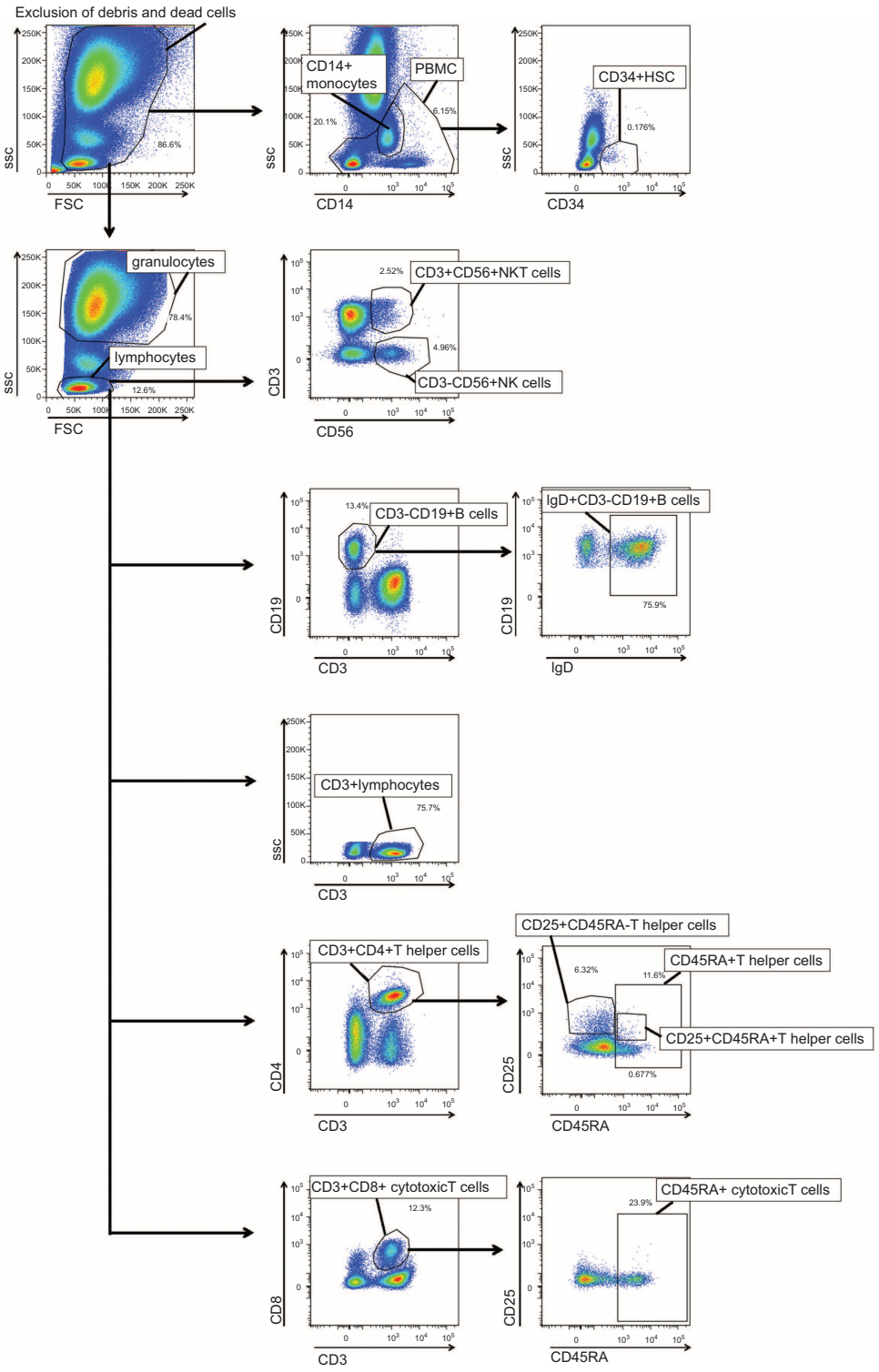
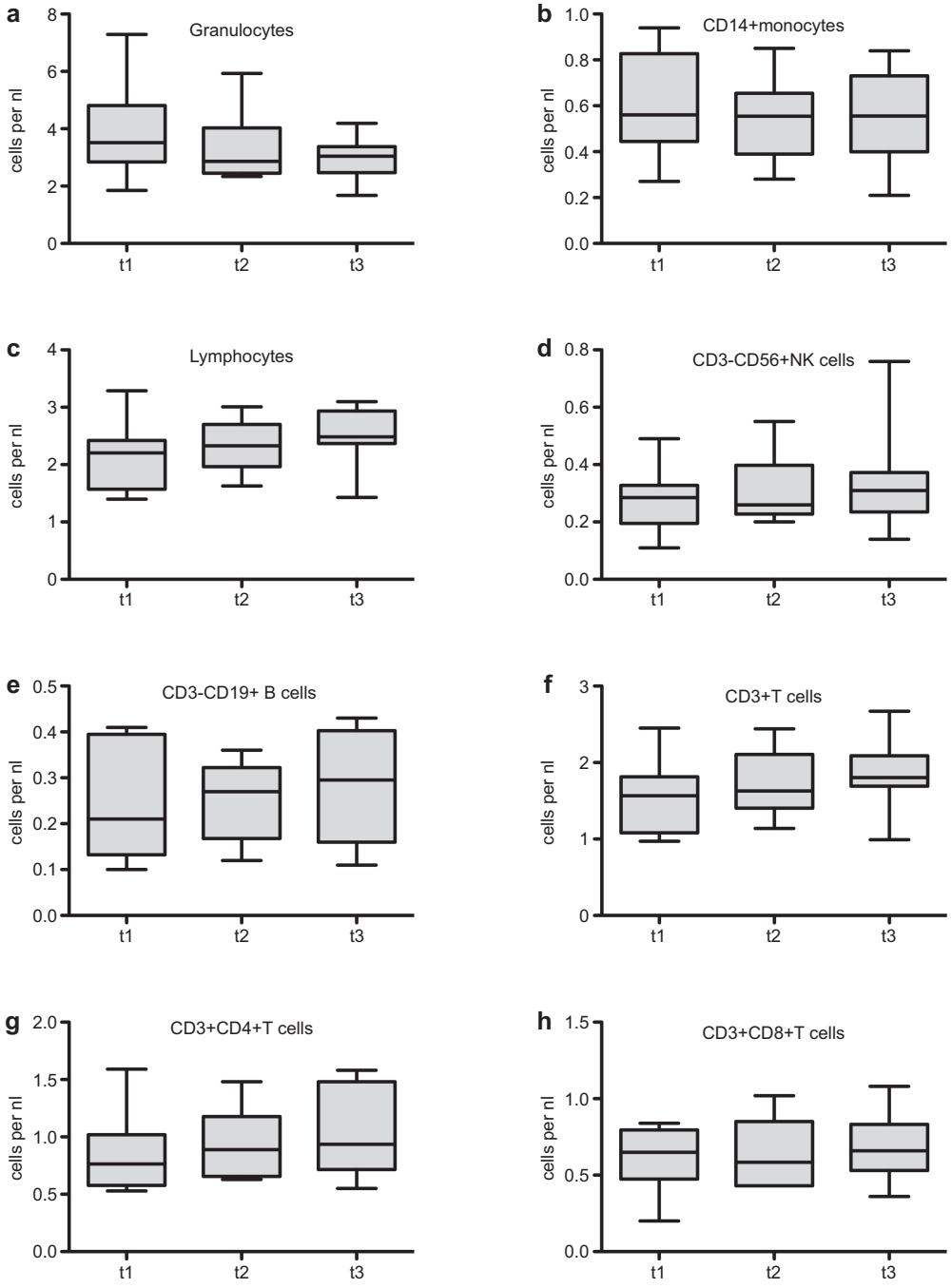
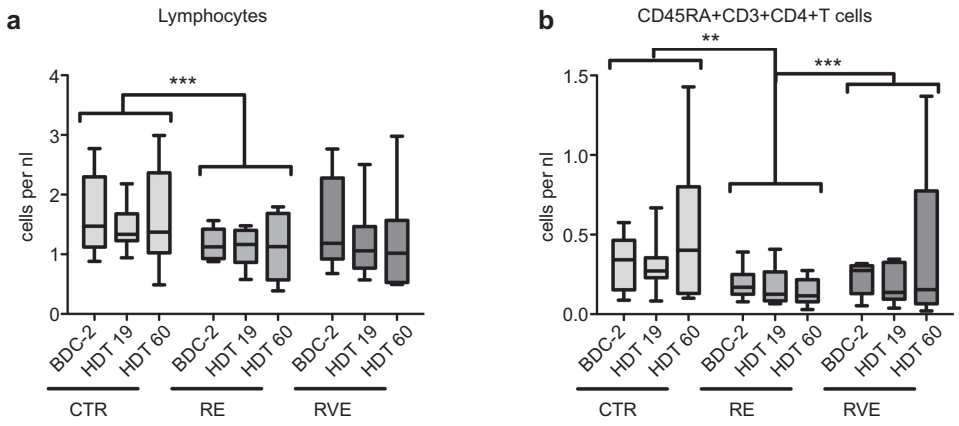


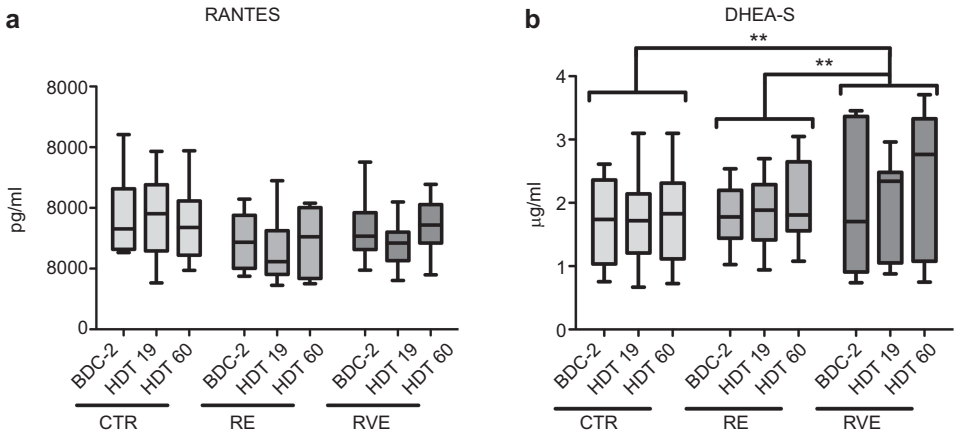
Supplemental Figure 1 FACS gating strategy. After the exclusion of debris and dead cells, a gate was set on PBMC and $CD14^+$ monocytes in a CD14/SSC plot. Within the PBMC gate, the $CD34^+$ HSC were gated in a CD34/SSC plot. Granulocyte and lymphocyte gates were set in a FSC/SSC plot. Within the lymphocyte gate further gates were set on $CD56^+CD3^+$ NKT and $CD56^+CD3^-$ NK cells in a CD56/CD3 plot. Within the lymphocyte gate, the $CD3^-CD19^+$ B cells were gated in a CD3/CD19 plot. Within the $CD3^-CD19^+$ B cells, a further gate was set on the IgD^+ cells in a IgD/CD19 plot. Furthermore, within the lymphocyte gate the $CD3^+$ T cells were gated in a CD3/SSC plot. Within the lymphocyte gate, the T helper cells were gated as $CD3^+CD4^+$ in a CD3/CD4 plot. Within the T helper cell population, further gates were set on $CD45RA^+$, $CD25^-CD45RA^+$ and $CD25^+CD45RA^+$ T helper cells. Within the lymphocyte gate, the cytotoxic T cells were gated as $CD3^+CD8^+$ in a CD3/CD8 plot. Within the cytotoxic T cell population, a further gate was set on $CD45RA^+$ cytotoxic T cells.



Supplemental Figure 2 Time-dependent effects in leukocyte subpopulations for a non-bed rest group (n=10). Blood samples were drawn on three different time points corresponding to the time gaps between BDC-2, HDT19 and HDT60. Leukocytes were analyzed flow cytometrically according to their morphology and different surface markers. Data are shown as box and whisker plots (box: median and the 25th and 75th percentiles; whiskers: minimum+maximum). No time-dependent differences were found in any of the selected parameters tested (**a**) granulocytes, (**b**) $CD14^+$ monocytes, (**c**) lymphocytes, (**d**) $CD3^-CD56^+$ NK cells, (**e**) $CD3^-CD19^+$ B cells, (**f**) $CD3^+$ T cells, (**g**) $CD3^+CD4^+$ T cells and (**h**) $CD3^+CD8^+$ T cells. Paired t-tests were performed and no significant values were detected.



Supplemental Figure 3 Exercise (RE+RVE pooled *versus* CTR) dependent time effects in leukocyte subpopulations. Blood samples were drawn on BDC-2, HDT19 and HDT60. Leukocytes were analyzed flow cytometrically according to their morphology and different surface markers. Then the cell counts were calculated on the basis of the leukocyte count conducted at the routine hospital laboratory undergoing regular quality controls. Data are shown as box and whisker plots (box: median and the 25th and 75th percentiles; whiskers: minimum+maximum). Exercise-dependent time effects were found in (**a**) lymphocytes and (**b**) $CD45RA^{+}CD3^{+}CD4^{+}$ T cells. Pairwise repeated measurement analyses were performed for the different exercise groups when the overall repeated measurement analysis revealed a significant time effect of exercise type; the statistical analysis refers to the difference in time course, $*P<0.05$, $**P<0.01$.



Supplemental Figure 4 Exercise-dependent time effects in plasma factors. Blood samples were drawn on BDC-2, HDT19 and HDT60. Plasma was analyzed by a multiplex suspension array or ELISA/RIA. Data are shown as box and whisker plots (box: median and the 25th and 75th percentiles; whiskers: minimum+maximum). Exercise-dependent time effects were found in (**a**) RANTES and (**b**) DHEA-S. Pairwise repeated measurement analyses were performed for the different exercise groups when the overall repeated measurement analysis revealed a significant time effect of exercise type; the statistical analysis refers to the difference in time course, $*P<0.05$, $**P<0.01$.

Supplemental Table 1 Distribution of non-smokers and former smokers (prior to the start of the BBR-2 study)

Cigarettes/day before bed rest	Number of subjects
0 (non-smokers)	19
<2 occasionally (not every day)	3
1–5	1
5–10	2
10–15	0
15–20	0
20–40	0
>40	0

Supplemental Table 2 Subject characteristics of the non-bed rest group

Subjects	Age	Size	Weight	BMI
1	40	182	75	22.6
2	25	182	71	21.4
3	34	190	84	23.3
4	41	183	79	24.7
5	42	175	74	24.2
6	27	182	92	27.8
7	29	172	68	23
8	39	191	107	29.3
9	32	184	80	23.6
10	32	178	80	25.2

Supplemental Table 3 Immune cells, cytokines, chemokines and neuroendocrine factors which show time-dependent effects (from BDC-2 to HDT 19 to HDT60) in repeated measurement analyses. The ANOVA was controlled for confounding by subject exercise, age, body mass index, cohort (first, second, third or fourth), smoker before study and interactions between these parameters. The interactions are marked with *. Confounding factors are listed. Significant *P* values (*P*<0.05) are shown. Where 'exercise' values (bold) or the 'exercise'×'other confounder' interaction values were significant on repeated measures, additional repeated measures (ANOVAs) comparing individual groups (i.e., CTR *versus* RE, CTR *versus* RVE and RE *versus* RVE) were performed (for results, see Supplementary Figures 3 and 4)

Parameters		
	Confounding factors	*P* value
Immune cell subpopulations		
Granulocytes		**0.028**
	Cohort	<**0.001**
	Smoker	<**0.001**
	Cohort*smoker	<**0.001**
Lymphocytes		**0.045**
	Cohort	<**0.001**
	Exercise*cohort	**0.002**
	Exercise*smoker	**0.010**
	Cohort*smoker	**0.002**
	BMI	**0.029**
$CD14^+$ monocytes	Cohort	**0.005**
Lymphocyte subpopulations		
T cells		
$CD3^+$	Cohort	**0.034**
T helper cells		
$CD3^+CD4^+$ T helper cells	Cohort	**0.003**
$CD45RA^+CD3^+CD4^+$ T helper cells	**Exercise**	**0.008**
	Cohort	<**0.001**
	Smoker	**0.021**
	Exercise*cohort	**0.006**
	BMI	**0.045**
$CD45RA^-CD25^+CD3^+CD4^+$ T helper cells	Cohort	**0.031**
Hematopoietic stem cells		
$CD34^+$ HSC	Cohort*smoker	**0.027**
NK cells		
$CD3^-CD56^+$ NK cells	Cohort	**0.004**
Cytokines and chemokines		
PDGF	Cohort	**0.034**
RANTES	**Exercise**	**0.007**
	Cohort	**0.034**
	Exercise*smoker	**0.029**
Neuroendocrine factors		
DHEA-S	Smoker	**0.038**
	Exercise*cohort	**0.017**
	Cohort*smoker	**0.046**