iScience, Volume *26*

Supplemental information

The role of dysregulated ghrelin/LEAP-2 balance in anorexia nervosa

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SUPPLEMENTAL INFORMATION

Figure	ANOVA	Mice per group	Factors	$F_{(DFn,DFd)}$	p-value
Body Weight (g)	Three-way	AL/ALW n=8	Time ****	$F_{(16,448)}$= 70,930	p<0,001
Figure 1A		FR/FRW n=8	FR ****	$F_{(1,28)}$= 73,060	p<0,001
			Running	$F_{(1,28)}$=0,580	p=0,452
			Time x FR ****	$F_{(16,448)}$=79,200	p<0,001
			Time x Running **	$F_{(16,448)}$=2,539	P<0,001
			FR x Running	$F_{(1,28)}$=2,059	p=0,162
			Time x FR x Running	$F_{(16,448)}$= 1,649	p=0,053
AG in food restriction (pmol/L)	Two-way	AL/ALW n=8	Running	$F_{(1,28)}$=0,386	p=0,539
Figure 1B		FR/FRW n=8	FR ****	$F_{(1,28)}$=40,630	p<0,001
			FR x Running	$F_{(1,28)}$=0,009	p=0,755
LEAP-2 in food restriction (pmol/L)	Two-way	AL/ALW n=8	Running	$F_{(1,28)}$=0,025	p=0,874
Figure 1C		FR/FRW n=8	FR ****	$F_{(1,28)}$=11,020	p=0,002
			FR x Running	$F_{(1,28)}$=0,011	p=0,917
AG/LEAP-2 molar ratio in food restriction	Two-way	AL/ALW n=8	Running	$F_{(1,28)}$=0,559	p=0,461
Figure 1D		FR/FRW n=8	FR ****	$F_{(1,28)}$=30,490	p<0,001
			FR x Running	$F_{(1,28)}$=0,651	p=0,426
AG in refed mice (pmol/L)	RM	FR n=8	Refeeding ****	$F_{(1,13)}$=26,370	p<0,001
Figure 1E		FRW n=7	Running	$F_{(1,13)}$=1,310	p=0,272
			Refeeding x Running	$F_{(1,13)}$=0,007	p=0,936
LEAP-2 in refed mice (pmol/L)	RM	FR n=8	Refeeding ****	$F_{(1,14)}$=17,630	p<0,001
Figure 1F		FRW n=8	Running	$F_{(1,14)}$=0,377	p=0,055
			Refeeding x Running	$F_{(1,14)}$=1,458	p=0,247
AG/LEAP-2 molar ratio in refed mice	RM	FR n=8	Refeeding ****	$F_{(1,13)}$=20,530	p<0,001
Figure 1G		FRW n=8	Running	$F_{(1,13)}$=0,193	p=0,668
			Refeeding x Running	$F_{(1,13)}$=1,041	p=0,326

Table S1. ANOVA table of preclinical data, related to Figure 1. Data represent mean±SEM and were analyzed using three-way ANOVA (A), two-way ANOVA (B-D) or RM ANOVA (E-G).

*** p<0.001, **** p<0.0001. AG: Acyl-ghrelin. LEAP-2: Liver-expressing Antimicrobial Peptide-2.

	V1 (n=30)	V2 (n=30)	Paired Student t-test
Age (years)	27,00±1,68	-	
Duration of illness (months)	92,27±17,70	-	
Days before inclusion	4,43±0,44	-	
Subtype			
AN-R	77,0% (n=23)	-	
AN-BP	23,3% (n=7)	-	
BMI (kg/m2) ****	14,22±0,24	20,11±0,09	t=26,6 df=29 p<0,0001
EDI-2 ****	90,64±9,00	62,94±7,73	t=5,21 df=29 p<0,0001
EAI **	25,61±2,36	21,51±2,04	t=2,90 df=29 p<0,01
Weekly Leisure Activity (hour) *	44,94±5,99	30,76±3,19	t=2,56 df=29 p=0,01
AG (pmol/L) ****	36,22±5,36	20,79±3,88	t=5,47 df=29 p<0,0001
LEAP-2 (pmol/L) *	3830±390,60	3095±303,60	t=2,06 df=29 p<0,05
AG/LEAP-2 molar ratio**	0,01±0,0001	0,007±0,0009	t=3,07 df=29 p<0,01

Table S2. Epidemiological, clinical, and biological data of 30 patients in the longitudinal follow-up study, related to Figure 3. Data represent mean±SEM and were analyzed using paired t-test: *p<0.05, **p<0.01, ****p<0.0001. AN-R: Anorexia Nervosa Restrictive subtype, AN-BP: Anorexia Nervosa, Binge Purge subtype. BMI: Body Mass Index. EDI-2: Eating Disorder Inventory-2, EAI: Exercise Addiction Index. AG: Acyl-ghrelin, LEAP-2: Liver Expressing Antimicrobial Peptide-2.

		Stable remission	Unstable Remission	Student or Mann-Whitney test
N		12	17	
Age (years)		25.58±1.63	28,00±2,76	p=0,879
Duration of illness (months)		96,67±27,62	94,35±24,83	p=0,939
BMI (kg/m2)	V1	14,66±0,35	13.9±0.33	p=0,140
	V2*	20,25±0,09	20.01±0.15	p=0,043
	V3****	20.07±0.44	16.58±0.35	p<0,001
Weight gain (% initial body weight)		39.23±4.21	45.08±2.51	p=0,27
EDI-2	V1	111.1±15.25	81.1±12.13	p=0,136
	V2	77.67±14.69	57.00±10.23	p=0,234
	V3	80.33±15.97	38.60±15.63	p=0,053
EAI	V1	30.83±4.88	21.06±2.58	p=0,085
	V2	23.88±4.78	19.93±2.07	p=0,70
	V3	22.83±3.00	13.55±3.01	p=0,061
AG (pmol/L)	V1	31.99±7.88	39.96±7.71	p=0,444
	V2	19.05±6.47	22.09±5.17	p=0,491
	V1 versus V2 in stable **			p=0,009
	V1 versus V2 in unstable ***			p<0,001
LEAP-2 (pmol/L)	V1	3469±672.3	4202±490.1	p=0,131
	V2	3291±506.1	3021±405.1	p=0,678
	V1 versus V2 in stable			p=0,640
	V1 versus V2 in unstable *			p=0,044
AG/LEAP-2 molar ratio	V1	0.011±0.003	0.011±0.002	p=0,913
	V2	0.006±0.001	0.008±0.001	p=0,394
	V1 versus V2 in stable *			p=0,043
	V1 versus V2 in unstable			p=0,078

Table S3. Epidemiological, clinical and biological data on patients evaluated 6 months after discharge, related to Figure 4. Patients were characterized with either stable remission

(n=12) if BMI≥18,5 or unstable remission (n=17) if BMI<18,5. Data represent mean±SEM and were analyzed using Student t-test or non-parametric Mann Whitney test depending on data distribution. *p<0.05, **p<0.01, *** p<0.001, **** p<0,0001. BMI: Body mass Index (kg/$m^{2)}$, AG: Acyl-ghrelin. LEAP-2: Liver-expressing Antimicrobial Peptide-2. V1: undernourished state at admission; V2: refed state at discharge.

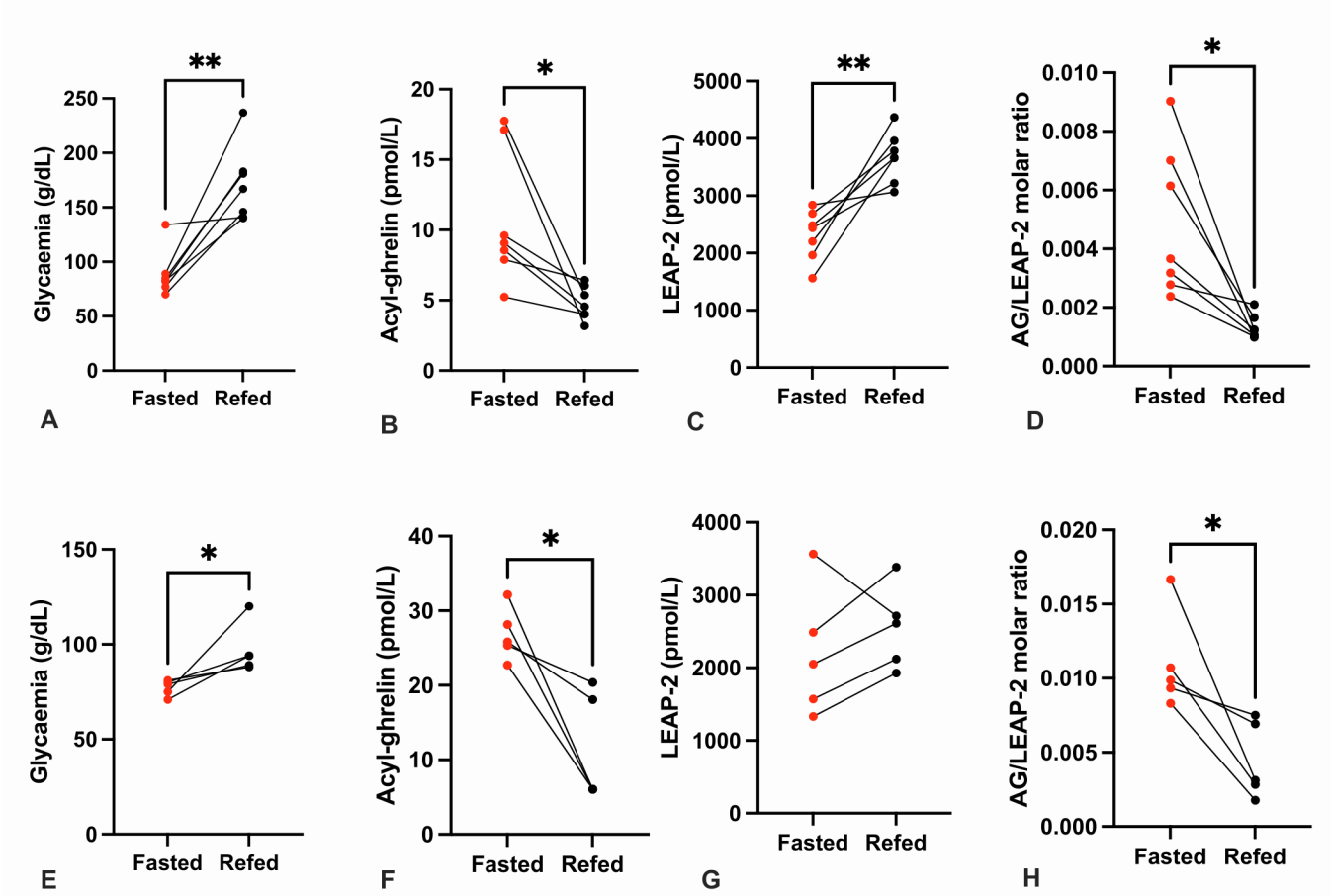

Figure S1. Longitudinal changes in plasma AG and LEAP-2 levels during acute fasting and refeeding in eight-week-old female mice (A-D) and in healthy women (E-H), related to the STAR Methods. Acute 48h fasting in female significantly decreased blood glucose levels (A) that was restored after 48h *ad libitum* refeeding. Plasma levels of AG were significantly decreased (B) but LEAP-2 levels (C) were increased with refeeding. AG/LEAP-2 molar ratio (D) was significantly higher in fasted compared to refed animals. Blood glucose and plasma AG and LEAP-2 levels were assayed in five healthy women after a 15-hours fast and 2 hours after a standard meal. Plasma levels of glucose (E) increased after a meal and AG (F) decreased. LEAP-2 levels increased after the meal in four participants out of five. AG/LEAP-2 molar ratio (H) were significantly higher in fasted compared to post-prandial state in humans. Data are presented as individual values. AG: Acyl-ghrelin. LEAP-2: Liver-expressing Antimicrobial Peptide-2. Statistical analysis: Paired t-test. *p<0.05, ** p<0.01.