Effects of restrictive cafeteria feeding and treadmill exercise on body composition, metabolic profile, locomotor activity, exploratory behaviour and HPA axis in obese rats.

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Supplementary Table S1. Cafeteria (CAF) diet composition for the first period of the experiment.

| Ingredient                | Week 1 | Week 4 | Week 8 |
|---------------------------|--------|--------|--------|
| Muffin                    | 3.25 g | 7 g    | 7 g    |
| Bacon                     | 3 g    | 6 g    | 8 g    |
| Carrot                    | 2.5 g  | 6 g    | 8 g    |
| Biscuits with pâté        | 3.5 g  | 5 g    | 5 g    |
| Biscuits with cheese      | 3.5 g  | 5 g    | 5 g    |
| Jellied sugared milk      | 25 g   | 40 g   | 45 g   |
| Chow                      | 15 g   | 15 g   | 25 g   |
| g of diet provided        | 56 g   | 84 g   | 103 g  |
| Kcal provided             | 131    | 197    | 246    |
Supplementary Table S2. Detailed treadmill training protocol for the progressive intensity increase from session 1 (week 1) until session 12 (week 2)

| Minute | S.1     | S.2     | S.3     | S.4     | S.5 – S.7 | S.8 – S.12 | S.13 onwards |
|--------|---------|---------|---------|---------|-----------|------------|--------------|
| 0      | 0 m/min | 0 m/min | 4 m/min | 4 m/min | 5 m/min   | 5 m/min    | 6 m/min      |
| 1      | 0 m/min | 0 m/min | 5 m/min | 5 m/min | 6 m/min   | 6 m/min    | 7 m/min      |
| 2      | 0 m/min | 0 m/min | 6 m/min | 6 m/min | 7 m/min   | 7 m/min    | 8 m/min      |
| 3      | 0 m/min | 0 m/min | 7 m/min | 7 m/min | 8 m/min   | 8 m/min    | 9 m/min      |
| 4      | 0 m/min | 0 m/min | 8 m/min | 8 m/min | 9 m/min   | 9 m/min    | 10 m/min     |
| 5      | 0 m/min | 0 m/min | 9 m/min | 9 m/min | 10 m/min  | 10 m/min   | 11 m/min     |
| 6      | 0 m/min | 5 m/min | 9 m/min | 9 m/min | 10 m/min  | 10 m/min   | 11 m/min     |
| 7      | 0 m/min | 6 m/min | 9 m/min | 10 m/min| 11 m/min  | 11 m/min   | 12 m/min     |
| 8      | 0 m/min | 7 m/min | 9 m/min | 10 m/min| 11 m/min  | 11 m/min   | 12 m/min     |
| 9 to 14| 0 m/min | 8 m/min | 9 m/min | 10 m/min| 11 m/min  | 11 m/min   | 12 m/min     |
| 15     | 4 m/min | 8 m/min | 9 m/min | 10 m/min| 11 m/min  | 11 m/min   | 12 m/min     |
| 16     | 5 m/min | 8 m/min | 9 m/min | 10 m/min| 11 m/min  | 11 m/min   | 12 m/min     |
| 17     | 6 m/min | 8 m/min | 9 m/min | 10 m/min| 11 m/min  | 11 m/min   | 12 m/min     |
| 18     | 7 m/min | 8 m/min | 9 m/min | 10 m/min| 11 m/min  | 11 m/min   | 12 m/min     |
| 19 to 24| 8 m/min  | 8 m/min  | 9 m/min | 10 m/min| 11 m/min  | 11 m/min   | 12 m/min     |
| 25 to 29| 8 m/min  | 8 m/min  | 9 m/min | 10 m/min| 11 m/min  | 11 m/min   | 12 m/min     |
| 30 ot 35| 8 m/min  | 8 m/min  | 7 m/min | 8 m/min  | 8 m/min   | 8 m/min    | 8 m/min      |
| Total distance (feet) | 400 ft | 660 ft | 860 ft | 920 ft | 1000 ft   | 1100 ft    | 1250 ft      |
| Total distance (meters) | 122 m | 201 m | 262 m | 280 m | 305 m     | 335 m      | 381 m       |
Supplementary Table S3. Statistical results for the ANOVA analysis of tissue weight

|                          | STD-CAF          |                |                | CAF-CAF          |                |                |
|--------------------------|------------------|----------------|----------------|------------------|----------------|----------------|
|                          | DIET             | EXE            | DIET*EXE       | DIET             | EXE            | DIET*EXE       |
|                          | F (1,34)         | p              | F (1,34)       | p                | F (1,32)       | p              | F (1,32)       | p                |
| Inguinal WAT (g)         | 65,156           | <0,001         | 0,000          | 0,994            | 1,030          | 0,317          | 4,711           | 0,038            | 0,814           | 0,374            | 3,464           | 0,072            |
| Retroperitoneal WAT (g)  | 141,496          | <0,001         | 3,527          | 0,069            | 0,252          | 0,619          | 9,314           | 0,005            | 1,099           | 0,302            | 0,023           | 0,881            |
| Mesenteric WAT (g)       | 51,068           | <0,001         | 2,133          | 0,153            | 0,000          | 0,996          | 6,710           | 0,014            | 0,649           | 0,427            | 0,721           | 0,402            |
| Epididymal WAT (g)       | 135,051          | <0,001         | 0,277          | 0,602            | 1,252          | 0,271          | 5,239           | 0,029            | 0,003           | 0,955            | 0,364           | 0,550            |
| Visceral WAT (g)         | 137,092          | <0,001         | 2,585          | 0,117            | 0,315          | 0,578          | 9,707           | 0,004            | 0,702           | 0,408            | 0,027           | 0,870            |
| Total WAT (g)            | 102,576          | <0,001         | 0,566          | 0,457            | 0,722          | 0,401          | 7,956           | 0,008            | 0,906           | 0,348            | 1,099           | 0,302            |
| Soleus muscle (g)        | 0,100            | 0,754          | 1,304          | 0,261            | 0,659          | 0,423          | 0,737           | 0,397            | 1,695           | 0,202            | 0,580           | 0,452            |
| Gastrocnemius muscle (g) | 0,759            | 0,390          | 0,423          | 0,520            | 0,204          | 0,654          | 5,014           | 0,032            | 0,768           | 0,387            | 0,009           | 0,923            |
| Adrenal gland (mg)       | 0,064            | 0,802          | 0,201          | 0,657            | 0,011          | 0,918          | 0,229           | 0,636            | 0,009           | 0,924            | 0,515           | 0,478            |
| Thymus (mg)              | 3,806            | 0,059          | 0,563          | 0,458            | 0,103          | 0,751          | 0,805           | 0,376            | 1,472           | 0,234            | 0,665           | 0,421            |
| Inguinal WAT (g/100g)    | 55,137           | <0,001         | 0,022          | 0,883            | 1,424          | 0,241          | 2,714           | 0,109            | 1,027           | 0,319            | 4,012           | 0,054            |
| Retroperitoneal WAT      | 136,984          | <0,001         | 6,169          | 0,018            | 0,622          | 0,436          | 6,697           | 0,014            | 1,193           | 0,283            | 0,135           | 0,716            |
| (g/100g)                 |                  |                |                |                  |                |                |                  |                  |                  |                  |                  |                  |
| Mesenteric WAT (g/100g)  | 32,888           | <0,001         | 3,546          | 0,068            | 0,013          | 0,912          | 5,231           | 0,029            | 0,783           | 0,383            | 2,398           | 0,131            |
| Epididymal WAT (g/100g)  | 118,349          | <0,001         | 1,162          | 0,289            | 3,326          | 0,077          | 1,793           | 0,190            | 0,096           | 0,758            | 0,223           | 0,640            |
| Visceral WAT (g/100g)    | 142,560          | <0,001         | 5,479          | 0,025            | 0,837          | 0,367          | 7,541           | 0,010            | 0,758           | 0,391            | 0,289           | 0,595            |
| Total WAT (g/100g)       | 100,955          | <0,001         | 1,323          | 0,258            | 1,348          | 0,254          | 5,776           | 0,022            | 1,198           | 0,282            | 1,224           | 0,277            |
| Soleus muscle (g/100g)   | 11,586           | 0,002          | 1,251          | 0,271            | 0,823          | 0,371          | 0,011           | 0,916            | 1,519           | 0,227            | 1,514           | 0,228            |
| Gastrocnemius muscle     | 31,851           | <0,001         | 0,100          | 0,753            | 0,186          | 0,669          | 0,254           | 0,618            | 0,091           | 0,765            | 0,256           | 0,616            |
| (g/100g)                 |                  |                |                |                  |                |                |                  |                  |                  |                  |                  |                  |
| Adrenal gland (mg/100g)  | 4,652            | 0,038          | 0,240          | 0,627            | 0,001          | 0,977          | 0,109           | 0,743            | 0,010           | 0,922            | 0,207           | 0,652            |
| thymus (mg/100g)         | 0,105            | 0,748          | 0,579          | 0,452            | 0,188          | 0,668          | 0,047           | 0,829            | 1,032           | 0,317            | 0,620           | 0,476            |
**Supplementary Table S4. Statistical results for the ANOVA analysis of metabolic serum parameters**

| Parameter                        | STD-CAF | CAF-CAFR |
|----------------------------------|---------|----------|
|                                 | DIET    | EXE      | DIET*EXE | DIET    | EXE      | DIET*EXE |
| F (1,32)                         | p       | F(1,32)  | p        | F (1,32) | p        | F(1,32)  | p        |
| Glucose (mg/L)                   | 11,900  | 0.002    | 0.618    | 0.438    | 1,230    | 0.276    | 1,570    | 0.219    | 2,940    | 0.096    | 0.019    | 0.892    |
| Triacylglycerides (mg/L)         | 13,250  | 0.001    | 0.001    | 0.973    | 0.618    | 0.437    | 1,211    | 0.279    | 0,513    | 0.479    | 0.000    | 0.993    |
| Cholesterol (mg/L)               | 4,402   | 0.044    | 5,044    | 0.032    | 0,759    | 0.390    | 5,920    | 0.021    | 3,034    | 0.091    | 0.005    | 0.945    |
| LDL-Cholesterol (mg/L)           | 6,242   | 0.018    | 0.240    | 0.628    | 0,035    | 0.852    | 0,625    | 0.435    | 0.035    | 0.852    | 0.485    | 0.491    |
| HDL-cholesterol (mg/L)           | 22,162  | 0.000    | 6,141    | 0.019    | 0.062    | 0.805    | 2,455    | 0.127    | 4,710    | 0.038    | 0.287    | 0.596    |
| NEFAs (mM)                       | 0.835   | 0.368    | 0.922    | 0.344    | 0.135    | 0.715    | 0.019    | 0.890    | 0.022    | 0.882    | 0.313    | 0.580    |
| insulin (µg/L)                   | 25,705  | 0.000    | 0.003    | 0.959    | 1.155    | 0.291    | 2,225    | 0.146    | 0.163    | 0.689    | 0.289    | 0.594    |
| HOMA-IR                          | 33,689  | 0.000    | 0.094    | 0.762    | 0.336    | 0.566    | 2,292    | 0.140    | 0.036    | 0.851    | 0.157    | 0.695    |
| Leptin (ng/mL)                   | 77,574  | 0.000    | 0.856    | 0.362    | 9.803    | 0.004    | 4,195    | 0.049    | 1.578    | 0.218    | 4,280    | 0.047    |
| Adiponectin (µg/mL)              | 13,053  | 0.001    | 0.052    | 0.821    | 3.071    | 0.089    | 0.294    | 0.591    | 0.053    | 0.819    | 1.437    | 0.239    |
| Leptin/adiponectin ratio         | 68,517  | 0.000    | 0.164    | 0.688    | 8.048    | 0.008    | 1,551    | 0.222    | 1,134    | 0.295    | 1,165    | 0.288    |
Supplementary Figure S5. Exercise decreased activity in the enclosed arms without affecting the open arms.

The first analysis of the EPM variables to evaluate the effects of CAF feeding and exercise upon total entries (Fig. 8.A), total distance travelled (Fig. 8.B) and time spent in the centre of the maze (Fig. 8.C) revealed CAF feeding to decrease the time spent in the centre of the maze \( [F(1,37)=5.978, p=0.020] \) (Fig. 8C). No other effects/interactions on these variables were found. The second analysis for comparing these variables between CAF and CAFR diet and exercise also revealed no effects/interactions.

We then proceeded to analyse the number of entries (Fig. 8.A), the distance travelled (Fig. 8.B) and the time spent into the open and enclosed arms (Fig. 8.C). The first analysis showed no effects/interactions on any of those variables. The second analysis reported an effect of exercise decreasing the number of entries and the distance travelled in the enclosed arms \( \text{entries: } F(1,36)=4.551, p=0.041; \text{ distance travelled: } F(1,36)=4.691, p=0.038 \), but not affecting the time spent in the enclosed arms. No other significant effects of the diet not ‘diet*exercise’ was found. These results taken together point to no major differences on anxiety-like behaviour as measured in the EPM between diet and exercise conditions.

Fig. S5. Anxiety-like behaviour in the Elevated Plus Maze test. A) Number of entries. B) Distance travelled (m). C) Time spent (seconds). * \( p<0.05 \) vs STD; # \( p<0.05 \) vs corresponding non-exercised group.
**Supplementary Table S6. Statistical results for the ANOVA analysis comparing STD and CAFR diets**

| Biometric parameters | Diet | Exercise |
|----------------------|------|----------|
| AC (cm)              | 1,37 | 25 <0.001 | 1,37 | 0,000 ns |
| **Tissues**          |      |          |      |          |
| Inguinal WAT (g)     | 1,37 | 50,625 <0.001 | 1,37 | 5,811 0,021 |
| Retroperitoneal WAT (g) | 1,37 | 89,701 <0.001 | 1,37 | 3,696 0,063 |
| Mesenteric WAT (g)   | 1,37 | 28,484 <0.001 | 1,37 | 0,612 ns |
| Epididymal WAT (g)   | 1,37 | 157,945 <0.001 | 1,37 | 2,636 ns |
| Abdominal WAT (g)    | 1,37 | 94,63 <0.001 | 1,37 | 2,727 ns |
| Total WAT (g)        | 1,37 | 84,802 <0.001 | 1,37 | 5,199 0,029 |
| Gastrocnemius (g)    | 1,37 | 3,922 0,056 | 1,37 | 0,413 ns |
| Retroperitoneal WAT (g/100g) | 1,37 | 74,148 <0.001 | 1,37 | 4,15 0,049 |
| Mesenteric WAT (g/100g) | 1,37 | 17,273 <0.001 | 1,37 | 0,596 ns |
| Abdominal WAT (g/100g) | 1,37 | 81,176 <0.001 | 1,37 | 3,122 0,086 |
| Total WAT (g/100g)   | 1,37 | 70,085 <0.001 | 1,37 | 5,876 0,021 |
| Gastrocnemius (g/100g) | 1,37 | 59,578 <0.001 | 1,37 | 0,037 ns |
| **Serum parameters** |      |          |      |          |
| Leptin (ng/mL)       | 1,37 | 20,727 <0.001 | 1,37 | 0,274 ns |
| Cholesterol (mg/L)   | 1,37 | 18,654 <0.001 | 1,37 | 5,515 0,025 |