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Chapter 2

Can whole-blood samples be stored over 24 hours without compromising stability of C-reactive protein, retinol, ferritin, folate, and fatty acids in epidemiologic research?

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Technical brief

Ideally, blood samples for biomarker measurement are collected centrally and processed immediately to avoid any unwanted changes in concentrations that could affect validity. In large-scale epidemiologic and clinical studies, however, this theoretical goal must give way to a more pragmatic approach.[1,2] The Amsterdam Born Children and their Development (ABCD) study is a clinically based cohort study of pregnant women in Amsterdam (the Netherlands) in which we use, for practical and ethical reasons, blood collection in conjunction with existing schemes of care, with samples subsequently sent to a central laboratory by mail or courier. As a consequence, delay times between collection and processing may exceed 24 h, to an incidental maximum of 96 h. Although the stabilities of the biomarkers of interest, i.e., C-reactive protein (CRP), retinol, ferritin, folate, and fatty acids (FAs) have been studied previously,[1-9] the variety of designs (e.g., storage ≤24 h) hampers the applicability of existing results to our research and similar studies. We therefore investigated the appropriateness of our standardized, practice-based approach of blood collection by assessing stability in samples stored up to 96 h (the maximum delay time possible when samples are sent by mail) with a focus on the first 28 h (the maximum delay time allowed in the ABCD study).

Blood samples were collected from 41 generally healthy female volunteers, 22–56 years of age. One woman was at that time 16 weeks pregnant, but because her measurements did not deviate, we decided not to exclude her from analysis. Written informed consent was obtained from all participants. For 20 women, 28 mL of blood was collected in seven 4-mL Vacuettes (Greiner BV) for the preparation of serum. For the other 21 women, 28 mL blood was collected in seven 4-mL Vacutainer EDTA tubes (Becton and Dickinson BV) for the preparation of plasma. One tube from each woman was centrifuged (1600*g* for 10 min) between 1 and 2 h after blood collection ($t_{0;}$ baseline), and the remaining tubes were stored in a cabinet at room temperature (~21° C) and centrifuged 2 (t_2), 4 (t_4) , 24 (t_{24}), 26 (t_{26}), 28 (t_{28}), or 96 (t_{96}) h after baseline. Time points were chosen to mimic courier and postal conditions seen in the ABCD study, 4 h being the anticipated maximum delay time when blood is sent by courier, 28 h being the anticipated maximum delay time when blood is sent by overnight-mail, and 96 h being the anticipated maximum delay time when blood is sent by mail but over the weekend.

Aliquots (1 mL) of plasma and serum were stored at −80°C and transferred on dry ice to collaborating laboratories for analysis within 1 week. In serum, CRP concentrations were measured by use of the Dade-Behring high-sensitivity assay.[10] Ferritin and folate concentrations were measured by immunoassay with chemiluminescence detection on the Advia Centaur System (Bayer Group),[11] and retinol was measured with an isocratic reversed-phase HPLC method (Waters Ltd.).[12] Intra- and interassay CVs, as determined previously, were 4.1% and 5.2%, respectively, for CRP; 2.5% and 5.4% for ferritin; 7.9% and 6.1% for folate; and 3.3% and 7.3% for retinol. The FA composition of plasma phospholipids was determined by capillary gas chromatography with flame ionization detection (HP5890 series II, Hewlett

Packard) as described by Al et al.[13] Absolute (mg/L of plasma) and relative (percentage of total phospholipid-associated FAs) amounts were calculated for the major saturated FAs and the major mono- and polyunsaturated FAs of the n-3, n-6, and n-9 families, each constituting ≥0.1% of total FAs. CVs varied from 1.3% for arachidonic acid (20:4n-6) to 5.6% for Mead acid (20:3n-9). For FA analysis, all paired samples were assayed in the same run.

After verification of the homogeneity of variance and identification of outliers [two measurements of folate processed at t_{26}, one baseline measurement of linoleic acid (18:2n-6), one baseline measurement of Mead acid (20:3n-9) and one measurement of adrenic acid (22:4n-6) at t_{24}, all excluded from analysis], we performed repeated-measures ANOVA to explore time effects over 96 h and to calculate the mean percentage change per time point. Because we assumed that the stability depended on both reliability and validity (because true changes over time can be established only if measurement error is small), we assessed stability at 28 h by calculating intraclass correlation coefficients[5] (two-way mixed-effect model with single-measure reliability; SPSS), and by computing Spearman rank correlations for a (n = 50) bootstrap sample. In the latter procedure, we took for all women random samples from all values and calculated correlation coefficients with baseline values. Because these correlations depend on both the distribution of the individual values and the stability, this measure provides a nonparametric estimate of the "noise" to be expected because of unknown variance of delay, in particular in regression-like analyses. Additional analyses of within- and between-subject CVs, assessing the magnitude of the variation attributable to time in relation to the variation between individuals,[1] can be viewed in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue1/.

The changes in concentration, reliability coefficients, and Spearman rank correlations for measured analytes are shown in **Table 2.1**. CRP was excluded from the reliability and validity analyses because we considered the number of observations above the clinically significant detection level (0.5 mg/L) insufficient (insufficient power). A graphic representation of changes across all time points is presented in the online Data Supplement. For CRP, retinol, and ferritin, changes in concentration during the 96-h storage period were small and not significant (≤10%, $P \geq 0.1$) For folate, the mean concentration changed significantly over time ($P = 0.037$), with the sharpest decrease (8.7%) in the first 2 h of storage. Reliability and validity measures were high for all serum analytes (intraclass correlation coefficient ≥ 0.96; Spearman rank correlation coefficient ≥ 0.80).

Significant changes over time were observed for most FAs, but only for linoleic acid and adrenic acid were changes ≥10%. The linoleic acid concentration decreased 14% over the 96-h storage period ($P \leq 0.001$), and the concentration of adrenic acid increased 16% ($P \leq 0.001$). For the majority of FAs, reliability coefficients were ≥0.97. For Mead acid, the reliability coefficient was lower (0.81). Spearman rank correlations were ≥0.93 for all FAs except Mead acid (0.78).

Our results demonstrate that a pragmatic approach for data collection does not affect

the stability of measurements of CRP, retinol, ferritin, and most FAs, because these markers showed limited variance within a 96-h storage period. For folate, linoleic acid, and adrenic acid, significant changes (≥10%) over time were observed, but these did not significantly affect measures of 28-h validity.

Although previous studies on the stability of CRP and ferritin showed results similar to ours,[3-6,8] studies on retinol are conflicting. Both Key et al.[1] and Hankinson et al.[2] have reported small decreases (≤5.0%), whereas Mejia et al.[7] observed no change in concentration. We also observed no significant changes, possibly as result of the storage of our samples in the dark to protect vitamin A from photodestruction.[14]

Folate is known for its sensitivity for both heat and light, and substantial decreases (>10%) during storage have been reported.[8,9] Zhang et al.,[9] who found a 15% decrease after 24 h of storage, concluded that this is within acceptable limits for clinical analysis. Similarly, we observed no relevant time-related changes in the 28-h stability analyses.

To our knowledge, no previous studies have reported on the stability of FAs in plasma phospholipid fraction. Generally, we observed only small changes in concentrations, which did not significantly affect coefficients of correlation. For Mead acid, however, reliability and validity coefficients were more influenced by variability in concentration, possibly because of the small magnitude of the individual values (<1%).[5] Surprisingly, we observed not only decreases in FA concentrations (assumingly attributable to FA peroxidation), but also increases. A second process may be ongoing during storage: the formation of erythrocyte microparticles. In vitro, glycolysis will lower the glucose concentration in erythrocytes, causing depletion of ATP. Consequently, the erythrocytes cannot maintain membrane integrity, and microparticles will be released.[15] Over time, the concentration of plasma phospholipid-associated FAs may thus become enriched with erythrocyte membrane phospholipids containing relatively high amounts of polyunsaturated FAs.

Our study has some limitations. One limitation is that we did not measure in duplicate to adjust for intraassay variation. In addition, serum samples were not analyzed within one run to minimize interassay variability. However, standardized procedures were used for analysis, with CVs ≤ 7.9% for serum analytes and ≤ 5.6% for plasma phospholipid-associated FAs. In addition, the reliability analyses showed high agreement between follow-up and baseline values. Another limitation is that samples were not randomized before analysis. Possible bias from order of draw, although unlikely, can therefore not be ruled out. A third limitation is that we were not able to test the reliability and validity of CRP over the 28-h storage period. However, changes in concentration were small (≤5.5%), which, as mentioned before, is in line with results from other studies.[3,5]

In conclusion, this study shows that in the context of epidemiologic studies investigating (nutritional) status during routine care, a pragmatic approach to blood collection may validly be applied to determine CRP, retinol, ferritin, folate, or FA status. Although storage will diminish the precision of estimates, standard (correlational) epidemiologic analyses will not be compromised in samples stored for a maximum of 28 h.

Table 2.1 Stability of serum analytes and fatty acids in plasma phospholipids: changes in concentration after storage for 2 to 96 h, and 28-h reliability and validity measures

	Mean (SE) baseline value	Change from baseline, %						*P*, time effect[a]	28-h reliability (ICC)[b]	28-h validity (Spearman)[c]
		+2h	+4h	+24h	+26h	+28h	+96h			
Serum analytes										
CRP,[d] mg/L (n = 6)	18.4 (14.2)	−3.0	−4.4	−5.5	−5.2	−2.9	−3.0	0.370	-	-
CRP,[e] mg/L (n = 5)	4.2 (0.9)	−10.0	−1.4	−6.2	−3.8	1.4	2.8	0.105	-	-
Retinol, µmol/L	3.01 (0.2)	2.7	0.8	1.7	0.4	1.8	1.1	0.311	.97	.96
Ferritin, µg/L	45 (11)	−0.4	−1.4	0.5	2.1	3.1	2.6	0.110	.99	.99
Folate, nmol/L	19.7 (2.9)	−8.7	−10.8	−13.6	−18.7	−11.5	−16.5	0.037	.97	.87
Fatty acids in plasma phospholipids, % by weight of total fatty acids										
16:0	29.16 (0.40)	−0.5	−0.2	0.7	0.7	0.6	2.4	<0.001	.98	.93
18:0	12.41 (0.27)	−0.4	0.1	2.0	2.0	1.7	6.0	<0.001	.98	.96
18:1n-9	8.39 (0.24)	−0.8	−1.0	−1.9	−1.7	−2.2	−3.8	<0.001	.97	.96
20:3n-9	0.38 (0.02)	6.1	7.1	2.3	0.1	0.5	4.9	0.256	.81	.78
18:2n-6	20.97 (0.59)	−0.4	−1.1	−4.7	−4.9	−5.3	−14.0	<0.001	.99	.97
20:3n-6	3.00 (0.14)	−0.4	−0.3	1.0	1.1	1.5	4.0	<0.001	.99	.98
20:4n-6	8.73 (0.30)	−0.2	−0.3	−0.1	0.0	0.2	1.4	0.021	.99	.98
22:4n-6	0.30 (0.01)	−0.2	0.9	3.1	4.1	4.8	16.2	<0.001	.99	.99
22:5n-6	0.21 (0.02)	0.2	−0.2	0.5	1.7	2.7	6.8	<0.001	.99	.97
18:3n-3	0.25 (0.01)	−0.9	−1.7	−4.7	−3.5	−4.9	−9.7	<0.001	.97	.95
20:5n-3	1.03 (0.17)	−0.2	−1.0	−1.9	−2.0	−1.7	−5.2	0.004	.99	.99
22:5n-3	0.78 (0.04)	0.5	0.9	3.0	3.6	4.3	9.6	<0.001	.99	.98
22:6n-3	3.56 (0.22)	0.0	0.1	1.7	2.0	2.6	5.8	<0.001	.99	.98

[a] *Huynh-Feldt correction in repeated measures ANOVA (accounting for unequal variances of differences across groups).*

[b] *Mean Spearman rank correlation for bootstrap sample of n = 50.*

[c] *ICC, intraclass correlation coefficient.*

[d] *Only six individuals with values above detection level (0.5 mg/L), power too low for reliability and validity measurements.*

[e] *One of the six individuals had extreme high values (baseline value, 89 mg/L), analyses repeated with five individuals.*

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Data Supplement

Table S2.1 Variability of serum analytes and phospholipid-associated fatty acids in samples stored up to 28 h

	CV(%)	
	Within	Between
Serumanalytes		
CRP,[a] mg/L (n = 6)	-	-
CRP,[b] mg/L (n = 5)	-	-
Retinol, µmol/L	0.9	22.5
Ferritin, µg/L	1.6	102.3
Folate, nmol/L	7.7	61.8
Fatty acids in plasma phospholipids, % by weight of total fatty acids		
16:0	0.5	5.7
18:0	1.2	9.7
18:1n-9	1.0	11.8
20:3n-9	3.2	23.1
18:2n-6	2.4	12.2
20:3n-6	1.2	20.7
20:4n-6	0.1	15.4
22:4n-6	2.4	21.8
22:5n-6	1.4	39.1
18:3n-3	2.4	25.1
20:5n-3	1.0	72.1
22:5n-3	1.5	23.0
22:6n-3	1.2	26.6

[a] *Only six individuals with values above detection level (0.5 mg/L), power too low for reliability and validity measurements.*

[b] *One of the six individuals had extreme high values (baseline value, 89 mg/L), analyses repeated with five individuals.*

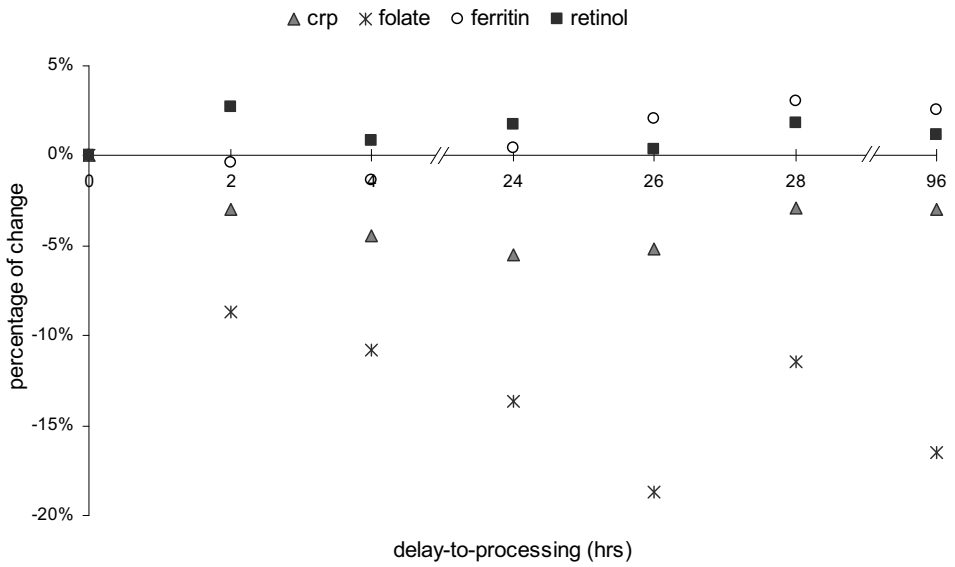

Figure S2.1 Mean percentage of change in concentration of serum analytes during storage of whole blood: C-reactive protein (n = 6), retinol (n = 20), ferritin (n = 20), and folate (n = 20).

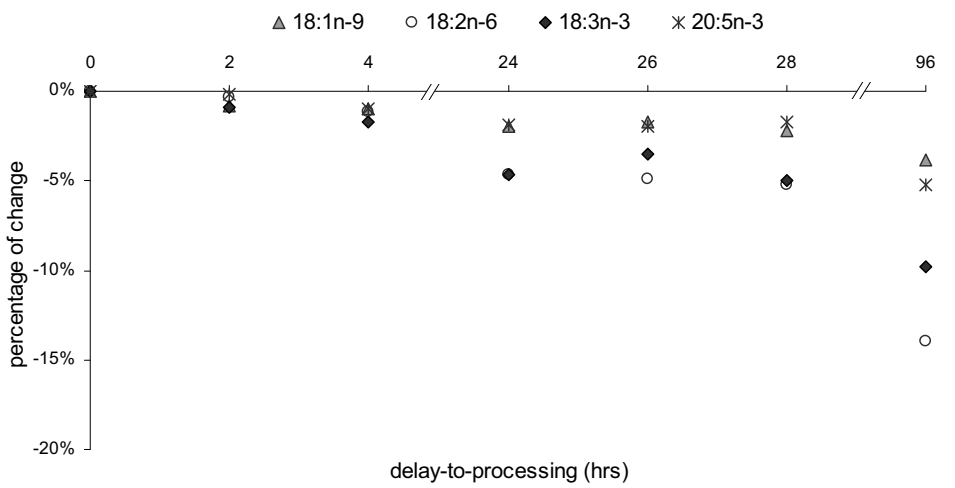

Figure S2.2 Mean percentage of change in concentration of plasma fatty acids in phospholipid fraction during storage of whole blood (n = 21): fatty acids showing a decrease in concentration.

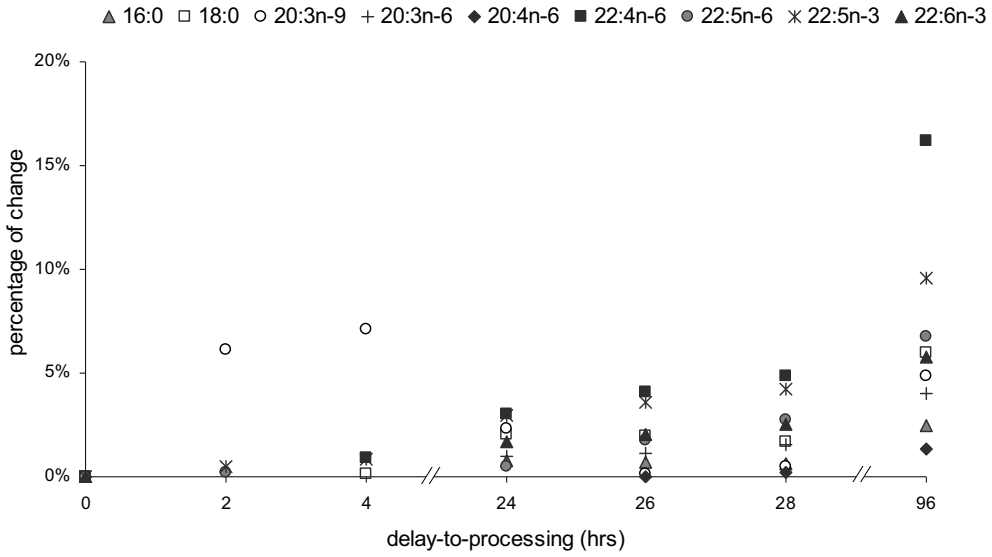

Figure S2.3 Mean percentage of change in concentration of plasma fatty acids in phospholipid fraction during storage of whole blood (n = 21): fatty acids showing an increase in concentration.

References

1. Key T, Oakes S, Davey G, et al. Stability of vitamins A, C, and E, carotenoids, lipids, and testosterone in whole blood stored at 4 degrees C for 6 and 24 hours before separation of serum and plasma. Cancer Epidemiol Biomarkers Prev 1996;5:811–4.
2. Hankinson SE, London SJ, Chute CG, et al. Effect of transport conditions on the stability of biochemical markers in blood. Clin Chem 1989;35:2313–6.
3. Aziz N, Fahey JL, Detels R, Butch AW. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. Clin Diagn Lab Immunol 2003;10:652–7.
4. Birgegard G. Serum ferritin: physiological and methodological studies. Clin Chim Acta 1980;103:277–85.
5. Giltay EJ, Geleijnse JM, Schouten EG, Katan MB, Kromhout D. High stability of markers of cardiovascular risk in blood samples. Clin Chem 2003;49:652–5.
6. Kubasik NP, Ricotta M, Hunter T, Sine HE. Effect of duration and temperature of storage on serum analyte stability: examination of 14 selected radioimmunoassay procedures. Clin Chem 1982;28:164–5.
7. Mejia LA, Arroyave G. Determination of vitamin A in blood. Some practical considerations on the time of collection of the specimens and the stability of the vitamin. Am J Clin Nutr 1983;37:147–51.
8. Chu SY, MacLeod J. Effect of three-day clot contact on results of common biochemical tests with serum. Clin Chem 1986;32:2100.
9. Zhang DJ, Elswick RK, Miller WG, Bailey JL. Effect of serum-clot contact time on clinical chemistry laboratory results. Clin Chem 1998;44:1325–33.
10. Ledue TB, Weiner DL, Sipe JD, Poulin SE, Collins MF, Rifai N. Analytical evaluation of particle-enhanced immunonephelometric assays for C-reactive protein, serum amyloid A and mannose-binding protein in human serum. Ann Clin Biochem 1998;35:745–53.
11. Owen WE, Roberts WL. Comparison of five automated serum and whole blood folate assays. Am J Clin Pathol 2003;120:121–6.
12. Talwar D, Ha TK, Cooney J, Brownlee C, O'Reilly DS. A routine method for the simultaneous measurement of retinol, alpha-tocopherol and five carotenoids in human plasma by reverse phase HPLC. Clin Chim Acta 1998;270:85–100.
13. Al MD, van Houwelingen AC, Kester AD, Hasaart TH, de Jong AE, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. Br J Nutr 1995;74:55–68.
14. Barreto-Lins MH, Campos FA, Azevedo MC, Flores H. A re-examination of the stability of retinol in blood and serum, and effects of a standardized meal. Clin Chem 1988;34:2308–10.
15. Lutz HU, Liu SC, Palek J. Release of spectrin-free vesicles from human erythrocytes during ATP depletion. I. Characterization of spectrin-free vesicles. J Cell Biol 1977;73:548–60.

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