Effects and blood concentrations of cobalt after ingestion of 1 mg/d by human volunteers for 90 d[1–3]

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ABSTRACT

Background: Over-the-counter cobalt supplements are available for sale in the United States, but little is known regarding their clinical effects and biokinetic distribution with long-term use.

Objective: We assessed blood kinetics, biochemical responses, and clinical effects in 5 adult men and 5 adult women who voluntarily ingested ~1.0 mg Co/d (0.080–0.19 mg Co · kg^{-1} · d^{-1}) of a commercially available cobalt supplement over a 3-mo period.

Design: Volunteers were instructed to take the cobalt dietary supplement in the morning according to the manufacturer's label. Blood samples were collected and analyzed for a number of biochemical variables before, during, and after dosing. Hearing, vision, cardiac, and neurologic functions were also assessed in volunteers before, during, and after dosing.

Results: After ~90 d of dosing, mean cobalt blood concentrations were lower in men than in women. Mean cobalt whole blood and serum concentrations in men were 20 μg/L (range: 12–33 μg/L) and 25 μg/L (range: 15–46 μg/L), respectively. In women, mean cobalt whole blood and serum concentrations were 53 μg/L (range: 6–117 μg/L) and 71 μg/L (range: 9–149 μg/L), respectively. Estimated red blood cell (RBC) cobalt concentrations suggested that cobalt was sequestered in RBCs during their 120-d life span, which resulted in a slower whole blood clearance compared with serum. The renal clearance of cobalt increased with the serum concentration and was, on average, lower in women (3.5 ± 1.3 mL/min) than in men (5.5 ± 1.9 mL/min). Sex-specific differences were observed in cobalt absorption and excretion. There were no clinically significant changes in biochemical, hematologic, and clinical variables assessed in this study.

Conclusion: Peak cobalt whole blood concentrations ranging between 9.4 and 117 μg/L were not associated with clinically significant changes in basic hematologic and clinical variables. This study was registered at clinicaltrials.gov as NCT01990794.

Am J Clin Nutr 2014;99:632–46.

INTRODUCTION

Cobalt is essential to human health in the form of vitamin B-12 (cobalamin) (1–3). For most of the general population, dietary intake (5–40 μg Co/d) represents the primary source of cobalt exposure (4). Dietary supplements that contain cobalt are available in the United States, with recommended doses that range from 0.2 to 1 mg Co/d (5–7). Some supplement manufacturers have suggested that cobalt helps improve fat and carbohydrate metabolism, protein synthesis, and red blood cell (RBC)[4] production (5–7). Although the US Food and Drug Administration (FDA) has not developed an acceptable daily intake for cobalt, the United Kingdom Expert Group on Vitamins and Minerals concluded that supplementation with 1400 μg Co/d was unlikely to cause adverse health effects in adults (8). The European Food Safety Authority has suggested a safe intake of 600 μg Co/d for noncarcinogenic effects (9), and Finley et al (10) concluded that a lifetime oral dose of 2100 μg Co/d should not pose a health hazard.

In the 1950s and 1960s, cobalt was used to treat various forms of anemia in children and adults (11–13). The therapeutic use of cobalt was associated with occasional side effects, primarily thyroid dysfunction in children and, less frequently, reversible vision and hearing impairment in adults (12, 14–18). Because of cobalt's inconsistent efficacy, its potential for adverse effects, and the advent of modern drugs to stimulate RBC production, cobalt is no longer prescribed as a therapeutic treatment for anemia (11, 19).

Historically, cobalt-toxicity concerns have focused on respiratory effects in workers who processed cobalt-containing alloys and salts (20). One notable exception occurred in the 1960s, when an unusual outbreak of cardiomyopathy was reported in a subset of heavy drinkers who consumed large quantities of beer to which $CoSO_4$ or $CoCl_2$ had been added as a foam stabilizer (21–23). Because much higher daily doses of cobalt were commonly used in therapeutic treatments without such reported effects, nutritional factors, ischemically modified albumin, and excessive alcohol intake were thought to have complicated the disease progression in these individuals (21, 24). Today, in addition to traditional dietary sources, human exposure to cobalt occurs through environmental contamination, occupational exposures, dietary supplements, and implanted medical devices, such as knee and hip replacements.

There is little information in the published literature regarding the cobalt body burden, steady state blood concentrations, or any clinical effects that may occur after long-term cobalt dietary supplemen-

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[4] Abbreviations used: ALT, alanine aminotransferase; CK-MB, creatine kinase–myocardial band; FDA, Food and Drug Administration; LTT, lymphocyte transformation test; MoM, metal-on-metal; OCT, optical coherence tomography; RBC, red blood cell; RNFL, retinal nerve fiber layer; SI, stimulation index; TSH, thyroid-stimulating hormone; T4, free thyroxine.

Received August 23, 2013. Accepted for publication December 31, 2013.
First published online February 5, 2014; doi: 10.3945/ajcn.113.071449.

tation (≥90 d). This study is the third study in a sequence of separate clinical cobalt dietary supplement ingestion studies. The first study showed that measurable increases in cobalt whole blood concentrations could be detected after 2 wk of oral cobalt supplementation (25). The second study evaluated steady state cobalt concentrations in the blood and assessed a number of biochemical variables after ~30 d of oral cobalt supplementation with 1 mg Co/d (26). However, the 30-d study did not evaluate renal clearance, which appreciably limited conclusions about the absorption, storage, and secretion of cobalt; also, the 30-d study did not perform any clinical assessments of hearing, vision, neurologic, or cardiac endpoints (26). Therefore, the purpose of this third study was to provide a more-thorough evaluation of cobalt blood kinetics as well as a more-detailed assessment of any potential health effects that may occur after long-term (continuous consumption from ≥8 to 12 wk) cobalt supplementation.

SUBJECTS AND METHODS

Subjects

The volunteer population consisted of 13 healthy adults, 10 of whom successfully completed the study. The 10 volunteers consisted of 5 adult men and 5 adult women who ingested 2 mL (~1.0 mg Co) of a liquid cobalt dietary supplement daily in the form of $CoCl_2$ for an average of 89 d. The dosing range for most volunteers was 88–90 d; one volunteer (volunteer J) ingested the supplement for 91 d (**Table 1**). The study protocol and informed consent forms were approved by the Western Institutional Review Board. Before study participation, volunteers provided informed consent, which included information about cobalt and its health effects. Volunteers were prescreened on the basis of study eligibility criteria. Each subject provided a review of his or her past medical history. Subjects were aware that no benefits to human health were to be expected as a result of participation and that the results might be used by nutrition scientists, by regulatory agencies, and in litigation proceedings. Exclusion criteria included the use of multivitamins or other dietary supplements while participating in the study, having a history of an allergy to cobalt, documented cardiovascular, thyroid, kidney, or liver disease, previous total joint replacement, insulin-dependent diabetes, or weight <45 kg. In addition, pregnant or lactating women were not enrolled in this study. All women of childbearing potential had a negative serum pregnancy test ≤7 d before starting the study.

Eleven of the 13 volunteers were Cardno ChemRisk employees. The primary reason for the use of Cardno ChemRisk employees was the ease of recruitment of volunteers with an understanding of scientific protocols, particularly regarding the importance of complying with study requirements such as timed urine collections and blood draws over an extended period of time. External blood draw centers and Quest laboratories were used for cobalt and biochemical analysis, independent physicians were used for all clinical assessments, and a third-party contractor was hired for volunteers to contact if they had any concerns or issues. Measured cobalt blood concentrations in the 90-d study were similar to those measured in the 30-d study, which was composed of non–Cardno ChemRisk employees (26).

Study design

Volunteers were instructed to take the cobalt dietary supplement in the morning according to the manufacturer's label, which suggested a serving of 1 mg Co (~2 mL)/d "in water or juice as maintenance." Liquid cobalt supplements were purchased online, and a sample from each bottle was sent to Chemical Solutions for confirmation of the cobalt content. Cobalt supplement concentrations were determined by using inductively coupled plasma mass spectrometry with a reporting limit of 1 μg/L. The mean cobalt concentration of 0.49 mg/mL (range: 0.40–0.53 mg/mL) was consistent with the manufacturer's label that indicated that 2 mL

TABLE 1
Study participant characteristics and individual cobalt doses

Study volunteer	Age	Weight	Cobalt concentration in supplement	Dose[1]	No. of blood draws completed
	y	*kg*	*μg/serving*[2]	*μg Co · kg*$^{-1}$ *· d*$^{-1}$	
Men					
A	29	74	960	13	16
B	51	94	1020	11	16
C	34	89	1040	12	16
D	28	133	1060	8	15[3]
E	24	84	1020	12	16
Average	33	95	1020	11	—
Women					
F	23	55	1020	19	16
G	44	81	1020	13	15[4]
H	23	71	1000	14	16
I	50	66	800	12	16
J	40	70	820	12	16
Average	36	68	932	14	—

[1] Total amount ingested ranged from 70.4 to 93.6 mg depending on total intake days.
[2] One serving equated to 2 mL.
[3] Volunteer D was unable to complete the day 8 or 9 blood draw.
[4] Volunteer G was missing data from the day 22, 23, or 24 blood draw because of an error in specimen collection or processing.

corresponded to ~1 mg Co. According to the manufacturer's label, the supplement contained water, cobalt chloride, and ionic trace, which "contains a full spectrum of 72 naturally occurring minerals and trace minerals." The amount of ionic trace per serving was 200 μg.

Volunteers either ingested the supplement directly from a syringe or ingested a full glass of water that contained the supplement. Volunteers were asked to maintain a weekly record of any health-related changes from their baseline conditions during the study period, including a 2-wk follow-up period. Participants followed their normal dietary habits but were asked to refrain from consuming multivitamins and energy or sports drinks that contained high amounts of vitamin B-12 (eg, 5 Hour Energy; Living Essentials LLC). Dietary logs were collected and reviewed for the consumption of foods known to contain high amounts of cobalt (4, 27–30) or dramatic changes in the diet during the course of the study. Because of the low average dietary intake of cobalt in the general population and low baseline cobalt blood concentrations for all study volunteers, the dietary cobalt content was not directly analyzed.

Specimen collection

A total of 15–16 blood samples were obtained from each volunteer at preselected intervals including 2 baseline (predosing) blood samples and 5 postdosing samples (**Figure 1**). Blood draws were conducted before 1200 at a local national blood draw center (Quest Diagnostics) by using stainless steel needles (Becton Dickinson and Co). For a complete blood count with differential analysis, venous blood was collected into a whole blood EDTA-coated (lavender-top) tube. Serum separator tubes (Becton Dickinson and Co) were used to collect venous blood for the following laboratory tests: lipid panel, comprehensive metabolic panel, creatine kinase–myocardial band (CK-MB) fraction, thyroid-stimulating hormone (TSH), free thyroxine (T4), total iron, and ferritin. For the cobalt whole blood analysis, venous blood was collected into an EDTA-coated (royal blue–top) trace-element tube [trace-element collection tubes (K_2 EDTA); Becton Dickinson and Co]. Blood draws conducted on day 1 (predose) and the last day of dosing (day 88, 89, or 90) were collected in a fasting state for lipids and glucose determination. Day 1 (predose) indicates that the variables were measured prior to cobalt administration on the first day of the dosing period.

For cobalt serum analysis, venous blood was collected into a no-additive (royal blue–top) trace-element tube (Monoject no-additive royal blue–top tube; Tyco Health Care Group). These samples were centrifuged and transferred into a trace metal–free plastic vial for additional analysis. Tubes without anticoagulants were drawn first, followed by serum tubes, and then whole blood tubes for trace-element analysis. The protocol for cobalt blood analysis specified method precision equal to a relative SD ≤20% and a method accuracy between 95% and 105% of known targets with a calibration range of 0.5–500 μg/L. A 24-h urine collection for cobalt analysis was performed at 3 time points selected to correspond to approximate steady state conditions (Figure 1). In addition, 3 volunteers (2 women and 1 man) collected 24-h urine samples for cobalt analysis at 1, 2, 6, and 10 wk postdosing.

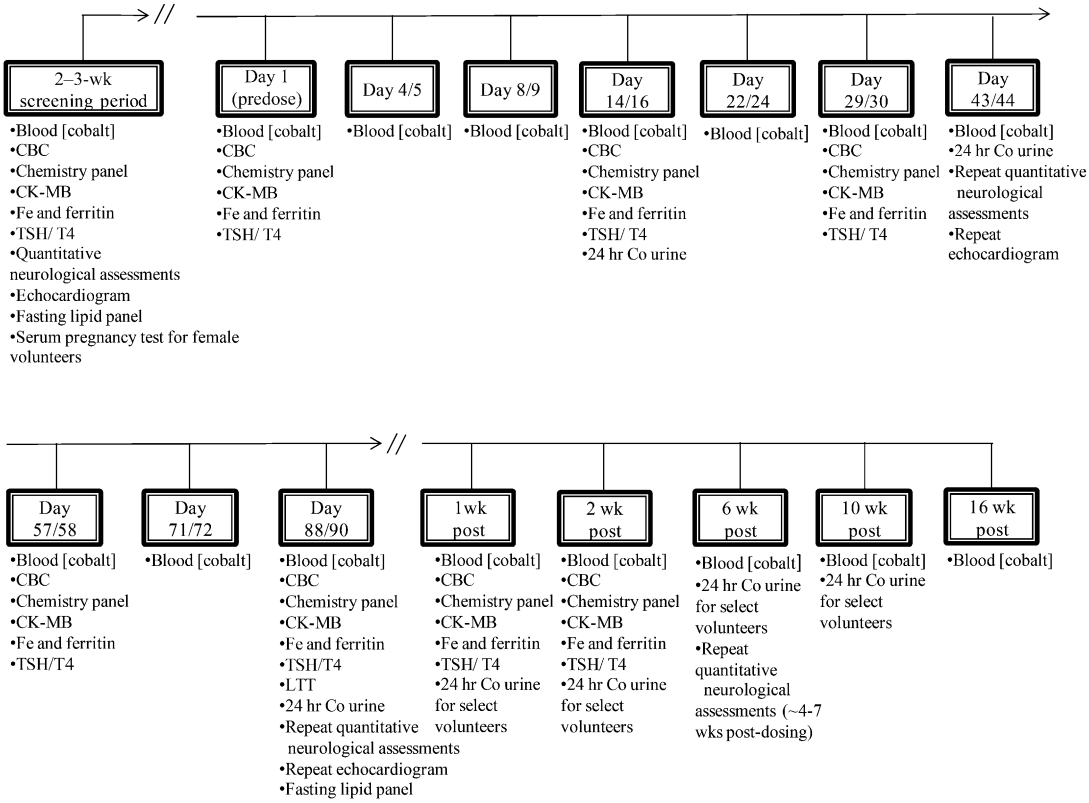

FIGURE 1. Study design. Dates of individual blood draws varied slightly (eg, Day 4/5 indicates that volunteers had their blood drawn after 4 or 5 d of dosing). Blood [cobalt]: serum and whole blood cobalt concentrations; CBC, complete blood count, including differential and platelets; CK-MB, creatine kinase–myocardial band; Fe, total iron; LTT, lymphocyte transformation test; TSH, thyroid-stimulating hormone; T4, free thyroxine.

Twenty-four–hour urine samples were collected in acid-washed trace-element–free plastic containers, and 10 mL was decanted into a 50-mL trace-element–free conical tube for analysis.

Blood analysis for trace elements

Whole blood for trace-element analysis was analyzed by Quest Diagnostics Nichols Institute (San Juan Capistrano, CA, or Valencia, CA). Serum for trace-element analysis was analyzed by the Quest Diagnostics Nichols Institute (Chantilly, VA). Urine for trace-element analysis was analyzed by Quest Diagnostics Nichols Institute (Valencia, CA). Cobalt concentrations in whole blood, serum, and urine were evaluated by using inductively coupled plasma mass spectrometry. The reporting limit for cobalt in whole blood was initially 0.5 μg/L but was lowered to 0.2 μg/L during the study. The reporting limit for cobalt in serum was initially 1.0 μg/L but was lowered to 0.1 μg/L during the study. The reporting limit for cobalt in urine was 0.5 μg/L.

Biochemical assessment and endpoint-specific examinations

Blood chemistries assessed during the study included a lipid panel, comprehensive metabolic panel, CK-MB, TSH, T4, complete blood count with differential, total iron, and ferritin (Figure 1). A sensitivity to metals before and after cobalt dosing was assessed by using an in vitro lymphocyte transformation test (LTT) (Orthopedic Analysis) with separated peripheral blood mononuclear cells collected from 30 to 40 mL peripheral whole blood. This assay evaluated potential metal allergies on the basis of proliferative actions of isolated cells determined by using [3H]-thymidine (Amersham International) incorporation rates after metal exposure. The average proliferation rate for each metal treatment was normalized to individual proliferation rates of nontreated control cells that generated a stimulation index (SI). According to the manufacture, an SI from 2 to 4 indicated mild reactivity, from 5 to 8 indicated moderate reactivity, and >8 indicated high reactivity to the metal.

Audiologic assessments including a pure-tone threshold determination at frequencies that ranged from 250 to 16000 Hz were performed with volunteers serving as their own baseline controls for receptive changes during the study (Figure 1). Decreases in hearing were considered clinically significant when one of the following 3 American Speech-Language-Hearing Association criteria were met: *1*) a $\geq$20-dB decrease in the pure-tone threshold at one test frequency, *2*) a $\geq$10-dB decrease at 2 adjacent test frequencies, or *3*) the loss of 3 consecutive test frequencies where responses were previously obtained (31). Pure-tone air thresholds were obtained by using an AC40 clinical audiometer (Interacoustics) with EAR 3A inserts (3M) and HDA 200 headphones (Sennheiser) (calibrated according to American National Standards Institute S3.6 2004 specifications). Pure-tone measurements were all conducted in a double-walled sound-treated room that met International Organization for Standardization 8253–1 standards of ambient sound pressure levels.

Two-dimensional and Doppler echocardiographic examinations (iE 33 or Sonos 5500; Philips) were used to assess cardiac anatomy, structure, and function during the study (Figure 1). The left-ventricular ejection fraction was measured, and an ejection fraction value $\geq$55% was considered normal for both men and women (32). Chamber volumes, valvular anatomy and function, wall motion, and ventricular function during systole and diastole were also assessed along with various chamber pressures.

Neurologic assessments performed during the study included a comprehensive neurologic examination, Romberg testing, motor nerve conduction studies, and sensory nerve conduction studies, which were typically of the lower extremity (Figure 1). Physical examinations were performed at the first 3 visits to assess gait, cranial nerves, touch, proprioception, pinprick, and vibration sense in the legs. Strength, coordination, muscle tone, and reflexes were also assessed during the physical examination. Motor and sensory conduction velocities and amplitudes were measured by using a Premier Plus electromyography machine (Teca). A peroneal nerve conduction velocity value $\geq$40 m/s was considered normal, and a sural sensory conduction velocity value $\geq$38 m/s was considered normal.

Ophthalmology studies included an assessment of visual acuity, slit lamp evaluations, and visual field testing (Figure 1). Visual field measurements, such as the visual field index, mean deviation, and pattern SD values, were determined to assess any possible changes in the visual field over time. The mean deviation expressed the overall sensitivity, averaged across the visual field, relative to a group of healthy, age-matched controls; the value was expressed in decibels (33). The pattern SD measured the degree to which the shape of the volunteer's measured field of vision departed from the normal age-corrected reference field; the value was expressed in decibels. The retinal nerve fiber layer (RNFL) thickness and optic nerve head were assessed by using optical coherence tomography (OCT) (Cirrus HD-OCT 4000 Series; Carl Zeiss Meditec Inc). A normal RNFL thickness was between $\leq$95th and $>$5th percentiles, borderline normal RNFL was reported to be between less than or equal to the fifth percentile and greater than the first percentile, and abnormal RNFL thickness was reported to be less than or equal to the first percentile in accordance with recommendations from the OCT manufacturer.

Statistical evaluations

Blood cobalt data are presented as mean cobalt whole blood or serum concentrations reported for a particular time point. Steady state conditions were determined by consecutive blood draw days on which the rate of increase in cobalt serum or whole blood concentration was $\leq 0.1\ \mu g \cdot L^{-1} \cdot d^{-1}$. To confirm steady state conditions, the average slope for the steady state period was calculated by using blood data from each individual and shown to be $\leq 0\ \mu g \cdot L^{-1} \cdot d^{-1}$ for both serum and whole blood. Some time points for 2 volunteers (volunteers F and G) were excluded from the steady state analysis because one volunteer reported a substantial change in her diet, and the other volunteer reported that she had changed the time of her supplement intake. A 2-phase exponential decay curve was used to fit a loss curve to cobalt whole blood and serum concentrations measured on the last day of dosing (day 88, 89, or 90) and to cobalt whole blood and serum concentrations measured at 1, 2, 6, 10, and 16 wk postdose. Statistical Analysis System software 9.2 (SAS Inc) was used to fit the model by using the software's nonlinear regression procedure. On the basis of reporting limits of the assay and known background concentrations of cobalt in the serum and whole blood of unexposed individuals, we set constraints of the exponential decay curve to plateau at 0.4 and 0.3 μg/L for cobalt in whole blood and serum, respectively.

The relations between serum and whole blood cobalt concentrations during dosing were assessed by using Student's 2-tailed paired *t* tests. Student's 2-tailed paired *t* tests were also conducted to analyze changes in serum and plasma biochemical data at each dosing and postdosing sampling point, and significance was assigned at $P < 0.05$. Unless otherwise indicated, the 2 baseline measurements were averaged for a single baseline value. Findings are presented as means ± SDs. The reference range for each variable was defined as the minimum and maximum reference values from a range provided by Quest Diagnostic Laboratories from a standard population of adult men and women. To test for changes in metal sensitivity by using the LTT, significant responses were also assessed by using the Student's 2-tailed paired *t* test; significance was assigned at $P < 0.05$.

Renal excretion was calculated to be the mass of cobalt excreted in the urine on a daily basis as a function of the cobalt serum concentration. Urinary excretion data were fit to the power law

$$y = a \times x^{b} \quad (1)$$

where y equals the daily urine cobalt excretion (μg/d), a is the intercept, x is the measured cobalt serum concentration, and b is the slope. Renal efficiency was calculated as the ratio of the urine cobalt concentration to the concurrent serum cobalt concentration (34).

RESULTS

Subject characteristics

Ten volunteers (5 men and 5 women) completed the required study procedures, and individual doses ranged from 8 to 19 $\mu g \cdot kg^{-1} \cdot d^{-1}$ (Table 1). No subjects reported forgetting to ingest the dietary supplement during the study.

Cobalt kinetics in whole blood, serum, and RBCs

On the last day of dosing, the mean whole blood cobalt concentration in women (53.3 μg/L; range: 6.2–117.2 μg/L) was ~2.6-times higher than in men (20.4 μg/L; range: 12.4–32.9 μg/L) (**Figure 2**; *see* Supplemental Table S1 under "Supplemental data" in the online issue). The difference in cobalt blood concentrations between men and women appreciably exceeded the 1.3-time difference in the administered dose (Table 1). However, this difference was not statistically significant at any time point, which was likely

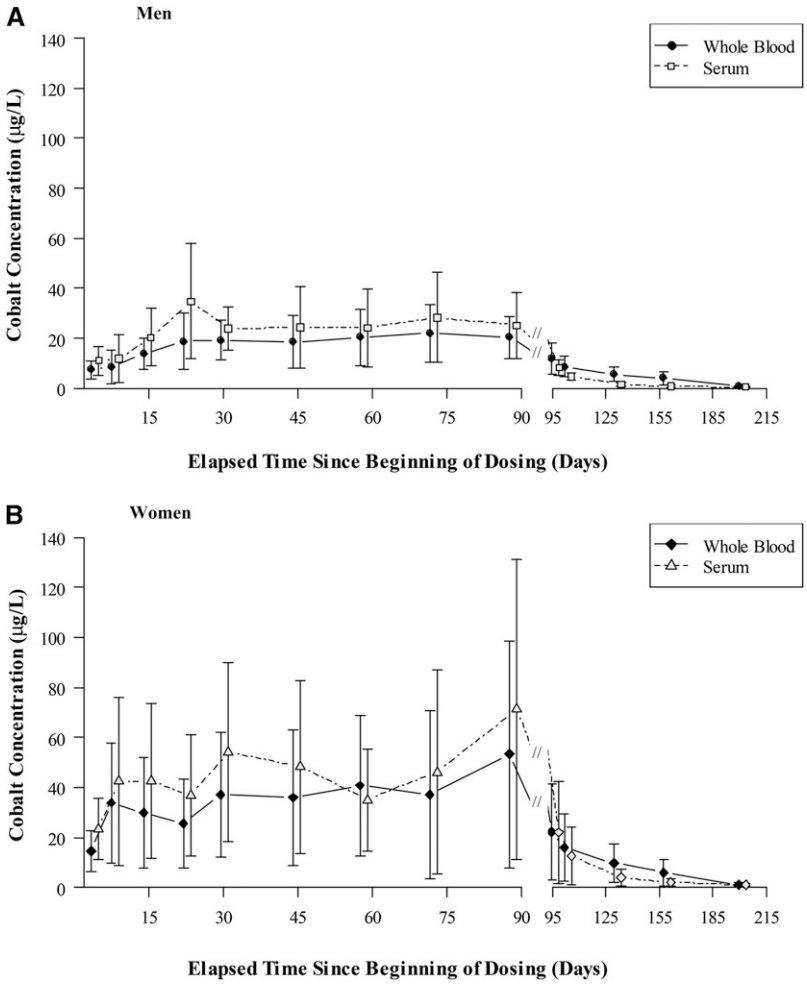

FIGURE 2. Time course of the mean (±SD) whole blood and serum cobalt concentrations after dietary supplementation with ~1.0 mg Co/d for an average of 89 d. A: Mean cobalt whole blood and serum concentrations in men (n = 5). B: Mean cobalt whole blood and serum concentrations in women (n = 5).

because of the interindividual variability in blood cobalt concentrations (Figure 2).

Baseline whole blood cobalt concentrations for most volunteers were shown to be equal to or less than the initial reporting limit of 0.5 μg/L (*see* Supplemental Table S1 under "Supplemental data" in the online issue). All predose serum cobalt concentrations were less than the initial reporting limit of 1 μg/L (*see* Supplemental Table S2 under "Supplemental data" in the online issue). Selected whole blood samples with elevated cobalt concentrations [such as in volunteers F (81.2 μg/L), G (117.2 μg/L), and H (42.6 μg/L)] were confirmed by a repeat analysis at Quest Diagnostic Laboratories. In addition, duplicate serum cobalt samples were sent to an outside laboratory (Applied Speciation) for a confirmatory analysis of most samples. In general, there was good agreement between cobalt serum concentrations reported by the 2 laboratories, and at most time points, there was no significant difference between cobalt serum concentrations reported by the 2 laboratories (*see* Supplemental Table S3 under "Supplemental data" in the online issue).

Serum cobalt concentrations were consistently higher than mean whole blood cobalt concentrations at all dosing periods (Figure 2), and serum cobalt concentrations were highly correlated with cobalt whole blood concentrations (R^2 = 0.92; slope: 1.245; men and women combined) (*see* Supplemental Figure S1A under "Supplemental data" in the online issue). Cobalt serum concentrations increased at an average rate of 1.2 μg/L for every 1-μg/L increase in whole blood during dosing. However, the mean difference between serum and whole blood cobalt concentrations during dosing was not statistically significant, which was most likely because of the large variability in the data set. On average, serum and whole blood cobalt concentrations reached apparent steady state conditions around days 20 and 35 for men and women, respectively, and steady state conditions were achieved by day 58 or 59 in all volunteers (Figure 2; *see* Supplemental Table S1 under "Supplemental data" in the online issue).

After the dosing phase ended, the relative fraction of cobalt in whole blood increased until it equaled and then consistently exceeded that in serum (Figure 2; *see* Supplemental Figure S1, B and C, under "Supplemental data" in the online issue). Cobalt elimination from serum and whole blood appeared to follow a 2-phase exponential decay curve, with an initial rapid elimination phase followed by a second slower elimination phase. The average elimination half-life for cobalt in serum and whole blood described by the first exponential term (T_1) was shown to be ~3 d. The average elimination half-life for cobalt in serum and whole blood from the second exponential term (T_2) was ~22 and ~36 d, respectively. The shorter half-life term T_1 corresponded to the elimination (a_1) of 52% and 76% of the cobalt in whole blood and serum concentration, respectively (**Tables 2** and **3**).

The estimated concentration of cobalt in RBCs was calculated by adjusting for concurrent serum cobalt concentrations and the average hematocrit for each volunteer. Steady state conditions appeared to be achieved in 20–60 d, and estimated RBC concentrations were remarkably consistent, with central tendency steady state concentrations of ~10–20 μg/L in most volunteers (**Figure 3**, A and B). The elimination of cobalt from RBCs appears to be linear with time (Figure 2, A and B). When RBC cobalt concentrations were normalized to the last day of dosing (day 88, 89, or 90), the inverse of the normalized slope in the 10 volunteers corresponded to an estimated RBC lifetime ($\pm$SD) of 120 $\pm$ 25 d (average R^2 = 0.87). Similarly, when the 10 normalized cobalt RBC concentrations were averaged at each postdosing time point and plotted against time, a distinct linear decreasing trend and ~120–125-d lifetime of the RBC on the basis of cobalt elimination were readily apparent (**Figure 4**).

Cobalt urine concentrations and renal clearance

Throughout dosing, the average cobalt urine concentration in women was ~2 to 5-times higher than the average cobalt urine concentration reported in men (*see* Supplemental Table S4 under "Supplemental data" in the online issue). Serum cobalt concentrations were correlated with urine cobalt concentrations (R^2 = 0.61, $P < 0.05$) for men and women combined. As shown in **Figure 5**, A and B, urinary cobalt excretion as a function of the serum cobalt concentration was fit reasonably well by a power law relation with a reasonable correlation between power law predicted and measured values for men (R^2 = 0.75) and women (R^2 = 0.85).

Women retained more cobalt than men did as reflected by the average renal clearance of cobalt for men and women during dosing

TABLE 2
Computed variables describing the retention of cobalt in whole blood[1]

Volunteer	a_1	λ_1	T_1	a_2	λ_2	T_2
A	0.27	0.22	3.1	0.73	0.017	42
B	0.48	0.10	7.0	0.52	0.015	47
C	0.67	0.30	2.3	0.33	0.021	33
D	0.51	0.28	2.5	0.49	0.019	36
E	0.44	0.23	3.0	0.56	0.022	31
F	0.65	0.37	1.9	0.35	0.018	38
G	0.62	0.24	2.8	0.38	0.018	39
H	0.73	0.31	2.3	0.27	0.021	33
I	0.38	3.90	0.2	0.62	0.024	29
J	0.41	0.25	2.8	0.59	0.020	35
Average	0.52	0.62	2.8	0.48	0.020	36

[1] Rate of cobalt elimination from whole blood fit best to a 2-phase exponential decay model with a biologic half-life of ~3 d (T_1) and 40 d (T_2). The elimination rate constant for phases 1 and 2 of the model is represented by λ_1 and λ_2, respectively. The fraction eliminated by each component of the model is represented by a_1 and a_2, respectively.

TABLE 3
Computed variables describing the retention of cobalt in serum[1]

Volunteer	a_1	λ_1	T_1	a_2	λ_2	T_2
A	0.89	0.11	6.4	0.11	0.019	37
B	0.89	0.14	5.0	0.11	0.016	43
C	0.62	0.33	2.1	0.38	0.063	11
D	0.80	0.26	2.7	0.20	0.044	16
E	0.71	0.23	3.0	0.29	0.035	20
F	0.79	0.35	2.0	0.21	0.036	19
G	0.70	0.23	3.0	0.30	0.037	19
H	0.82	0.33	2.1	0.18	0.034	21
I	0.64	3.71	0.2	0.36	0.062	11
J	0.76	0.16	4.3	0.24	0.028	25
Average	0.76	0.58	3.08	0.24	0.037	22

[1] Rate of cobalt elimination from serum fit best to a 2-phase exponential decay model with a biologic half-life of ~3 d (T_1) and 20 d (T_2). The elimination rate constant for phases 1 and 2 of the model is represented by λ_1 and λ_2, respectively. The fraction eliminated by each component of the model is represented by a_1 and a_2, respectively.

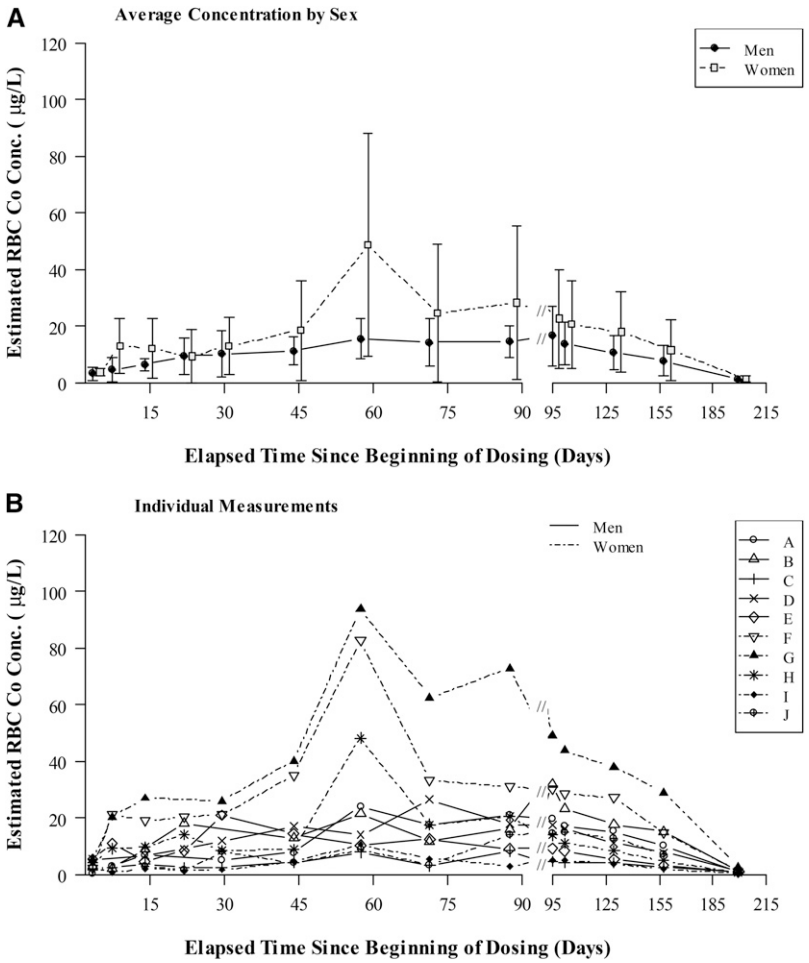

FIGURE 3. Time course of the mean (±SD) and individual estimated RBC cobalt concentrations after dietary supplementation with ~1.0 mg Co/d for an average of 89 d. A: Mean estimated RBC cobalt concentration in men (n = 5) and women (n = 5). Error bars represent SDs of cobalt RBC concentrations. B: Estimated individual RBC cobalt concentration (n = 10). Co, cobalt; Conc., concentration; RBC, red blood cell.

of 5.5 ± 1.9 and 3.5 ± 1.3 mL/min, respectively. Observed differences in renal clearance likely reflected differences in individual (unadjusted for the surface area) estimated glomerular filtration rates of 120 mL/min (range: 101–143 mL/min) in men and 99 mL/min (range: 84.6–126 mL/min) in women as well as related differences in average daily urine production volumes of 2900 mL (range: 800–5700 mL) in men and 1800 mL (range: 660–3100 mL) in women. The average renal efficiency (the ratio of urine to serum cobalt concentrations) for men and women throughout dosing was similar at 3.4 ± 2.5 and 3.3 ± 2.1, respectively.

Blood biochemical profiles

Baseline blood values for biochemical testing were compared with values obtained after ~30 d (day 29 or 30), 60 d (day 57 or 58), and 90 d (day 88, 89, or 90) of dosing as well as at 1 and 2 wk postdosing. The ingestion of cobalt during the 90-d study period did not produce any clinically important changes in serum biochemical or hematologic variables (**Tables 4** and **5**; *see* Supplemental Figures S2–S16 under "Supplemental data" in the online issue).

Mean ferritin concentrations in the men were significantly decreased from baseline on days 29 or 30 (30%), 57 or 58 (36%), and 88, 89, or 90 (37%), and a significant decrease in mean creatinine concentrations (2%) was observed at the end of dosing. However, ferritin and creatine concentrations reported in men remained within standard reference ranges throughout dosing

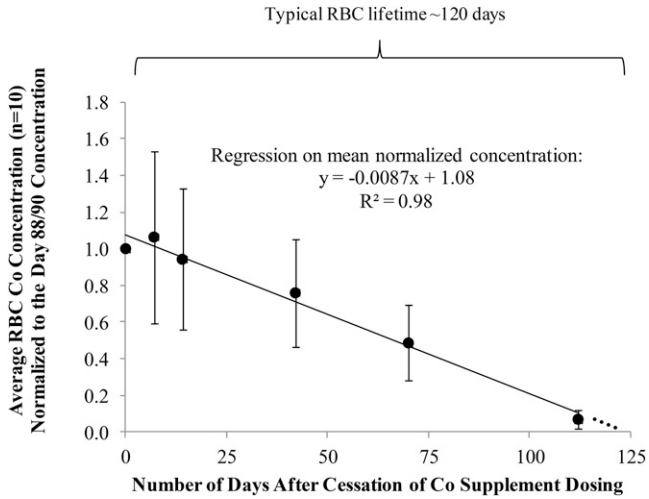

FIGURE 4. Average RBC Co concentrations in men and women combined normalized to the day 88, 89, or 90 concentrations (n = 10). Co, cobalt; RBC, red blood cell.

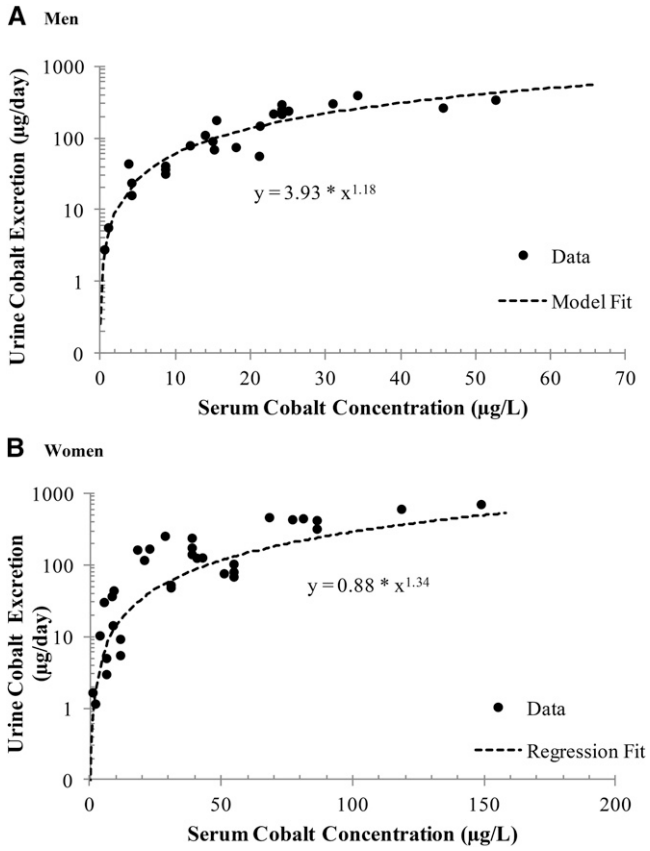

FIGURE 5. Relation between the serum cobalt concentration and urinary cobalt excretion in men ($n = 5$) (A) and women ($n = 5$) (B). Each data point represents an individual measurement. Urinary cobalt excretion as a function of the serum cobalt concentration was fit reasonably well by a power law relation with a reasonable correlation between power law predicted and measured values for men ($R^2 = 0.75$) and women ($R^2 = 0.85$).

(*see* Supplemental Figure S2 and S13 under "Supplemental data" in the online issue). With respect to women, there was a significant decrease in mean hemoglobin (3%) and alanine aminotransferase (ALT) (23%) concentrations on the last day of dosing (day 88, 89, or 90). A significant increase in CK-MB concentrations (69%) was also noted on the last day of dosing. However, all volunteers were within normal reference ranges for hemoglobin, CK-MB, and ALT concentrations at all time points during cobalt supplementation (*see* Supplemental Figures S6, S12, and S14 under "Supplemental data" in the online issue). Although there was not a significant decrease in mean ferritin concentrations over time for women, a decrease in mean concentrations was observed starting at the 1-wk predose and day 1 (predose) draws (*see* Supplemental Figure S2 under "Supplemental data" in the online issue).

No adverse clinical effects attributable to metal sensitization were observed in any of the 10 volunteers who completed the study. Accordingly, the LTT showed no significant change for most of the metals tested with the exception of molybdenum, for which the SI after dosing increased to mild reactivity (**Figure 6**). The SI of 2 volunteers (E and J) indicated a mild increase in cobalt reactivity at the end of the study (SI: 3 and 2.3, respectively). Neither of these volunteers had particularly high blood cobalt concentrations. Three volunteers (E, F, and I) exhibited an increase in nickel sensitization, but no change in cobalt sensitization was observed.

Endpoint-specific examinations

Sequential examinations before, during, and after dosing did not detect any clinically significant echocardiographic, hearing, or vision changes (**Tables 6–9**; *see* Supplemental Figure S17 under "Supplemental data" in the online issue). To assess for optic nerve atrophy, the RNFL thickness and optic nerve head were evaluated in both the right and left eyes by using OCT (Tables 8 and 9). All volunteers remained within this normative range throughout the study, except volunteer H, whose total RNFL thickness in her left eye was above the 95% percentile throughout the entire study.

Physical examinations revealed preserved muscle strength and tendon reflexes, with no signs of motor deficits throughout the study. No abnormalities of motor nerve velocities (motor nerve conduction velocities were $\geq$40 m/s) or amplitudes were noted (**Table 10**; *see* Supplemental Figures S18 and S19 under "Supplemental data" in the online issue). However, changes in sural sensory nerve amplitudes were observed during the dosing period (Table 10; *see* Supplemental Figure S20 under "Supplemental data" in the online issue), but no consistent pattern of change was noted. Changes in sensory nerve conduction velocities also appeared to be stochastic with no consistent trend over time (*see* Supplemental Figure S21 under "Supplemental data" in the online issue).

Self-reporting of adverse events

No serious adverse events were reported by any volunteer during the study period. Volunteer B developed ear pain and tinnitus associated with sinusitis after the first 2 wk of dosing but remained in the study. Three women volunteers (not shown in Table 1) were withdrawn from the study after self-reported migraine headaches ($n = 1$) or rashes ($n = 2$) within the first 2 wk of the study. A clear association between cobalt exposure and self-reported adverse events could not be determined because several confounding factors were identified. *See* Supplemental Table S5 and S6 for more details regarding self-reported adverse events.

DISCUSSION

This study provided a unique opportunity to evaluate cobalt blood kinetics and potential health status changes after the ingestion of an over the counter cobalt supplement. After a daily ingestion of $\sim$1 mg Co for up to 90 d, the average steady state whole blood and serum cobalt concentrations were $\sim$56 times greater than background cobalt serum and whole blood concentrations, which are typically $<$0.5 μg (35–44). Cobalt is primarily cleared by the kidneys, and patients with renal failure experience appreciably elevated blood and serum cobalt concentrations compared with those of healthy individuals exposed to the same internal or external dose (45, 46). Our data indicated that, in healthy individuals, the renal excretion of cobalt was mediated by a saturable reabsorption process, particularly at serum concentrations slightly above background concentrations (Figure 5). The average ratio between urine and serum cobalt concentrations (renal efficiency) decreased after

TABLE 4
Blood clinical indexes for men[1]

Variable	Baseline (n = 10)	Days 29 and 30 (n = 5)	Days 57 and 58 (n = 5)	Days 88 and 90 (n = 5)	1 wk postdose (n = 5)	2 wk postdose (n = 5)	Reference range
WBC ($10^3/\mu$L)	6.79 ± 1.12	6.42 ± 0.79	6.94 ± 0.94	6.34 ± 0.75	7.1 ± 1.78	6.42 ± 0.46	3.8–10.8
RBC ($10^6/\mu$L)	4.71 ± 0.2	4.70 ± 0.20	4.81 ± 0.18	4.72 ± 0.32	4.77 ± 0.24	4.79 ± 0.21	4.2–5.1
Hematocrit (%)	45.1 ± 2.53	45.0 ± 2.54	46.3 ± 2.75	45.0 ± 2.9	45.8 ± 2.39	45.7 ± 1.11	38.5–50
Hemoglobin (g/dL)	14.9 ± 0.93	14.8 ± 0.82	15.1 ± 0.74	14.9 ± 0.76	14.9 ± 0.78	15.0 ± 0.4	13.2–15.5
Protein (g/dL)	7.26 ± 0.30	7.24 ± 0.30	7.16 ± 0.31	7.14 ± 0.11	7.28 ± 0.33	7.32 ± 0.41	6.2–8.3
Albumin (g/dL)	4.63 ± 0.32	4.60 ± 0.37	4.56 ± 0.23	4.48 ± 0.25	4.60 ± 0.3	4.56 ± 0.3	3.6–5.1
TSH (mIU/L)	2.35 ± 1.72	3.35 ± 3.82	3.11 ± 3.65	2.24 ± 2.02	2.30 ± 1.93	2.33 ± 2.35	0.4–4.5
T4 (ng/dL)	1.15 ± 0.15	1.16 ± 0.13	1.16 ± 0.13	1.12 ± 0.08	1.15 ± 0.10	1.14 ± 0.05	0.8–1.8
Total iron (μg/dL)	122 ± 26.4	119 ± 39.5	105 ± 47.1	82.6 ± 32.6	115 ± 37.1	111 ± 42.8	40–175
Ferritin (ng/mL)[2]	136 ± 77.3	95.2 ± 57.5[a]	86.6 ± 59.6[a]	85.2 ± 53.1[a]	74.6 ± 50.3[a]	90.4 ± 66.0[a]	20–380
CK-MB (ng/mL)[2]	1.58 ± 0.68	0.97 ± 0.95	0.92 ± 0.85	1.23 ± 0.82	1.25 ± 1.08	1.42 ± 1.53	0–5.0
Creatinine (mg/dL)	1.05 ± 0.09	1.01 ± 0.16	1.04 ± 0.11	0.99 ± 0.10[a]	1.02 ± 0.12	1.05 ± 0.10	0.6–1.35
ALT (U/L)	30.6 ± 19.3	26.2 ± 13.5	32.0 ± 19.3	29.4 ± 17.1	29.8 ± 15.6	29.8 ± 14.5	6–60
AST (U/L)	22.9 ± 4.26	21.0 ± 4.06	22.4 ± 7.02	23.4 ± 6.5	23.6 ± 4.72	25.8 ± 9.60	10–40
HDL cholesterol (mg/dL)[3]	50.2 ± 14.1	—	—	50.6 ± 11.9	—	—	≥40
Total cholesterol (mg/dL)[3]	179 ± 26.6	—	—	188 ± 46.6	—	—	125–200
Triglycerides (mg/dL)[3]	113 ± 51.1	—	—	122 ± 71.5	—	—	<150
Glucose (mg/dL)	89.9 ± 13	83.4 ± 9.76	81.2 ± 26.1	87.4 ± 13.8	90.6 ± 2.97	79.4 ± 9.5[a]	65–99

[1] Data are represented as the means ± SDs of individual results. To calculate the mean, one-half of the reporting limit was used for values reported to be less than the reporting limit. Individual baseline values for the 1-wk predose draw and the day 1 (predose) draw were averaged unless otherwise noted. Student's 2-tailed paired t test was used to assess the effects of dietary cobalt supplementation on blood chemical indexes. The reference range is defined as the minimum and the maximum reference value from Quest Diagnostic Laboratories as determined in adult men. Significant difference in blood chemical indexes before and after cobalt dosing at specific time points ($P < 0.05$) is noted by a superscript lowercase letter a; however, the mean value after dosing was within the reference range. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK-MB, creatine kinase–myocardial band fraction; RBC, red blood cell; TSH, thyroid-stimulating hormone; T4, free thyroxine; WBC, white blood cell.

[2] The average baseline is the mean ± SD of the 1-wk predose draw because there was a significant difference between the 1-wk predose draw and the day 1 (predose) draw; as such, the 2 baseline values were not averaged ($n = 5$).

[3] The average baseline is the mean ± SD of the 1-wk predose draw because there was not a second baseline draw for this variable ($n = 5$).

TABLE 5
Blood clinical indexes for women[1]

Variable	Baseline (n = 10)	Days 29 and 30 (n = 5)	Days 57 and 58 (n = 5)	Days 88 and 90 (n = 5)	1 wk postdose (n = 5)	2 wk postdose (n = 5)	Reference range
WBC ($10^3/\mu L$)	6.46 ± 0.94	6.44 ± 1.09	6.36 ± 0.88	5.98 ± 1.24	6.1 ± 1.51	6.34 ± 0.92	3.8–10.8
RBC ($10^6/\mu L$)[2]	4.42 ± 0.28	4.28 ± 0.18	4.3 ± 0.21	4.32 ± 0.27	4.42 ± 0.37	4.38 ± 0.31	3.8–5.1
Hematocrit (%)	41.5 ± 1.68	40.9 ± 1.15	41.0 ± 1.14	40.9 ± 2.00	41.7 ± 2.17	40.9 ± 2.42	35–45
Hemoglobin (g/dL)	13.5 ± 0.43	13.3 ± 0.50	13.2 ± 0.37	13.1 ± 0.31[a]	13.4 ± 0.75	13.1 ± 0.61	11.7–15.5
Protein (g/dL)	7.11 ± 0.33	7.00 ± 0.35	7.08 ± 0.28	7.02 ± 0.38	6.96 ± 0.48	7.04 ± 0.29	6.2–8.3
Albumin (g/dL)	4.39 ± 0.22	4.32 ± 0.16	4.34 ± 0.17	4.28 ± 0.19	4.20 ± 0.27	4.32 ± 0.25	3.6–5.1
TSH (mIU/L)	1.73 ± 0.77	1.53 ± 1.03	1.65 ± 1.08	2.30 ± 1.90	1.73 ± 0.90	1.55 ± 0.63	0.4–4.5
T4 (ng/dL)	1.10 ± 0.11	1.16 ± 0.11	1.12 ± 0.11	1.08 ± 0.18	1.22 ± 0.13[a]	1.06 ± 0.11	0.8–1.8
Total iron (μg/dL)	88.4 ± 12.6	77.4 ± 21.5	84.6 ± 19.4	79.2 ± 21	77.6 ± 51.4	64 ± 11.6[a]	40–175
Ferritin (ng/mL)	51.3 ± 40.4	32.0 ± 24.2	27.4 ± 23.2	21.6 ± 14.7	34.6 ± 43.4[a]	24.8 ± 18.7	10–232
CK-MB (ng/mL)	0.61 ± 0.37	0.80 ± 0.45	0.59 ± 0.35	1.03 ± 0.56[a]	0.81 ± 0.36	1.09 ± 0.48[a]	0–5.0
Creatinine (mg/dL)	0.79 ± 0.09	0.78 ± 0.07	0.77 ± 0.08	0.80 ± 0.06	0.84 ± 0.09[a]	0.82 ± 0.13	0.5–1.1
ALT (U/L)	14.8 ± 4.19	13.6 ± 4.39	14.8 ± 6.61	11.2 ± 3.11[a]	12.6 ± 3.85	15.8 ± 4.87	6–60
AST (U/L)	18.8 ± 4.63	17.6 ± 3.51	20.2 ± 8.87	15.8 ± 4.66	16.8 ± 5.36[a]	20.6 ± 6.66	10–40
HDL cholesterol (mg/dL)[3]	85.2 ± 19.0	—	—	78.4 ± 23.1	—	—	≥46
Total cholesterol (mg/dL)[3]	209 ± 31.7	—	—	220 ± 54.6	—	—	125–200
Triglycerides (mg/dL)[3]	105 ± 41.3	—	—	95.6 ± 29.6	—	—	<150
Glucose (mg/dL)[2]	88.2 ± 7.53	82.2 ± 10.1	86.4 ± 9.4	83.2 ± 9.91[a]	79.6 ± 14.5	88.4 ± 15.1	65–99

[1] Data are represented as the means ± SDs of individual results. To calculate the mean, one-half of the reporting limit was used for values reported to be less than the reporting limit. Individual baseline values for the 1-wk predose draw and the day 1 (predose) draw were averaged unless otherwise noted. Student's 2-tailed paired t test was used to assess the effects of dietary cobalt supplementation on blood chemical indexes. The reference range is defined as the minimum and the maximum reference value from Quest Diagnostic Laboratories as determined in adult women. Significant difference in blood chemical indexes before and after cobalt dosing at specific time points ($P < 0.05$) is noted by a superscript lowercase letter a; however, the mean value after dosing was within the reference range. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK-MB, creatine kinase–myocardial band fraction; RBC, red blood cell; TSH, thyroid-stimulating hormone; T4, free thyroxine; WBC, white blood cell.

[2] The average baseline is the mean ± SD of the 1-wk predose draw because there was a significant difference between the 1-wk predose draw and the day 1 (predose) draw; as such, the 2 baseline values were not averaged ($n = 5$).

[3] The average baseline is the mean ± SD of the 1-wk predose draw because there was not a second baseline draw for this variable ($n = 5$).

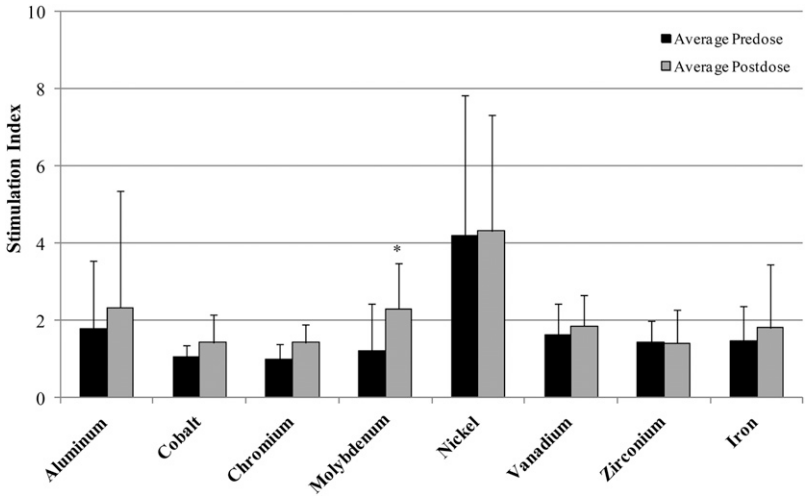

FIGURE 6. Normalized lymphocyte proliferation response to various metals before and after cobalt dietary supplementation with approximately 1 mg Co/d. The graph illustrates the averaged normalized lymphocyte transformation response to each metal in men and women combined (n = 10). Error bars denote the SD of the data. *Significant changes in the lymphocyte response before and after dosing, $P < 0.05$ (paired Student's t test).

dosing stopped, which suggested the renal conservation of cobalt at lower serum concentrations. Furthermore, our findings were consistent with observed increases in cobalt clearance and efficiency in metal-on-metal (MoM) hip-implant patients with increasing serum cobalt concentrations (34). The difference in absorption between men and women implied by the difference in 24-h urinary cobalt excretion during dosing may have been due to a greater iron demand in women because cobalt and iron are thought to share a common intestinal uptake mechanism. Studies have shown that iron deficiency increases cobalt absorption in both animals and humans (47–51). In addition, fertile women require more iron than infertile women because of increased iron needs associated with menstruation and substantial iron demands of pregnancy. Therefore, the fertility status and timing of menstruation may also be contributing factors in the variability of cobalt absorption observed between men and women as well as in women themselves. Similarly, individuals who exercise regularly require more iron compared with sedentary individuals because of greater energy demands during prolonged periods of physical activity, a higher turnover of erythrocytes, and an increased loss of iron in sweat and urine (52). After dosing was complete, cobalt urine concentrations rapidly decreased in tandem with the rapid drop in cobalt serum concentrations, which indicated that cobalt was rapidly cleared from the blood by similar mechanisms in men and women once exposure ceased.

Cobalt serum and whole blood times to steady state and elimination kinetics observed in this 90-d study (Figure 2) were consistent with results of previous studies and provided additional evidence of steady state conditions being achieved within ~2 to 8 wk of supplementation. For example, Finley et al (26) observed steady state conditions for 7 of 10 volunteers within 31 d continuous exposure to ~1 mg Co/d. Similarly, Duckham and Lee (12) reported stable cobalt serum concentrations (400–1000 μg/L) after 2 mo continuous oral treatment with 6.18–12.36 mg Co/d in anephric patients. These findings suggested that the

TABLE 6
Comparison of left and right ventricular function in volunteers before and after cobalt supplementation[1]

Variable	Prestudy	Study midpoint	Study completion	Reference range
LVID (ed) (cm)	4.77 ± 0.52	4.75 ± 0.55	4.65 ± 0.44	3.7–5.6
LVID (es) (cm)	3.15 ± 0.38	2.97 ± 0.49	2.95 ± 0.55	1.8–4.2
RVID (ed) (cm)	2.08 ± 0.46	2.02 ± 0.38	2.16 ± 0.35	0.7–3.03
Ao Root (ed) (cm)	2.99 ± 0.38	3.03 ± 0.45	3.01 ± 0.44	2.0–4.0
IVS (ed) (cm)	1.07 ± 0.19	1.06 ± 0.22	1.05 ± 0.13	0.6–1.1
IVS (es) (cm)	1.40 ± 0.24	1.46 ± 0.24	1.48 ± 0.24	0.8–2.0
LVPWs (ed) (cm)	0.94 ± 0.12	0.95 ± 0.11	0.99 ± 0.13	0.6–1.1
LVPWs (es) (cm)	1.49 ± 0.19	1.47 ± 0.18	1.52 ± 0.15	0.8–2.0
LVEF (2D est) (%)	65.9 ± 4.43	66.8 ± 4.21	65.0 ± 2.54	>55
LA (es) (cm)	3.15 ± 0.21	3.34 ± 0.34	3.13 ± 0.44	1.9–4.0
LA volume index (mL/m^2)	23.3 ± 4.27	24.2 ± 5.33	24.0 ± 6.08	16–28

[1] All values are means ± SDs for men and women combined (n = 10). Student's 2-tailed paired t test was used to assess effects of dietary cobalt supplementation; there were no statistical differences between baseline values and study-midpoint and -completion values ($P > 0.05$). Ao Root, aortic dissection; ed, end diastolic; es, end systolic; IVS, thickness of interventricular septum; LA, left atrium; LVEF, left ventricular ejection fraction; LVID, left ventricular inner dimension; LVPW, left ventricular wall; RVID, right ventricular inner dimension; 2D est, 2-dimensional estimate.

TABLE 7
Comparison of hearing function in volunteers before, during, and after cobalt supplementation[1]

Time point and ear	250 Hz	500 Hz	1000 Hz	2000 Hz	3000 Hz	4000 Hz	6000 Hz	8000 Hz	12,000 Hz	16,000 Hz
	dB	*dB*	*dB*	*dB*	*dB*	*dB*	*dB*	*dB*	*dB*	*dB*
Baseline										
Left	10.0 ± 4.7	10.0 ± 4.1	9.0 ± 3.2	9.0 ± 5.7	7.0 ± 7.5	10.5 ± 9.0	10.0 ± 8.5	5.5 ± 7.6	9.5 ± 13.0	24.0 ± 21.2
Right	8.0 ± 6.3	6.0 ± 6.1	7.5 ± 2.6	9.5 ± 4.4	8.5 ± 4.7	6.0 ± 5.7	12.0 ± 8.9	10.0 ± 7.8	7.5 ± 10.1	25.5 ± 24.9
Study midpoint										
Left	9.0 ± 3.9	8.5 ± 4.7	6.5 ± 4.1	8.5 ± 6.7	7.5 ± 6.8	9.5 ± 10.1	11.0 ± 9.1	8.5 ± 8.8	8.5 ± 11.8	21.0 ± 22.6
Right	8.0 ± 6.7	7.5 ± 6.8	5.0 ± 4.1[a]	9.5 ± 6.0	8.0 ± 4.2	6.0 ± 5.2	11.0 ± 9.9	7.5 ± 7.2	9.0 ± 8.1	25.0 ± 24.7
Study completion										
Left	7.5 ± 3.5	6.5 ± 4.7	6.5 ± 4.7	7.0 ± 6.8	8.0 ± 7.5	8.5 ± 10.5[a]	9.0 ± 6.9	9.0 ± 7.1[a]	9.5 ± 12.4	22.0 ± 20.0
Right	6.0 ± 5.7	5.5 ± 5.0	7.5 ± 4.2	7.5 ± 6.3	5.0 ± 3.3[a]	7.5 ± 5.4	10.0 ± 9.7	7.5 ± 5.4	8.0 ± 7.9	24.0 ± 22.2

[1] All values are means ± SDs for men and women combined (n = 10). Student's 2-tailed paired t test was used to assess effects of dietary cobalt supplementation, and significant differences were not observed unless noted by a superscript lowercase letter a ($P < 0.05$).

dosing duration required to achieve a steady state and steady state blood concentrations themselves may be sensitive to individual characteristics.

Whole blood cobalt elimination times in our study were consistent with Finley et al (26) and Curtis et al (46) who reported that whole blood cobalt concentrations decreased by 50% or 52% 1 wk postdosing and 86% by 10 wk postdosing after daily doses of ~1 mg Co for 1 mo or ~12.5 mg Co for 2 wk, respectively. Our data, which were consistent with previously reported human data (26, 46), suggested that steady state blood concentrations are achieved within 2 to 8 wk of daily cobalt ingestion, and after the cessation of dosing, elimination from the blood is relatively rapid. The near-baseline concentrations ~120 d after dosing ended were consistent with the RBC lifetime. These data on cobalt elimination should be useful for future refinements of kinetic models describing cobalt distribution in humans. In addition, whole blood cobalt concentrations, which represent contributions of storage in RBC and protein-bound cobalt in circulating serum, may be a useful metric for the assessment of risk of systemic effects after long-term cobalt exposure (24).

Comprehensive blood screening tests, including an assessment of serum biochemical variables commonly used as biomarkers of cardiac (CK-MB), liver (ALT and aspartate aminotransferase) and kidney (serum creatinine) function, showed no clinically significant changes after the oral ingestion of ~1 mg Co/d for an average of 89 d. Changes in thyroid function (elevated TSH and decreased T4) and hematologic variables (increased RBC, hemoglobin, and hematocrit) have been identified as sensitive clinical indicators of the cobalt response in humans (10, 53, 54), and individual values for RBC, TSH, and T4 were largely within reference ranges at all sampling points during this study (*see* Supplemental Figures S4, S9, and S10 under "Supplemental data" in the online issue). For one woman volunteer on the 90th day of dosing (volunteer J), her TSH concentration was greater than the reference range, and the T4 concentration was at the lower end of the reference range, but there was no change in her RBC, hemoglobin, or hematocrit concentrations (*see* Supplemental Figures S4B, S5B, S6B, S9B, and S10B under "Supplemental data" in the online issue).

Dilative cardiomyopathy has been reported in humans after oral exposure to cobalt that resulted from the consumption of large amounts of beer containing a cobalt-additive foaming agent (21–23, 55, 56). In our study, sequential cardiac exams did not detect any clinically significant echocardiographic changes,

TABLE 8
Values of the right eye for select RNFL and OHN variables[1]

	Prestudy	Study midpoint	Study completion
RNFL			
Average RNFL thickness (μm)	97.10 ± 7.89	97.00 ± 7.89	96.50 ± 7.81
OHN			
Rim area (mm^2)	1.29 ± 0.23	1.38 ± 0.23	1.31 ± 0.19
Disc area (mm^2)	1.65 ± 0.30	1.71 ± 0.33	1.59 ± 0.20
Average C:D ratio	0.43 ± 0.12	0.41 ± 0.13	0.40 ± 0.12
Vertical C:D ratio	0.41 ± 0.13	0.40 ± 0.12	0.38 ± 0.13
Cup volume (mm^3)	0.09 ± 0.06	0.08 ± 0.05	0.08 ± 0.05
VFI			
VFI (%)	99.40 ± 0.70	99.30 ± 0.48	99.60 ± 0.52
Mean deviation (dB)	−0.22 ± 1.01	0.29 ± 0.85	0.26 ± 0.82[a]
PSD (dB)	1.44 ± 0.31	1.37 ± 0.30	1.26 ± 0.16

[1] All values are means ± SDs for men and women combined (n = 10). Student's 2-tailed paired t test was used to assess effects of dietary cobalt supplementation, and significant differences were not observed unless noted by a superscript lowercase letter a ($P < 0.05$). C:D, cup:disc; OHN, optic nerve head; PSD, pattern SD; RNFL, retinal nerve fiber layer; VFI, visual field index.

TABLE 9
Values of the left eye for select RNFL and OHN variables[1]

	Prestudy	Study midpoint	Study completion
RNFL			
Average RNFL thickness (μm)	97.00 ± 8.67	96.30 ± 8.64	96.50 ± 9.01
OHN			
Rim area (mm^2)	1.35 ± 0.23	1.39 ± 0.29	1.36 ± 0.25
Disc area (mm^2)	1.73 ± 0.31	1.74 ± 0.42	1.63 ± 0.27
Average C:D ratio	0.44 ± 0.11	0.42 ± 0.12	0.40 ± 0.11
Vertical C:D ratio	0.40 ± 0.10	0.39 ± 0.11	0.36 ± 0.10
Cup volume (mm^3)	0.08 ± 0.04	0.07 ± 0.04	0.07 ± 0.04
VFI			
VFI (%)	99.30 ± 0.82	99.50 ± 0.97	99.70 ± 0.48
Mean deviation (dB)	−0.25 ± 0.78	0.02 ± 0.69	−0.09 ± 0.89
PSD (dB)	1.38 ± 0.30	1.28 ± 0.13	1.26 ± 0.16

[1] All values are means ± SDs for men and women combined (n = 10). Student's 2-tailed paired t test was used to assess effects of dietary cobalt supplementation; there were no significant differences ($P > 0.05$). C:D, cup:disc; OHN, optic nerve head; PSD, pattern SD; RNFL, retinal nerve fiber layer; VFI, visual field index.

including changes in the left-ventricular size (left-ventricular diameters at diastole and systole, left-ventricular internal dimension at diastole, and left-ventricular internal dimension at systole) and systolic function (ejection fraction) (Table 6), which indicated no cardiac damage in healthy individuals. Certain neurologic effects, such as reversible hearing and vision impairment, have also been described in isolated case reports involving therapeutic ingestion of cobalt as well as metal ion release from cobalt-containing orthopedic prostheses (12, 14, 57–61). In our study, the audiometric testing showed no clinically significant changes in sound recognition at any of the frequencies tested (Table 7; *see* Supplemental Figure S17 under "Supplemental data" in the online issue). In addition, no clinically significant changes in visual acuity or visual field measurement were noted, and no detectable optic nerve injury was observed after an average of 89 d cobalt supplementation (Tables 8 and 9).

Effects on the peripheral nervous system have also been reported, generally at relatively high cobalt serum or whole blood concentrations (>250 μg/L) (57, 59, 61–66). A few reports have suggested that similar symptoms may occur in MoM hip-implant patients at lower cobalt serum concentrations (<30 μg/L) (66–68). Although motor nerve conduction assessments for amplitude and velocity were normal in our volunteers, a general decrease in the sensory nerve conduction velocity and amplitude at the completion of the study compared with baseline readings was noted. However, no consistent trends were discernible. For example, at study completion (day 88, 89, or 90), 7 of 10 volunteers (A, C, D, F–I) experienced a slight decrease in the sural sensory amplitude, averaging 27% below baseline (range: 11–44%), whereas 3 other volunteers (B, E, and J) experienced an average increase of 28% (range: 9–47%). At study completion (day 88, 89, or 90), 9 of 10 volunteers who completed the study exhibited an average decrease of 16% in sural sensory nerve conduction velocities (range: 3–42% decrease), but there was no correlation between whole blood cobalt concentrations and changes in the sural sensory nerve conduction velocity (*see* Supplemental Figure S22 under "Supplemental data" in the online issue). The absence of an apparent relation with whole blood or serum cobalt concentrations greatly diminished the likelihood that the observed trends were associated with cobalt administration. Although none of the volunteers in our study were considered anemic, decreases in ferritin concentrations associated with repeated blood draws were noted for all volunteers, which may have caused electrophysiologic changes in the sural sensory nerve conduction, which is an effect that has been associated with iron deficiency anemia (69).

The primary limitation of our study was its relatively small sample size, which may have affected our ability to identify subtle changes in biological indexes identifiable in larger populations. However, we believe the study was sufficiently robust because we carefully evaluated effects of cobalt exposure for ~90 d on hematologic and thyroid variables, both of which have been identified as sensitive markers of cobalt exposure.

TABLE 10
Values of the sural sensory and peroneal motor variables[1]

	Prestudy	Study midpoint	Study completion	1 mo postdose
Sural sensory				
Amplitude (μV)	24.2 ± 14.8	23.4 ± 14.9	20.7 ± 12.4	28.3 ± 24.2
Velocity (m/s)	42.4 ± 5.8	39.9 ± 7.4	36.4 ± 7.0[a]	43.3 ± 7.8
Peroneal motor				
Amplitude (mV)	5.1 ± 2.3	5.5 ± 2.3	5.6 ± 3.0	—
Velocity (m/s)	58.7 ± 13.7	64.1 ± 18.7	63.2 ± 24.8	—

[1] All values are means ± SDs for men and women combined (n = 10). Student's 2-tailed paired t test was used to assess effects of dietary cobalt supplementation, and significant differences were not observed unless noted by a superscript lowercase letter a ($P < 0.05$, paired t test for prestudy compared with indicated study time).

Neither variable showed any clinically significant change during our study. For example, Davis and Fields (70) observed polycythemia in 6 men with increased RBC counts of 0.5–1.19 million (mean: 0.96 million) and increased hemoglobin concentrations of 0.9–1.6 g/dL after 7–22 d of receiving ~68 mg Co/d (one man initially received a slightly lower dose of 54 mg Co/d). Our pairwise comparisons had a power of 80% to detect a mean increase in RBC count of 0.34 million (σ = 0.2 million; n = 5) and a mean increase in hemoglobin concentration of 0.85 g/dL (σ = 0.5 g/dL; n = 5), which were less than the minimum increases detected in men who experienced polycythemia in Davis and Fields (70). Therefore, our sample size and analysis appeared to have been sufficient to identify a clinically meaningful response to cobalt exposure had it occurred as defined by previous observations of cobalt-related hematologic effects over short periods of exposure. In addition, our volunteers were all healthy adults, and thus, similar results may not occur in individuals with chronic preexisting health conditions.

In 2011, the US FDA issued a draft guidance for industry regarding notification and safety assessments for new dietary ingredients in dietary supplements (71). The draft guidance of new dietary ingredients indicated that clinical trials ≥90 d in duration with defined endpoints are useful for the assessment of the safety of chronic supplement ingestion. Furthermore, the US FDA Redbook on human studies of food and food additives indicated that human chronic administration usually corresponds to a continuous consumption ≥8–12 wk after safety has been shown in short-term clinical studies (72). Therefore, the data collected in this study should be useful for a health assessment after extended or chronic cobalt-dosing periods. In addition, our findings provide a comparison group for populations currently exposed to cobalt, such as individuals with MoM hip implants.

We thank Chris Ronk, Meghan Glynn, and Paul Scott for their assistance with the statistical analysis of data. We also thank Brent Kerger for his helpful discussions regarding cobalt kinetics.

The authors' responsibilities were as follows—BET, DAG, JMO, and KMU: conducted the research; BET and KMU: analyzed data; BET, DAG, and KMU: had primary responsibility for the final content of the manuscript; and all authors: designed the research, wrote the manuscript, and read and approved the final manuscript. All of the authors are employed or contracted by Cardno ChemRisk, which is a consulting firm that provides scientific advice to the government, regulatory agencies, corporations, law firms, and various scientific and professional organizations. Cardno ChemRisk has been engaged by DePuy Orthopedics Inc, which is a manufacturer of prosthetic devices. DePuy Orthopedics Inc authorized the funding of the study, but neither DePuy Orthopedics Inc nor their lawyers offered scientific comment, guidance, or suggestions on the protocol. This manuscript was prepared and written exclusively by the authors without review or comment by employees or counsel of DePuy Orthopedics Inc.

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