Supporting Information

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Fig. S1. Experimental outline of the study and representative HP $^{13}$C dataset. (A) CTRL and 0.2% CPZ animals were imaged using $T_2$-weighted MRI and $^{13}$C MRSI of HP [1-$^{13}$C]pyruvate at four distinct time points: week 0, 4, 6, and after a 6-wk period of standard chow diet to allow recovery (W0, W4, W6, and W6 + W6 recovery). A subset of animals was killed at each time point for immunofluorescence analysis and enzyme activity assays. (B) $T_2$-weighted MR image of a mouse head is overlaid with the grid used for HP $^{13}$C MR acquisition. The region of interest (corpus callosum) is highlighted in red (white box). Corresponding stack plot of HP $^{13}$C spectra show HP [1-$^{13}$C]pyruvate delivery and subsequent HP [1-$^{13}$C]lactate production over time in the voxel of interest [3-s temporal resolution, 16 time points (TP)]. All dynamic spectra (shown in black) are summed (shown in red) and integrals of HP [1-$^{13}$C]pyruvate and HP [1-$^{13}$C]lactate calculated for each imaging session and each animal.
Fig. S2. Contrast-enhanced MRI following CPZ diet. (A) Representative $T_1$-weighted MR images of a CTRL mouse and a mouse at W4 CPZ, prior to (pre-contrast) and after (post-contrast) injection of a gadolinium-based contrast agent (arrow indicates the time of contrast agent injection), showing characteristic enhancement of surrounding tissues and blood vessels on postcontrast images. Time courses of signal intensity (B) for the corpus callosum and the thalamus, showing an increase in both regions following contrast agent injection in CTRL and W4 CPZ mice. Quantitative analyses of the area under the curve (C), slope (D), and relative signal enhancement (E) showed no significant differences between CTRL and W4 CPZ mice ($n=3$ mice per group). All values are reported as mean ± SD, except $D$, which displays mean ± SEM. AU, arbitrary unit.
Fig. S3. Immunofluorescence staining of the corpus callosum, thalamus, and cortex following CPZ diet and evaluation of PDH activity in the thalamus. (A) Representative immunofluorescence images of MPs (Iba-1, red) and PDK1 (green) in the thalamus and cortex showing no expression of PDK1, and hence no colocalization with Iba-1 at any time point of interest (n = 3 mice per group). (Scale bar: 100 μm.) (B) Representative immunofluorescence staining of astrocytes (GFAP, red) and OPCs (PDGFR-α, red) showing no colocalization with PDK1 staining (green) in the corpus callosum at any time points of interest (n = 3 mice per group). (Scale bar: 100 μm.) (C) Quantitative analyses of PDH enzyme activity in the thalamus revealed no significant changes following CPZ diet (n = 4–7 mice per group). (D) Immunofluorescence staining for LDH-A and nuclear staining (Hoechst) show that LDH-A is not detected in the corpus callosum following W4 of CPZ diet. LDH-A expression was found in the CTRL area CA-1 of the hippocampus (n = 3 mice per group). (Magnification: 20×.)
Fig. S4. Immunofluorescence staining and HP [1-13C]pyruvate delivery in CX3CR1GFP/GFP mice following W4 of CPZ diet. (A) Representative immunofluorescence staining for cell infiltration (Hoechst, blue), myelin (MBP, red), astrocytes (GFAP, red), and MPs (Iba-1, red) shows no evident changes between CX3CR1GFP/GFP mice that received a CTRL chow or W4 of CPZ diet. (Scale bar: 100 μm.) HP [1-13C]pyruvate levels in CX3CR1GFP/GFP were not significantly different between CTRL and CPZ groups over time in (B) the corpus callosum, (C) the thalamic area, and (D) the neck region (n = 6 mice per group). All values are reported as mean ± SD. A.U., arbitrary unit.