

Supplementary material

Animals and dietary regimen.

Four-week-old male C57BL/6J mice were purchased from SAS (Saint-Germain-Nuelles, France). The mice were fed a standard chow (CHOW) *ad libitum* starting at the age of seven weeks. The standard chow diet derives 9% of its energy from fat, 58% from carbohydrates and 33% from protein and comprises 3% fat and 5% sucrose. The CHOW (V1534) diet was purchased from Ssniff (Soest, Germany). All mice were housed in laboratory cages at 21-23 °C with 50-60% humidity (10-15 exchanges per h) with tap water and diet provided *ad libitum* for sixteen weeks. For tissue collection, the mice were anesthetized with isoflurane and further sacrificed by cervical dislocation.

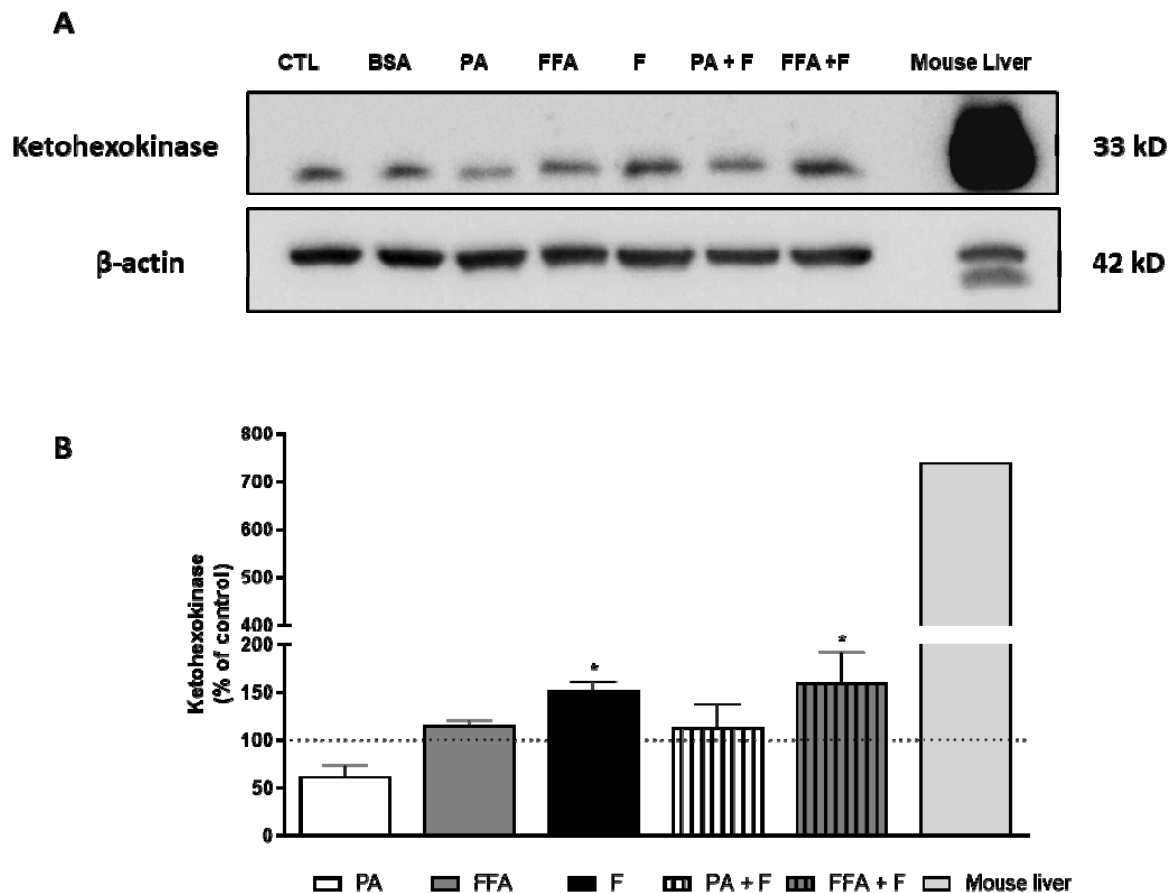


Fig. S1: Effect of supra-physiological concentration of fructose on hepatic fructokinase protein level. (a) Typical Western blot result of whole cell homogenates showing the protein level of Ketoheokinase (KHK) in cells treated with palmitic acid (PA, 0.5 mM) or a mix of free fatty acids (FFA, 0.25 mM) in the presence or absence of F (F, 10 mM) for 24 h. Mouse liver homogenate (30 µg) was used as positive control, as KHK is highly expressed in liver. This blot was inverted and contrast-optimized for visualization purposes. Quantification of the bands was performed using the original blots. (b) Quantification of KHK protein levels under the different treatment conditions normalized to β-actin levels and for the control group (100% marked by a dotted line). Data are the mean ± SEM of four independent experiments, and the

results normalized on the control condition (CT = 100 %, marked by a dotted line). Significance was accepted with *P < 0.05 for comparisons between treatment vs CTL or 0.1 % BSA.

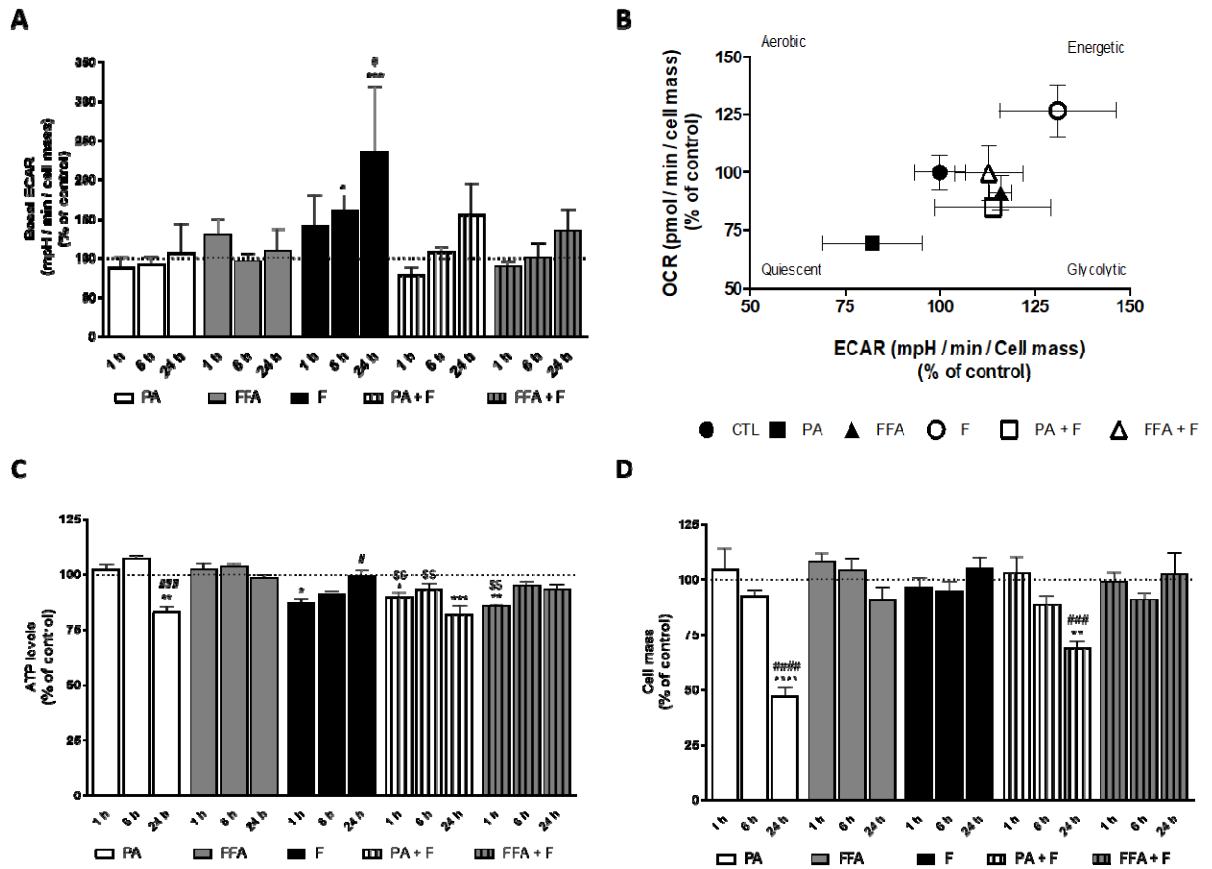


Fig. S2: Time-dependent effects of fatty acid excess on extracellular acidification, ATP levels and cell mass. (a) Basal extracellular acidification rate of HepG2 cells treated with palmitic acid (PA, 0.5 mM) or a mix of free fatty acids (FFA, 0.25 mM) in the presence or absence of fructose (F, 10 mM) for 1, 6 and 24 h. (b) The average mitochondrial basal OCR was plotted against the average basal ECAR (data points taken from Fig. 5b and S2a) in cells submitted to the treatments described above. (c) Same as panel A but now for cell mass density. Data are the mean \pm SEM of four independent experiments, and the results normalized on the control condition (CT = 100 %, marked by a dotted line). Significance was accepted with *P < 0.05, **P < 0.01, ***P < 0.0005, ****P < 0.0001 for comparisons between treatment vs CTL (BSA 0,01g/mL) and *P < 0.05, ***P < 0.0005, ****P < 0.0001 for comparisons during time in the same group (24 and 6 h vs 1 h). Significance for additional fructose effect as accepted with ^{ss}P < 0.01.