

**Table S1.** Associations of insomnia severity and relevant covariates with levels of serum biomarkers.

Biomarker level	Unit of measurement	n	Effect	b	b		β	p	VIF
					95% CI				
					LL	UL			
Immunologic									
α1-antitrypsin	mg/L	175							
			Insomnia severity	-.717	-7.121	5.686	-.020	.825	1.402
			Depression severity	.755	-2.659	4.170	.040	.663	1.433
			Antidepressant use	-3.457	-51.358	44.444	-.016	.887	2.125
			Gender	18.049	-8.856	44.954	.103	.187	1.061
			Age	-.964	-2.195	.267	-.136	.124	1.347
			Cohort of origin	-15.318	-51.873	21.237	-.091	.409	2.128
Calprotectin <sup>a</sup>	μg/mL	178							
			Insomnia severity	.021	-.020	.062	.091	.311	1.402
			Depression severity	-.002	-.025	.020	-.020	.825	1.441
			Antidepressant use	.232	-.075	.539	.165	.138	2.142
			Gender	.014	-.159	.187	.013	.871	1.064
			Age	.001	-.007	.009	.031	.726	1.349
			Cohort of origin	.115	-.121	.351	.106	.338	2.154
cAMP	pmol/mL	176							
			Insomnia severity	-.290	-1.223	.644	-.050	.541	1.400
			Depression severity	-.061	-.569	.447	-.020	.812	1.427
			Antidepressant use	1.464	-5.547	8.475	.042	.681	2.139
			Gender	-11.242	-15.227	-7.257	-.397	.000	1.067
			Age	.119	-.065	.303	.102	.204	1.340
			Cohort of origin	.189	-5.208	5.585	.007	.945	2.149
Endothelin-1 <sup>a</sup>	pg/mL	177							
			Insomnia severity	-.019	-.037	-.002	-.183	.033	1.404
			Depression severity	.009	-.001	.019	.161	.065	1.446
			Antidepressant use	-.013	-.146	.120	-.021	.846	2.140
			Gender	-.065	-.140	.010	-.126	.091	1.063
			Age	.007	.003	.010	.324	.000	1.348
			Cohort of origin	.104	.002	.207	.212	.046	2.149
Myeloperoxidase <sup>a</sup>	ng/mL	178							
			Insomnia severity	.010	-.034	.055	.041	.650	1.402
			Depression severity	.000	-.024	.024	.000	.996	1.441
			Antidepressant use	.286	-.051	.622	.186	.095	2.142
			Gender	-.006	-.195	.184	-.005	.953	1.064
			Age	-.004	-.012	.005	-.074	.400	1.349
			Cohort of origin	.089	-.170	.348	.075	.499	2.154
Resistin <sup>a</sup>	ng/mL	178							
			Insomnia severity	-.016	-.045	.013	-.096	.266	1.402
			Depression severity	-.007	-.023	.009	-.077	.377	1.441
			Antidepressant use	.042	-.175	.259	.040	.703	2.142
			Gender	.073	-.049	.196	.088	.238	1.064

Thromboxane <sup>a</sup>	ng/mL	178	Age	-.009	-.015	-.004	-.273	.001	1.349
			Cohort of origin	-.009	-.176	.158	-.012	.912	2.154
			Insomnia severity	-.030	-.109	.049	-.067	.448	1.402
			Depression severity	.001	-.042	.043	.002	.978	1.441
			Antidepressant use	.485	-.108	1.078	.176	.109	2.142
			Gender	-.254	-.588	.080	-.116	.136	1.064
			Age	-.012	-.027	.003	-.134	.124	1.349
			Cohort of origin	.349	-.107	.805	.166	.133	2.154
			Insomnia severity	-.018	-.038	.001	-.166	.062	1.406
			Depression severity	.008	-.002	.019	.141	.116	1.437
TNFαR2 <sup>a</sup>	ng/mL	177	Antidepressant use	.027	-.119	.172	.039	.719	2.140
			Gender	-.074	-.156	.008	-.136	.078	1.060
			Age	.002	-.002	.006	.079	.362	1.346
			Cohort of origin	-.004	-.116	.108	-.008	.939	2.150
			Insomnia severity	-.123	-.433	.187	-.070	.435	1.418
			Depression severity	.223	.065	.380	.238	.006	1.449
			Antidepressant use	.214	-2.096	2.524	.020	.855	2.141
			Gender	-.854	-2.022	.314	-.099	.151	1.066
			Age	.118	.062	.173	.333	.000	1.384
			Cohort of origin	-.555	-2.378	1.268	-.068	.548	2.161
Zonulin <sup>b</sup>	ng/mL	176	Insomnia severity	-.148	-.725	.430	-.044	.615	1.402
			Depression severity	.029	-.283	.340	.016	.856	1.441
			Antidepressant use	.828	-3.509	5.166	.041	.707	2.142
			Gender	-.161	-2.606	2.285	-.010	.897	1.064
			Age	.086	-.027	.198	.130	.134	1.349
			Cohort of origin	-1.787	-5.123	1.549	-.115	.292	2.154
			Insomnia severity	-.225	-.736	.286	-.077	.386	1.402
			Depression severity	.045	-.230	.321	.029	.745	1.441
			Antidepressant use	-.429	-4.266	3.408	-.024	.826	2.142
			Gender	.343	-1.821	2.506	.024	.755	1.064
Neurotrophic BDNF	ng/mL	178	Age	.081	-.018	.180	.140	.109	1.349
			Cohort of origin	-1.976	-4.927	.976	-.145	.188	2.154
			Insomnia severity	-.225	-.736	.286	-.077	.386	1.402
			Depression severity	.045	-.230	.321	.029	.745	1.441
			Antidepressant use	-.429	-4.266	3.408	-.024	.826	2.142
			Gender	.343	-1.821	2.506	.024	.755	1.064
			Age	.081	-.018	.180	.140	.109	1.349
			Cohort of origin	-1.976	-4.927	.976	-.145	.188	2.154
			Insomnia severity	-.225	-.736	.286	-.077	.386	1.402
			Depression severity	.045	-.230	.321	.029	.745	1.441
BDNF free	ng/mL	178	Antidepressant use	-.429	-4.266	3.408	-.024	.826	2.142
			Gender	.343	-1.821	2.506	.024	.755	1.064
			Age	.081	-.018	.180	.140	.109	1.349
			Cohort of origin	-1.976	-4.927	.976	-.145	.188	2.154
			Insomnia severity	-.225	-.736	.286	-.077	.386	1.402
			Depression severity	.045	-.230	.321	.029	.745	1.441
			Antidepressant use	-.429	-4.266	3.408	-.024	.826	2.142
			Gender	.343	-1.821	2.506	.024	.755	1.064
			Age	.081	-.018	.180	.140	.109	1.349
			Cohort of origin	-1.976	-4.927	.976	-.145	.188	2.154
BDNF total	ng/mL	178	Insomnia severity	-.106	-.617	.406	-.036	.684	1.402
			Depression severity	.068	-.208	.343	.043	.629	1.441
			Antidepressant use	.380	-3.458	4.218	.021	.845	2.142
			Gender	.038	-2.126	2.202	.003	.972	1.064
			Age	.076	-.024	.175	.131	.134	1.349
			Cohort of origin	-1.420	-4.372	1.533	-.104	.344	2.154
			Insomnia severity	-.106	-.617	.406	-.036	.684	1.402
			Depression severity	.068	-.208	.343	.043	.629	1.441
			Antidepressant use	.380	-3.458	4.218	.021	.845	2.142
			Gender	.038	-2.126	2.202	.003	.972	1.064
EGF	pg/mL	177	Age	.076	-.024	.175	.131	.134	1.349
			Cohort of origin	-1.420	-4.372	1.533	-.104	.344	2.154
			Insomnia severity	-.106	-.617	.406	-.036	.684	1.402
			Depression severity	.068	-.208	.343	.043	.629	1.441
			Antidepressant use	.380	-3.458	4.218	.021	.845	2.142
			Gender	.038	-2.126	2.202	.003	.972	1.064
			Age	.076	-.024	.175	.131	.134	1.349
			Cohort of origin	-1.420	-4.372	1.533	-.104	.344	2.154
			Insomnia severity	-.106	-.617	.406	-.036	.684	1.402
			Depression severity	.068	-.208	.343	.043	.629	1.441

Neuropeptide	Substance P <sup>a</sup>	pg/mL	175	Insomnia severity	-5.792	-24.025	12.441	-.056	.531	1.424
				Depression severity	4.173	-5.637	13.984	.076	.402	1.468
				Antidepressant use	77.142	-58.631	212.914	.123	.264	2.180
				Gender	-36.102	-111.968	39.764	-.072	.349	1.060
				Age	-2.582	-6.063	.899	-.126	.145	1.340
				Cohort of origin	119.375	14.808	223.943	.247	.026	2.179
Neuroendocrine	Cortisol <sup>b</sup>	µg/dL	176	Insomnia severity	-.009	-.031	.013	-.071	.434	1.405
				Depression severity	.006	-.006	.018	.084	.361	1.435
				Antidepressant use	-.048	-.213	.117	-.064	.566	2.134
				Gender	.019	-.075	.114	.032	.686	1.082
				Age	.000	-.005	.004	-.020	.827	1.355
				Cohort of origin	-.014	-.141	.113	-.025	.826	2.142
Metabolic	Acetyl-L-carnitine <sup>a</sup>	ng/mL	178	Insomnia severity	.056	-.404	.517	.019	.809	1.405
				Depression severity	-.108	-.357	.141	-.069	.392	1.431
				Antidepressant use	-2.017	-5.359	1.324	-.114	.235	2.139
				Gender	.144	-1.804	2.093	.010	.884	1.061
				Age	-.107	-.202	-.013	-.183	.026	1.349
				Cohort of origin	3.247	.564	5.930	.238	.018	2.147
	Apolipoprotein A1	mg/mL	177	Insomnia severity	.001	-.021	.023	.006	.942	1.399
				Depression severity	-.002	-.014	.010	-.027	.764	1.441
				Antidepressant use	-.071	-.235	.094	-.092	.398	2.140
				Gender	.145	.052	.238	.236	.002	1.064
				Age	.002	-.003	.006	.060	.486	1.347
				Cohort of origin	-.071	-.197	.056	-.120	.272	2.150
	Leptin <sup>a</sup>	ng/mL	178	Insomnia severity	-.020	-.083	.044	-.043	.539	1.402
				Depression severity	.035	.001	.069	.145	.043	1.441
				Antidepressant use	.525	.049	1.001	.189	.031	2.142
				Gender	1.333	1.065	1.601	.600	.000	1.064
				Age	.016	.004	.029	.179	.010	1.349
				Cohort of origin	.298	-.068	.664	.140	.110	2.154
	Prolactin <sup>a</sup>	µIU/mL	178	Insomnia severity	.009	-.030	.048	.036	.653	1.402

Depression severity	.001	-.020	.022	.007	.930	1.441
Antidepressant use	.311	.019	.604	.210	.037	2.142
Gender	.199	.034	.364	.167	.019	1.064
Age	-.014	-.022	-.006	-.289	.000	1.349
Cohort of origin	.372	.147	.597	.327	.001	2.154

Note. BDNF = brain-derived neurotrophic factor; cAMP = cyclic adenosine monophosphate; EGF = epidermal growth factor; LL = lower limit; TNF $\alpha$ R2 = tumour necrosis factor  $\alpha$  receptor 2; UL = upper limit; VIF = variance inflation factor. A significant *b*-coefficient indicates the  $\beta$ -coefficient is also significant. *b* represents unstandardised regression coefficients;  $\beta$  indicates standardised regression coefficients.

<sup>a</sup> Ln-transformation was applied.

<sup>b</sup> Heteroscedasticity-adjusted confidence interval was computed.

**Table S2.** Associations of insomnia severity and relevant covariates with levels of urine biomarkers.

Biomarker level	Unit of measurement	n	Effect	b	b		β	p	VIF
					95% CI				
					LL	UL			
Immunologic									
α1-antitrypsin <sup>a</sup>	μg/L	174	Insomnia severity	.025	-.041	.091	.060	.454	1.415
			Depression severity	.018	-.016	.051	.082	.302	1.404
			Antidepressant use	.106	-.375	.587	.044	.664	2.340
			Gender	.273	.002	.545	.136	.048	1.045
			Age	.003	-.009	.015	.040	.590	1.258
			Cohort of origin	-.721	-1.048	-.393	-.436	.000	2.256
			Calprotectin <sup>a b</sup>	ng/mL	174	Insomnia severity	-.017	-.119	.084
Depression severity	.045	-.007	.096			.099	.089	1.404	
Antidepressant use	.017	-.727	.761			.003	.963	2.340	
Gender	2.825	2.379	3.271			.672	.000	1.045	
Age	.008	-.014	.030			.049	.454	1.258	
Cohort of origin	-1.148	-1.607	-.690			-.333	.000	2.256	
cGMP <sup>a</sup>	pmol/mL	174	Insomnia severity			-.007	-.045	.031	-.030
			Depression severity	.011	-.009	.030	.093	.286	1.404
			Antidepressant use	-.290	-.569	-.011	-.231	.042	2.340
			Gender	-.075	-.232	.083	-.071	.350	1.045
			Age	-.007	-.014	.000	-.154	.064	1.258
			Cohort of origin	-.314	-.505	-.124	-.361	.001	2.256
			HVEM <sup>a</sup>	ng/mL	174	Insomnia severity	-.010	-.050	.031
Depression severity	.011	-.010				.032	.088	.306	1.404
Antidepressant use	-.110	-.408				.188	-.081	.468	2.340
Gender	-.254	-.422				-.086	-.221	.003	1.045
Age	-.007	-.014				.001	-.139	.089	1.258
Cohort of origin	-.278	-.481				-.075	-.295	.008	2.256
Isoprostane-2 <sup>a</sup>	ng/mL	173				Insomnia severity	.008	-.035	.051
			Depression severity	.007	-.015	.029	.053	.545	1.408
			Antidepressant use	.025	-.291	.341	.018	.874	2.339
			Gender	-.338	-.517	-.159	-.279	.000	1.042
			Age	-.009	-.018	-.001	-.190	.022	1.266
			Cohort of origin	-.137	-.353	.078	-.138	.209	2.254
			Lipocalin-2 <sup>a</sup>	ng/mL	173	Insomnia severity	.073	-.018	.164
Depression severity	.021	-.025				.068	.072	.370	1.417
Antidepressant use	-.074	-.738				.590	-.022	.827	2.337
Gender	.743	.367				1.120	.267	.000	1.044

LTB4 <sup>a</sup>	pg/mL	174	Age	.004	-.013	.021	.038	.611	1.268
			Cohort of origin	-.968	-1.420	-.515	-.425	.000	2.251
			Insomnia severity	.001	-.035	.037	.005	.959	1.415
			Depression severity	.008	-.010	.027	.078	.383	1.404
			Antidepressant use	-.255	-.522	.011	-.217	.060	2.340
			Gender	-.195	-.346	-.045	-.196	.011	1.045
			Age	-.001	-.008	.006	-.020	.808	1.258
			Cohort of origin	-.157	-.338	.025	-.192	.090	2.256
			Insomnia severity	-.012	-.079	.055	-.031	.719	1.413
			Depression severity	.009	-.025	.043	.046	.596	1.397
Resistin <sup>a</sup>	ng/mL	173	Antidepressant use	-.148	-.636	.340	-.067	.550	2.337
			Gender	-.295	-.571	-.018	-.158	.037	1.048
			Age	-.017	-.029	-.005	-.222	.008	1.263
			Cohort of origin	-.442	-.775	-.110	-.289	.009	2.253
			Insomnia severity	.003	-.041	.047	.011	.901	1.415
			Depression severity	.009	-.014	.032	.066	.438	1.404
			Antidepressant use	-.022	-.346	.301	-.015	.892	2.340
			Gender	-.349	-.532	-.167	-.276	.000	1.045
			Age	-.006	-.015	.002	-.125	.122	1.258
			Cohort of origin	-.283	-.504	-.063	-.273	.012	2.256
Thromboxane <sup>a</sup>	ng/mL	174	Insomnia severity	.003	-.041	.047	.011	.901	1.415
			Depression severity	.009	-.014	.032	.066	.438	1.404
			Antidepressant use	-.022	-.346	.301	-.015	.892	2.340
			Gender	-.349	-.532	-.167	-.276	.000	1.045
			Age	-.006	-.015	.002	-.125	.122	1.258
			Cohort of origin	-.283	-.504	-.063	-.273	.012	2.256
Neurotrophic EGF <sup>a</sup>	ng/mL	174	Insomnia severity	-.007	-.048	.034	-.027	.746	1.415
			Depression severity	.009	-.012	.030	.072	.385	1.404
			Antidepressant use	-.023	-.322	.277	-.016	.881	2.340
			Gender	-.088	-.257	.081	-.074	.305	1.045
			Age	-.019	-.027	-.012	-.398	.000	1.258
			Cohort of origin	-.237	-.441	-.033	-.242	.023	2.256
Midkine <sup>a b</sup>	pg/mL	172	Insomnia severity	.025	-.026	.076	.084	.336	1.413
			Depression severity	.004	-.021	.029	.025	.760	1.408
			Antidepressant use	-.147	-.500	.207	-.088	.414	2.338
			Gender	-.634	-.842	-.426	-.447	.000	1.046
			Age	-.011	-.020	-.001	-.184	.030	1.271
			Cohort of origin	-.067	-.299	.165	-.058	.569	2.253
Neuropeptide Substance P <sup>b</sup>	pg/mL	170	Insomnia severity	-1.710	-8.455	5.034	-.038	.617	1.416
			Depression severity	2.896	-.736	6.527	.125	.117	1.387
			Antidepressant use	-43.715	-112.676	25.247	-.168	.212	2.376
			Gender	-32.718	-62.808	-2.628	-.150	.033	1.045
			Age	-.322	-1.589	.946	-.037	.617	1.263
			Cohort of origin	-110.077	-160.200	-59.954	-.618	.000	2.303

Neuroendocrine									
Aldosterone <sup>a</sup>	ng/mL	171							
			Insomnia severity	.001	-.044	.046	.004	.965	1.403
			Depression severity	.003	-.020	.027	.026	.773	1.406
			Antidepressant use	.142	-.195	.478	.096	.408	2.365
			Gender	.093	-.096	.281	.074	.333	1.044
			Age	-.013	-.021	-.005	-.257	.003	1.256
			Cohort of origin	.004	-.224	.232	.004	.970	2.278
Cortisol <sup>a b</sup>	µg/dL	174							
			Insomnia severity	.020	-.038	.078	.064	.508	1.415
			Depression severity	-.014	-.040	.013	-.089	.301	1.404
			Antidepressant use	-.151	-.610	.308	-.088	.517	2.340
			Gender	-.095	-.323	.134	-.065	.416	1.045
			Age	-.013	-.023	-.002	-.211	.015	1.258
			Cohort of origin	-.036	-.319	.247	-.031	.799	2.256
Metabolic									
Acetyl-L-carnitine <sup>a</sup>	ng/mL	174							
			Insomnia severity	-.048	-.096	.001	-.168	.055	1.415
			Depression severity	.032	.007	.057	.219	.012	1.404
			Antidepressant use	.205	-.150	.561	.128	.256	2.340
			Gender	-.153	-.354	.048	-.113	.134	1.045
			Age	-.001	-.010	.008	-.009	.909	1.258
			Cohort of origin	-.117	-.359	.125	-.105	.342	2.256

Note. cGMP = cyclic guanosine monophosphate; EGF = epidermal growth factor; HVEM = herpes virus entry mediator; LL = lower limit; LTB4 = leukotriene B4; UL = upper limit; VIF = variance inflation factor. A significant *b*-coefficient indicates the  $\beta$ -coefficient is also significant. *b* represents unstandardised regression coefficients;  $\beta$  indicates standardised regression coefficients.

<sup>a</sup> Ln-transformation was applied.

<sup>b</sup> Heteroscedasticity-adjusted confidence interval was computed.

**Table S3.** Intra- and inter-assay reliability as reported by vendors of assay kits used for biomarker measurement.

Biomarker	Sample(s)	Vendor	Intra-assay CV	Inter-assay CV
Immunologic				
$\alpha$ 1-antitrypsin	Serum and urine	Immundiagnostik GmbH, Bensheim, Germany	<8%	<11%
Calprotectin	Serum and urine	Sanbio B, Hycult biotech, Uden, The Netherlands	<6%	<5%
cAMP	Serum	R&D systems Europe Ltd., Abingdon, United Kingdom	<4%	<9%
cGMP	Urine	R&D systems Europe Ltd., Abingdon, United Kingdom	<7%	<9%
Endothelin-1	Serum	R&D systems Europe Ltd., Abingdon, United Kingdom	<3%	<7%
HVEM	Urine	Ray Biotech Inc., Norcross, GA, USA	<10%	<12%
Isoprostane-2	Urine	Northwest Life Science Specialties, LLC, Vancouver, WA, USA	U/A	U/A
Lipocalin-2	Urine	Ray Biotech Inc., Norcross, GA, USA	<10%	<12%
LTB4	Urine	R&D systems Europe Ltd., Abingdon, United Kingdom	<5%	<9%
Myeloperoxidase	Serum	R&D systems Europe Ltd., Abingdon, United Kingdom	<3%	<10%
Resistin	Serum and urine	R&D systems Europe Ltd., Abingdon, United Kingdom	<5%	<9%
Thromboxane	Serum and urine	R&D systems Europe Ltd., Abingdon, United Kingdom	<6%	<7%
TNF $\alpha$ R2	Serum	Ray Biotech Inc., Norcross, GA, USA	<10%	<12%
Zonulin	Serum	Immundiagnostik GmbH, Bensheim, Germany	<5%	<9%
Neurotrophic				
BDNF	Serum	Promega Benelux BV, Leiden, The Netherlands	U/A	U/A
BDNF free	Serum	Promega Benelux BV, Leiden, The Netherlands	U/A	U/A
BDNF total	Serum	Promega Benelux BV, Leiden, The Netherlands	U/A	U/A
EGF	Serum and urine	Ray Biotech Inc., Norcross, GA, USA	<10%	<12%
Midkine	Urine	Cellmid Limited, Perth, Australia	U/A	U/A
Neuropeptide				
Substance P	Serum and urine	R&D systems Europe Ltd., Abingdon, United Kingdom	<7%	<12%
Neuroendocrine				
Aldosterone	Urine	LDN, Germany	<6%	<8%
Cortisol	Serum and urine	R&D systems Europe Ltd., Abingdon, United Kingdom	<7%	<14%
Metabolic				
Acetyl-L-carnitine	Serum and urine	Abbexa Ltd, United Kingdom	<10%	<12%
Apolipoprotein A1	Serum	R&D systems Europe Ltd., Abingdon, United Kingdom	<4%	<7%
Leptin	Serum	Ray Biotech Inc., Norcross, GA, USA	<10%	<12%
Prolactin	Serum	Diagnostics Biochem Canada Inc.	<10%	<12%

Note. BDNF = brain-derived neurotrophic factor; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; EGF = epidermal growth factor; HVEM = herpes virus entry mediator; LTB4 = leukotriene B4; TNF $\alpha$ R2 = tumour necrosis factor  $\alpha$  receptor 2; U/A = unavailable.