

Toxicological assessment of arsenic containing phosphatidylcholines in HepG2 cells

Hannah Finke,^a Viktoria K. Wandt,^{a,b} Franziska Ebert,^a Nikolaus Guttenberger,^c Ronald A. Glabonjat,^c Michael Stiboller,^{a,c} Kevin A. Francesconi,^c Georg Raber^c and Tanja Schwerdtle^{a,b,*}

^aDepartment of Food Chemistry, Institute of Nutritional Science, University of Potsdam, Nuthetal, 14558, Germany

^bTraceAge-DFG Research Unit on Interactions of Essential Trace Elements in Healthy and Diseased Elderly, Potsdam-Berlin-Jena, Germany

^cInstitute of Chemistry – Analytical Chemistry, NAWI Graz, University of Graz, Universitätsplatz 1, 8010 Graz, Austria

*corresponding author

Supplemental Material

Material and Methods

Stability PC 733

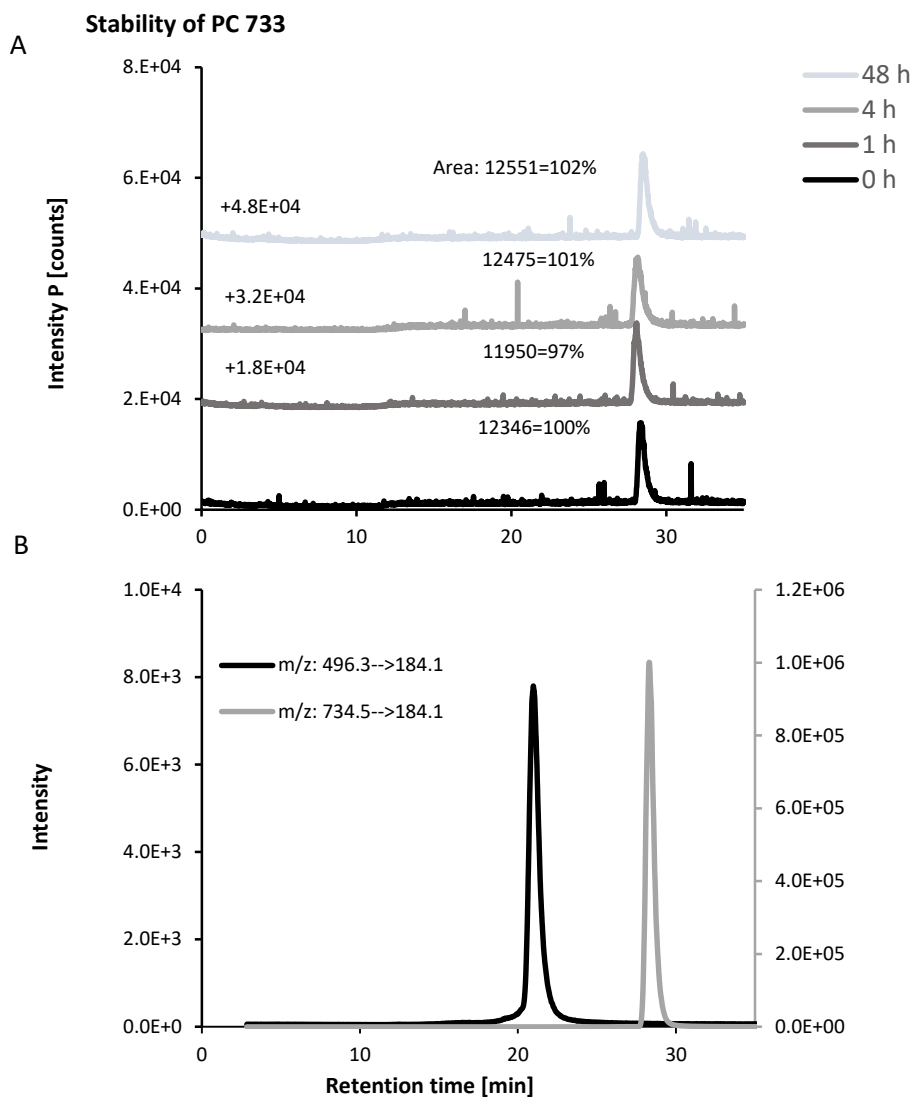
Stability of PC 733 was studied by incubation of cell media with 10 µM PC 733 and taking aliquots after 0, 1, 4, and 48 h. Afterwards media was directly injected to HPLC (1200 Infinity, Agilent) and phosphorous was detected with ICPMS (Agilent). A solution containing 1 µg/L Rh was continuously introduced post column for normalization. Standards of PC 733 and 1-Palmitoyl2-hydroxy-sn-glycero-3-PC (*lyso*-PC 495) (10 µM in water) were also measured *via* ESIMS (6495, Agilent). Parameters are displayed in supplemental Table S1, results are found in Supplemental Figure S1. Relative quantification was performed with Origin Pro (OriginLab, Northampton, MA, USA) after normalization to Rh standard (Supplemental Table S2).

Supplemental Table S1: Parameters of HPLC-ICPMS/ESIMS measurements of cell media incubated with 50 µM PC 733

	MEDIA SAMPLES	STANDARDS
HPLC		
COLUMN	YMC PFP Triart, 250*3 mm, 3 µM	
COLUMN OVEN [°C]	40	
FLOW [ML/MIN]	0.25	
SOLVENTS	A: 10 mM NH ₄ Ac in H ₂ O; B: 10 mM NH ₄ Ac in MeOH	
GRADIENT	0-3 min: 20-80% B 3-20 min: 80-100% B 20-35 min: 100% B 25-36 min: 20% B Postrun: 5 min	
INJECTION VOLUME [µL]	20	1 (<i>lyso</i> -PC 495), 20 (PC 733)
SPLIT	20 %: ICP-MS/MS 80 %: Waste	20 %: ICP-MS/MS 80 %: ESI-MS/MS
ICPMS		
FORWARD POWER [W]	1550	
PLASMA GAS [L/MIN]	15	
OPTION GAS FLOW (20 % V/V OXYGEN) [L/MIN]	0.2	
NEBULIZER	MicroMist	
NEBULIZER FLOW [L/MIN]	0.65	

S/C TEMP [°C]	2
SAMPLE DEPTH [MM]	10
REACTION GAS FLOW OXYGEN [L/MIN]	0.3
INTERNAL STANDARD	Rh: 103→103
ANALYTE PHOSPHOROUS	P: 31→47
ESIMS	
MODE	- ESI+
DRYING GAS TEMPERATURE [°C]	- 14
NEBULIZER [PSI]	- 20
SHEATH GAS TEMPERATURE [°C]	- 250
SHEATH GAS FLOW [L/MIN]	- 11
CAPILLARY [V]	- 3000
NOZZLE [V]	- 1500
ION FUNNEL: RF HIGH [V]/ RF LOW [V]	- 200/100
MASS TRANSITIONS (MULTIPLE REACTION MONITORING)	
PC 733 [M+H] ⁺	- 734.56 → 184.1
LYSO-PC 495 [M+H] ⁺	- 496.3→184.1

Results



Supplemental Figure S1: A) HPLC-ICPMS chromatogram of cell media incubated with PC 733 after different incubation times (0, 1, 4, 48 h). B) HPLC-ESIMS/MS chromatogram of lyso-oxo-PC 495 (10 μ M, 28.9 min) and PC 733 (50 μ M, 21.5 min) standards. Retention time adjusted 2.85 min after split.

Stock solution and media without cells incubated with oxo-AsPC 839 and thioxo-AsPC 855, quantitative Data

Supplemental Table S2: Quantification of identified As species in cell media without cells incubated with 50 μ M oxo-AsPC 839 (A) or thioxo-AsPC 855 (B) for 0, 1, 4, and 48 h in μ g As/L and % of total As measured via HPLC-ICPMS. Identification in parallel to analytes of media with cells depicted in Table 1 (Main Paper) (exact masses and $\Delta m/m$ measured via HPLC-HR-ESIMS) and Table S4 (fragments and $\Delta m/m$ measured via HPLC-HR-ESIMS). Compounds were identified in parallel to media with cells, see Table S5.

A	Front/ oxo-AsFA 250	oxo- AsFA 278	oxo- AsFA 306	lyso-oxo- AsPC 601 (1)	lyso-oxo- AsPC 601 (2)	oxo-AsFA 348	oxo-AsFA 362	oxo-AsPC 839	thioxo-AsPC 855
Retention time [min]	2.9	4.3	6.9	8.4	8.7	9.9	10.7	14.5	16.6
	μ g As/L (% of total As)								
Stock solution 5mM (dilution 1: 500)	<0.5	0.9 (0.05)	2.5 (0.14)	597.2 (32)	239 (13)	<0.5	46.9 (3)	929 (52)	<0.5
Media without cells, 50 μ M oxo-AsPC 839, 0 h	0.5 (0.02)	<0.5	1.5 (0.07)	122 (5.4)	1202 (53)	16.7 (0.7)	468 (21)	452 (20)	<0.5
Media without cells, 50 μ M oxo-AsPC 839, 1 h	<0.5	<0.5	1.5 (0.06)	115 (4.7)	1135 (46)	23.7 (1.0)	712 (29)	483 (20)	1.6 (0.06)
Media without cells, 50 μ M oxo-AsPC 839, 4 h	<0.5	<0.5	1.3 (0.05)	112 (4.3)	1183 (45)	27.9 (1.1)	1012 (39)	267 (10)	2.4 (0.09)
Media without cells, 50 μ M oxo-AsPC 839, 48 h	0.8 (0.02)	0.7 (0.02)	1.9 (0.05)	140 (3.9)	1502 (41)	42.5 (1.2)	1895 (52)	33.5 (1)	6.1 (0.2)

B	lyso- oxo-AsPC 601 (1)	lyso- oxo-AsPC 601 (2)	oxo-AsFA 362	thioxo- AsFA 350	oxo- AsPC 839	thioxo- AsFA 378	thioxo- AsPC 855
Retention time [min]	8.4	8.7	10.7	13.9	14.3	14.9	16.6
	μ g As/L (% of total As)						
Stock solution 5mM (dilution1: 500)	2.6 (0.2)	1.7 (0.2)	1.7 (0.2)	74.4 (7)	81.1 (8)	<0.5	902.5 (85)
Media without cells, 50 μ M thioxo-AsPC 855, 0 h	1.2 (0.1)	10.2 (0.9)	11.1 (1)	3.7 (0.4)	136 (11)	26 (2)	1005 (84)
Media without cells, 50 μ M thioxo-AsPC 855, 1 h	1.1 (0.1)	10.1 (0.5)	21.9 (1)	3.8 (0.2)	153 (8)	77 (4)	1674 (86)
Media without cells, 50 μ M thioxo-AsPC 855, 4 h	1.2 (0.1)	12.8 (1.0)	39.1 (3)	3.7 (0.3)	117 (8)	201 (14)	1066 (73)
Media without cells, 50 μ M thioxo- AsPC 855, 48 h	6 (0.2)	54.2 (1.6)	234 (7)	<0.5	100 (3)	2127 (64)	756 (23)

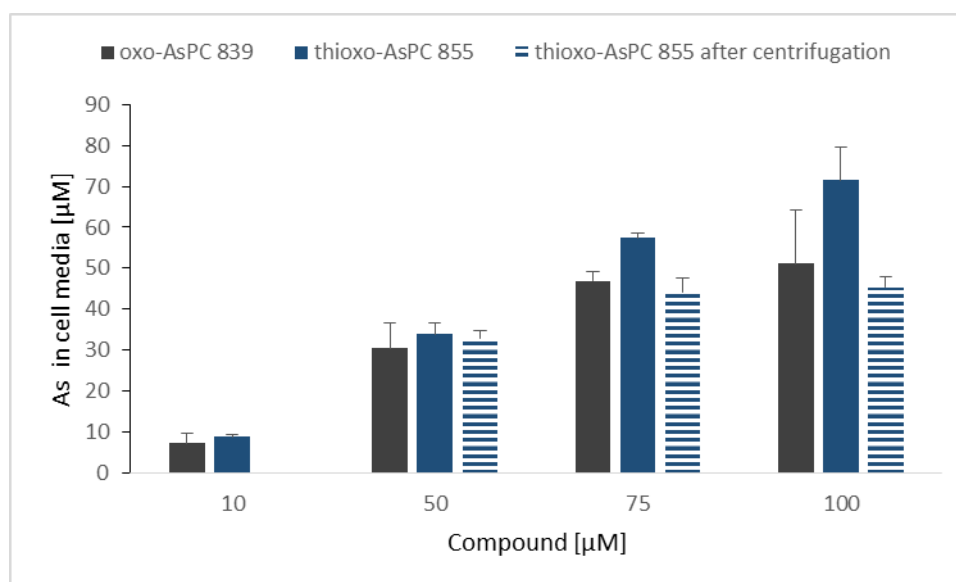
Bioavailability, numerical data

Supplemental Table S3: Cellular As (n=3, mean $\pm$ SD) measured *via* ICP-MS/MS after microwave-assisted acid digestion of HepG2 cells incubated with different concentrations of oxo-AsPC 839 and thioxo-AsPC 855. Data on cellular As after oxo-AsFA 362 incubation for 48 h in HepG2 cells for comparison.¹ -: not investigated. We observed a precipitate for 75 μ M and 100 μ M thioxo-AsPC 855.

Compound \ Incubated concentration [μ M]	Cellular As [μ M]			
	10	50	75	100
AsPC 839	122 $\pm$ 40	1112 $\pm$ 146	2038 $\pm$ 375	4485 $\pm$ 1019
thioxo-AsPC 855	32 $\pm$ 15	293 $\pm$ 115	431 $\pm$ 90	405 $\pm$ 162
oxo-AsFA 362	157 $\pm$ 18	1909 $\pm$ 191	-	7552 $\pm$ 310

- 1 S. Meyer, G. Raber, F. Ebert, L. Leffers, S. M. Müller, M. S. Taleshi, K. A. Francesconi and T. Schwerdtle, *Toxicol. Res.*, 2015, **4**, 1289–1296.

Total As in cell media, effect of centrifugation of media total As



Supplemental Figure S2: Total As (n=3, mean $\pm$ SD) in cell media incubated with oxo-AsPC 839 and thioxo-AsPC 855 after microwave-assisted acid digestion. Media of thioxo-AsPC 855 was centrifuged additionally 10 min at 16060xg.

Fragmentation of As-Species of incubation cell-media of HepG2 cells after incubation with oxoAsPC 839 and cell media and pellets of HepG2 cells incubated with thioxo-AsPC 855.

	Compound	Ret. time [min]	As [ng]		Experim. mass [u] [M+H] ⁺	Molecular Formula	$\Delta m/m$ [ppm]	MS/MS frag.	$\Delta m/m$ [ppm]	MS/MS frag.	$\Delta m/m$ [ppm]	MS/MS frag.	$\Delta m/m$ [ppm]
			pellet 1 pellet 2	medium 1 (48h) medium 2 (48h)									
Incubation oxo-AsPC 839	Front/ oxo-AsFA 250	2.88		270 315	251.0619	C ₉ H ₁₉ AsO ₃	-1.5	C ₂ H ₆ As	3.0	C ₂ H ₆ OAs	0.2		
	oxo-AsFA278	4.32		66.9 77.1	279.0932	C ₁₁ H ₂₃ AsO ₃	-1.5	C ₂ H ₆ As	2.6	C ₂ H ₆ OAs	3.8		
	oxo-AsFA 306	6.87		22.8 25.2	307.1246	C ₁₃ H ₂₇ AsO ₃	-1	C ₂ H ₆ As	4.2	C ₂ H ₆ OAs	1.1		
	lyso-oxo- AsPC 601 (1)	8.40		324 324	[M+2H] ²⁺ ; 301.6425	C ₂₅ H ₅₃ AsNO ₈ P	-3.4	C ₁₇ H ₃₆ O ₃ As	0.2	C ₂ H ₆ As	5.2	C ₅ H ₁₅ O ₄ NP	0.5
	lyso-oxo- AsPC 601 (2)	8.67		2907 2892									
	oxo-AsFA 334	9.06		Nq.	335.1555	C ₁₅ H ₃₁ AsO ₃	-1.9	C ₂ H ₆ As	4.3	C ₂ H ₆ OAs	1.4		
	oxo-AsFA 348	9.90		63.6 58.8	349.1712	C ₁₆ H ₃₃ AsO ₃	-1.9	C ₂ H ₆ As	2.5	C ₂ H ₆ OAs	0.3		
	oxo-AsFA362	10.65		3780 3681	363.1861	C ₁₇ H ₃₅ AsO ₃	-3.8	C ₂ H ₆ As	3.6	C ₂ H ₆ OAs	2.0		
	oxo-AsPC 839	14.24		36.0 26.4	[M+2H] ²⁺ ; 420.7572	C ₄₁ H ₈₉ AsNO ₉ P	-2.7	C ₁₇ H ₃₆ O ₃ As	0.3	C ₂ H ₆ OAs	3.3	C ₅ H ₁₅ O ₄ NP	0.9
	thioxo-AsPC 855	16.55		17.4 18.6	Identified by retention time								
Incubation thioxo-AsPC 855	front		0.4 1.6	87.3 85.2									
	oxo-AsFA 220	3.63	6.8 7.6	135 134	221.0148	C ₇ H ₁₃ AsO ₃	-2.5	C ₂ H ₆ As	3.1	C ₂ H ₆ OAs	1.6		
	thioxo- AsFA238	5.13	-	117 124	239.0079	C ₇ H ₁₅ AsO ₂ S	-1.0	C ₂ H ₆ As	3.8	C ₂ H ₆ AsS	2.2		
	lyso-oxo- AsPC 601 (1)	8.4	-	-	[M+2H] ²⁺ ; 301.6431	C ₂₅ H ₅₃ AsNO ₈ P	-1.5	C ₁₇ H ₃₆ O ₃ As	-2.2	C ₂ H ₆ As	2.9	C ₅ H ₁₅ O ₄ NP	0.2
	lyso-oxo- AsPC 601 (2)	8.71	0.6 0.5	15.3 12.3									
	thioxo-AsFA 294	10.49	-	60.6 69.3	295.0709	C ₁₁ H ₂₃ AsO ₂ S	-1.5	C ₂ H ₆ As	3.6	C ₂ H ₆ AsS	-0.4		
	oxo-AsFA 362	10.71	17.3 10.1	273 201	363.1863	C ₁₇ H ₃₅ AsO ₃	-3.4	C ₂ H ₆ As	4.9	C ₂ H ₆ OAs	2.4		
	thioxo- AsFA322	12.48	Nq. 0.4	95.7 84.6	323.1012	C ₁₃ H ₂₇ AsO ₂ S	-2.6	C ₂ H ₆ As	3.2	C ₂ H ₆ AsS	0.1		
	thioxo-AsFA 350	13.86	0.6 1.0	34.8 41.7	351.1325	C ₁₅ H ₃₁ AsO ₂ S	-2.5	C ₂ H ₆ As	3.4	C ₂ H ₆ AsS	0.1		
	oxo-AsPC 839	14.26	6.2 3.7	96.6 100.8	[M+2H] ²⁺ ; 420.7571	C ₄₁ H ₈₉ AsNO ₉ P	-2.9	C ₁₇ H ₃₆ O ₃ As	-0.2	C ₂ H ₆ As	3.1	C ₅ H ₁₅ O ₄ NP	-0.1
	thioxo-AsFA 378	14.89	28.5 19.1	2715 2499	379.1635	C ₁₇ H ₃₅ AsO ₂ S	-2.9	C ₂ H ₆ As	3.6	C ₂ H ₆ AsS	-0.4		
	thioxo-AsPC 855	16.55	10.4 5.4	351 402	Identified by retention time			C ₂ H ₆ As	0.5			C ₅ H ₁₅ O ₄ NP	0.1

Table S4:

Quantitative As (ng) and identified experimental masses of [M+H]⁺ determined by HPLC-ICPMS and HPLC-HR-ESIMS of As species, respectively. Section1: HepG2 cell media incubated with 50 μ M oxo-AsPC 839 for 48 h; section 2: HepG2 cell pellets and cell media incubated with 50 μ M thioxo-AsPC 855 for 48 h. -: <0.5 μ g/L; Nq. not quantified due to lack of peak separation. Oxo-AsFA 250 could not be separated from other early eluting arsenicals. Quantitative amounts of two separate pellets and media (3mL) are shown individually. Suggested structures are shown in Chart 1 in main paper.

Quantitative data cell-media after different time points

Table S5: Quantification of identified As species in cell media incubated with 50 μM oxo-AsPC 839 (A) or thioxo-AsPC 855 (B) for 0, 1, 4, and 48 h. Exact masses measured via HPLC-HR-ESIMS, concentrations in $\mu\text{g As/L}$ and % of total As measured via HPLC-ICPMS. Fragments and $\Delta m/m$ are depicted in Table 1 of the Main Paper. Thioxo-AsPC 855 was assigned due to retention time and external standard, $n=2$, shown separately.

A	Front/oxo-AsFA 250	Oxo-AsFA 278	Oxo-AsFA 306	lyso-oxo-AsPC 601 (1)	lyso-oxo-AsPC 601 (2)	oxo-AsFA 348	oxoAsFA 362	oxo-AsPC 839	thioxo-AsPC 855
Retention time [min]	2.9	4.3	6.9	8.4	8.7	9.9	10.7	14.2	16.6
	$\mu\text{g As/L}$ (% of total As)								
Media with cells, 50 μM oxo-AsPC 839, 0 h	<0.5	<0.5	1.3 (0.07) 0.9 (0.05)	111 (5.7) 94 (5.7)	995 (50) 841 (51)	15.0 (0.77) 11.5 (0.70)	449 (23) 366 (22)	388 (20) 332 (20)	<0.5 0.8 (0.05)
Media with cells, 50 μM oxo-AsPC 839, 1 h	0.5 (0.02) <0.5	<0.5	1.5 (0.06) 1.6 (0.07)	113 (4.5) 110 (4.6)	1119 (46) 1105 (46)	20.3 (0.84) 21.6 (0.91)	715 (30) 717 (30)	449 (19) 443 (19)	1.2 (0.05) 0.8 (0.03)
Media with cells, 50 μM oxo-AsPC 839, 4 h	1.7 (0.07) 1.6 (0.06)	<0.5	1.7 (0.07) 1.7 (0.07)	119 (4.7) 116 (4.5)	1169 (46) 1187 (46)	24.5 (0.97) 22.5 (0.89)	1025 (40) 1029 (40)	203 (8) 192 (8)	1.4 (0.06) 1.2 (0.05)
Media with cells, 50 μM oxo-AsPC 839, 48 h	89.6 (3.6) 105 (4.3)	22.3 (0.9) 25.7 (1.1)	7.6 (0.30) 8.4 (0.34)	108 (4.3) 108 (4.3)	986 (39) 963 (39)	21.2 (0.85) 19.6 (0.79)	1260 (51) 1227 (50)	12 (0.5) 8.8 (0.4)	5.8 (0.23) 6.2 (0.25)

B	Front	oxo-AsFA 220	thioxo-AsFA 238	lyso-oxo-AsPC 601 (1)	lyso-oxo-AsPC 601 (2)	thioxo-AsFA 294	oxo-AsFA 362	thioxo-AsFA 322	thioxo-AsFA 350	oxo-AsPC 839	thioxo-AsFA 378	thioxo-AsPC 855
Retention time [min]	3.6	5.1	8.4	8.7	10.5	10.7	12.5	13.9	14.3	14.9	16.6	
	μg As/L (% of total As)											
Media with cells, 50 μM thioxo-AsPC 855, 0 h	<0.5	<0.5	<0.5	0.6 (0.04) <0.5	6.4 (0.4) 3.1 (0.43)	<0.5	14.1 (0.9) 7.0 (1.0)	<0.5	3.7 (0.2) 3.1 (0.4)	167 (11) 97 (13)	28.7 (1.9) 13.0 (1.8)	1300 (86) 609 (83)
Media with cells, 50 μM thioxo-AsPC 855, 1 h	<0.5	<0.5	<0.5	0.5 (0.04) <0.5	5.1 (0.4) 4.8 (0.3)	<0.5	16.8 (1.2) 15.5 (1.1)	<0.5 (0.05)	2.7 (0.2) 2.4 (0.2)	126 (9) 122 (9)	62.4 (4.5) 67.8 (4.7)	1171 (85) 1224 (85)
Media with cells, 50 μM thioxo-AsPC 855, 4 h	<0.5	<0.5	<0.5	0.5 (0.04) 0.6 (0.05)	5.2 (0.4) 5.5 (0.5)	<0.5	29.2 (2.1) 25.2 (2.3)	1.7 (0.1) 2.8 (0.3)	3.1 (0.2) 1.6 (0.2)	113 (8) 93 (9)	169 (12) 162 (15)	1093 (77) 782 (73)
Media with cells, 50 μM thioxo-AsPC 855, 48 h	29.1 (2.2) 28.4 (2.3)	44.9 (3.4) 44.5 (3.6)	38.9 (2.9) 41.3 (3.3)	<0.5	5.1 (0.4) 4.1 (0.3)	20.2 (1.5) 23.1 (1.8)	90.9 (6.9) 67.1 (5.4)	31.9 (2.4) 28.2 (2.3)	11.6 (0.9) 13.9 (1.1)	32.2 (2.5) 33.6 (2.7)	905 (68) 833 (67)	117 (9) 134 (11)