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J Appl Lab Med. Author manuscript; available in PMC 2021 March 11.

Published in final edited form as:
J Appl Lab Med. 2018 July 01; 3(1): 100–108. doi:10.1373/jalm.2017.025478.

Long-Term Stability of 18 Nutritional Biomarkers Stored at −20 °C and 5 °C for up to 12 Months

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Abstract

Background: Consistent information on long-term storage stability for a broad range of nutritional biomarkers is lacking. We investigated the stability of 18 biomarkers stored at suboptimal temperatures (−20 °C and 5 °C) for up to 12 months.

Methods: Multiple vials of serum or whole blood pools (3 concentrations) were stored at −20 °C or 5 °C, removed from the −20 °C freezer after 3, 6, 9, and 12 months and from the 5 °C refrigerator after 6 and 12 months, and placed into a −70 °C freezer until analysis at study completion. Vials stored continuously at −70 °C were used as the reference condition for optimal storage. We measured 18 biomarkers: 4 iron status, 1 inflammation, 8 water-soluble vitamin, and 5 fat-soluble vitamin. For each temperature, we calculated geometric mean concentrations and average percent changes of geometric means across pools relative to the reference condition estimated from a linear mixed model.

Results: Most biomarkers (13 of 18) showed no difference in concentration after 12 months of storage at −20 °C. Serum ferritin (1.5%), soluble transferrin receptor (−1.7%), and folate (−10.5%) showed small to moderate significant changes at 6 months, but changes were acceptable based on biologic variability. Serum pyridoxal-5′-phosphate (−18.6% at 9 months) and vitamin C (−23% at 6 months) showed large and unacceptable changes at −20 °C. All serum fat-soluble vitamins and iron status indicators, vitamin B12, total homocysteine, and methylmalonic acid showed acceptable changes when stored at 5 °C for up to 12 months.

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Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

C.M. Pfeiffer, H. Chen, and R.L. Schleicher designed the research and H. Chen conducted the research. M.R. Sternberg and H. Chen performed the statistical analysis. H. Chen, M.R. Sternberg, C.M. Pfeiffer and R.L. Schleicher were involved in the data analysis and interpretation. H. Chen drafted the manuscript. C.M. Pfeiffer, M.R. Sternberg, and R.L. Schleicher provided critical revision of the manuscript for important intellectual content. C.M. Pfeiffer has primary responsibility for final content. All authors have read and approved the manuscript.

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official views or positions of the US federal government including the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

Conclusions: Overall, we found good long-term stability for multiple nutritional biomarkers stored at suboptimal temperatures.

Storage of serum at −70 °C or lower is customary to maintain long-term integrity of biomarkers. However, −70 °C freezers are rare in low- and middle-income countries and sometimes in clinical settings. Sample integrity could be affected at higher storage temperatures. Few studies have assessed storage stability at −20 °C and 5 °C for multiple panels of nutritional biomarkers (1–3) (Table 1). Most reports have focused on 1 nutritional biomarker or 1 panel (4–15). Furthermore, typically only short-term storage at 5 °C was assessed. The CDC provides technical assistance to international surveys that collect, process, and store samples, often under suboptimal conditions. In a previous study, we assessed whether nutritional biomarkers are affected by delays in whole blood processing and freezing of serum, simulating suboptimal field conditions (1). In this study, we addressed long-term sample stability by exposing multiple pools to frozen storage at −20 °C and refrigerated storage at 5 °C for up to 12 months.

METHODS

We used in-house prepared serum pools (3 concentrations). For folate, we also used whole blood pools. For C-reactive protein (CRP)[2] and vitamin C (VIC), we had only 2 concentrations. Pools were prepared by combining native serum from blood donors obtained from a commercial blood bank, without any additives. We amended VIC (with ascorbic acid) and vitamin D pools (with 25-hydroxyvitamin D_2) to increase endogenous concentrations. Multiple sets of vials were stored at −20 °C (−24 °C to −19 °C) and 5 °C (3–5 °C) (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.jalm.org/content/vol3/issue1). Vials were removed from the freezer after 3, 6, 9, and 12 months and from the refrigerator after 6 and 12 months and placed into a −70 °C freezer until the end of the 1-year study period when all conditions were analyzed in the same analytical run. Vials stored continuously at −70 °C for the study period were used as the reference condition for optimal storage.

The study included 4 iron status [ferritin (FER), soluble transferrin receptor (sTfR), iron, and unbound iron-binding capacity], 1 inflammation (CRP), 8 water-soluble vitamin [serum folate (S-FOL), whole blood folate (WB-FOL), pyridoxal-5′-phosphate (PLP), 4-pyridoxic acid, vitamin B12 (B12), total homocysteine (tHcy), methylmalonic acid (MMA), and VIC], and 5 fat-soluble vitamin (vitamin A or retinol, vitamin E or α-tocopherol, γ-tocopherol, 25-hydroxyvitamin D_3, and 25-hydroxyvitamin D_2) biomarkers. Each condition was analyzed in replication: 3 vials × 2 replicates/vial for n = 6 per pool, except for the 6- and 12-month conditions at −20 °C (6 vials × 2 replicates/vial for n = 12 per pool). Assuming a log-normal underlying distribution, there was 80% power to detect a 5% change from the reference condition using a 2-sided paired *t*-test at $\alpha = 0.05$ when the CV of the difference was <10%. Samples were analyzed at the CDC using validated methods (see Table 1 in the online Data Supplement). Except for WB-FOL measured by the microbiologic assay, all methods

[2]**Nonstandard abbreviations:** CRP, C-reactive protein; VIC, vitamin C (ascorbic acid); FER, ferritin; sTfR, soluble transferrin receptor; S-FOL, serum folate; WB-FOL, whole blood folate; PLP, pyridoxal-5′-phosphate; B12, vitamin B12; tHcy, total homocysteine; MMA, methylmalonic acid.

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satisfied optimum or desirable quality specifications for analytical imprecision based on biologic variation (16). In addition to the study samples, each analytical run contained multiple concentrations of internal quality control samples prepared in duplicate and measured at the beginning and end of each run to verify that results were within predefined acceptability limits.

We transformed the data logarithmically ($\log_{10}$) to account for the increasing variance with increasing concentration. For each biomarker, SAS software (version 9.2, SAS Institute) was used to estimate a linear mixed model after a $\log_{10}$ transformation. Each model contained time and pool as fixed effects and vial as a random effect. The least-squares estimates (95% CIs) for the time effect were back-transformed to provide a pairwise least-squares geometric means (95% CI) to the reference condition. Pairwise comparisons were based on F-tests ($\alpha = 0.05$) with denominator degrees of freedom determined by a Satterthwaite adjustment.

RESULTS

The pools spanned a range of typical concentrations seen in presumably healthy persons in the US population (see Table 2 in the online Data Supplement). In pools stored at –20 °C for up to 12 months, FER and sTfR showed small (<2%) but significant changes at 6 months (FER) and 12 months (sTfR), respectively (Table 2). However, these changes were within the optimum quality specifications for bias based on biologic variability (16) (see Table 1 in the online Data Supplement). S-FOL (–10.5%), PLP (–18.6%), and VIC (–23%) showed moderate to large significant decreases at 6 months (S-FOL and VIC) and 9 months (PLP) (Table 2). Although the change in S-FOL was within the desirable bias, the changes in PLP and VIC exceeded the minimum bias and were, therefore, unacceptable (see Table 1 in the online Data Supplement). After 12 months of storage, PLP and VIC concentrations decreased further to 33.3% and 49.3%, respectively (Table 2). None of the fat-soluble vitamins showed significant changes after 12 months at –20 °C; however, we observed a borderline significant interaction between time and pool for vitamin A ($P = 0.049$).

In pools stored at 5 °C for up to 12 months, FER and iron showed small (<5%) but significant changes at 6 months (Table 2). The changes appeared to increase slightly at 12 months (FER, 2.4%; iron, 9.2%), but none of the changes exceeded the minimum bias and, thus, were acceptable (see Table 1 in the online Data Supplement). All water-soluble vitamin biomarkers except for 4-pyridoxic acid showed significant changes after 12 months at 5 °C (Table 2). The changes for B12, tHcy, and MMA were small (<7%), acceptable, and not yet significant after 6 months. However, S-FOL (–61.5%) and PLP (–70.2%) showed large, significant, and unacceptable decreases already after 6 months that decreased further after 12 months (S-FOL, –77.8%; PLP, –95.1%). Whereas we observed a significant interaction between time and pool for PLP ($P < 0.0001$), the changes at 6 months [–71.3% (low), –67.9% (medium), –71.2% (high)] and 12 months [–97.8% (low), –90.9% (medium), –94.1% (high)] were similar for each pool (data not shown). None of the fat-soluble vitamins showed significant changes after 12 months at 5 °C (Table 2).

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DISCUSSION

This study provides updated information on long-term storage stability up to 12 months at 2 suboptimal temperatures, −20 °C and 5 °C, for 3 groups of nutritional biomarkers commonly measured in micronutrient surveys (see Table 3 in the online Data Supplement). The fat-soluble vitamins showed remarkable stability at both temperatures, which agrees with previous reports regarding storage at −20 °C (12–15), but provides new information for storage at 5 °C. In 1 previous report, vitamin A and E concentrations in EDTA plasma were shown to decline by 20% to 50% after 1 year at −20 °C, possibly because of the EDTA anticoagulant (3). It should be noted that fat-soluble micronutrients such as the carotenoids are less stable (12), and proper long-term storage at −70 °C is required if those compounds are to be measured.

The iron status indicators showed overall good stability with relatively small and acceptable changes at both temperatures (see Table 3 in the online Data Supplement). These findings confirm previous reports of long-term frozen (4–6) and short-term refrigerated (1) storage. The consistency of the small increase in FER at 6 and 12 months at both temperatures and in iron at 6 and 12 months at 5 °C suggests that the effects are real rather than random variability. Evaporation is unlikely the cause because the same vials and storage locations were used for all biomarkers.

The water-soluble vitamin biomarkers—and within this group S-FOL, PLP, and VIC—showed the least stability (see Table 3 in the online Data Supplement). Storage at −20 °C resulted in significant but acceptable decreases in S-FOL but unacceptable decreases in PLP and VIC. Storage at 5 °C resulted in unacceptable decreases in S-FOL and PLP (VIC was not analyzed at this condition). Similar (3) and even larger (7, 8) decreases in S-FOL at −20 °C were reported previously, possibly because of longer (29 years) storage time (7) or the specific assay used (8). Reports on PLP stability are mixed (3, 7, 10). VIC was reported to be stable in acidified heparin plasma for 20 days at −20 °C followed by a significant decline (11). Consistent with previous reports (1, 7, 8), B12, tHcy, MMA, and 4-pyridoxic acid showed good stability in our study at both temperatures. Stability of WB-FOL at −20 °C was reported for up to 10 weeks (9). Our data suggest up to 1-year stability at −20 °C.

We assessed the acceptability of change based on biologic variation (16). Most previous reports did not use objective quality specifications; however, 1 report specified the stability criteria as the deviation from the initial measurement being <2 times the long-term assay CV (6). Previously we used a combination of biologic and analytic variation (1). However, a larger number of samples are needed for that approach; otherwise, the acceptability thresholds are too wide. Because of limited resources, we performed this study with a few well-characterized pools. We used samples stored at −70 °C as the reference condition and analyzed all conditions together to minimize assay variability. This was possible because of prior knowledge about the long-term stability of the studied biomarkers at −70 °C from published reports (4, 8, 11, 12) and in-house data.

Although we found good long-term stability for multiple nutritional biomarkers stored at suboptimal temperatures, surprisingly even for some biomarkers stored at 5 °C, we urge

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researchers to store samples at the lowest temperature possible to allow future analyses of unplanned biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

The authors thank the staff of the Nutritional Biomarkers Branch at the Division of Laboratory Sciences for conducting the laboratory analyses for this study.

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IMPACT STATEMENT

Researchers and clinicians who may not have access to optimally stored biological samples will benefit from updated stability information for a broad range of nutritional biomarkers. The same conditions were applied across biomarkers, and assay variability was minimized by analyzing all conditions together; thus, results can be easily compared across multiple water- and fat-soluble vitamin biomarkers and iron status indicators. The data, produced by the use of well-controlled and precise analytical methods, showed overall good storage stability, in some cases even for refrigerated storage, which was surprising.

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Table 1.

Summary of previous reports on storage stability of nutritional biomarkers.[a]

Author, year (*reference*)	Biomarker(s) studied	Storage conditions	Findings	Comments
Drammeh, 2008 (7)	FER, sTfR S-FOL, B12, VIC VIA, VIE, 25OHD	Serum samples from 35 blood donors (15 men, 20 women, all 18 years of age) stored at 11 °C for up to 14 days compared with samples stored at –70 °C; all conditions analyzed together at the end of the study in the same batch	No significant changes for FER and sTfR at any time point Significant decrease for VIC (5%) after 2 days; significant decrease for S-FOL (5%) after 7 days Significant increases for VIE (1%) and 25OHD (7%) after 7 days (but no change after 10 and 14 days) and for VIA (3%) after 14 days	Clinically acceptable change based on combined analytical imprecision and intraindividual biologic variability: 5.5% for FER, 4.1% for sTfR, 11.7% for S-FOL, 5.3% for B12, 14.9% for VIC, 3.4% for VIA, 3.9% for VIE, and 3.9% for 25OHD
lhara, 2004(2)	S-FOL, B12, VIC VIA, VIE	Vitamin-enriched lyophilized serum preparation stored at –20 °C for up to 1 year	No evidence of degradation of S-FOL, B12, VIC No evidence of degradation of VIA; small degradation for VIE (less than between-day analytic variation)	Because the analytic stability of methods during study period was unclear, assay values were corrected with the use of standard solutions (VIC, VIA, and VIE) or manufacturer's control serum (S-FOL and B12)
Ocke, 1995 (2)	S-FOL, PLP, B12	EDTA plasma samples from 55 blood donors (male and female white individuals 20–55 years of age) stored at –20 °C for up to 4 years (3, 6, 12, 24, 36, 48 months) compared	Small decrease in S-FOL (20%) after 1 year, constant after that; significant fluctuations for PLP and B12 within ±20%	Except for S-FOL, the decreases differed largely across subjects after suboptimal storage
	VIA, VIE, 25OHD	with freshly analyzed samples; all samples analyzed in duplicate at the end of each time point; storage time at time of analysis differed across donors because it took 3 months to collect from 55 donors	Large decreases in VIA (20–30% after 1 year, >65% after 2 years) and VIE (50% after 1 year, >90% after 2 years); significant fluctuations for 25OHD within ±20%	EDTA plasma used in this study may explain some of the differences in findings compared with other studies using serum
Jansen, 2013(4)	FER, sTfR, iron, UIBC	Serum samples (for FER, iron, and UIBC) and citrate plasma samples (for sTfR) from 16 blood donors stored at –20 °C, –70 °C, or –196 °C for up to 1 year; reference at time 0	Constant concentrations (±10% of time 0) for all 3 temperatures over the 1-year period	Storage at –20 °C and –70 °C for up to 1 year validated relative to ideal storage at –196 °C
Gislefoss, 2007 (5)	Iron	Serum samples from 3 different groups of 130 male blood donors (40–49 years of age) stored in the Janus Serum Bank at –25 °C for 25 and 2 years compared with 1-month-old samples	Nonsignificant and numerically small differences in group median values	Cross-sectional approach; assumption that only negligible changes in serum components had taken place in the background population over the 25-year period
Donnelly, 1995(6)	Iron	Two serum pools from healthy adult volunteers (21–55 years of age) stored at 22 °C for 48 hours, 4°C for 14 days, or –20 °C for 4 months, compared with freshly prepared serum	Stable at all 3 temperatures for the specified times	Stability criteria: deviation from initial measurement less than twice the long-term CV for the assay
Hustad, 2012(7)	S-FOL,[b] PLP, 4-PA, B12, tHcy, MMA	Serum samples from 5 different groups of 130 male blood donors (40–49 years of age) stored in the Janus Serum Bank at –25 °C for 29, 17, 6, and 4 years compared with freshly collected samples	After 29 years of storage, large decreases (>50%) in S-FOL and PLP, small decrease (<25%) in 4-PA, small increase (<15%) in B12, minimal increases (<5%) in tHcy and MMA	Cross-sectional approach; assumption that only negligible changes in serum components had taken place in the background population over the 29-year period
Jansen, 2012(8)	S-FOL, B12	Serum samples from 16 blood donors stored at –20 °C, –70 °C, or –1 96 °C for up to 1 year; reference at time 0	Small (<20%) and large (~50%) decrease in S-FOL after 14 days and 1 month at –20 °C, respectively; constant S-	Rank order of folate concentrations in samples was not affected

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Author, year (*reference*)	Biomarker(s) studied	Storage conditions	Findings	Comments
		Also assessed short-term stability for storage at 4°C for up to 4 days	FOL from 1–12 months and constant B12 concentrations for all storage conditions; similar stability at –70 °C and –196 °C for both analytes Small (<20%) decrease in S-FOL and constant B12 concentrations after 4 days at 4°C	Storage at –70 °C for up to 1 year validated relative to ideal storage at –196 °C
O'Broln, 1997 (9)	WB-FOL	EDTA-whole blood samples from 3 donors stored at –20 °C for up to 10 weeks and analyzed weekly Also assessed short-term stability as intact blood or hemolysate stored at 4 °C, 22 °C, 37 °C for up to 7 days	Stable with between-assay CVs of 12.0%, 8.6%, and 12.6% after 10 consecutive weekly assays Maximum folate stability at 4°C with intact blood (<20% decrease after 7 days); >20% decrease with intact blood stored at 22 °C for 4 days or at 37 °C for 1 day and with hemolysate stored at 22 °C for 1 day	
Borschel, 1987(10)	PLP	Plasma samples (n = 11) stored at –30°Cfor 1–2 years and analyzed originally by a macromethod and later by a micromethod that has been demonstrated to perform equlvalent to the macromethod	Stable with comparable mean ± SD concentrations for the micromethod (152 ± 30 pmol/mL) and macromethod (145 ± 26 pmol/mL)	Indirect demonstration of stability
Karlsen 2007(11)	VIC	Heparin plasma (acidified and nonacidified) stored at –70 °C or –20 °C for up to 2 years	No degradation observed in acidified plasma stored at –70 °C for 80 days; 6.8% decline observed after 1 year, 14% after 2 years Similar degradation pattern in nonacidified samples at –70 °C and acidified samples at –20 °C (no degradation for 20 days followed by a significant decline) Complete degradation in nonacidified plasma stored at –20 °C for 3 months	No information provided on number of samples used for each experiment
Craft, 1988(12)	VIA, VIE	Plasma samples stored at –20 °C for 5 and 15 months and compared with samples stored at –70 °C	No degradation observed at either condition	No information provided on number of samples used for storage at –20 °C
Gunter, 1988(13)	VIA, VIE	Serum samples (n = 238) stored at –20 °C for 7–13 months and reanalysls values (fresh aliquot) compared with original values	For VIA, stored values were virtually same as fresh values ($R^2 = 0.91$) For VIE, stored values were about 10%–30% lower than fresh values ($R^2 = 0.62$)	No information provided on assay stability over period of study
Driskel, 1985(14)	VIA	Serum samples (n = 86) stored at –20 °C for 5–8 years and analyzed originally by a colorimetric method and later by HPLC-UV	Similar coefficient of correlation between values for fresh and frozen-stored samples ($r = 0.71$) compared with values for HPLC and colorimetric method (r=0.78, n = 15)	Indirect demonstration of stability
Norris, 1986(15)	25OHD	Heparin plasma (n = 11) stored at –18 °C for 11 months and compared with samples analyzed after 14 days	Small (<10%) decrease	

[a] $25OHD_2$, 25-hydroxyvitamin D_2; $25OHD_3$, 25-hydroxyvitamin D_3; 4PA, 4-pyridoxic acid; HPLC-UV, high performance liquid chromatography with ultraviolet detection; γ-TOC, γ-tocopherol; UIBC, unsaturated iron-binding capacity; VIA, vitamin A (retinol); VIE, vitamin E (α-tocopherol).

[b] 5-Methyltetrahydrofolate, the main circulating folate form in serum, was assessed.

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Table 2.

Change in geometric mean biomarker concentrations for QC pools stored frozen at −20 °C or refrigerated at 5 °C for up to 12 months.[a]

	Storage at −20° C for up to 12 months[b]					Storage at 5 ° C for up to 12 months[b]				
	Change at 12 months		Time point of change[d]			Change at 12 months		Time point of change[d]		
Biomarker[c], unit	Mean (95% CI), %	P value	Month	Mean (95% CI), %	P value	Mean (95% CI), %	P value	Month	Mean (95% CI), %	P value
Iron status indicators										
FER, ng/mL	1.2 (0.0, 2.4)	0.0468	6	1.5(0.3,27)	0.0161	2.4(0.6,4.1)	0.0093	6	1.8(0.1,3.5)	0.0436
sTfR, mg/L	−1.7 (−3.1,−0.2)	0.0233	12	AS[e]	AS	1.8 (−0.2,3.8)	0.07	NA[f]	NA	NA
Iron, μ/dL	0.0 (−4.0,4.2)	0.99	NA	NA	NA	9.2(4.4,14.3)	0.0005	6	47(0.1,9.5)	0.0465
UIBC, μ/dL	−1.6 (−5.4, 2.5)	0.44	NA	NA	NA	2.7 (−7.7,14.4)	0.61	NA	NA	NA
CRP, mg/L	−0.3 (−2.4,1.9)	0.80	NA	NA	NA	−1.4 (—3.5, 0.7)	0.18	NA	NA	NA
Water-soluble vitamins										
S-FOL, nmol/L	−11.7 (−20.1,−2.4)	0.0156	6	−10.5 (−19.0, −1.0)	0.0311	−77.8 (−81.2,−73.9)	<0.0001	6	−61.5 (−67.3,−54.8)	<0.0001
WB-FOL, nmol/L	−4.50 (−10.4,1.7)	0.15	NA	NA	NA	NS[g]	NS	NS	NS	NS
PLP, nmol/L	−33.3 (−43.8,−20.9)	<0.0001	9	−18.6 (−33.2,−0.8)	0.0420	−95.1 (−96.4,−93.4)	<0.0001	6	−70.2 (−78.0,−59.6)	<0.0001
4-PA, nmol/L	−4.4 (−9.9,1.6)	0.15	NA	NA	NA	1.7 (−2.3,5.7)	0.40	NA	NA	NA
B12, pg/mL	2.0 (−0.3,4.3)	0.09	NA	NA	NA	3.3(0.1,67)	0.044	12	AS	AS
Hcy, μmol/L	2.1 (−11.0,−17.0)	0.77	NA	NA	NA	6.2(1.5,11.1)	0.012	12	AS	AS
MMA, nmol/L	1.0 (−1.5,3.5)	0.42	NA	NA	NA	6.6(2.2,11.2)	0.0048	12	AS	AS
VIC, mg/dL	−49.3 (−60.9,−34.2)	<0.0001	6	−23.0 (−40.7,−0.1)	0.0493	NS	NS	NS	NS	NS
Fat-soluble vitamins										
VIA, μ/dL	−1.2 (−3.5,1.0)	0.27	NA	NA	NA	−2.2 (−4.8,0.6)	0.117	NA	NA	NA
VIE, μ/dL	−1.0 (−2.9,1.0)	0.33	NA	NA	NA	−1.8 (−3.8,0.2)	0.069	NA	NA	NA
γ-TOC, μ/dL	−0.3 (−2.0,1.4)	0.73	NA	NA	NA	−0.3 (−2.0,1.4)	0.736	NA	NA	NA
$25OHD_3$, nmol/L	−0.8 (−4.0, 2.6)	0.65	NA	NA	NA	0.9 (−2.3,4.3)	0.564	NA	NA	NA
$25OHD_2$ nmol/L	2.5 (−5.9,11.7)	0.57	NA	NA	NA	7.1 (−3.2,18.4)	0.178	NA	NA	NA

[a]QC, quality control; $25OHD_2$, 25-hydroxyvitamin D_2; $25OHD_3$, 25-hydroxyvitamin D_3; 4PA, 4-pyridoxic acid; γ-TOC, γ-tocopherol; UIBC, unsaturated iron-binding capacity; VIA, vitamin A (retinol); VIE, vitamin E (α-tocopherol).

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[b]Reference samples were stored at −70 °C until analysis at study completion (about 12 months); samples for the −20 °C storage conditions were stored at −20 °C for 3, 6, 9, and 12 months and then at −70 °C for about 9, 6, 3, and 0 months until analysis; samples for the 5 °C storage conditions were stored at 5 °C for 6 and 12 months and then at −70 °C for about 6 and 0 months until analysis; all conditions were analyzed in the same analytical run; 3 vials and 2 replicates/vial (n = 6) were analyzed for the following conditions: reference, 3 months, and 9 months at −20 °C, 6 months and 12 months at 5 °C; 6 vials and 2 replicates/vial (n = 12) were analyzed for the following conditions: 6 months and 12 months at −20 °C.

[c]All biomarkers were measured in serum except for whole blood folate.

[d]Indicates first time point of significant change in biomarker concentration.

[e]AS, as shown to the left in column “Change at 12 months.”

[f]NA, no significant change observed up to 12 months of storage.

[g]NS, no samples available for analysis