SUPPLEMENTAL TABLES

Supplemental Table 1. Biomarker results

Biomarker	N	Mean	SD	Minimum	Maximum
CYC Baseline	143	-0.03	0.76	-7.16	1.92
CYC 16wk	114	0.22	1	-1.64	8.37
CYC 44wks	98	0.45	0.78	-1.4	2.87
ERRα Baseline	136	-0.14	1.52	-10.42	2.53
ERRα 16wks	106	-0.34	3.37	-18.85	17.5
ERRα 44wks	86	-1.11	3.84	-25.89	2.44
PGC-1a Baseline	130	0.02	2.32	-12.85	8.18
PGC-1a 16wks	92	-0.04	1.2	-5.08	5.03
PGC-1a 44wks	82	0.48	1.45	-2.06	6.53
PRDX3 Baseline	146	-0.05	0.9	-6.25	2.6
PRDX3 16wks	115	0.2	1.27	-1.72	10.22
PRDX3 44wks	100	0.37	0.96	-1.35	6.15
IL-6 Baseline	204	1.14	1.04	0.3	10.5
IL-6 16wks	196	0.99	0.76	0.2	8
IL-6 44wks	187	0.93	0.82	0.2	10
8OHDG Baseline	202	0.36	0.48	0.02	3.19
8OHDG 16wks	190	0.4	0.59	0.02	4.82
8OHDG 44wks	178	0.41	0.53	0.02	2.76

Data on biomarker results at each time point. The mean refers to the mean fractional change (FC) for leukocyte gene expression data (CYC, ERRα, PGC-1a, and PRDX3). Actual values are shown for plasma IL-6 (pg/ml) and for urine 8OHdG (ng/ml, normalized to creatine). N: Numbers of subjects with data for each biomarker at each time point. SD: standard deviation. For gene expression data, samples with cycle threshold (Ct) values greater than 36.68 were excluded as this fell within the range of no RNA control samples. Gene expression in these samples was considered undetectable, which could reflect either very low levels of expression or technical problems. A separate analysis of subjects with excluded samples was conducted to assess for the possibility of bias introduced from excluding these samples (see results). Samples also are missing due to subject drop outs or failure to collect samples. For IL-6 and 8OHdG, samples that thawed during shipping also were excluded.

Supplemental Table 2. Gene expression assays

Gene Symbol	Gene Name	RefSeq	Amplicon Length
RNA18S5	RNA; 18S ribosomal 5 (HOUSEKEEPING)	NR_003286.2	90
HPRT1	hypoxanthine phosphoribosyltransferase 1 (HOUSEKEEPING)	NM_000194.2	100
PPARGC1A (PGC-1α)	peroxisome proliferator-activated receptor gamma; coactivator 1 alpha	NM_013261.3	83
CYCS	cytochrome c; somatic	NM_018947.5	152
ESRRA (ERRα)	estrogen-related receptor alpha	NM_004451.3	146
PRDX3	peroxiredoxin 3	NM_006793.3;NM_014098.3	146

Gene expression assays used for qRT-PCR. Samples were considered suitable for qRT-PCR if there was at least 400ng of total RNA at a purity level of at least 1.6 using the optic density (OD) ratio of 260:280nm. Total RNA was reverse transcribed using the TaqMan® High Capacity cDNA Synthesis Kit (Applied Biosystems). Amplifications were performed in triplicate on a validated Applied Biosystems 7900HT real-time thermal cycler. Fold change calculations were performed using a standard delta-delta cycle threshold (Ct) method subtracting an average Ct of housekeeping gene expression (HPRT1 and RN18S1) from the Ct for the target gene at each time point, and then calculating the fold-change for the delta-delta Ct. Levels of expression at baseline are expressed as the fold-change relative to the mean delta Ct for all baseline samples. For results at 16 and 44 weeks, the fractional changes rather than fold changes from BL are shown, and were calculated by subtracting 1 for all fold-changes above 1, and adding one for all fold-changes below -1. This results in data being continuous across 0 and avoids a gap from -1 to 1 that exists for fold-change values.

Supplemental table 3: Correlations of biomarker results at different time points

	Baseline versus	
	16 weeks	**44 weeks**
PGC-1α	0.66 (p<0.0001)	0.77 (p<0.0001)
CYC	0.16 (p=0.10)	0.26 (p=0.01)
ERRα	0.33 (p<0.001)	0.20 (p=0.06)
PRDX3	0.17 (p=0.06)	0.15 (p=0.15)
IL-6	0.71 (p<0.0001)	0. 71 (p<0.0001)
8OHdG	0.57 (p<0.0001)	0.79 (p<0.0001)

To assess the reliability of the data, we analyzed each biomarker for a correlation between subjects' baseline results with their results at 16 and 44 weeks. These correlations suggest a degree of stability over time in individuals in levels of these biomarkers. On the other hand, the weaker correlation for PGC-1α target genes across different time points does not necessarily imply technical problems with the measurements as there likely is a degree of true biological variation in gene expression levels at different time points in the same subjects. The parentheses include p-values for H_0: rho=0.

Supplemental table 4: Intraclass Correlations (ICC)

Biomarker	Batch	ICC
CYC		0.79
ESRR		0.63
PGC-1alpha		0.56
PRDX		0.87
8OHDG	**Batch=1**	0.98
8OHDG	**Batch=2**	0.99
8OHDG	**Batch=3**	0.88
IL-6		0.48

ICCs are shown for each of the biomarkers based on data from analyses of multiple aliquots from the same subject and time points for each biomarker (n = 2 to 5 replicates per subject; n = 2 - 9 subjects per biomarker).

Supplemental Figure

Association of storage time (in days) with measured IL-6 levels (pg/ml). r = 0.095; p = 0.019.