

**Iron metabolism regulation in females and males exposed to simulated microgravity:  
results from the randomized trial AGBRESA - Horeau et al.**

*Online Supplementary Material*

**Supplemental Table 1. Baseline characteristics at BDC-13<sup>1</sup> in each experimental group**

	<b>CTL</b>	<b>cAG</b>	<b>iAG</b>
<b>N</b>	<b>8 (m=6/ f=2)</b>	<b>8 (m=5/ f=3)</b>	<b>7 (m=5/ f=2)</b>
<b>Age (years)</b>	34.58 ± 7.99	32.53 ± 9.86	34.21 ± 10.95
<b>Height (cm)</b>	177.04 ± 7.27	172.50 ± 8.05	174.10 ± 10.52
<b>Weight (kg)</b>	79.40 ± 12.67	71.80 ± 10.15	71.40 ± 4.51
<b>BMI (kg/m<sup>2</sup>)</b>	25.18 ± 2.58	24.00 ± 1.71	23.64 ± 1.71

<sup>1</sup>Values are presented as mean ± SD for each group, (CTL: n=8, cAG: n=8, iAG: n=7).

<sup>2</sup>BDC: *Baseline Data Collection*; BMI: *Body Mass Index*; CTL: *Control*; cAG: *continue Artificial Gravity*; iAG: *intermittent Artificial Gravity*.

**Supplemental Table 2. Daily nutrient intake<sup>1</sup>**

	<b>BDC all groups</b>	<b>HDT Ctrl<sup>2</sup></b>	<b>HDT cAG<sup>3</sup></b>	<b>HDT iAG<sup>4</sup></b>	<b>R+ all groups</b>
<b>Total Energy (kcal/d)</b>	2610 ± 379	2431 ± 365	2215 ± 362	2266 ± 323	2673 ± 417
<b>Protein (g/d)</b>	89.2 ± 11.4	93.56 ± 16.58	84.02 ± 13.54	85.69 ± 6.51	88.39 ± 13.19
<b>Protein (g/kgBW/d)</b>	1.2 ± 0.02	1.17 ± 0.1	1.18 ± 0.08	1.19 ± 0.05	1.19 ± 0.07
<b>Protein (% of total energy)</b>	13.99 ± 1.34	15.61 ± 1.82	15.43 ± 1.27	15.52 ± 1.6	13.53 ± 1.5
<b>Total fat (g/d)</b>	90.61 ± 14.08	83.88 ± 14.26	75.77 ± 13.77	77.74 ± 12.32	92.97 ± 17
<b>Total fat (% of total energy)</b>	31.98 ± 0.97	31.56 ± 1.72	31.38 ± 1.46	31.49 ± 1.17	31.98 ± 1.92
<b>Monosaturated fatty acids (g/d)</b>	31.57 ± 6.53	29.06 ± 5.74	26.19 ± 5.76	26.99 ± 5.34	34 ± 8.82
<b>Monosaturated fatty acids (% of total energy)</b>	11.11 ± 1.25	10.95 ± 1.48	10.83 ± 1.28	10.92 ± 1.17	11.66 ± 1.85
<b>Saturated fatty acids (g/d)</b>	24.16 ± 4.42	22.22 ± 4.99	20 ± 4.64	20.52 ± 4.23	24.38 ± 4.65
<b>Saturated fatty acids (% of total energy)</b>	8.55 ± 1.03	8.36 ± 1.38	8.29 ± 1.31	8.33 ± 1.23	8.43 ± 1.12
<b>Polyunsaturated fatty acids (g/d)</b>	30.67 ± 6.41	28.59 ± 7.96	25.94 ± 7.03	26.5 ± 6.93	30.43 ± 6.89
<b>Polyunsaturated fatty acids (% of total energy)</b>	10.84 ± 1.66	10.74 ± 2.37	10.76 ± 2.31	10.75 ± 2.35	10.47 ± 1.61
<b>Carbohydrates (g/d)</b>	329.2 ± 54.33	299.69 ± 46.03	273.71 ± 48	280.82 ± 48.13	339.94 ± 58.93
<b>Carbohydrates (% of total energy)</b>	51.16 ± 1.69	50.01 ± 2.25	50.09 ± 2	50.05 ± 2.01	51.59 ± 2.18
<b>Total Fiber (g/d)</b>	37.13 ± 5.59	34.1 ± 5.86	33.98 ± 4.79	33.11 ± 4.65	38.57 ± 6.27
<b>Fluid (ml/d)</b>	3741.64 ± 472.28	4027.42 ± 488.38	3557.29 ± 488.93	3611.98 ± 228.65	4358.77 ± 707.62
<b>Fluid (ml/kgBW/d)</b>	50.53 ± 2.02	51.04 ± 3.73	50.22 ± 1.21	50.2 ± 2.27	58.94 ± 7.11
<b>Calcium (mg/d)</b>	1080.7 ± 22.97	1080.53 ± 65.4	1081.86 ± 39.4	1081.96 ± 31.87	1084.82 ± 30.52
<b>Chloride (mg/d)</b>	4951.07 ± 444.07	4725.48 ± 473.26	4678.94 ± 457.4	4731.45 ± 357.58	4972.43 ± 591.78
<b>Sodium (mg/d)</b>	2950.79 ± 92.16	2905.93 ± 203.26	2885.02 ± 223.73	2918.86 ± 152.6	2952.06 ± 230.81
<b>Sodium (mmol/kgBW/d)</b>	1.76 ± 0.26	1.63 ± 0.29	1.8 ± 0.27	1.77 ± 0.14	1.76 ± 0.28

<b>Potassium (mg/d)</b>	3918.34 ± 552.68	3715.89 ± 460.91	3560.37 ± 478.97	3586.89 ± 399.01	3971.78 ± 565.67
<b>Fluoride (mg/d)</b>	2.16 ± 0.54	2 ± 0.75	2.11 ± 0.73	2.11 ± 0.71	2.12 ± 0.49
<b>Jodine (µg/d)</b>	203.46 ± 73.24	208.55 ± 59.9	206.43 ± 55.44	208.75 ± 52.57	194.69 ± 68.56
<b>Copper (µg/d)</b>	1915.38 ± 293.09	1904.21 ± 343.26	1807.92 ± 329.56	1835.75 ± 313.09	2023.63 ± 332.23
<b>Magnesium (mg/d)</b>	445.1 ± 48	448.35 ± 67.36	431.13 ± 57.65	436.85 ± 53.94	465.67 ± 52.44
<b>Iron (mg/d)</b>	19.73 ± 6.97	19.55 ± 6.95	20.54 ± 7	20.06 ± 6.87	21.1 ± 7.28
<b>Phosphorus (mg/d)</b>	1522.27 ± 160.47	1542.21 ± 240.7	1453.18 ± 204.42	1479.21 ± 151.27	1526.94 ± 191.06
<b>Zinc (mg/d)</b>	13.17 ± 3.8	13.54 ± 4.49	12.98 ± 4.51	13.18 ± 4.58	13.71 ± 3.8
<b>Vitamin B1, Thiamin (mg/d)</b>	1.53 ± 0.31	1.53 ± 0.28	1.53 ± 0.27	1.52 ± 0.26	1.59 ± 0.34
<b>Vitamin B2, Riboflavin (mg/d)</b>	1.87 ± 0.41	1.8 ± 0.52	1.78 ± 0.49	1.77 ± 0.49	1.92 ± 0.4
<b>Vitamin B3, Niacin equivalent (mg/d)</b>	31.5 ± 6.9	32.84 ± 8.54	30.52 ± 7.85	31.06 ± 6.87	31.24 ± 7.13
<b>Vitamin B5, Pantothenic Acid (mg/d)</b>	5.56 ± 0.79	5.8 ± 1.18	5.45 ± 1.17	5.52 ± 1.08	5.6 ± 0.83
<b>Vitamin B6 (mg/d)</b>	2.19 ± 0.58	2.47 ± 0.48	2.43 ± 0.47	2.41 ± 0.46	2.29 ± 0.57
<b>Vitamin B7, Biotin (µg/d)</b>	47.75 ± 7.56	50.18 ± 11.73	47.8 ± 11.38	48.14 ± 11.1	51.48 ± 9.35
<b>Vitamin B9, Folate (µg/d)</b>	731.29 ± 135.82	740.38 ± 162.46	750.75 ± 156.54	755.06 ± 154.06	730.31 ± 129.86
<b>Vitamin B12, Cobalamin (µg/d)</b>	6.52 ± 2.03	5.63 ± 2.35	5.37 ± 2.07	5.48 ± 2.09	6.41 ± 2.08
<b>Retinol equivalent (µg/d)</b>	1293.79 ± 674.26	1663.28 ± 1019.75	1764.47 ± 1141.28	1718.24 ± 1122.32	1364.31 ± 689.68
<b>Vitamin A Retinol (mg/d)</b>	0.32 ± 0.12	0.38 ± 0.13	0.34 ± 0.12	0.35 ± 0.12	0.33 ± 0.12
<b>Vitamin C (mg/d)</b>	230.17 ± 64.91	199.88 ± 98.36	202.15 ± 96.25	201.94 ± 100.67	238.41 ± 62.19
<b>Vitamin D (µg/d), (diet + supplementation)</b>	29.19 ± 2.84	28.61 ± 2.71	28.26 ± 2.54	28.28 ± 2.51	28.99 ± 3.13
<b>Vitamin E (mg/d)</b>	25.86 ± 6.59	22.44 ± 6.95	20.79 ± 6.45	21.05 ± 6.51	26.83 ± 7.44
<b>Vitamin K (µg/d)</b>	189.8 ± 94.33	210.65 ± 124.7	205.59 ± 129.14	209.56 ± 132.34	196.04 ± 94.53

<sup>1</sup> Values are presented as mean ± SD for each group.

<sup>2</sup> CON (n=8): Control group

<sup>3</sup> AG (n=8): continuous artificial gravity (30 min/day).

<sup>4</sup> iAG (n=7): intermittent artificial gravity (6x5 min/day).

**Supplemental Table 3. Impact of artificial gravity on blood and RBC parameters before**

	CON <sup>3</sup>		cAG <sup>4</sup>		iAG <sup>5</sup>		SOURCE OF VARIATION <sup>2</sup>		
	BDC-3	HDT60	BDC-3	HDT60	BDC-3	HDT60	TIME	GRAVITY	INTERACTION
RBC (10 <sup>6</sup> /μL)	4.83 ± 0.6	5.13 ± 0.7	4.54 ± 0.2	4.69 ± 0.4	4.53 ± 0.5	4.76 ± 0.5	<b>P=0.001</b>	NS	NS
Hemoglobin (g/dL)	14.2 ± 1.5	14.9 ± 1.7	13.7 ± 1.1	14.1 ± 1.5	13.4 ± 1.6	14.1 ± 1.0	<b>P&lt;0.001</b>	NS	NS
Hematocrit (%)	41.0 ± 4.3	42.6 ± 4.9	39.7 ± 3.0	40.4 ± 4.0	39.3 ± 4.0	40.6 ± 3.0	<b>P=0.031</b>	NS	NS
Reticulocyte (%)	0.80 ± 0.3	0.81 ± 0.3	0.84 ± 0.2	0.88 ± 0.2	0.87 ± 0.2	0.82 ± 0.2	NS	NS	NS
MCV (fL)	85.2 ± 5.6	83.5 ± 4.7	87.4 ± 3.1	86.2 ± 3.0	86.8 ± 4.4	85.6 ± 3.6	<b>P&lt;0.001</b>	NS	NS
RDW (%)	12.5 ± 0.6	12.1 ± 0.3	12.3 ± 0.7	12.4 ± 0.4	13.0 ± 1.5	12.8 ± 1.4	NS	NS	NS
MCH (pg)	29.5 ± 2.1	29.3 ± 1.8	30.2 ± 1.2	30.1 ± 2.1	29.5 ± 1.7	29.8 ± 1.4	NS	NS	NS
MCHC (g/dL)	34.6 ± 0.5	35.1 ± 0.6	34.5 ± 0.8	35.0 ± 0.5	33.9 ± 0.7	34.9 ± 0.8	<b>P&lt;0.001</b>	NS	NS

**bed rest (BDC-6), and after 6 and 57 days of bed rest (HDT6 and HDT57)<sup>1</sup>**

<sup>1</sup> Values are presented as mean ± SD for each group.

<sup>2</sup> Data were analyzed with Two-way ANOVA for repeated measures.

<sup>3</sup> CON (n=8): Control group

<sup>4</sup> AG (n=8): continuous artificial gravity (30 min/day).

<sup>5</sup> iAG (n=7): intermittent artificial gravity (6x5 min/day).

**Supplemental Table 4. Impact of artificial gravity on lean mass before bed rest (BDC-4),**

	CON <sup>3</sup>		cAG <sup>4</sup>		iAG <sup>5</sup>		SOURCE OF VARIATION <sup>2</sup>		
	BDC-4	HDT60	BDC-4	HDT60	BDC-4	HDT60	TIME	GRAVITY	INTERACTION
<b>Total body weight (kg)<sup>3,4</sup></b>	80.0 ± 12.9	77.8 ± 12.8	72.1 ± 10.1	70.5 ± 10.1	72.4 ± 5.3	71.0 ± 6.2	NS	<b>P=0.025</b>	NS
<b>Absolute total lean mass (kg)</b>	56.0 ± 7.9	52.8 ± 8.1	51.8 ± 11.0	48.4 ± 10.9	51.5 ± 9.4	49.0 ± 9.7	NS	<b>P=0.019</b>	NS
<b>Relative total lean mass (g/kg)</b>	704.1 ± 63.7	681.1 ± 84.3	714.0 ± 84.2	682.0 ± 59.1	708.2 ± 77.6	684.2 ± 81.0	NS	NS	NS
<b>Absolute lower limb lean mass (kg)</b>	19.0 ± 2.6	16.8 ± 2.9	17.5 ± 3.7	15.4 ± 3.7	16.8 ± 2.7	15.1 ± 2.9	NS	<b>P=0.007</b>	NS
<b>Relative lower limb lean mass (g/kg)</b>	239.5 ± 18.2	216.7 ± 20.2	241.4 ± 30.2	216.0 ± 30.2	237.8 ± 18.7	216.8 ± 23.0	<b>P=0.027</b>	NS	NS

**and after 60 days of bed rest (HDT60)<sup>1</sup>**

<sup>1</sup> Values are presented as mean ± SD for each group.

<sup>2</sup> Data were analyzed with Two-way ANOVA for repeated measures.

<sup>3</sup> CON (n=8): Control group

<sup>4</sup> AG (n=8): continuous artificial gravity (30 min/day).

<sup>5</sup> iAG (n=7): intermittent artificial gravity (6x5 min/day).

**Supplemental Table 5. Iron regulation before bed rest (BDC-6), and after 6 and 57 days**

	MALES (n=16)				FEMALES (n=7)			
	BDC-6	HDT6	HDT57	One-way RM ANOVA <sup>2</sup>	BDC-6	HDT6	HDT57	One-way RM ANOVA <sup>2</sup>
<b>Serum iron (μmol/l)</b> <sup>2,3</sup>	17.33 ± 4.5	21.58 ± 6.05 <sup>*</sup>	19.19 ± 4.54	<b>P=0.012</b>	14.10 ± 2.6	17.91 ± 6.14	13.23 ± 5.21	<b>NS</b>
<b>Transferrin (g/l)</b>	2.46 ± 0.2	2.33 ± 0.22 <sup>*</sup>	2.25 ± 0.24 <sup>***</sup>	<b>P&lt;0.001</b>	2.66 ± 0.3	2.59 ± 0.30	2.47 ± 0.19	<b>NS</b>
<b>Transferrin Saturation (%)</b>	28.43 ± 7.3	37.63 ± 11.8 <sup>**</sup>	34.31 ± 7.98 <sup>*</sup>	<b>P=0.002</b>	21.53 ± 3.5	27.84 ± 8.99	21.39 ± 8.64	<b>NS</b>
<b>Serum hepcidin (nmol/l)</b>	5.2 ± 2.4	7.1 ± 3.5 <sup>**</sup>	5.2 ± 3.4	<b>P=0.002</b>	3.2 ± 3.4	3.1 ± 1.87	2.57 ± 2.65	<b>NS</b>
<b>Serum ferritin (μg/l)</b>	101.8 ± 56.8	103.8 ± 54.8	92.4 ± 52.9	<b>NS</b>	38.7 ± 32.1	40.0 ± 26.5	30.3 ± 22.5	<b>NS</b>
<b>sTfR (mg/l)</b>	1.15 ± 0.2	1.09 ± 0.3	1.22 ± 0.3	<b>NS</b>	1.12 ± 0.9	1.26 ± 0.2	1.12 ± 0.2	<b>NS</b>

of bed rest (HDT6 and HDT57)<sup>1</sup>

<sup>1</sup> Values are presented as mean ± SD for both sexes, (n=16 males, n=7 females).

<sup>2</sup> Data were analyzed with one-way ANOVA for repeated measures for each group.

For all significant effect, Tukey's post hoc tests were used to locate differences between baseline and each time points:

<sup>3</sup> Males compared to baseline: \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

## Supplemental Figure 1. Participant flow chart diagram

