

Supplemental Experimental Procedures

In Exp 3, mice were allocated to one of two experimental groups. HF (the control group $n=12$ mice) or the HF+BCAA (the experimental group, $n=12$). As above HF mice were provided free access to the same high fat diet containing 60 kJ% fat (D12492) and drank water ad libitum starting at $t=-1$ wk (i.e., a week before $t=0$). As in exp 1 and 2, this group continued to receive water throughout the study. The HF+BCAA also received the high fat diet and water for the first week. However at $t=0$ wk their water was switched to BCAA containing water (109 mmol/L each of Leu, Ile and Valine). The Leu concentration used was lower in Exp 3 because the limit of Leu solubility is altered when all three BCAA are dissolved. During the last few weeks, calorimetry, locomotor activity, and insulin tolerance were measured. On one occasion, blood was taken after the 5h period of food deprivation.

Supplemental Results

Body weight, food intake and fluid intake. Adding BCAA to the drinking water did not affect body weight, food intake or daily fluid intake between the HF and HF+Leu groups, though it caused slightly less than a doubling of Leu intake (Supplemental Figs 7 and 8 and data not shown).

VO_2 , RQ and locomotor activity. O_2 consumption, RQ and locomotor activity were measured after wk 16 of BCAA supplementation. There was no effect of BCAA supplementation on these parameters when the HF and HF+Leu groups were compared (Supplemental Fig 9).

Plasma total cholesterol. Supplemental Table 1 shows that BCAA supplementation did not significantly improve the plasma

cholesterol concentration however there was a trend toward lower mean values in the HF+Leu compared to the HF group.

Fasting glucose and insulin sensitivity.

Fasting plasma glucose concentrations were not significantly different after chronic BCAA supplementation between the HF and HF+Leu groups (Supplementary Table 2). An insulin tolerance test was performed after 14 wk of high fat feeding and BCAA supplementation to assess insulin sensitivity (Supplemental Fig 10). No significant changes in insulin sensitivity were suggested by these tests between the HF and HF+Leu groups.

SUPPLEMENTAL TABLE 1 Plasma total cholesterol in HF and HF+BCAA mice from Exp 3¹

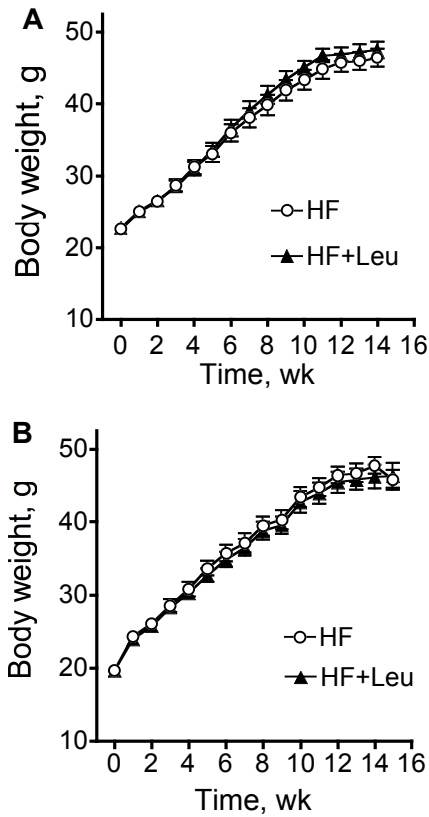
Exp	Group	Total Cholesterol, $mmol/L^{-1}$	n	P
3	HF	3.9±0.1	11	0.26
	HF+BCAA	3.8±0.2	11	

¹Results are mean and standard error, n of determinations. The n shown is lower than n of mice because of insufficient plasma. Mice were food deprived over-night but continued to receive BCAA in the water

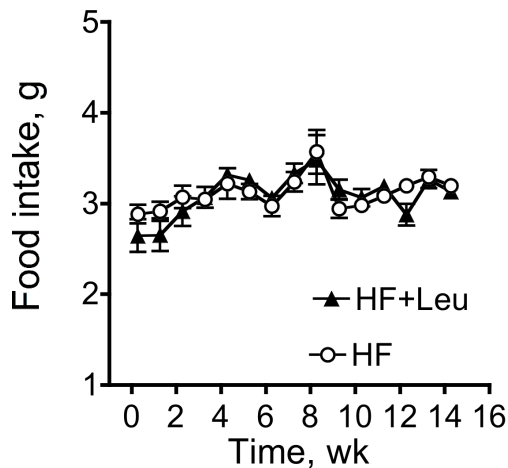
SUPPLEMENTAL TABLE 2 Whole blood glucose in HF and HF+BCAA mice from Exp 3¹

Exp	Group	Glucose, $mmol/L$	n	p-value
3	HF	12.2±0.4	12	0.27
	HF+BCAA	12.8±0.4	12	

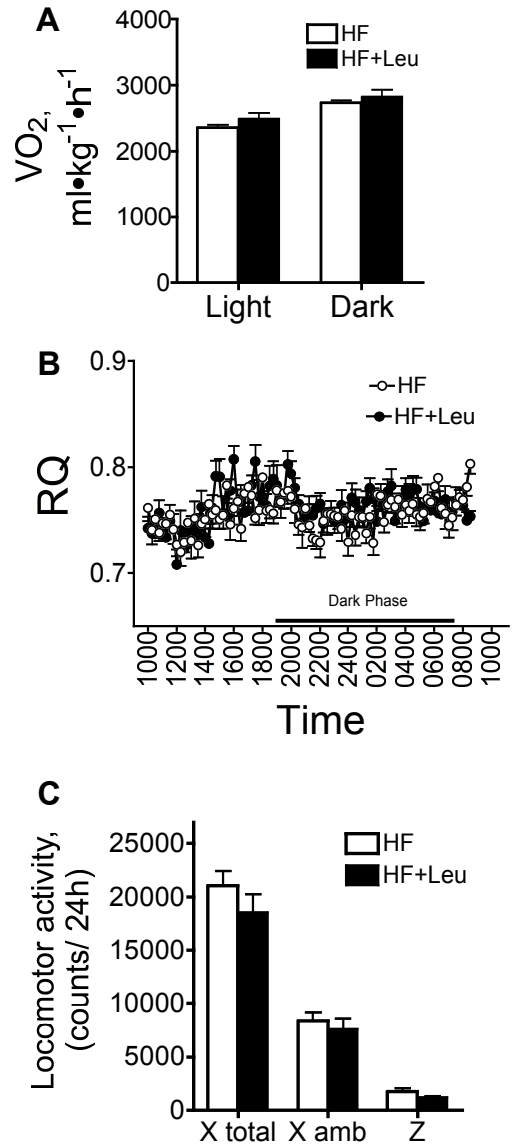
¹ Mice were food deprived for 5 h and all switched to water before the glucose measurements during wk 12. The results are mean and standard errors



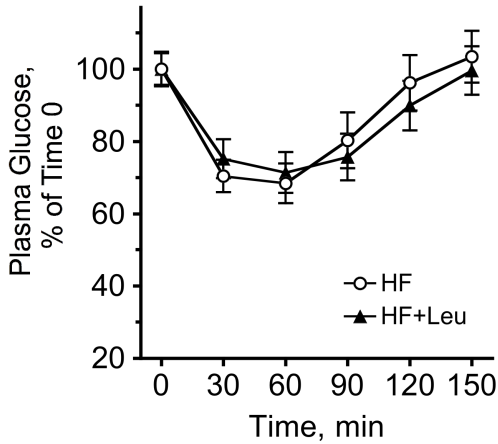
SUPPLEMENTAL FIGURE 1. Body weights of HF and HF+Leu mice in Exp 1 (A) and Exp 2 (B). Data are mean \pm SE. The n = 12 (A) or 15 (B).



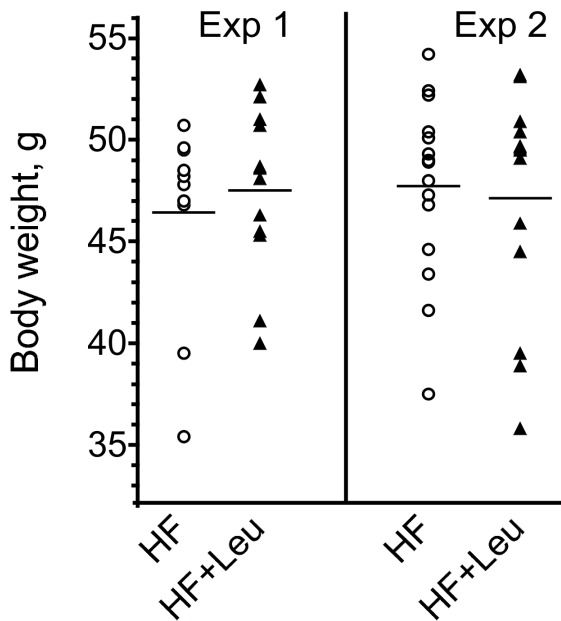
SUPPLEMENTAL FIGURE 2. Food intake of HF and HF+Leu mice in Exp 2. Data are mean \pm SE and n=15. Similar results were observed in Exp 1 (data not shown).



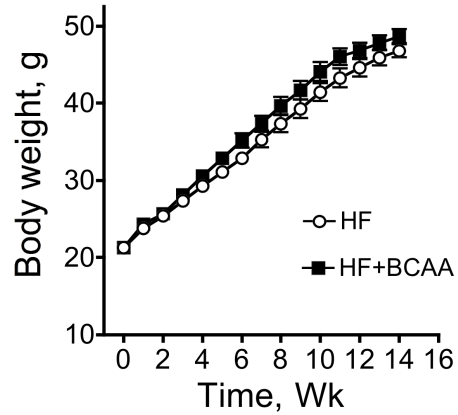
SUPPLEMENTAL FIGURE 3. VO_2 (A), RQ (B) and locomotor activity (C) in Exp 2 HF and HF+Leu mice. Oxygen consumption (VO_2 , Panel A), RQ (B) and locomotor activity (C) were measured after wk 14 wk. Data are mean \pm SE and n=15.



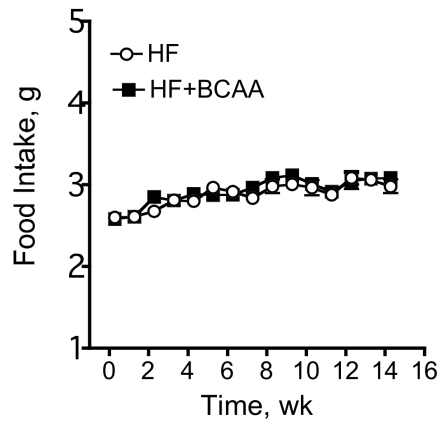
SUPPLEMENTAL FIGURE 4. Effect of Leu supplementation on insulin sensitivity after the 5h of food deprivation of HF and HF+Leu mice in Exp 1. Blood glucose was measured before and after intraperitoneal injection of Insulin (0.75 mU per gram body weight). Data are mean± SE and n=12. Similar results were observed in Exp 2 (data not shown).



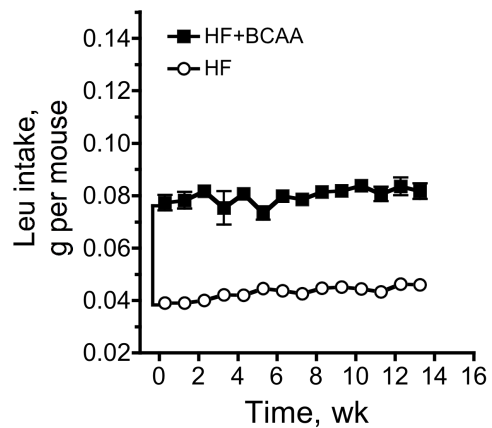
SUPPLEMENTAL FIGURE 5. Individual wk 14 body weights are plotted for the HF and HF+Leu group using data from exp 1 (n=12) and 2 (n=15). The symbols represent individual body weights and the horizontal line indicates the mean body weight.



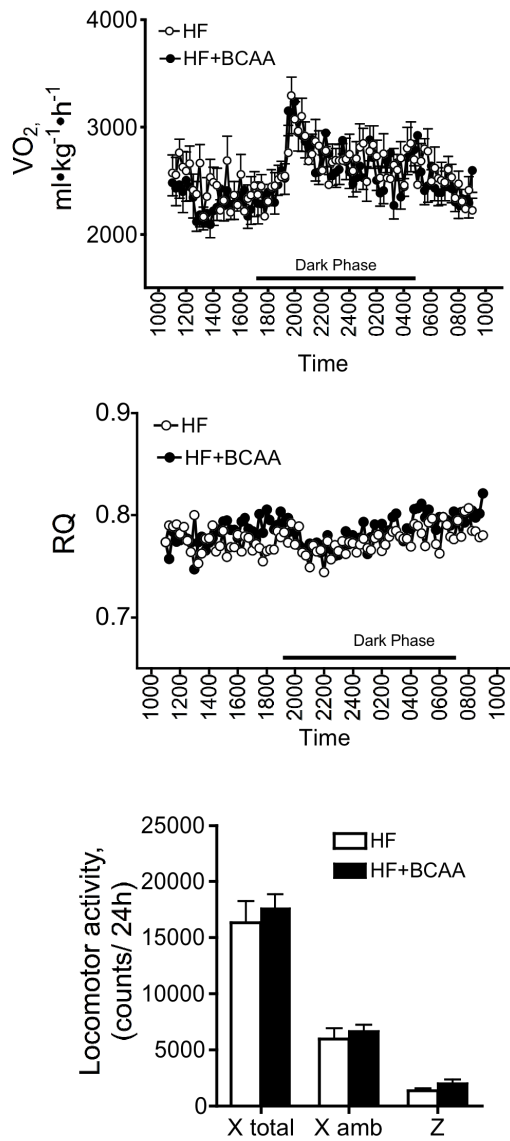
SUPPLEMENTAL FIGURE 6. Body weights of HF and HF+BCAA mice in Exp 3. Data are mean± SE, n = 12.



SUPPLEMENTAL FIGURE 7. Food intakes in HF and HF+BCAA mice in Exp 3. Data are mean± SE, n=12.



SUPPLEMENTAL FIGURE 8. Leu intakes of HF and HF+BCAA mice in Exp 3. Data are mean± SE, n = 12. All of the HF+Leu means are statistically different from HF, p<0.05.



SUPPLEMENTAL FIGURE 9. VO_2 (A), RQ (B) and locomotor activity (C) of HF and HF+Leu mice in Exp 3. Oxygen consumption (A), RQ (B) and locomotor activity (C) were measured after wk 14 wk. Data are mean \pm SE, n=15.