

Table S1: Dietary fatty acid composition

| <b>Fatty acid</b>           | <b>LFD</b> | <b>HFD</b> |
|-----------------------------|------------|------------|
| C12:0 and less              | No data    | 3.20%      |
| Myristic Acid 14:0          | No data    | 0.90%      |
| Palmitic Acid 16:0          | 0.20%      | 7.10%      |
| Stearic Acid 18:0           | 0.10%      | 9.30%      |
| Arachidic acid 20:0         | No data    | 0.30%      |
| Palmitoleic Acid 16:1       | No data    | 0.10%      |
| Oleic Acid 18:1             | 2.40%      | 12.00%     |
| Gadoleic Acid 20:1          | trace      | 0.10%      |
| Linoleic Acid 18:2 n6       | 0.80%      | 2.00%      |
| α Linolenic Acid 18:3 n3    | 0.40%      | 0.70%      |
| Arachadonic Acid 20:4 n6    | No data    | No data    |
| EPA 20:5 n3                 | No data    | trace      |
| DHA 22:6 n3                 | No data    | No data    |
| Total n3                    | 0.45%      | 0.74%      |
| Total n6                    | 0.76%      | 2.05%      |
| Total Mono Unsaturated Fats | 2.46%      | 12.20%     |
| Total Polyunsaturated Fats  | 1.21%      | 2.79%      |
| Total Saturated Fats        | 0.28%      | 20.92%     |

Table S2: Exercise training protocol

| Week | Day  | Speed and Time   | Week | Day  | Speed and Time   |
|------|------|------------------|------|------|------------------|
| 1    | Mon  | 12m/min (30min)  | 4    | Mon  | 15 m/min (50min) |
|      | Tues | 12m/min (30min)  |      | Tues | 15 m/min (55min) |
|      | Wed  | 12m/min (30min)  |      | Wed  | 15 m/min (60min) |
|      | Thu  | 13m/min (30min)  |      | Thu  | 16 m/min (60min) |
|      | Fri  | 13m/min (30min)  |      | Fri  | 16 m/min (60min) |
|      | Sat  | -                |      | Sat  | -                |
|      | Sun  | -                |      | Sun  | -                |
| 2    | Mon  | 14 m/min (30min) | 5    | Mon  | 16 m/min (60min) |
|      | Tues | 14 m/min (30min) |      | Tues | 16 m/min (65min) |
|      | Wed  | 14 m/min (40min) |      | Wed  | 16 m/min (65min) |
|      | Thu  | 14 m/min (40min) |      | Thu  | 16 m/min (70min) |
|      | Fri  | 15 m/min (40min) |      | Fri  | 17 m/min (70min) |
|      | Sat  | -                |      | Sat  | -                |
|      | Sun  | -                |      | Sun  | -                |
| 3    | Mon  | 15 m/min (40min) | 6    | Mon  | 17m/min(70min)   |
|      | Tues | 15 m/min (45min) |      | Tues | 17 m/min (70min) |
|      | Wed  | 15 m/min (45min) |      | Wed  | 18 m/min (70min) |
|      | Thu  | 15 m/min (50min) |      | Thu  | 18 m/min (70min) |
|      | Fri  | 15 m/min (50min) |      | Fri  | 19 m/min (70min) |
|      | Sat  | -                |      | Sat  | -                |
|      | Sun  | -                |      | Sun  | -                |

**Figure S1: Hypothalamic lipid accumulation in response to obesity in ob/ob mice.**

The hypothalamus was excised from LFD and ob/ob mice and analysed for total lipid content. (A) Phospholipid content, dialkylphosphatidylcholine (diAPC), lysophosphatidylcholine (LPC), odd chain phosphatidylcholine (odd PC), phosphatidylcholine (PC), alkylphosphatidylcholine (PC(O)), alkenylphosphatidylcholine (PC(P)), phosphatidylethanolamine (PE), alkylphosphatidylethanolamine (PE(O)), alkenylphosphatidylethanolamine (plasmalogen) (PE(P)), phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylserine (PS). (B) Sterol lipid content, cholesterol ester (CE) and cholesterol (COH). Sphingolipids dihydroceramide (dh Cer), ceramide (Cer), sphingomyelin (SM), hydroxyphingomyelin (SM(OH)), G<sub>M3</sub> ganglioside (GM3), monohexosylceramide (MHC), dihexosylceramide (DHC) and trihexosylceramide (THC). Glycerolipid content, bis(monoacylglycero)phosphate (BMP), diacylglycerol (DAG) and triacylglycerol (TAG). LFD, white bars, ob/ob black bars. *n*=14 LFD, *n*=6 ob/ob, \**p*<0.05 vs. LFD

**Figure S2: Hypothalamic lipid accumulation in response to exercise during high fat feeding.**

The hypothalamus was excised from HFD and HFD Ex mice and analysed for total lipid content. (A) Phospholipid content, dialkylphosphatidylcholine (diAPC), lysophosphatidylcholine (LPC), odd chain phosphatidylcholine (odd PC), phosphatidylcholine (PC), alkylphosphatidylcholine (PC(O)), alkenylphosphatidylcholine (PC(P)), phosphatidylethanolamine (PE), alkylphosphatidylethanolamine (PE(O)), alkenylphosphatidylethanolamine (PE(P)), phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylserine (PS). (B) Sterol lipid content, cholesterol ester (CE) and cholesterol (COH). Sphingolipids dihydroceramide (dh Cer), ceramide (Cer), sphingomyelin (SM), hydroxyphingomyelin (SM(OH)), G<sub>M3</sub> ganglioside (GM3), monohexosylceramide (MHC), dihexosylceramide (DHC) and trihexosylceramide (THC). Glycerolipid content, bis(monoacylglycero)phosphate (BMP), diacylglycerol (DAG) and triacylglycerol (TAG). HFD, white bars, HFD Ex black bars. *n*=9 HFD, *n*=10 HFD Ex, \**p*<0.05 vs. HFD.

**Figure S3: Hypothalamic fatty acid content in DAG and TAG.**

Hypothalamus DAG and TAG content were analysed for the total amount of saturated (Sat), monounsaturated (Mono) and polyunsaturated (Poly) fatty acids. DAG (A) and TAG (C) fatty acid content in LFD vs. ob/ob mice. LFD, white bars, ob/ob black bars.  $n=14$  LFD,  $n=6$  ob/ob. DAG (B) and TAG (D) fatty acid content in HFD vs. HFD Ex mice. HFD, white bars, HFD Ex black bars.  $n=9$  HFD,  $n=10$  HFD Ex.

**Figure S4: Fatty acid composition of lipids in the hypothalamus relative to dietary lipid availability.**

Hypothalamus DAG (A), TAG (B) and PC (C) lipid content were analysed for the amount of saturated (Sat), monounsaturated (Mono) and polyunsaturated (Poly) fatty acids expressed as a percentage of the total lipid pool. This was compared against the percentage composition of fatty acid types in the low fat (LFD) and high fat diets (HFD). LFD white bars, HFD black bars.  $n=14$  LFD,  $n=9$  HFD.