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LETTERS TO THE EDITOR

N-Octanoylated Ghrelin Levels in Cord and Neonatal Blood

To the editor:

I read with interest the article by Kitamura *et al.* (1) correlating ghrelin concentration in cord and neonatal blood with fetal growth and energy balance. I would like to bring to your attention some methodological concerns that have arisen.

Ghrelin is a peptide of 28 amino acids, and it requires posttranslational n-octanoyl modification of a serine residue for its activity (2, 3). This acylation enables the binding to type 1a GH secretagogue receptor that is essential for the biological functions of the hormone (2). Non-octanoylated ghrelin does not activate type 1a GH secretagogue receptor (4, 5), and it is presumed to be inactive (2).

The polyclonal antibodies used by the authors to measure plasma ghrelin do not distinguish octanoylated and nonoctanoylated ghrelin, and, as a consequence, the total ghrelin level was measured. However, a monoclonal antibody specific for the active form of ghrelin (with the octanoyl group on serine 3) is commercially available (Linco Research, St. Charles, MO; catalog no. GHRA-88HK). I believe that the measurement of total ghrelin should always be associated to the determination of the biologically active ghrelin concentration. Special precautions are required when the active form of ghrelin is measured because it is very unstable and labile in plasma; the samples should be kept in ice and processed as quickly as possible after blood is withdrawn; the plasma should be acidified.

The authors found no correlation between total ghrelin and GH in cord blood. Also, no relationship was observed between ghrelin concentrations in neonates and mean daily body weight gain during the first month of life. I believe that these observations should be confirmed by measuring the active n-octanoylated ghrelin levels.

In conclusion, further studies are required to elucidate the role of active n-octanoylated ghrelin during fetal and neonatal life and to evaluate the correlation of its levels with GH secretion.

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References

1. **Kitamura S, Yokota I, Hosoda H, Kotani Y, Matsuda J, Naito E, Ito M, Kangawa K, Kuroda Y** 2003 Ghrelin concentration in cord and neonatal blood: relation to fetal growth and energy balance. J Clin Endocrinol Metab 88:5473–5477
2. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K** 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660
3. **Hosoda H, Kojima M, Matsuo H, Kangawa K** 2000 Purification and characterization of rat des-Gln14-ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. J Biol Chem 275:21995–22000
4. **Hosoda H, Kojima M, Matsuo H, Kangawa K** 2000 Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. Biochem Biophys Res Commun 279:909–913
5. **Bednarek MA, Feighner SD, Pong SS, McKee KK, Hreniuk DL, Silva MV, Warren VA, Howard AD, Van Der Ploeg LH, Heck JV** 2000 Structure-function studies on the new growth hormone-releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of the growth hormone secretagogue receptor 1a. J Med Chem 43:4370–4376

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Authors' Response: N-Octanoylated Ghrelin Levels in Cord and Neonatal Blood

To the editor:

We thank Dr. Ferrero for the interest in our work (1). As mentioned, the measurement of an active n-octanoylated ghrelin is important. However, we must consider instability of this form. We have measured both active and total ghrelin concentrations in cord and neonatal blood. We used two types of RIA systems that we have developed (2). A RIA using antirat ghrelin N-terminal [1-11] measures the octanoylated active form of ghrelin. It was termed N-RIA. We also used an antirat ghrelin C-terminal [13-28], measuring total form ghrelin; it was termed C-RIA. The antiserum used in this assay exhibited 100% cross-reactivity with rat or human ghrelin, respectively. No significant cross-reactivity with other peptides was observed. Because the active form of ghrelin is very unstable, plasma samples were always collected in chilled tubes containing EDTA·2Na (1 mg/ml) and aprotinin (500 U/ml), separated at 4 C immediately, and added 1/10 of 1 N HCl before freezing.

There was excellent correlation between active and total ghrelin concentrations in venous cord blood ($r = 0.81$; $P < 0.0001$). Ghrelin concentration (N-RIA) negatively correlated with IGF-I concentration but did not correlate significantly with GH concentration. In the neonate, we could not find a significant correlation between ghrelin concentration (N-RIA) and mean daily body weight gain during the first month of life.

These results are comparable to our previous observations using the C-RIA (3). Because other correlation studies in our article showed similar results even with the N-RIA, we used the C-RIA to avoid the effect of instability of the active form of ghrelin. Our results suggest that the regulation of fetal ghrelin concentration may not originate in the fetal GH axis, but rather in fetomaternal energy transport.

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References

1. **Ferrero S** 2004 N-octanoylated ghrelin levels in cord and neonatal blood. J Clin Endocrinol Metab 89:4768
2. **Hosoda H, Kojima M, Matsuo H, Kangawa K** 2000 Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. Biochem Biophys Res Commun 279:909–913
3. **Kitamura S, Yokota I, Hosoda H, Kotani Y, Matsuda J, Naito E, Ito M, Kangawa K, Kuroda Y** 2003 Ghrelin concentration in cord and neonatal blood: relation to fetal growth and energy balance. J Clin Endocrinol Metab 88:5473–5477

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Should Serum Calcitonin Be Routinely Measured in Patients with Thyroid Nodules—Will the Law Answer before Endocrinologists Do?

To the editor:

In addition to the studies reviewed by Hodak and Burman (1) in their editorial question about calcitonin measurements in patients with thyroid nodules, another recent *JCEM* study (2) has addressed the clinical

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aspects of the issue without providing an answer. But there is also a legal aspect of this issue that can be stated as follows: Is it medically negligent to omit calcitonin measurement in the evaluation of the patient with a thyroid nodule? Negligence can usually be defended against by demonstrating compliance with the standard of care. In many malpractice trials, the standard of care is that of the average practitioner of the class, and North American endocrinologists do not routinely measure calcitonin in the serum of patients with thyroid nodules (3). However, there is another standard of care in medical jurisprudence, the standard of reasonable care, which is finding increasing application in our courts (4). This standard was developed and applied by the Supreme Court of Washington in the case of *Helling v. Carey* (5). In that case, Barbara Helling sued two ophthalmologists for failure to diagnose glaucoma. When they tested her eye pressure at age 32 for the first time, she was diagnosed with glaucoma, which had greatly impaired her vision. Ophthalmology standards did not then require routine pressure tests for glaucoma for patients under 40 yr of age.

The medical-legal question at trial was whether the ophthalmologists' compliance with their profession's standard protected them from liability. While two lower courts said yes, the Washington Supreme Court said no. The Court's reasoning could be used as an argument for measuring calcitonin in the serum of patients with thyroid nodules: "Although the incidence of glaucoma in the age range of the plaintiff is approximately one in 25,000, this alone should not be enough to deny her a claim. Where its presence can be detected by a simple, well-known [relatively inexpensive], harmless test, where the results of the test are definitive, where the disease can be successfully arrested by early detection, and where its effects are irreversible if undetected over a substantial period of time, liability should be imposed upon defendants even though they did not violate the standard existing within the profession of ophthalmology. . . . We therefore hold. . . . . that the reasonable standard that should have been followed. . . . . was the timely giving of this simple, harmless. . . . . test to this plaintiff and that, in failing to do so, the defendants were negligent. . . . "

The standard of care for thyroid nodules that has been established by thyroid disease experts and their professional associations does not include the measurement of serum calcitonin in North America, although it arguably does in Europe (3, 6). But what is reasonable care? Perhaps it would be prudent for endocrinologists to answer this question before the law does it for us?

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References

1. **Hodak SP, Burman KD** 2004 The calcitonin conundrum—is it time for routine measurement of serum calcitonin in patients with thyroid nodules. J Clin Endocrinol Metab 89:511–514
2. **Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, Miccoli P, Iacconi P, Basolo F, Pinchera A, Pacini F** 2004 Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. J Clin Endocrinol Metab 89:163–168
3. **Bonnema SJ, Bennedbaek FN, Ladenson PW, Hegedus L** 2002 Management of the nontoxic multinodular goiter: a North American survey. J Clin Endocrinol Metab 87:112–117
4. **Peters PG** 2000 The quiet demise of deference to custom: malpractice law at the millennium. Washington Lee Law Review 57:163–205
5. *Helling v. Carey* 1974 83 Wash. 2d 514
6. **Bonnema SJ, Bennedbaek FN, Wiersinga WM, Hegedus L** 2000 Management of the nontoxic multinodular goitre: a European questionnaire study. Clin Endocrinol (Oxf) 53:5–12

doi: 10.1210/jc.2004-0314

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Authors' Response: Should Serum Calcitonin Be Routinely Measured in Patients with Thyroid Nodules—Will the Law Answer before Endocrinologists Do?

To the editor:

We thank Dr. Deftos for his comments (1) and for noting the legal implications raised in our editorial regarding whether serum calcitonin measurements should be routinely determined in patients with a thyroid nodule(s). Our conclusion was that at present there was insufficient medical evidence to recommend routine calcitonin measurement, at least in the United States where pentagastrin is not available. Dr. Deftos extends our discussion and suggests that perhaps there is legal precedence relating to this circumstance (and others) because the Supreme Court of Washington has ruled in the case of *Helling v. Carey* that the appropriate standard should be "reasonable care" rather than "customary or standard care."

We do not believe that the legal criteria of "reasonable care" as related to glaucoma evaluation apply to calcitonin screening in patients with thyroid nodules. In his letter, Dr. Deftos cites the crux of the *Helling v. Carey* decision: ". . . where [the presence of a disease] can be detected by a simple [relatively inexpensive], well known, harmless test, where the results of the test are definitive, where the disease can be successfully arrested by early detection, and where its effects are irreversible if undetected over a substantial period of time, liability should be imposed upon defendants even though they did not violate the standard existing within the profession." This argument hinges on the presence of a *definitive* test—one that when performed, provides a certain and conclusive diagnosis. Serum calcitonin screening in this context is complex rather than simple, and the results are not always definitive. The measurement of calcitonin is, in fact, harmless, but the test has many false-positive results, and calcitonin levels may be difficult to interpret, especially when the baseline serum calcitonin measurement is between 10 and 100 pg/ml. Because a thyroid fine-needle aspiration of these lesions is frequently not helpful and because the ultimate therapeutic treatment of suspected medullary carcinoma is total thyroidectomy with appropriate surgical dissection, the consequences of taking action on a false-positive test are extreme. As we note in our editorial, there are circumstances where calcitonin testing may add to an already extant level of clinical suspicion. In this setting, the test may be contributory and useful. However, for routine screening of patients with thyroid nodules, we maintain that testing of basal calcitonin levels does not meet the noted requirement of "definitive testing." Indeed, in this situation, measuring and interpreting calcitonin levels seems too complex to apply the legal criteria espoused in *Helling v. Carey*.

Because Dr. Deftos did raise the issue of standards for medical malpractice, we would like to address this broader area as well. The medical malpractice legal standard of care has developed through the court system; currently most states have a codified standard of care statute or explicitly prescribed case law. An example of a typical state statute is Virginia's medical malpractice statute; "To establish a *prima facie* case of medical malpractice, the plaintiff must establish: 1) the applicable standard of care; 2) that the standard has been violated; and 3) that there is a causal relationship between the violation and the alleged harm. Under Virginia law, the definition of standard of care is ". . . that degree of skill and diligence practiced by a reasonably prudent practitioner in the field of practice or specialty in the Commonwealth of Virginia" (2). Courts have occasionally explored nontraditional legal remedies with respect to the standard of care issue (3). One such case is *Helling v. Carey* where a greater duty of care was imposed under the caveats noted above (4). Washington courts narrowly construe the *Helling* holding within its exact language and emphasize the individual facts of the case, thereby limiting its broader utilization (5). Overall, "unless they contract to do more, the law exacts of physicians and surgeons in the practice of their profession only that they possess and exercise that reasonable degree of skill, knowledge, and care ordinarily possessed and exercised by members of their profession under similar circumstances" (6).

We certainly agree with Dr. Deftos that further discussion of the legal

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aspect of medical care is important and relevant for endocrinologists. We also believe that screening guidelines should be derived based on published medical evidence in consideration of relevant social, legal, and economic applications. However, at present, we do not think there is a sufficient medical or legal basis to recommend calcitonin screening in all patients with thyroid nodule(s).

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References

1. **Deftos LJ** 2004 Should serum calcitonin be routinely measured in patients with thyroid nodules—will the law answer before endocrinologists do? J Clin Endocrinol Metab 89:4768–4769
2. Va. Code Ann. § 8.01-581.20(A)
3. **Markowitz MD** 2001 Charles medical standard of care jurisprudence as evolutionary process: implications under managed care. 2 Yale J Health Policy Law, Ethics 59:69
4. *Helling v. Carey*, 1974 83 Wn. 2d 514, 520–522 (Utter, J., concurring)
5. *Fullerton v. Sacred Heart Med. Ctr.*, 2003 Wash. App. LEXIS 984, at 7–9
6. 61 Am Jur 2d Physicians, Surgeons, and Other Healers §188

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Authors' Response: Should Serum Calcitonin Be Routinely Measured in Patients with Thyroid Nodules—Will the Law Answer before Endocrinologists Do?

We are grateful to Dr. Deftos for the letter (1) that gives us the opportunity to make some observations regarding routine measurement of serum calcitonin (Ct) in patients with thyroid nodule(s). In our opinion, there are several lines of evidence (reviewed in Refs. 2 and 3) that support the serum Ct measurement in association with fine-needle aspiration cytology (FNAC) as the most accurate tests for discovering medullary thyroid carcinoma (MTC). In addition, our experience on more than 10,000 patients demonstrates that when MTC is diagnosed by Ct screening, it has a better outcome likely due to the statistically significant lower stage of the disease at the diagnosis (2). Unfortunately, Hodak and Burman (3) could not discuss this issue because our paper was published just before their editorial. It is known that MTC is the most aggressive differentiated thyroid tumor, with a survival rate of 50% at 10 yr, and that the best prognostic factor is the stage of disease, with intrathyroidal tumors showing the best prognosis (4). The possibility of performing an early MTC diagnosis offered by the routine serum Ct measurement must be taken into account if we want to improve our capability to cure a disease that can be definitively cured only by the completeness of the initial surgical treatment. It is in fact well known that nowadays conventional chemotherapy and radiotherapy are ineffective to cure advanced MTC (5).

Serum Ct measurement is a reliable test when performed in referral centers that have accurately defined their own cut-off values for basal and pentagastrin-stimulated Ct. A detailed description of the method and its practical usefulness is very well described in the "Laboratory Medicine Practice Guidelines for the Diagnosis and Monitoring of Thyroid Disease," recently published in the official journal of the American Thyroid Association and validated by a total of 84 independent reviewers from different countries (6).

One of the main arguments against the routine use of serum Ct measurement is the possibility of false-positive tests, particularly when baseline values are between 10 and 100 pg/ml. This is not the real scenario when the test is strictly applied to patients with thyroid nodules but not to other benign thyroid disorders, such as Hashimotos' thyroiditis, where confounding factors still exist. In our experience in more than 10,000 patients, all presenting with thyroid nodule(s), false-positive results were very rare, and, rather than false positive, they were due to other well-known benign conditions associated with increased serum Ct concentrations, such as renal failure, that can be easily recognized by careful medical history. Regarding the problem of the "gray zone", *i.e.* low elevation of serum Ct, the results of pentagastrin stimulation, or of calcium stimulation if pentagastrin is not available (6), are usually sufficient to achieve the correct diagnosis, provided that each center has a well-established reference normal range (6). In addition, even if at the end of such a work-up the final diagnosis is still not evident (very few cases), there is the possibility to measure Ct directly in the FNA effluent. This test integrates the result of FNAC and would establish the final diagnosis with great accuracy.

Admittedly the problem of false-positive values of Ct measurement due to methodological artifacts or to Ct increases unrelated to MTC does exist. For this reason the results of basal and stimulated Ct measurements should be carefully weighed against the clinical diagnosis and the FNAC pattern.

Finally, because the incidence of sporadic MTC may be influenced by the genetic and/or environmental factors that may differ in Europe and North America, it would be interesting and informative to carry out prospective studies on serum Ct screening of thyroid nodules in North America.

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References

1. **Deftos LJ** 2004 Should serum calcitonin be routinely measured in patients with thyroid nodules—will the law answer before endocrinologists do? J Clin Endocrinol Metab 89:4768–4769
2. **Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, Miccoli P, Iacconi P, Basolo F, Pinchera A, Pacini F** 2004 Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. J Clin Endocrinol Metab 89:163–168
3. **Hodak SP, Burman KD** 2004 Editorial: the calcitonin conundrum—it is time for routine measurement of serum calcitonin in patients with thyroid nodules. J Clin Endocrinol Metab 89:511–514
4. **Gharib H, McConahey WM, Tiegs RD, Bergstralh EJ, Goellner JR, Grant CS, van Heerden JA, Sizemore GW, Hay ID** 1992 Medullary thyroid carcinoma: clinicopathologic features and long-term follow-up of 65 patients treated during 1946 through 1970. Mayo Clin Proc 67:934–940
5. **Moley JF** 2003 Medullary thyroid carcinoma. Curr Treat Options Oncol 4:339–347
6. **Demers LM, Spencer CA** 2003 Laboratory medicine practice guidelines: calcitonin (Ct) and RET proto-oncogene measurement. Thyroid 13:68–74

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Low Vitamin B12 and Bone Loss: A Role for Folate Deficiency

To the editor:

Stone *et al.* (1) have recently reported that in older women low serum levels of vitamin B12 are associated with increased hip bone loss. They did not find a relation between vitamin B12 and bone mineral density (BMD) or its change in time, as should have been expected, but only that women in the first quintile of vitamin B12 values (levels $\leq$ 280 pg/ml) experience a greater bone loss than those of the other four quintiles. The study is weakened by the much more frequent intake of unspecified multivitamins in the group of women of the upper four vitamin B12 quintiles (50% *vs.* 18%). Vitamin B12 and its coenzyme folic acid are important for homocysteine metabolism, and their deficiency is associated with an increase of homocysteine levels. Familial hyperhomocysteinemia is associated with skeletal abnormalities, and recent data have shown that more modest elevation of homocysteine is also asso-

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ciated with a higher risk of osteoporotic fractures (2, 3). Similarly, a higher risk of osteoporosis is associated with mutations of genes involved in homocysteine metabolism, leading to hyperhomocysteinemia (4). Interestingly, this event was reported only in women depleted of folates and not explained only by homocysteine levels (4). It is surprising that the authors never mentioned this possibility and, worse than that, that they did not measure homocysteine and folate levels in their samples. By a cross-sectional investigation performed in younger postmenopausal women, we have recently reported that lumbar spine BMD is linearly and positively related to serum levels of folate but not vitamin B12 or homocysteine (5). The lowest quartile of folate was associated not only with the lowest BMD values, but also with the lowest levels of vitamin B12 and the highest values of homocysteine. However, at multivariate analysis, only levels of folate and weight were the significant determinants of BMD. Recent data suggest that folate may exert both cardiovascular and bone effects independent of homocysteine (6, 7). Both vitamin B12 and folate have been reported to decrease with age. Accordingly, vitamin B12-deficient individuals are very likely also folate-deficient individuals. We wonder whether the conclusion of Stone *et al.* (1) would have been different in case homocysteine, and in particular folate, were evaluated. For possible future inferential studies aimed to the prevention of osteoporosis, it is critical to know whether it is vitamin B12 or folate levels that are related to bone mineralization, and which one of the two has the major impact in the general population.

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References

1. **Stone KL, Bauer DC, Sellmeyer D, Cummings SR** 2004 Low serum vitamin B-12 levels are associated with increased hip bone loss in older women: a prospective study. J Clin Endocrinol Metab 89:1217–1221
2. **van Meurs JBJ, Dhonukshe-Rutten RAM, Pluijm SMF, van der Klift M, de Jonge R, Lindemans J, de Groot LCPGM, Hofman A, Witteman JCM, van Leeuwen JPTM, Breteler MMB, Lips P, Pols HAP, Uitterlinden AG** 2004 Homocysteine levels and the risk of osteoporotic fracture. N Engl J Med 350:2033–2041
3. **McLean RR, Jacques PF, Selhub J, Tucker KL, Samelson EJ, Broe KE, Hannan MT, Cupples A, Kiel DP** 2004 Homocysteine as predictive factor for hip fracture in older women. N Engl J Med 350:2042–2049
4. **McLean RR, Karasik D, Selhub J, Tucker KL, Ordovas JM, Russo GT, Cupples LA, Jacques PF, Kiel DP** 2004 Association of a common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene with bone phenotypes depends on plasma folate status. J Bone Miner Res 19:410–418
5. **Cagnacci A, Baldassari F, Rivolta G, Arangino S, Volpe A** 2003 Relation of homocysteine, folate and vitamin B12 to bone mineral density of postmenopausal women. Bone 33:956–999
6. **Doshi SN, McDowell IFW, Moat SJ, Payne N, Durrant HJ, Lewis MJ, Goodfellow J** 2002 Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering. Circulation 105:22–26
7. **McFarlane SI, Muniyappa R, Shin JJ, Bahtiyar G, Sowers JR** 2004 Osteoporosis and cardiovascular disease: brittle bones and boned arteries, is there a link? Endocrine 23:1–10

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Authors' Response: Low Vitamin B12 and Bone Loss: A Role for Folate Deficiency

To the editor:

In our cohort of women aged 65 and older, we found that women with lower serum vitamin B12 levels experience more rapid bone loss from the hip than women with higher levels of vitamin B12 (1). Specifically, participants with baseline serum vitamin B12 levels no greater than 280 pg/ml (207.2 pmol/liter) experienced an average rate of total hip bone loss of 1.6% (0.2 to 2.8%) annually compared with a loss rate of 0.2% (−0.2% to 0.5%) among women with levels above 280 pg/ml.

Drs. Cagnacci *et al.* (2) make reference to their own recently published cross-sectional study linking serum folate levels, but not vitamin B12 levels, to spine bone mineral density (BMD) in younger postmenopausal women (3) and question whether we may have found folate levels, had we measured them, to be more strongly associated with rates of hip bone loss in our cohort. Unfortunately, this question cannot be answered based on available data. Despite previous studies supporting a direct effect of B12 deficiency (4), we emphasize in our manuscript that other unmeasured dietary correlates of vitamin B12 intake may account for the observed association between serum vitamin B12 levels and rates of bone loss. Only a randomized trial will provide definitive evidence of a causal association of vitamin B12 (or folate) levels with reduction in rates of bone loss or fracture.

In addition, we would like to point out several important differences between our study and the study of Cagnacci *et al.* (3). Our study is prospective, with rates of change in hip BMD as the primary outcome. In contrast, the Cagnacci study was cross-sectional, with spine BMD as the outcome. Furthermore, our population is considerably older than that of Cagnacci *et al.* (3). This is an important distinction given that B12 deficiency, although rare in younger healthy people, is considerably more prevalent in the elderly (5, 6). These fundamental differences in both study population and design could easily explain the apparent divergence in results of the two studies.

Cagnacci *et al.* (3) have also questioned why we did not observe a significant trend in rates of bone loss across quintiles of vitamin B12 levels. We disagree that this would be the expected result. Tissue deficiency of vitamin B12 may only occur when levels fall below a particular threshold. Above that threshold, there may be no benefit of having higher levels of B12. Therefore, we appropriately examined both linear and threshold relationships of vitamin B12 and bone loss. Because there is a lack of consensus as to what level of B12 should be considered as clinically low, we chose to use the lowest quintile (≤280 pg/ml in our cohort). We believe that this is reasonable because it has been demonstrated that many individuals with tissue deficiency of B12 have serum B12 levels in what might be considered in the low-normal range (200–300 pg/ml) (5).

Finally, Cagnacci *et al.* (3) have pointed out that vitamin B12 plays a role in homocysteine metabolism, and given the evidence of association of genetic hyperhomocysteinemia with skeletal abnormalities, this could be a potential mechanism for the relationship of low vitamin B12 and rates of bone loss. We agree that it would have been useful to also have homocysteine measures in our cohort, particularly given recent data on homocysteine levels and fracture (7), and we mentioned this limitation in the discussion. Although we hope that future studies will further the understanding of the mechanism for the relationship between vitamin B12 and bone outcomes, this was beyond the scope of our observational study. Nonetheless, before any formal recommendations can be made concerning supplementation with vitamin B12 or folate for prevention of bone loss, supporting evidence from randomized trials is needed.

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References

1. **Stone KL, Bauer DC, Sellmeyer D, Cummings SR** 2004 Low serum vitamin B-12 levels are associated with increased hip bone loss in older women: a prospective study. J Clin Endocrinol Metab 89:1217–1221
2. **Cagnacci A, Cannoletta M, Baldassari F, Volpe A** 2004 Low vitamin B12 and bone loss: a role for folate deficiency. J Clin Endocrinol Metab 89:4770–4771
3. **Cagnacci A, Baldassari F, Rivolta G, Arangino S, Volpe A** 2003 Relation of homocysteine, folate, and vitamin B12 to bone mineral density of postmenopausal women. Bone 33:956–959
4. **Goerss JB, Kim CH, Atkinson EJ, Eastell R, O'Fallon Melton III LJ** 1992 Risk of fractures in patients with pernicious anemia. J Bone Miner Res 7:573–579
5. **Lindenbaum J, Rosenberg IH, Wilson PWF, Stabler SP, Allen RH** 1994

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Prevalence of cobalamin deficiency in the Framingham elderly population. Am J Clin Nutr 60:2–11
6. **Carmel R, Green R, Jacobsen DW, Rasmussen K, Florea M, Azen C** 1999 Serum cobalamin, homocysteine, and methylmalonic acid concentrations in a multiethnic elderly population: ethnic and sex differences in cobalamin and metabolite abnormalities. Am J Clin Nutr 70:904–910
7. **McLean RR, Jacques PF, Selhub J, Tucker KL, Samelson EJ, Broe KE, Hannan MT, Cupples LA, Kiel DP** 2004 Homocysteine as a predictive factor for hip fracture in older persons. N Engl J Med 350:2042–2049

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Two Cases of Thyroid Carcinoma That Were Not Stimulated by Recombinant Human Thyrotropin

To the editor:

In their recent article in *JCEM*, Driedger and Kotowycz (1) reported that ^{131}I uptake by thyroid carcinoma metastases and thyroglobulin production was not stimulated by recombinant human TSH (rhTSH). Potential mechanisms that were proposed included variation in receptor binding sites of rhTSH compared with endogenous TSH and serial exposure to rhTSH exerting progressive selection pressure on the TSH receptor (TSHR). Although changes in the TSHR are likely to be responsible for the variation in response seen, it is important to consider a role for the difference in circulating thyroid hormone levels between thyroid hormone withdrawal and rhTSH administration.

Levels of circulating T_4 and T_3 were not reported by Driedger and Kotowycz (1). This may also be a potential factor in the variation of response of thyroid carcinoma to stimulation with hypothyroidism compared with rhTSH. *In vitro*, T_3 has been shown to down-regulate the expression of TSHR gene (2). Although thyroid hormone receptor expression is reduced in thyroid cancers (3), evidence exists for T_3 exerting its effect on TSHR by thyroid hormone receptor-independent means (2). In the presence of higher circulating T_3 levels when thyroid hormone is not withdrawn, the potential exists for T_3 to reduce TSHR expression. This would result in a reduced response to exogenous TSH, as manifested by reduced iodine uptake and thyroglobulin production.

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References

1. **Driedger AA, Kotowycz N** 2004 Two cases of thyroid carcinoma that were not stimulated by recombinant human thyrotropin. J Clin Endocrinol Metab 89: 585–590
2. **Chen S-T, Lin J-D, Lin K-H** 2002 Characterization of a thyroid hormone-mediated short-loop feedback control of TSH receptor gene in an anaplastic human thyroid cancer cell line. J Endocrinol 175:459–465
3. **Takano T, Miyauchi A, Yoshida H, Nakata Y, Kuma K, Amino N** 2003 Expression of TRβ1 mRNAs with functionally impaired mutations is rare in thyroid papillary carcinoma. J Clin Endocrinol Metab 88:3447–3449

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Author's Response: Two Cases of Thyroid Carcinoma That Were Not Stimulated by Recombinant Human Thyrotropin

To the editor:

We agree with Dr. Depczynski (1) that free T_3 could act to down-regulate the expression of the TSH receptor in patients receiving radioiodine therapy under recombinant TSH-stimulated conditions. Our patients were both maintained on TSH-suppressive doses of L-thyroxine, and their free T_3 values were at the upper end of the normal range. For patient CD, who never had I-131 uptake under recombinant TSH stimulation, TSH receptor down-regulation is a reasonable additional mechanism for the failure to concentrate I-131. However, this mechanism would not explain the progressive loss of iodine avidity and thyroglobulin production in patient MB. We accept the point that several mechanisms might act to reduce iodine uptake in thyroid cancer metastases.

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Reference

1. **Depczynski B, Lillioja S** 2004 Two cases of thyroid carcinoma that were not stimulated by recombinant human thyrotropin. J Clin Endocrinol Metab 89:4772

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Association of Glutathione Peroxidase Activity with Insulin Resistance and Dietary Fat Intake during Normal Pregnancy

To the editor:

In a recent article, Chen *et al.* (1) reported a significant increase in erythrocyte glutathione peroxidase activity during pregnancy. The same article also reported higher glutathione peroxidase activity in Blacks and significant positive correlations with fasting insulin, C-peptide, and insulin resistance. Increased glutathione peroxidase activity during pregnancy is a very surprising finding in light of the many previous reports that selenium status declines significantly during normal pregnancy. This paper by Chen *et al.* (1) seems to be the first report of increased glutathione peroxidase (or any other parameter of selenium status) during pregnancy.

Lower blood selenium concentrations and glutathione peroxidase activities have been reported in pregnant women relative to prepregnancy levels (2–5) and relative to nonpregnant control women (6–9). Glutathione peroxidase activity in erythrocytes (5) and selenium concentration in hair (10) are lower in pregnant women, and amniotic fluid selenium concentrations decline throughout pregnancy (11), suggesting that a real decrease in selenium status occurs during pregnancy. The retention of stable isotopes of selenium is greater in pregnant women than in nonpregnant controls (12), suggesting that the decreased selenium status is driven by an increased requirement for selenium, presumably to support fetal growth.

It seems far more likely that the apparent increase in glutathione peroxidase activity was a result of their choice to express the activity relative to the hemoglobin concentration. It is well known that hemoglobin concentration declines during normal pregnancy (13); however, these authors did not report the hemoglobin data. The paper also does not mention what prenatal nutritional supplements were administered to, or used by, these pregnant women. If supplements were not given, then hemoglobin concentrations very likely declined (14–16) and would account for the apparent increase in glutathione peroxidase activity. If supplements were given and they contained selenium and/or vitamin E (which may improve selenium utilization) (17), then the supplements may have been the source of a real increase in glutathione peroxidase activity, as has been observed before (18). The apparent racial differences can also be explained by noting that hemoglobin is lower in African-American women than in Caucasian women (19) and anemia is more prevalent in Black women (20–22), which would cause the ratio of glutathione peroxidase to hemoglobin to appear higher in African-Americans relative to Caucasians.

The authors could have reported the glutathione peroxidase activity as a simple concentration to show that the effect was independent of a change in hemoglobin, or they could have reported the hemoglobin data to show that it did not change. Alternatively, they could have included hemoglobin concentration as a covariate in the statistical analysis of the

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glutathione peroxidase/hemoglobin ratios. Because these data were not reported and none of these issues were raised in the article, it would seem appropriate to address them now by publishing this letter and the authors' response.

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References

1. **Chen X, Scholl TO, Leskiw MJ, Donaldson MR, Stein TP** 2003 Association of glutathione peroxidase activity with insulin resistance and dietary fat intake during normal pregnancy. J Clin Endocrinol Metab 88:5963–5968
2. **McGlashan ND, Cook SJ, Melrose W, Martin PL, Chelkowska E, von Witt RJ** 1996 Maternal selenium levels and sudden infant death syndrome (SIDS). Aust N Z J Med 26:677–682
3. **Butler JA, Whanger PD, Tripp MJ** 1982 Blood selenium and glutathione peroxidase activity in pregnant women comparative assays in primates and other animals. Am J Clin Nutr 36:15–23
4. **Hinks LJ, Ogilvy SA, Hambidge KM, Walker V** 1989 Maternal zinc and selenium status in pregnancies with a neural tube defect or elevated plasma alpha fetoprotein. Br J Obstet Gynaecol 96:61–66
5. **Zachara BA, Wardak C, Didkowski W, Maciag A, Marchaluk E** 1993 Changes in blood selenium and glutathione concentrations and glutathione peroxidase activity in human pregnancy. Gynecol Obstet Invest 35: 12–17
6. **Ferrer E, Alegria A, Barbera R, Farre R, Lagarda MJ, Monleon J** 1999 Whole blood selenium content in pregnant women. Sci Total Environ 227: 139–143
7. **Navarro M, Lopez H, Perez V, Lopez MC** 1996 Serum selenium levels during normal pregnancy in healthy Spanish women. Sci Total Environ 186:237–242
8. **Wasowicz W, Wolkanin P, Bednarski M, Gromadzinska J, Sklodowska M, Grzybowska K** 1993 Plasma trace element (Se, Zn, Cu) concentrations in maternal and umbilical cord blood in Poland—relation with birth weight, gestational age, and parity. Biol Trace Elem Res 38:205–215
9. **Gromadzinska J, Wasowicz W, Krasomski G, Broniarczyk D, Andrijewski M, Rydzynski K, Wolkanin P** 1998 Selenium levels, thiobarbituric acid-reactive substance concentrations and glutathione peroxidase activity in the blood of women with gestosis and imminent premature labour. Analyst 123:35–40
10. **Guvenc H, Karatas F, Guvenc M, Kunc S, Aygun AD, Bektas S** 1995 Low levels of selenium in mothers and their newborns in pregnancies with a neural tube defect. Pediatrics 95:879–882
11. **Karunanithy R, Roy AC, Ratnam SS** 1989 Selenium status in pregnancy studies in amniotic fluid from normal pregnant women. Gynecol Obstet Invest 27:148–150
12. **Swanson CA, Reamer DC, Veillon C, King JC, Levander OA** 1983 Quantitative and qualitative aspects of selenium utilization in pregnant and nonpregnant women: an application of stable isotope methodology. Am J Clin Nutr 38:169–180
13. **Bashiri A, Burstein E, Sheiner E, Mazor M** 2003 Anemia during pregnancy and treatment with intravenous iron: review of the literature. Eur J Obstet Gynecol Reprod Biol 110:2–7
14. **Mahomed K** 2000 Iron and folate supplementation in pregnancy. Cochrane Database Syst Rev:CD001135
15. **Sloan NL, Jordan E, Winikoff B** 2002 Effects of iron supplementation on maternal hematologic status in pregnancy. Am J Public Health 92:288–293
16. **Dawson EB, McGanity WJ** 1987 Protection of maternal iron stores in pregnancy. J Reprod Med 32:478–487
17. **Combs Jr GF, Combs SB** 1986 The role of selenium in nutrition. San Diego: Academic Press
18. **Hawkes WC, Alkan FZ, Oehler L** 2003 Absorption, distribution and excretion of selenium from beef and rice in healthy North American men. J Nutr 133: 3434–3442
19. **Hunter GR, Weinsier RL, McCarthy JP, Enette Larson-Meyer D, Newcomer BR** 2001 Hemoglobin, muscle oxidative capacity, and VO2max in African-American and Caucasian women. Med Sci Sports Exerc 33:1739–1743
20. **Semba RD, Shah N, Klein RS, Mayer KH, Schuman P, Vlahov D** 2002 Prevalence and cumulative incidence of and risk factors for anemia in a multicenter cohort study of human immunodeficiency virus-infected and -uninfected women. Clin Infect Dis 34:260–266
21. **Bodnar LM, Scanlon KS, Freedman DS, Siega-Riz AM, Cogswell ME** 2001 High prevalence of postpartum anemia among low-income women in the United States. Am J Obstet Gynecol 185:438–443
22. **Leshan L, Gottlieb M, Mark D** 1995 Anemia is prevalent in an urban, African-American adolescent population. Arch Fam Med 4:433–437

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Authors' Response: Association of Glutathione Peroxidase Activity with Insulin Resistance and Dietary Fat Intake during Normal Pregnancy

To the editor:

In his letter, Hawkes (1) suggests that our findings on glutathione peroxidase (GPx) during pregnancy were the result of residual confounding by changes in maternal hemoglobin with gestation and by differences in hemoglobin between ethnic groups. Consequently, we reexamined our data using absolute erythrocyte GPx activity in lieu of computing the ratio of GPx to hemoglobin, the usual method of expressing the concentration (2–4). These results confirmed our original findings. The absolute GPx activity increased significantly between entry to care and the third trimester (2196 mU/ml at entry *vs.* 2382 mU/ml, third trimester; $P < 0.001$), and the effect persisted even when hemoglobin was used as a covariate in the models. Ethnic differences in absolute GPx activity were present both at entry and in the third trimester. At entry, African-Americans (2344 $\pm$ 65 mU/ml) had absolute levels of GPx that were significantly higher ($P < 0.01$) than Hispanics (2079 $\pm$ 60 mU/ml) but not whites (2166 $\pm$ 114 mU/ml). During the third trimester, GPx levels in African-American gravidas exceeded both ethnic groups ($P < 0.02$) [2515 $\pm$ 60 mU/ml (African-Americans), 2316 $\pm$ 56 mU/ml (Hispanics), 2207 $\pm$ 103 mU/ml (whites)]; use of hemoglobin as a covariate did not alter this result. On both occasions, African-American women had significantly higher absolute GPx activity, despite having lower levels of hemoglobin.

Although it may be true that GPx concentrations are lower in pregnancy, that result usually pertains when the controls are not pregnant (2, 4, 5); our aim was not to compare pregnant to nonpregnant subjects but rather to examine changes during pregnancy. Prior studies of pregnancy have been inconsistent and have reported small decreases with gestation (3, 6) as well as increases that were not statistically significant (2, 7). The sample sizes in these studies were quite small. As far as we are aware, our study is the largest to date.

We agree that changes in Selenium (Se) during pregnancy may influence GPx activity. Selenium-dependent GPx contains Se as a covalently bound selenocysteine. However, other factors such as the level of glutathione and whether the patient is taking supplements are also likely to be important in the regulation of GPx activity during pregnancy. A total of 86% of our subjects were taking multivitamin capsules during trimester 3. When we examined the relationship between GPx and multivitamin use, subjects at the extremes showed differences. Gravidas who took a daily multivitamin by trimester 3 had significantly greater levels ($P < 0.03$) of GPx (27.60 $\pm$ 0.50 mU/mg hemoglobin) than those who did not take a supplement (25.4 $\pm$ 0.9 mU/mg hemoglobin), suggesting that they are better able to cope with oxidative stress during late pregnancy.

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References

1. **Hawkes WC** 2004 Association of glutathione peroxidase activity with insulin resistance and dietary fat intake during normal pregnancy. J Clin Endocrinol Metab 89:4772–4773
2. **Hinks LJ, Ogilvy-Stuart A, Hambidge KM, Walker V** 1989 Maternal zinc and selenium status in pregnancies with a neural tube defect or elevated α-fetoprotein. Br J Obstet Gynaecol 96:61–66
3. **Zachara BA, Wardak C, Didkowski W, Maciag A, Marchaluk E** 1993 Changes

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in blood selenium and glutathione concentrations and glutathione peroxidase activity in human pregnancy. Gynecol Obstet Invest 35:12–17

4. **Gromadzinska J, Wasowicz W, Krasomski G, Broniarczyk D, Andrijewski M, Rydzynski K** 1998 Selenium levels, thiobarbituric acid-reactive substance concentrations and glutathione peroxidase activity in the blood of women with gestosis and imminent premature labour. Analyst 123:35–40
5. **Arikan S, Konukoglu D, Arikan C, Akcay T, Davas I** 2001 Lipid peroxidation and antioxidant status in maternal and cord blood. Gynecol Obstet Invest 51:145–149
6. **Mihailovic M, Cvetkovic M, Ljubic A, Kosanovic M, Nedeljkovic S, Jovanovic I, Pesut O** 2000 Selenium and malondialdehyde content and glutathione peroxidase activity in maternal and umbilical cord blood and amniotic fluid. Biol Trace Elem Res 73:47–54
7. **Loverro G, Greco P, Capuano F, Carone D, Cormio G, Selvaggi L** 1996 Lipoperoxidation and antioxidant enzymes activity in pregnancy complicated with hypertension. Eur J Obstet Gynecol Reprod Biol 70:123–127

doi: 10.1210/jc.2004-1200