are present in regions of high osteogenic activity[16]. Beta-2-adrenergic receptors have been identified in human[17,46] and rat[17] osteoblast-like cells and it is proposed that NE acts through osteoblasts via these receptors to stimulate bone resorption. The role of NE in stimulating bone resorption was suggested by the increased bone resorption that was observed in mouse calvaria *in vitro*[17] and in humans with phaeochromocytoma[47]. It is probable that the high plasma thyroid hormone concentration is the main contributor to the increased bone resorption seen in HT rats. It has been shown that there is an upregulation of beta-2-adrenergic receptors in subcutaneous adipocytes from patients with hyperthyroidism[48]. If a similar upregulation of beta-2-adrenergic receptors occurs in osteoblasts of HT animals then low osseous NE concentrations may contribute to the increased bone resorption in HT animals.

In conclusion, previous research predicts that thyroid hormone directly stimulates receptors on osteoblasts to increase both osteoblastic bone formation and osteoclastic bone resorption[5,49-51]. Osseous nerves, however, also affect bone remodeling and thyroid hormone is an integral factor in the maintenance of such nerves. The results presented in the current experiments show that thyroid hormone status modulates levels of two neural-associated substances, NGF and NE, within most bones studied. The possibility therefore arises that the influence of thyroid hormone on the nerves that supply bone could be another physiologic or pathophysiologic pathway for the action of thyroid hormone on bone in either euthyroid or hyperthyroid states.

Acknowledgments

The authors gratefully acknowledge the funding provided by the La Trobe University, Faculty of Health Sciences Research Grants (No. 00/A6) and the Australian Research Council (13049).

References

1. Mosekilde L, Eriksen EF, Charles P. Effects of thyroid hormone on bone and mineral metabolism. Endocrinol Metab Clin North Am 1990; 19:35-63.
2. Bijlsma JWJ, Duursma SA, Roelofs JMM, der Kindren PJ. Thyroid function and bone turnover. Acta Endocrinol (Copenh) 1983; 104:42-49.
3. Allain TJ, McGregor AM. Review: Thyroid hormones and bone. J Endocrinol 1993; 139:9-18.
4. Klaushofer K, Varga F, Glantschnig H, Fratzl-zelman N, Czerwenka E, Leis HJ, Koller K, Peterlik M. The regulatory role of thyroid hormones in bone cell growth and differentiation. Intern Con Prog Bone Min Res 1995; (suppl): 1996S-2003S.
5. Siddiqi A, Monson JP, Wood DF, Besser GM, Burrin, JM. Serum cytokines in thyrotoxicosis. J Clin Endocrinol Metab 1999; 84:435-439.
6. Garcia-Castellano JM, Diaz-Herrera P, Morcuende JA. Is bone a target-tissue for the nervous system? New advances on the understanding of their interactions. Iowa Orthop J 2000 20:49-58.
7. Barakat-Walter I. Role of thyroid hormones and their receptors in peripheral nerve regeneration. J Neurobiol 1999; 40:541-559.
8. Frenkel SR, Guerra LA, Mitchell OG, Singh IJ. Nerve growth factor in skeletal tissues of the embryonic chick. Cell Tissue Res 1990; 260:507-511.
9. Grills BL, Schuijers JA. Immunohistochemical localization of nerve growth factor in fractured and unfractured rat bone. Acta Orthop Scand 1998; 69:415-419.
10. Asaumi D, Nakanishi T, Asahara H, Inoue H, Takigawa. Expression of neuropeptides and their receptors (TRK) during fracture healing. Bone 2000; 26:625-633.
11. Jehan F, Naveilhan P, Neveu I, Harvie D, Dicou KE, Brachet P, Wion D. Regulation of NGF, BDNF and LNGFR gene expression in ROS 17/2.8 cells. Mol Cell Endocrinol 1996; 116:149-156.
12. Grills BL, Schuijers JA, Ward AR. Topical application of nerve growth factor improves fracture healing in rats. J Orthop Res 1997; 15:235-242.
13. Eppley BL, Snyderes RV, Winkelmann TM, Roufa DG. Efficacy of nerve growth factor in regeneration of mandibular nerve. J Oral Maxillofac Surg 1991; 49:61-68.
14. Walker P, Weichsel ME Jr, Guo SM, Fisher DA, Fisher DA. Radioimmunoassay for mouse nerve growth factor (NGF). Effects of thyroxine administration on tissue NGF levels. Brain Res 1980; 186:331-341.
15. Black MA, Pope L, Lefebvre F-A, Lefebvre YA, Walker P. Thyroid hormones precociously increase nerve growth factor gene expression in the submandibular gland of neonatal mice. Endocrinology 1992; 130:2083-2090.
16. Sisask G, Bjurholm A, Ahmed M, Kreicbergs A. The development of autonomic innervation in bone and joints of the rat. J Auton Nerv Syst 1996; 59:27-33.
17. Moore RE, Smith CK, Bailey CS, Voelkel EF, Tashjian AH Jr. Characterization of beta-adrenergic receptors on rat and human osteoblast-like cells and demonstration that beta-receptor agonists can stimulate bone resorption in organ culture. Bone Miner 1993; 23:301-315.
18. Christensen NJ. Plasma noradrenaline and adrenaline in patients with thyrotoxicosis and myxoedema. Clin Sci Mol Med 1973; 45:163-173.
19. Mano T, Sakamoto H, Fujita K, Makino M, Kakizawa H, Nagata M, Kotake M, Hamada M, Uchimura K, Hayakawa N, Hayashi R, Nakai A, Itoh M, Kuzuya H, Nagasaka A. Effects of thyroid hormone on catecholamine and its metabolite concentrations in rat cardiac muscle and cerebral cortex. Thyroid 1998; 8: 353-358.
20. Frost HM. From Wolff's Law to the Utah paradigm: Insights about bone physiology and its clinical applications. Anat Rec 2001; 262:398-419.
21. Finkelman RD, Eason AL, Rakijian DR, Tutundzhyan Y, Hardesty RA. Elevated IGF-II and TGF-beta concentrations in human calvarial bone: potential mechanism for increased graft survival and resistance to osteoporosis. Plast Reconstr Surg 1994; 93:732-738.
22. Finkelstein DI, Dooley PC, Luff AR. Recovery of muscle after different periods of denervation and treatments. Muscle Nerve 1993; 16:769-777.
23. Fitzsimons DP, Herrick RE, Baldwin RM. Isomyosin distribution in rodent muscles: effects of altered thyroid state. J Appl Physiol 1990; 69:321-329.
24. Grills BL, Gallagher JA, Allan EA, Yumita S, Martin TJ. Identification of plasminogen activator in osteoclasts. J Bone Miner Res 1990; 5:499-505.
25. Bancroft JD, Stevens A. Theory and practice of histological techniques, 4th edition. Churchill Livingstone, New York; 1996:332-334.
26. Bennett AD, Taglialatela G, Preze-Polo R, Hulsebosh CE. NGF levels decrease in the spinal cord and dorsal root ganglion after spinal hemisection. Neuroreport 1999; 10:889-893.
27. Schuijers JA, Walker DW, Browne CA, Thornburn GD. Effect of hypoxemia on plasma catecholamines in intact and immunosympathectomized fetal lambs. Am J Physiol 1986; 251:R893-R900.
28. Davies DT. Assessment of rodent thyroid endocrinology: Advantages and pitfalls. Comp Hematol Intern 1993; 3:142-152.
29. Carter WJ, Lynch ME. Effect of clenbuterol on recovery of muscle mass and carcass protein content following experimental hyperthyroidism in old rats. Comp Biochem Physiol Comp Physiol 1994; 108:387-394.

30. Grofte T, Wolthers T, Jensen DS, Moller N, Jorgensen JOL, Orskov H, Vilstrup H. Hepatic amino nitrogen conversion and organ N-contents in hypothyroidism, with thyroxine replacement, and in hyperthyroid rats. J Hepatol 1997; 26:409-416.
31. Allain TJ, Thomas MR, McGregor AM, Salisbury JR. A histomorphometric study of bone changes in thyroid dysfunction in rats. Bone 1995; 16:505-509.
32. Melsen F, Mosekilde L. Morphometric and dynamic studies of bone changes in hyperthyroidism. Acta Pathol Microbiol Scand 1977; 85:141-150.
33. Alberius P, Skagerberg G. Adrenergic innervation of the calvarium of the neonatal rat. Its relationship to the sagittal suture and developing parietal bones. Anat Embryol (Berl) 1990; 182:493-498.
34. Bjurholm A, Kreicbergs A, Terenuis L, Goldstein M, Schultzberg M. Neuropeptide Y-, tyrosine hydroxylase- and vasoactive intestinal polypeptide-immunoreactive nerves in bone and surrounding tissues. J Auton Nerv Syst 1988; 25:119-125.
35. Simmons DJ, Grynpas MD, Rosenberg GD. Maturation of bone and dentin matrices in rats flown on the Soviet biosatellite Cosmos 1887. FASEB J 1990; 4:29-33.
36. Miyamoto A, Shigematsu T, Fukunaga T, Kawakami K, Mukai C, Sekiguchi C. Medical baseline data collection on bone and muscle change with space flight. Bone 1998; 22(5 Suppl): 79S-82S.
37. Giordano T, Pan JB, Casuto D, Watanabe S, Arneric SP. Thyroid hormone regulation of NGF, NT-3 and BDNF RNA in the adult rat brain. Brain Res Mol Brain Res 1992; 16:239-245.
38. Suwanwalaikorn S, Ongphiphadhanakul B, Braverman LE, Baran DT. Differential responses of femoral and vertebral bones to long-term excessive L-thyroxine administration in adult rats. Eur J Endocrinol 1996; 134:655-659.
39. Levi-Montalcini R, Angeletti PU. The nerve growth factor. Physiol Rev 1968; 48:534-569.
40. Nakanishi T, Takahashi K, Aoki C, Nishikawa K, Hattori T, Taniguch S. Expression of nerve growth factor family neurotrophins in a mouse osteoblastic cell line. Biochem Biophys Res Comm 1994; 198:891-897.
41. Uebergang T, Thomas R, Gillespie M, Grills B. Nerve growth factor (NGF) and NGF receptor mRNA expression in UMR 201 and UMR 106.01 cells. Proc ANZBMS 1999; 9:63.
42. Bayliss RIS, Edwards OM. Urinary excretion of free catecholamines in Graves' disease. Endocrinology 1971; 49:167-173.
43. Beaven MA, Costa E, Brodie BB. The turnover of norepinephrine in thyrotoxic and nonthyrotoxic mice. Life Sci 1963; 4:241-246.
44. Gripois D, Fernandez C. Effects of thyroid hormones on the evolution of monoamine oxidase activity in the brain and heart of the developing rat. Enzyme 1977; 22:378-384.
45. Lyles GA, Callingham BA. The effects of thyroid hormones on monoamine oxidase in the rat heart. J Pharm Pharmacol 1974; 26:921-930.
46. Togari A, Arai M, Mizutani S, Mizutani S, Koshihara Y, Nagatsu T. Expression of mRNAs for neuropeptide receptors and beta-adrenergic receptors in human osteoblasts and human osteogenic sarcoma cells. Neurosci Lett 1997; 233:125-128.
47. Skrabanek P. Catecholamines cause the hypercalciuria and hypercalcaemia in phaeochromocytoma and in hyperthyroidism. Med Hypotheses 1977; 3:59-62.
48. Hellstrom L, Wahrenberg H, Reynisdottir S, Arner P. Catecholamine-induced adipocyte lipolysis in human hyperthyroidism. J Clin Endocrinol Metab 1997; 82:159-66.
49. Britto JM, Fenton AJ, Holloway WR, Nicholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 1994; 134:69-176.
50. Allain TJ, Chambers TJ, Flanagan AM, McGregor AM. Tri-iodothyronine stimulates rat osteoclastic bone resorption by an indirect effect. J Endocrinol 1992; 133:327-331.
51. Glass CK, Holloway JM. Regulation of gene expression by the thyroid hormone receptor. Biochim Biophys Acta 1990; 1032:157-176.