Loss of RAGE Defense: A Cause of Charcot Neuroarthropathy?

Kara A. Witzke, phd[1]
Aaron I. Vinik, md, phd[2]
Lisa M. Grant[2]
William P. Grant, dpm[3]

Henri K. Parson, phd[2]
Gary L. Pittenger, phd[2]
Niculina Burcus, ms[2]

OBJECTIVE—This study investigated the relationship between circulating soluble receptor for advanced glycation end products (sRAGE) and parameters of bone health in patients with Charcot neuroarthropathy (CNA).

RESEARCH DESIGN AND METHODS—Eighty men (aged 55.3 ± 9.0 years), including 30 healthy control subjects, 30 type 2 diabetic patients without Charcot, and 20 type 2 diabetic patients with stage 2 (nonacute) CNA, underwent evaluations of peripheral and autonomic neuropathy, nerve conduction, markers of bone turnover, bone mineral density, and bone stiffness of the calcaneus.

RESULTS—CNA patients had worse peripheral and autonomic neuropathy and a lower bone stiffness index than diabetic or control individuals (77.1, 103.3, and 105.1, respectively; $P < 0.05$), but no difference in bone mineral density ($P > 0.05$). CNA subjects also had lower sRAGE levels than control (162 vs. 1,140 pg/mL; $P < 0.01$) and diabetic (162 vs. 522 pg/mL; $P < 0.05$) subjects, and higher circulating osteocalcin levels.

CONCLUSIONS—CNA patients had significantly lower circulating sRAGE, with an accompanying increase in serum markers of bone turnover, and reduced bone stiffness in the calcaneus not accompanied by reductions in bone mineral density. These data suggest a failure of RAGE defense mechanisms against oxidative stress in diabetes. Future studies should determine if medications that increase sRAGE activity could be useful in mitigating progression to CNA.

Diabetes Care 34:1617–1621, 2011

Charcot neuroarthropathy (CNA) is a progressive, degenerative process that usually occurs in the ankle and midfoot. Although not exclusive to patients with diabetes, this condition will develop in ~1 in 600 patients with diabetes and 1 in 100 with neuropathy, although no current large-scale epidemiologic study has reported the true incidence of CNA (1,2). Clinical manifestations are marked by tissue and bone inflammation, bone destruction, resorption, and eventual joint deformity.

CNA is likely caused by a complex interaction between predisposing factors, including the presence of diabetes, neuropathy, and an intact peripheral circulation that provides the ability to mount an inflammatory response (3). The "neurovascular theory" of CNA, proposed by Charcot in 1868, suggests that bony changes result from damage to the central nervous system that directly controls bone nutrition and leads to uncontrolled inflammation. The "neurotraumatic theory" proposed shortly thereafter, however, postulates that CNA may be triggered by external trauma to the foot that sets the inflammatory response in motion. In either case, the progression of inflammation eventually leads to bone lysis, microfracture, and bone deformity.

The work of Mabilleau and Edmonds (4) suggests both theories have merit, and that although osteoclastic resorption mediated by receptor activator for nuclear factor-κB ligand (RANKL) occurs in acute Charcot osteoarthropathy, a RANKL-independent pathway mediated by proinflammatory cytokines may also be important (5).

The formation of advanced glycation end products (AGEs), driven by hyperglycemia and oxidative stress, is an important biochemical abnormality that accompanies diabetes and inflammation in general. An increase in AGE-modified collagen has been detected in tissues with the slowest turnover, such as the cortical bone of diabetic and old rats (6). This modified collagen may play a role in the pathogenesis of osteopenia present in patients whose diabetes is poorly controlled (7). AGEs also stimulate apoptosis of human mesenchymal stem cells (8) and osteoblast apoptosis through a nuclear factor-κB–independent mechanism that further limits bone formation (9). A proposed mechanism through which diabetes induces apoptosis by stimulating AGEs is via *N*-(carboxymethyl)lysine collagen, mediated through receptor for advanced glycation end products (RAGE), the pattern recognition receptor for AGE. RAGE is constitutively expressed but is induced by reactions known to initiate inflammation. RAGE has been suggested to increase the activation of RANKL, leading to enhanced osteoclastogenesis and impaired matrix mineralization (10,11).

We have previously shown an increase in skin blood flow in the affected foot of Charcot patients (12). In addition, we have suggested derangements in Charcot collagen structure of the Achilles' tendon, perhaps caused by accumulation of AGE cross-links (13,14), and a reduction in calcaneal bone stiffness despite normal foot bone mineral density (BMD) values in Charcot patients (15). These findings correlate CNA pathogenesis with bone quality, rather than bone density, which may be a more important predictor of fracture than bone density alone (11).

Our previous observations and the suggestion by others that RAGE-enhanced

---

osteoclastogenesis impairs matrix mineralization led us to investigate the relationship between circulating sRAGE and parameters of bone health in patients with CNA. We hypothesized that CNA patients would display more severe neuropathy, worse bone health, and decreased sRAGE than diabetic patients without CNA or healthy control individuals.

RESEARCH DESIGN AND METHODS

For this cross-sectional study, we recruited men with type 2 diabetes (with and without CNA), and attempted to age-match them with healthy control subjects. Inclusion criteria for the diabetic participants (with and without CNA) included ambulatory men aged between 40 and 80 years with established type 2 diabetes for more than 5 years and diabetes-derived nerve damage. Exclusion criteria included a documented history of vitamin D deficiency, thyroid disease, renal disease, or other metabolic diseases known to affect bone, as well as prior oral or intravenous antiresorptive drug use. Charcot patients had to be ambulatory and in the nonacute, convalescence stage (stage 2) of the disease, which is different from acute stage (stage 1) that is characterized by osseous resorption, soft-tissue swelling, and joint effusions (16). Control subjects were healthy, with no known chronic disease. This study was approved by the Eastern Virginia Medical School Institutional Review Board, and all subjects provided written consent before participating in this study.

The inclusion criteria for the study were met by 80 men, comprising 30 healthy control subjects, 30 type 2 diabetic patients without Charcot, and 20 type 2 diabetic patients with stage 2 CNA. Many of the CNA patients had, however, already undergone stabilization of the fractured or dislocated joints with beaming screws and other means of metallic fixation. Descriptive characteristics for all subjects are reported in Table 1. Subjects' evaluation consisted of an analysis of fasting blood chemistries; a complete neurologic examination, including quantitative sensory, autonomic nerve function, and nerve conduction tests; a bone density assessment; and quantitative ultrasound imaging of bilateral calcanei.

Nerve conduction studies

Lower limb nerve conduction parameters were measured bilaterally by surface stimulation of the sural, peroneal, and tibial nerves. Measurements included peroneal and tibial motor nerve amplitude, conduction velocity, distal onset latency, and F wave. All motor amplitudes were measured baseline to peak, and latencies were taken as onset latency. Motor conduction velocity was calculated as total conduction velocity (not segmental) using onset latencies throughout. Sensory amplitude was taken as baseline to peak, latency was reported as onset latency, and conduction velocity was calculated across the single segment using onset latency (Neuromax, Excel-Tech Ltd., Oakville, ON, Canada).

Quantitative sensory tests

Quantitative sensory testing was performed using the Medoc device according to previously published methods (17). Warm and cold thermal thresholds and heat and cold pain were tested using the TSA2001/VSA3001 computer-driven sensory measuring device (Medoc, Durham, NC). Touch pressure was measured using graded Semmes-Weinstein monofilaments (18). Vibration perception threshold was quantified using the Medoc device and the "method of limits" protocol, where repeated, increasing vibratory stimulation is applied to the plantar aspect of the dominant great toe. Subjects pushed a hand-held button as soon as they detected the vibration. The mean result of six trials was used in all analyses.

Autonomic function tests

Autonomic function was assessed by three tests using the ANX-3.0 Autonomic Monitor: heart rate variability during deep breathing at six breaths per min (E/I ratio) and R/R variation in response to the Valsalva maneuver and postural change (ANSAR Medical Technologies, Inc., Philadelphia, PA) (19).

BMD

BMD scans of the whole body, bilateral proximal femurs, and bilateral feet were obtained using dual-energy X-ray absorptiometry (GE Lunar Prodigy Advance, GE Healthcare, Waukesha, WI). Scans were performed according to the manufacturer's protocols, with the exception of the feet, for which no standardized protocol exists. Therefore, we developed a technique that used the "hand" mode, where a subject sat on the edge of the scan bed with the knee bent at 90° and the foot flat on the bed. The laser marker was positioned at the midline of the ankle, at the level of the lateral malleolus. During analysis, the region of interest was selected manually and included

Table 1—Patient demographics

Variable	Control subjects ($n = 30$)	Diabetic subjects ($n = 30$)	Charcot subjects ($n = 20$)
Duration of diabetes (months)	—	133 ± 80	178 ± 100
Age (years)	52.9 ± 7.7 (40–69)	$57.8 \pm 8.8^*$ (41–73)	$57.9 \pm 10.1^*$ (43–78)
Race			
Black	10	15	3
White	19	15	16
Asian	1	0	0
Hispanic	0	0	1
Weight (kg)	89.4 ± 15.2	$99.9 \pm 18.8^*$	$110.0 \pm 23.4^*$
BMI (kg/m^2)	28.8 ± 6.2	$32.9 \pm 7.0^*$	$33.7 \pm 5.6^*$
Waist-to-hip ratio	0.94 ± 0.06	$1.01 \pm 0.05^*$	$1.02 \pm 0.06^*$
BP (mmHg)			
Systolic	132 ± 13.8	137.9 ± 14.4	$149.7 \pm 19.7^*$
Diastolic	82.3 ± 10.5	82.6 ± 12.5	83.1 ± 7.4
HbA$_{1c}$ (%)	6.1 ± 1.0	7.0 ± 2.9	$8.3 \pm 1.6^*$
Glucose (mg/dL)	84.6 ± 26.1	$147.0 \pm 77.0^*$	$171.4 \pm 107.9^*$
Total calcium (mg/dL)	8.9 ± 0.6	8.9 ± 0.6	8.8 ± 0.6
Parathyroid hormone intact (pg/mL)	32.5 ± 24.1	18.1 ± 15.4†	38.3 ± 19.7
Vitamin D 25OH (ng/mL)	19.4 ± 9.7	16.4 ± 8.1	17.9 ± 9.6

Continuous data are expressed as mean $\pm$ SD (range), and categoric data as n. $^*P < 0.05$ vs. control subjects. †$P < 0.05$ vs. control and Charcot subjects.

only the metatarsals and phalanges to exclude any metal fixators.

Test-retest reliability for this measure is high ($r = 0.96$, $n = 31$), and the coefficient of variation is <1% for our laboratory. This corresponds well to other published studies of reproducibility of BMD using the hand mode (20), and is also comparable with other regional analyses of bone, where coefficients of variation typically range from 0.5 to 1.2%. Because no reference database currently exists for scans of the feet, diabetic patients were compared with sex- and age-matched control subjects.

Calcaneal stiffness

Calcaneal stiffness was determined using quantitative ultrasound imaging (GE Achilles Express, Waukesha, WI). Bone stiffness is automatically computed using an algorithm supplied by the manufacturer: stiffness index = $0.67 \times$ BUA $\pm$ $0.28 \times$ SOS $- 420$, where BUA is broadband ultrasound attenuation and SOS is the speed of sound through the tissues (GE Healthcare). Because bone stiffness predicts fracture independent of BMD, it is considered to be a measure of bone quality that also accounts for factors related to fracture that might not be detected by a measure of BMD alone.

Blood measures

Fasting blood samples were obtained by antecubital venipuncture into serum separator tubes and separated immediately by refrigerated centrifugation. The serum was stored at $-80°C$ according to instructions required by the manufacturers of the enzyme-linked immunosorbent assay (ELISA) kit. Samples were subsequently evaluated in duplicate for sRAGE by the Quantikine RAGE enzyme-linked immunoassay (R&D Systems, Minneapolis, MN); cross-linked *N*-telopeptides of type 1 (NTx) collagen, as a measure of bone resorption (Osteomark NTx ELISA/EIA, Wampole Laboratories, Princeton, NJ); and osteocalcin, as a measure of bone formation (ALPCO Diagnostics, Salem, NH).

Statistical analysis

Group differences on each dependent measure were evaluated using one-way ANOVA with a least significant differences post hoc analysis. Pearson-product moment correlations were also performed to determine significant relationships among variables. A multiple linear stepwise regression analysis was run to help identify factors that may distinguish those

who develop CNA from those who do not. All analyses were run using SPSS 18.0 software (SPSS Inc., Chicago, IL) with significance set at $P < 0.05$.

RESULTS—We evaluated 80 adult men, aged 55.3 ± 9.0 years, consisting of 30 healthy control subjects, 30 type 2 diabetic patients, and 20 type 2 diabetic patients diagnosed with stage 2 CNA. According to the classification scheme of Sanders and Frykberg (16) to describe neuropathic osteoarthropathy patterns of joint involvement, 28% of CNA patients displayed pattern 2 (tarsometatarsal/Lisfranc's joint involvement), 45% displayed pattern 3 (midtarsal and naviculo-cuneiform joint involvement), and 27% displayed pattern 4 (ankle and subtalar joint involvement). No patients were classified as pattern 1 (forefoot) or pattern 5 (calcaneus), which might have otherwise interfered with the measurement of BMD or calcaneal stiffness, or both.

CNA patients had similar duration of diabetes but higher HbA$_{1c}$ levels than their counterparts with diabetes (8.3 ± 1.6 vs. 7.0 ± 2.9%, Table 1). Fasting serum blood urea nitrogen and creatinine values for all diabetic and CNA patients were within the normal reference ranges. CNA and diabetic patients were slightly older, had a greater BMI, and a higher

waist-to-hip ratio than control subjects. Systolic blood pressure was higher in CNA subjects than in diabetic or control participants.

Diabetic and CNA patients showed more peripheral nerve damage than control subjects, and CNA subjects exhibited the highest degree of sympathetic and parasympathetic dysfunction and small and large fiber neuropathy compared with the other study groups (Table 2).

BMD of the proximal femur was slightly higher at the femoral neck and greater trochanter for diabetic patients than for CNA or control individuals, but BMD of the feet was not significantly different across groups (Table 3). Calcaneal stiffness was similar for diabetic and control subjects but was markedly reduced in the CNA patients ($P < 0.01$). These results indicate that ultrasound imaging may detect features that are not detectable by BMD scans using dual-energy X-ray absorptiometry.

Osteocalcin was only elevated in CNA, and NTx did not differ across groups. CNA patients displayed sRAGE values more than seven times lower than control subjects and more than three times lower than diabetic patients ($P < 0.05$). Across all subjects, there was a significant positive correlation between the calcaneal bone stiffness index and sRAGE

Table 2—Peripheral and autonomic neuropathy tests

Variable	Control subjects n = 30	Diabetic subjects n = 30	Charcot subjects n = 20
Tibia-ankle			
Amplitude (μV)	8.9 ± 4.1	4.3 ± 3.8*	0.6 ± 1.0†
Latency (msec)	4.7 ± 0.7	4.2 ± 1.8	3.6 ± 5.5
Conduction velocity (m/sec)	45.4 ± 3.7	35.4 ± 15.2*	7.7 ± 13.1†
F-wave (msec)	54.7 ± 6.4	47.5 ± 23.3	17.5 ± 29.7†
Peroneal-ankle			
Amplitude (μV)	4.8 ± 2.2	2.6 ± 2.3*	0.1 ± 0.3†
Latency (msec)	14.1 ± 2.1	13.6 ± 5.2	8.9 ± 9.4†
Conduction velocity (m/sec)	44.5 ± 3.3	35.6 ± 13.1*	16.4 ± 17.0†
F-wave (msec)	42.2 ± 22.1	38.8 ± 27.2	4.3 ± 17.2†
Positive sural response	30	28	4
Vibration perception (μ)	10.6 ± 11.1	31.7 ± 27.3*	100.5 ± 31.5†
Pressure perception (g)	2.9 ± 0.4	3.1 ± 0.3	5.9 ± 1.2†
Cold sensation (°C)	5.5 ± 2.7	7.7 ± 5.2	20.9 ± 9.9†
Warm sensation (°C)	7.1 ± 3.1	10.3 ± 3.2*	14.9 ± 3.2†
Cold pain	24.5 ± 9.0	26.4 ± 6.6	30.6 ± 3.5†
Warm pain	14.0 ± 3.3	15.4 ± 2.5*	17.5 ± 1.0†
Valsalva ratio	1.43 ± 0.25	1.36 ± 0.31	1.18 ± 0.28†
Parasympathetic deep breathing	611 ± 13	617 ± 13	632 ± 18†
Sympathetic Valsalva response	714 ± 17	713 ± 16	717 ± 20

Continuous data are expressed as mean ± SD; categoric data as *n*. *$P < 0.05$ vs. control subjects. †$P < 0.05$ vs. diabetic and control subjects.

Table 3—Blood biomarkers, bone density, and stiffness

Variable	Control subjects $n = 30$	Diabetic subjects $n = 30$	Charcot subjects $n = 20$
NTx (nmol/L)	13.6 ± 5.5	11.4 ± 5.7	13.8 ± 8.2
Osteocalcin (ng/mL)	1.29 ± 0.96	1.23 ± 0.68	2.29 ± 2.57*
sRAGE (pg/mL)	1140 ± 471	522 ± 660†	162 ± 67*
BMD (g/cm^2)			
Femoral neck	1.004 ± 0.141	1.048 ± 0.184‡	0.904 ± 0.219
Greater trochanter	1.080 ± 0.157	1.116 ± 0.165‡	0.987 ± 0.185
Total hip	1.018 ± 0.129	1.037 ± 0.150	0.955 ± 0.252
Foot	0.754 ± 0.081	0.792 ± 0.105	0.788 ± 0.120
Calcaneal stiffness index	105.2 ± 14.0	103.3 ± 18.6	77.1 ± 26.2*

Data are presented as mean ± SD. *$P < 0.05$ vs. diabetic and control subjects. †$P < 0.05$ vs. control subjects. ‡$P < 0.05$ vs. Charcot subjects.

($r = 0.35$, $P < 0.01$) and a negative correlation between stiffness index and osteocalcin ($r = -0.39$, $P < 0.001$). These results demonstrate a cross-sectional relationship between an impaired AGE defense, increased bone turnover, and reduced bone stiffness.

In multiple linear stepwise regression analysis, when measures of large and small fiber neuropathy, autonomic neuropathy, blood measures, BMD, and bone stiffness were entered into the model, 74.5% of group membership was predicted by vibration perception, pressure perception, and sRAGE ($r^2 = 0.745$, $P < 0.001$). Similarly, vibration perception and sympathetic Valsalva response accounted for 50.3% of the variability in bone stiffness ($r^2 = 0.503$, $P < 0.001$), indicating a possible relationship of both large fiber and autonomic neuropathies in the progression to CNA.

CONCLUSIONS—The two most important findings from this study were *1*) an indication that Charcot patients have an impaired RAGE defense mechanism with an accompanying increase in serum markers of bone turnover, and *2*) reduced bone stiffness in the calcaneus, not accompanied by reductions in bone mineral density.

In this study, sRAGE values in CNA patients were reduced by 86%, but the diabetic patients had a 50% reduction compared with control subjects. Hyperglycemia generates higher levels of AGE, but without adequate sRAGE to bind AGE, it and other ligands tend to accumulate in tissues with slow turnover, such as tendon, skin, bone, amyloid plaques, and cartilage (21). Accumulation of AGE has been shown to reduce osteoblastic activity via an increase in RAGE that increases RANKL activation that leads to enhanced osteoclastogenesis and impaired matrix mineralization (10,11,22). This cascade predisposes bone to fracture. Although our biomarker for bone resorption (NTx) was not different between groups, this does not preclude the possibility that bone may be inadequately mineralized and not contributing to bone strength. Studies have demonstrated that bone toughness and stiffness are affected by AGE cross-link formation, and the elastic modulus is independent of other determinants of bone strength such as bone density and microarchitecture (23,24). Our finding that bone stiffness detected by quantitative ultrasound imaging was markedly lower in CNA subjects, despite BMD values in the feet and proximal femur within normal reference ranges, supports the notion that bone stiffness is not necessarily correlated with BMD in certain disease states.

Osteocalcin, a marker of bone formation, was only elevated in CNA subjects. Elevated bone formation activity in Charcot patients is likely the result of an accumulation of microfractures, and an excessive attempt at bone repair to maintain bone integrity of the foot. So taken together, our finding that CNA patients display higher levels of bone formation markers and reduced sRAGE may suggest that CNA patients are susceptible to AGE-mediated osteoblast apoptosis, thereby limiting their ability to repair bone. This represents another possible pathway linking CNA to bone loss besides the RANKL/osteoprotegerin pathway. It would be valuable to investigate whether certain drugs that raise RAGE levels (e.g., ACE inhibitors, statins, and glitazones) (25), may also have a role in the prevention of Charcot progression in patients with severe neuropathy.

We have shown, along with others, that Charcot patients display significantly worse autonomic neuropathy (both sympathetic and parasympathetic) than diabetic or control subjects. This is an important finding, because it has recently been suggested that the presence of CNA may coincide with a loss of sympathetic activity that allows shunt vessels to open, which increases perfusion of the feet and may speed bone resorption (12). Simple autonomic function tests may indeed be useful tools to detect an increased predisposition to developing CNA in those with diabetes.

A limitation of our study was the slightly older age of the diabetic and CNA patients compared with control subjects, despite an attempt to age-match subjects. Although BMD is age-dependent, BMD did not differ between the groups. In addition, bone stiffness and RAGE levels were still different between diabetic and CNA individuals, despite the presence of diabetes for a similar duration. Therefore, we were not concerned that these comparisons were confounded by the 5-year difference in average age between the diabetic and CNA subjects and the control group. Although all of our CNA patients were in the chronic stage 2 of the disease, we did include patients who had recently undergone operative stabilization. This type of stabilization, however, does not represent a "cure" or even improvement of the underlying disease process. It is possible, however, that postsurgical CNA patients may possess a different biochemical profile than presurgical patients. None of our CNA patients had involvement of the calcaneus or metatarsals, so we were confident that our measures of bone stiffness and BMD were accurate. Lastly, we did not evaluate classic inflammation or oxidative stress markers, which would have added significantly to the argument that reductions in sRAGE promote inflammation or oxidative stress, or both.

In summary, we show that there is a spectrum of sRAGE deficiency wherein diabetes reduces sRAGE levels by 50%, suggesting a role in the complications of diabetes. Furthermore, CNA patients who have progressed to stage 2 have an 86% reduction in sRAGE and extremely advanced somatic and autonomic neuropathy that may prevent a normal defense against oxidative stress and may predispose them to reductions in bone integrity and strength. To further elucidate the deleterious effects of CNA on

collagen and bone quality, future studies could evaluate components of bone quality such as bone microarchitecture and mineralization from calcaneal biopsy specimens of CNA patients. In addition, it would be very valuable to fully explore the protective potential of medications that elevate RAGE on bone formation pathways in this vulnerable population.

References

1. Sinha S, Munichoodappa CS, Kozak GP. Neuro-arthropathy (Charcot joints) in diabetes mellitus (clinical study of 101 cases). Medicine (Baltimore) 1972;51:191–210
2. Fabrin J, Larsen K, Holstein PE. Long-term follow-up in diabetic Charcot feet with spontaneous onset. Diabetes Care 2000;23:796–800
3. Jeffcoate W. The causes of the Charcot syndrome. Clin Podiatr Med Surg 2008;25:29–42, vi
4. Mabilleau G, Edmonds ME. Role of neuropathy on fracture healing in Charcot neuro-osteoarthropathy. J Musculoskelet Neuronal Interact 2010;10:84–91
5. Mabilleau G, Petrova NL, Edmonds ME, Sabokbar A. Increased osteoclastic activity in acute Charcot's osteoarthropathy: the role of receptor activator of nuclear factor-kappaB ligand. Diabetologia 2008;51:1035–1040
6. Katayama Y, Akatsu T, Yamamoto M, Kugai N, Nagata N. Role of nonenzymatic glycosylation of type I collagen in diabetic osteopenia. J Bone Miner Res 1996;11:931–937
7. Levin ME, Boisseau VC, Avioli LV. Effects of diabetes mellitus on bone mass in juvenile and adult-onset diabetes. N Engl J Med 1976;294:241–245
8. Kume S, Kato S, Yamagishi S, et al. Advanced glycation end-products attenuate human mesenchymal stem cells and prevent cognate differentiation into adipose tissue, cartilage, and bone. J Bone Miner Res 2005;20:1647–1658
9. Alikhani M, Alikhani Z, Boyd C, et al. Advanced glycation end products stimulate osteoblast apoptosis via the MAP kinase and cytosolic apoptotic pathways. Bone 2007;40:345–353
10. Macaione V, Aguennouz M, Rodolico C, et al. RAGE-NF-kappaB pathway activation in response to oxidative stress in facioscapulohumeral muscular dystrophy. Acta Neurol Scand 2007;115:115–121
11. Yaturu S. Diabetes and skeletal health. J Diabetes 2009;1:246–254
12. Shapiro SA, Stansberry KB, Hill MA, et al. Normal blood flow response and vasomotion in the diabetic Charcot foot. J Diabetes Complications 1998;12:147–153
13. Vashishth D. Advanced glycation end-products and bone fractures. IBMS BoneKEy 2009;6:268–278
14. Grant WP, Sullivan R, Sonenshine DE, et al. Electron microscopic investigation of the effects of diabetes mellitus on the Achilles tendon. J Foot Ankle Surg 1997;36:272–278; discussion 330
15. Witzke KA, Vinik AI. Diabetic neuropathy in older adults. Rev Endocr Metab Disord 2005;6:117–127
16. Sanders L, Frykberg RG. Diabetic neuropathic osteoarthropathy. In *The High Risk Foot in Diabetes Mellitus*. Frykberg RG, Ed. New York, NY, Churchill Livingstone, 1991, p. 569
17. Vinik AI, Erbas T, Park TS, Pierce KK, Stansberry KB. Methods for evaluation of peripheral neurovascular dysfunction. Diabetes Technol Ther 2001;3:29–50
18. Vinik AI, Suwanwalaikorn S, Stansberry KB, Holland MT, McNitt PM, Colen LE. Quantitative measurement of cutaneous perception in diabetic neuropathy. Muscle Nerve 1995;18:574–584
19. Vinik AI, Ziegler D. Diabetic cardiovascular autonomic neuropathy. Circulation 2007;115:387–397
20. Peel NF, Spittlehouse AJ, Bax DE, Eastell R. Bone mineral density of the hand in rheumatoid arthritis. Arthritis Rheum 1994;37:983–991
21. Franke S, Siggelkow H, Wolf G, Hein G. Advanced glycation endproducts influence the mRNA expression of RAGE, RANKL and various osteoblastic genes in human osteoblasts. Arch Physiol Biochem 2007;113:154–161
22. McCarthy AD, Etcheverry SB, Bruzzone L, Lettieri G, Barrio DA, Cortizo AM. Nonenzymatic glycosylation of a type I collagen matrix: effects on osteoblastic development and oxidative stress. BMC Cell Biol 2001;2:16
23. Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int 2010;21:195–214
24. Viguet-Carrin S, Roux JP, Arlot ME, et al. Contribution of the advanced glycation end product pentosidine and of maturation of type I collagen to compressive biomechanical properties of human lumbar vertebrae. Bone 2006;39:1073–1079
25. Yan SF, Ramasamy R, Schmidt AM. The RAGE axis: a fundamental mechanism signaling danger to the vulnerable vasculature. Circ Res 2010;106:842–853