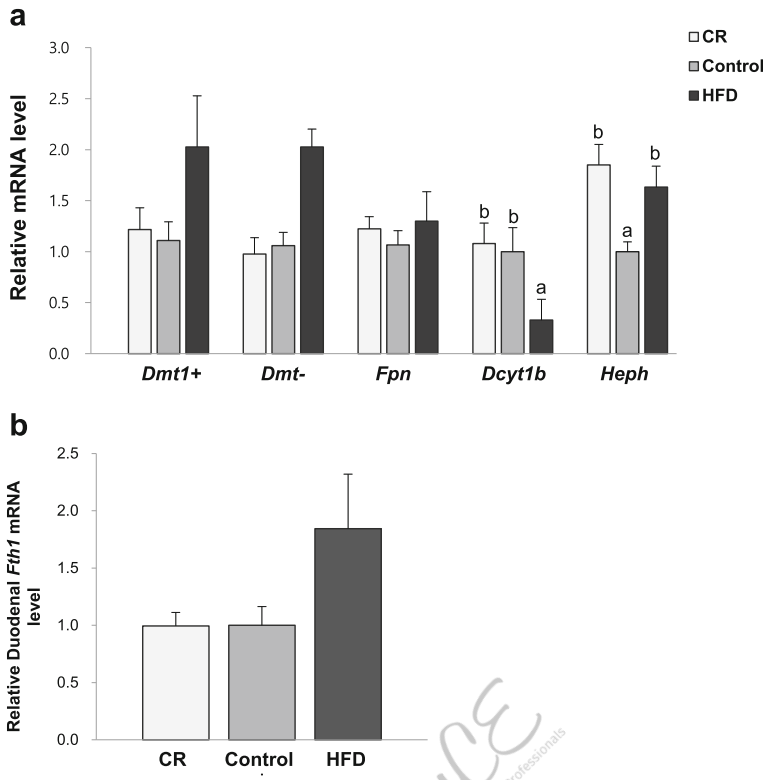

Fig. 5 Relative duodenal mRNA levels of ferritin and enzymes involved in iron transport: **a** *Dmt1*, *Fpt(ferroportin)*, *Heph*(*hephaestin)*, and *Dcyt1b* mRNA levels; **b** *Fth1(ferritin)* mRNA levels. Data are presented as mean ± SEM, $n = 7–12$ for each group. [ab]Different letters indicate $P < 0.05$. One-way ANOVA followed by Fisher's LSD multiple comparison was used to determine significant difference. All values were normalized to the levels of housekeeping gene *Gapdh* and expressed as relative mRNA level compared to the average level of the Control group

could ameliorate hepcidin expression in response to LPS. In this study, although *Mcp-1* and *Tnf-α* mRNA levels were higher in the HFD group, indicative of increased inflammatory response, hepcidin levels were lower than the other groups. Lower iron levels in the HFD group could have overridden the effect of these inflammatory mediators on hepcidin expression. Thus, hepcidin levels seemed to be mainly regulated by bodily requirements for iron in this study.

Expression of iron absorption related enzymes in duodenum tissue was determined to investigate the association between adiposity and iron status. mRNA levels of *Dcyt1b* and *Heph*, which is located at both sides of enterocyte and facilitate iron transport, were highest in the CR group. Dcyt1b is the ferric reductase that converts ferric iron to the ferrous state that enables iron to enter the apical side of enterocyte through the Dmt1, and hephaestin is the ferroxidase at the basolateral side of the enterocyte [41, 42]. Calorie restriction could have promoted iron absorption by upregulating those duodenal enzymes that enable the physical state of iron to enter the enterocyte. Sonnweber et al. [19] reported lowered *Dcyt1b* and *Heph* mRNA levels in obese mice compared with control mice. They suggested that decreased levels of ferroxidases were associated with iron deficiency in obesity. In this study, *Dcyt1b* expression was lowest in the HFD group and it showed significant negative correlation with white adipose tissue amount ($r = -0.509$), however, hephaestin level was upregulated in the HFD group compared with the Control group. Considering that hephaestin is affected by stored iron amount rather than by instant dietary iron intake [41], low iron status in the HFD group could have influenced *Heph* expression and led to higher expression in the HFD group. However, further research is needed to identify the relationship between adiposity and duodenal iron absorption and to find obesity related factors that regulate expression of iron transport enzymes.

Conclusions

This study confirmed that liver iron status has an inverse relationship with body adiposity. More importantly, we showed that liver iron levels could be upregulated in low adiposity through mild calorie restriction. Low inflammatory state induced by calorie restriction could be responsible for higher hepatic iron storage. Upregulated duodenal iron transport enzymes appeared to contribute to higher hepatic iron levels in the CR group.

Abbreviations

ANOVA: One-way analysis of variance; BMP: Bone morphogenetic protein; CR: Calorie restriction; CRP: C-reactive protein; DCYT1B: Duodenum cytochrome 1b; DMT1: Divalent metal transporter1; ECL: Enhanced chemiluminescence; HFD: High fat diet; IFN-γ: Interferon gamma;
IL-6: Interleukin-6; LPS: Lipopolysaccharide; LSD: Least significant difference; MCP-1: Monocyte chemoattractant protein 1; ROS: Reactive oxygen species; SPF: Specific pathogen free; TNF-α: Tumor necrosis factor alpha

Competing interests

The authors declare that they have no competing interests.

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