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Modest Effects of a Resistance Training Protocol Against Obesity and Changes in Glucose Metabolism Caused by High Carbohydrate Diet in Male Swiss Mice

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HIGHLIGHTS

- High-carbohydrate diet caused obesity and impaired glucose metabolism in mice.
- The obese phenotype was not prevented by simultaneous resistance training.
- Training had only mild effects on systemic and liver glucose metabolism alterations.
- Training variables are suggested as important determinants of exercise efficacy.

Abstract: Imbalanced feeding and reduced physical activity cause obesity and metabolic impairments. It was investigated whether an obese phenotype and alterations of glucose metabolism because of a high-carbohydrate diet (HCD) in mice could be prevented by a protocol of high-intensity interval resistance training (HIIRT). Male Swiss mice were distributed in groups: CoS (n=24) control, sedentary and fed with standard rodent chow; ObS (n=20) obese, sedentary, and ObT (n=20) obese, trained, both fed with HCD. The training sessions (group ObT) were made on vertical ladder with 90% of the maximal load of each animal corrected weekly. After eight weeks, *in vivo* glucose monitoring tests, tissue and blood analyses (n=10 animals per group) and *in situ* liver perfusion (n=10-14 animals per group) were carried out. HCD significantly increased food ingestion and adiposity; caused liver lipid accumulation, high blood glucose and glucose intolerance; and diminished the liver output of glucose, lactate, pyruvate and nitrogen. Despite the markedly improved training performance of the ObT mice, adiposity or liver fat were not significantly changed, and HIIRT was only mildly successful as a preventive agent against the changes of glucose metabolism (restoring glucose output only with glycerol and lactate, and pyruvate output with alanine) and lipid profile (such as triglycerides, HDL, VLDL and TyG index), suggesting an insufficient energy expenditure of the designed protocol. These observations indicate that excess adiposity does not compromise high-intensity resistance training, but training format, modality, intensity and frequency are important determinants of exercise efficacy against obesity and metabolic impairments.

Keywords: glucose metabolism; interval resistance training; high carbohydrate diet; mouse; liver perfusion.

INTRODUCTION

Energy metabolism is kept at a physiologically appropriate condition through a delicate dynamic balance between food ingestion and the use, storage and replacement of energy substrates. This balance, or energy homeostasis, involves the Central Nervous System (CNS), visceral nerves, several hormones, and the tissues, of which skeletal muscle, white adipose tissue (WAT) and liver are the most prominent, the latter the key organ for energy homeostasis in general and glucose homeostasis in particular [1-7].

Under normal circumstances, energy homeostasis is flexible enough to adapt itself to changes in nutrient intake or energy expenditure, the daily feeding-fasting cycle being the most common instance of this modulation. Another example is the metabolic rearrangement that takes place when skeletal muscles are active and demanding much more energy than at rest [1,6-10].

Marked imbalances between food ingestion and energy expenditure, however, compromise metabolic flexibility and energy homeostasis. A positive energy balance results in obesity, a pathology of varied etiology, but mostly linked to increased caloric ingestion and/or sedentarism, resulting in excessive buildup of fat depots, development or worsening of many clinical and pathological conditions in several organs and tissues, and increased rates of morbidity and mortality [1,7,11-18].

Energy-rich foods ingested at levels higher than energy expenditure cause significant alterations in the liver, especially because of the excess fat in this organ. Liver steatosis prompts a pro-inflammatory environment and favors oxidative stress due to the release of cytokines and other bioactive substances that can potentially disturb carbohydrate and lipid metabolism by the liver, and leads, in the long run, to non-alcoholic fatty liver disease (NAFLD) [6,8,19-21], also named MAFLD (metabolic associated fatty liver disease) [12].

Among the many interventions aimed at minimizing obesity and its comorbidities, appropriate eating habits and regular physical exercise stand out as essential tools. The vast literature about the benefits of physical activity, both in healthy people and in patients having varied clinical conditions, including overweight and obesity [1,8,9,14,22], makes its prophylactic and therapeutic merits unquestionable.

This study aimed at observing the effects of eight weeks of a high carbohydrate diet (HCD) on adiposity, plasmatic parameters, liver lipids, and systemic and hepatic glucose metabolism in sedentary male Swiss mice. In addition, the possible preventive effects of high-intensity interval resistance training (HIIRT) were tested.

MATERIAL AND METHODS

Animals and Diets

The experimental procedures were carried out according to the National Council of Animal Control and Experimentation (CONCEA, Brazil) and were approved by the Ethics Commission on the Use of Animals (CEUA) of the Institution (protocol 3522250722).

Sixty-four male Swiss mice were kept in individual plastic boxes in an environment with air exhaustion, controlled temperature (23 ± 2 °C) and photoperiod (light/dark cycles of 12 h/12 h). The supply of food and water was continuous and free. During acclimation all the mice were fed with irradiated pelletized standard chow for rodents (Nuvilar CR-1; Quimtia, Brazil).

At the start of the experimental period, the 30-day old mice were randomly assigned to the following groups, which were accompanied for eight weeks: CoS (n=24) fed with standard chow and not trained (sedentary control group); ObS (n=20) fed with HCD and not trained (sedentary obese group); ObT (n=20) fed with HCD and trained (trained obese group) (Figure 1).

CoS (n=24)	8 weeks – standard chow, not trained	In vivo tests (n=10)
		Liver perfusion (n=14)
ObS (n=20)	8 weeks – high carbohydrate diet, not trained	In vivo tests (n=10)
		Liver perfusion (n=10)
ObT (n=20)	8 weeks – high carbohydrate diet, trained	In vivo tests (n=10)
		Liver perfusion (n=10)

Figure 1. Experimental design.

From day one of the first week of intervention, groups ObS and ObT were fed with HCD, which was a pelletized mixture of standard chow, condensed milk, crystallized sugar, and water [23]. It was prepared and kept in the refrigerator for no more than a week. The standard chow given to the CoS group and the HCD given to groups ObS and ObT are shown in Table 1.

Table 1. Diets of groups CoS, ObS and ObT.

Component	CoS – standard chow	ObS/ObT – HCD
Kcal (/100 g dry weight)	402	428
Carbohydrates (g/100 g dry weight)	57.5	68
Protein (g/100 g dry weight)	30.0	16
Fat (g/100 g dry weight)	12.5	16

Follow-up of Groups

Food intake and body mass (b.m.) were recorded once a week during the eight-week intervention. Food intake was calculated as the difference between the food supplied and that remaining after 24 hours and was expressed as g/10 g b.m. per day.

Nasoanal length (in cm) was measured at the end of the interventions. The Lee index [$(\sqrt[3]{\text{g b.m./cm nasoanal length}}) \times 1000$] and the Body Mass Index (BMI) [$\text{g b.m./}(\text{cm nasoanal length})^2$] were calculated as indirect assessments of adiposity [24-26].

Habituation, Maximal Load Tests and Training

These were made on a vertical ladder designed for mice. The fishing sinkers used as loads were placed in a plastic tube fixed at the animal's tail.

Habituation to the ladder consisted of three climbing repeats, without load, for three alternate days of the week preceding the training period. At each climb, the animals were left free to explore the ladder and reach the resting chamber at the top. This procedure was applied to the three groups.

The maximal load (ML) incremental test was conducted on Mondays of the training weeks to determine the load for the training sessions of group ObT [27,28]. In the first ML test the mice made a first climb with 90% of their b.m. as initial load. Then, 8 g was added, and a new climb was made. There was a one-minute interval between successive attempts. This was repeated until exhaustion, established as the incapacity of the animal to climb the ladder after three non-painful stimuli to its tail. The ML was the highest load (in g) carried for the entire length of the ladder. From the second week onwards, the initial load of the ML test was 100% ML of the previous week.

The mice of groups CoS and ObS made the ML test on weeks 1 and 8; it was used 90% b.m. as initial load on both tests.

Group ObT performed two weekly training sessions (Wednesdays and Fridays) during the same eight weeks of the HCD. Each session was structured in three rounds, each one composed of complete climbing repeats until exhaustion, with a two-minute interval between rounds, and all were conducted with 90% ML of the week. This training protocol will be referred to as HIIRT – high-intensity interval resistance training [27].

In vivo Blood Glucose Monitoring

For the tests described below, blood glucose from samples collected through a puncture at the tip of the tail was determined through test-strips and glucometer (Optium Exceed®; Abbott, Brazil) during regular time intervals as described below. For the training blood glucose, the mice were in the fed state (immediate post-prandial metabolic state). For the glucose, insulin and adrenaline tolerance tests (GTT, ITT and ATT, respectively) the mice were subjected to a six-hour food deprivation (8 am-2 pm, post-absorptive metabolic state). Ten mice of each group were used in these tests (Figure 1) and in the collection of biological material.

At the 8th week of the interventions, blood glucose was determined before and after an ML test. Samples of blood were collected immediately before and at the end of the ML test, and three and 10 minutes later.

At the 9th week of intervention, the animals were subjected to an oral GTT. They were given a gastric gavage of glucose (1.5 g/kg b.m.) and blood glucose was determined before gavage (time zero) and 5, 10, 15, 20, 25, 30, 45 and 60 minutes after the glucose load.

The same mice were subjected to the ITT 48 hours after the GTT. They were given an intraperitoneal injection of regular insulin (Novolin®; Novo Nordisk, Brazil; 1 IU/kg b.m.) and blood glucose was determined at times 0, 5, 10, 15, 20, 25 and 30 minutes, time zero being immediately before insulin injection.

After 48 hours of the ITT, the animals were given an intraperitoneal injection of adrenaline (Adren®; Hipolabor, Brazil) at the dose of 0.5 mg/kg b.m. Blood glucose was determined at times 0, 5, 10, 15, 20, 25 and 30 minutes, time zero being immediately before adrenaline injection.

Collection of Biological Material and Biochemical Determinations

After resting in a quiet room for one hour after the ATT, the mice were euthanatized [2:1 (vol:vol) mixture of ketamine (0.116 g/mL) and xylazine (0.023 g/mL), volume 0.1 mL, intramuscular] and the following tissues were removed and weighed as representatives of lean and fat mass: liver, heart, kidneys, gastrocnemius skeletal muscle, visceral (mesenteric, periepididymal, retroperitoneal) WAT and subcutaneous (inguinal) WAT.

Liver samples were stored at – 80 °C to determine hepatic lipids. The liver lipids were extracted using chloroform and methanol [23,29-32]. Briefly, total lipids were measured through gravimetry and then dissolved in chloroform and isopropanol (1:2, vol:vol). Liver content of triglycerides (TG) was determined with commercial kit (Gold Analisa Diagnóstica, Brazil).

Blood was collected for biochemical determinations. Total cholesterol, HDL, TG, aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined (Gold Analisa commercial kits). The VLDL content was calculated as TG/5 and the LDL content was given by $TC - (HDL + VLDL)$ [33]. The atherogenic index was given as CT/HDL . The triglycerides glucose index (TyG index), an indirect measurement of insulin resistance that employs fasting blood glucose and triglycerides, was calculated as $\ln(\text{triglycerides} \times \text{glucose})/2$ [34,35].

In situ Liver Perfusion

The remaining mice of each group (Figure 1) were anesthetized [2:1 (vol:vol) ketamine and xylazine, volume 0.05 mL, intramuscular]. Next, the liver was exposed, a cannula was inserted and fixed by ligature in the portal vein (entrance route) and another in the inferior cava vein below the liver (exit route). Once cannulated, the liver was perfused with Krebs/Henseleit bicarbonate buffer (KH, pH 7.4, 37 °C). and the abdominal blood vessels below the liver were sectioned. The liver mass was estimated as 4% b.m. and the KH buffer flux was adjusted to 4 mL/min per g liver. Next, the thorax was opened, the diaphragm sectioned and the inferior cava vein above the diaphragm ligated.

After 30 minutes of stabilization, samples of the effluent fluid were collected through the inferior cava vein every 5 minutes, the first collection set as time zero. During collection, the liver was sequentially perfused as follows: 20 minutes with KH buffer (basal perfusion); KH buffer containing glycerol 4 mM, lactate 4 mM, alanine 4 mM and adrenaline 1 μ M (20 minutes each; stimulated perfusions). At the end of the perfusion the liver was removed and weighed.

Glucose, lactate, pyruvate and total nitrogen (urea + ammonia) were determined in the samples of the perfusion effluent fluid through colorimetric or enzymatic methods. Gold Analisa commercial kits and reaction media freshly prepared were used.

Statistics

The experimental design for this investigation indicated a value of E of 27 for each experimental protocol (n=10/group, three groups), higher than the recommended upper limit of 20 [36]. However, *in vivo* experiments and *in situ* liver perfusion are prone to extreme variability of results from animal to animal within a given group.

The data sets in figures are expressed as box (25%-75% percentiles) and whiskers (min and max values) showing the mean value by a horizontal line; or as mean $\pm$ standard error of the mean (SEM) in tables. They were subjected to Kolmogorov-Smirnov and/or Shapiro-Wilk normality tests. Experimental groups were compared through one-way ANOVA with Tukey *post hoc* (ANOVA/Tukey) or Kruskal-Wallis with Dunns *post hoc* (KW/Dunns). Sequential data within a group were compared through repeated measures ANOVA with Tukey *post hoc* (RM ANOVA/Tukey) for multiple records or paired t test for two records, 5% being the significance level adopted for all the statistical comparisons ($p < 0.05$). Statistics and graph construction were carried out using Prism® version 5.0 (GraphPad, USA).

RESULTS

Mice Given HCD Ingested More Food, Gained More Body Mass, and Stored More WAT

The relative food ingestion of the mice during the eight weeks of intervention is illustrated in Figure 2A. At the first week, the ObS and ObT mice ingested about 3.0 g/10 g b.m. per day, while for CoS the mean was 2.2. Ingestion decreased progressively, especially in the HCD-fed groups, so that the relative ingestion during week 8 was 1.5, 1.8 and 1.9 times lower than week 1 in groups CoS, ObS and ObT, respectively.

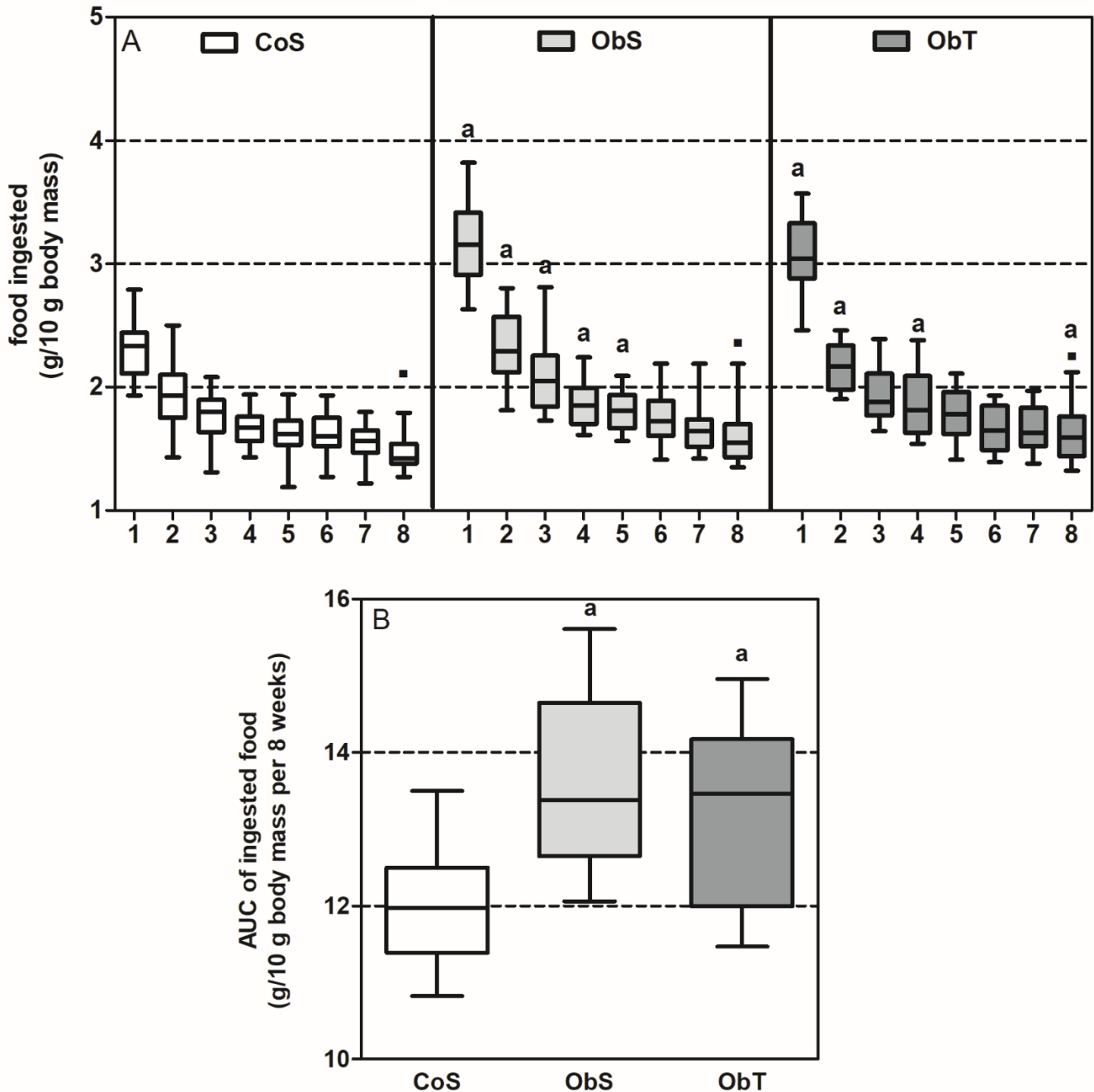


Figure 2. Food ingestion of groups CoS, ObS and ObT.

(A) Relative food ingestion during eight weeks; $p < 0.0001$ vs week 1 of the group (▪), paired t test; $p < 0.0001$ to $p < 0.05$ vs CoS (a), ANOVA/Tukey. (B) AUC of ingested food; $p < 0.0001$ vs CoS (a), ANOVA/Tukey. Data shown as box and whiskers; $n = 20-24$ /group.

The relative food ingestion during the eight weeks, expressed as area under curve (AUC, Figure 2B), was 18% and 11% higher in groups ObS and ObT, respectively, than in group CoS, but the HCD-fed groups did not differ from one another.

At the first week, body mass was similar in all the groups (Figure 3A). At the 8th week, body mass was about 7-8 g higher in groups ObS and ObT than in group CoS. That is, ObS and ObT had a body mass gain 16-20% higher than CoS. The final body mass was not different between the HCD-fed groups. Week-by-week statistical comparisons are not shown in Figure 3A.

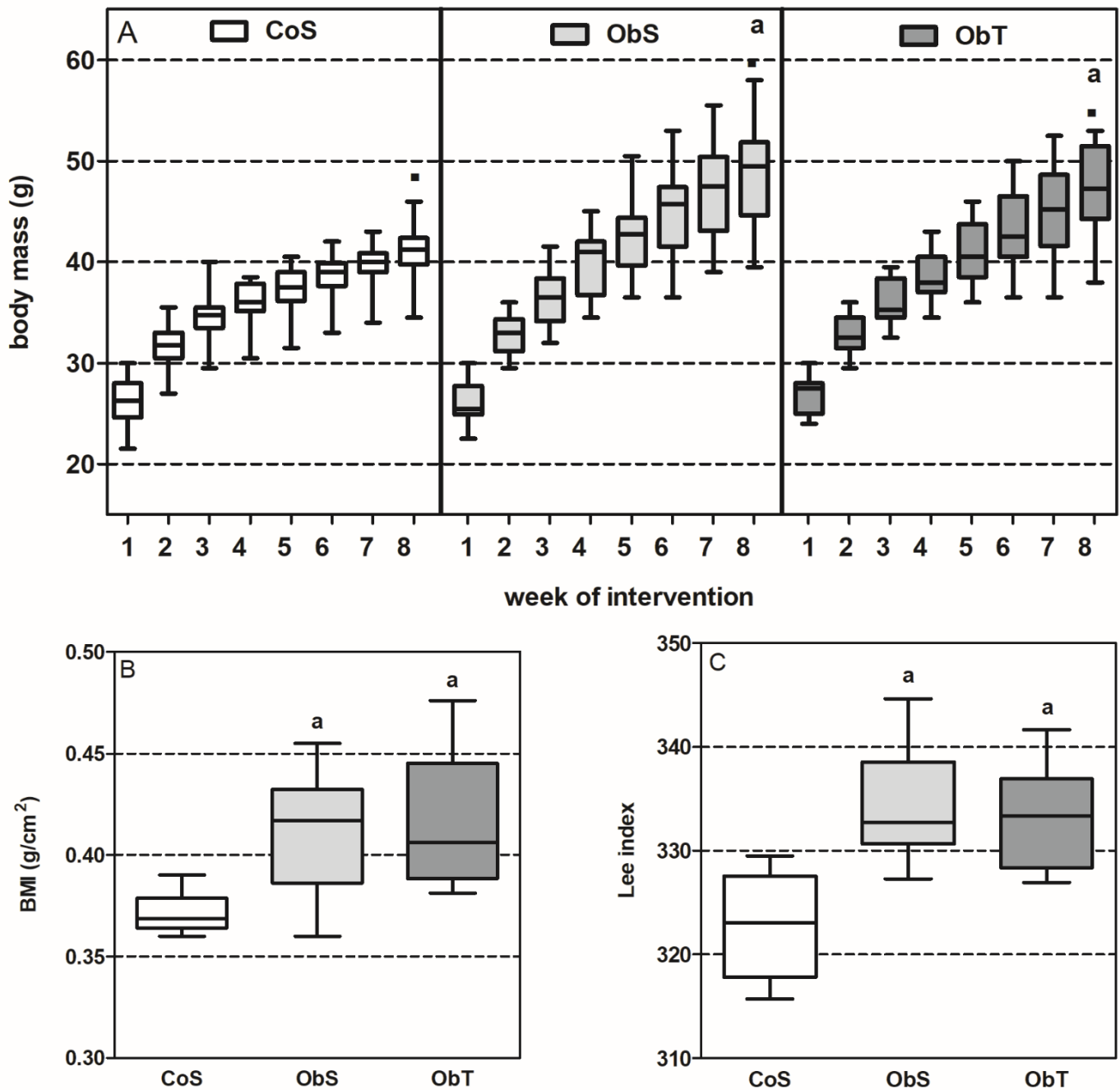


Figure 3. Body mass of groups CoS, ObS and ObT.

(A) Progression of body mass during eight weeks; $p < 0.0001$ vs week 1 of the group (▪), paired t test; $p < 0.0001$ vs CoS (a), ANOVA/Tukey. (B) Body mass index; $p < 0.0001$ vs CoS (a), ANOVA/Tukey. (C) Lee index; $p < 0.001$ vs CoS (a), ANOVA/Tukey. Data shown as box and whiskers; $n = 20-24$ /group.

Groups ObS and ObT had higher BMI (Figure 3B) and Lee index (Figure 3C) than group CoS. Significant differences of BMI or Lee index were not found between groups ObS and ObT. Based on the data of Figure 3, the HCD proved efficient as obesogenic diet and the mice of groups ObS and ObT were considered obese.

The mass of tissues and organs are in Table 2. All the WAT depots were increased in the HCD-fed groups, which in turn were similar to one another. The organs were similar in all the groups.

Table 2. White adipose tissues (WAT) and organs of groups CoS, ObS and ObT.

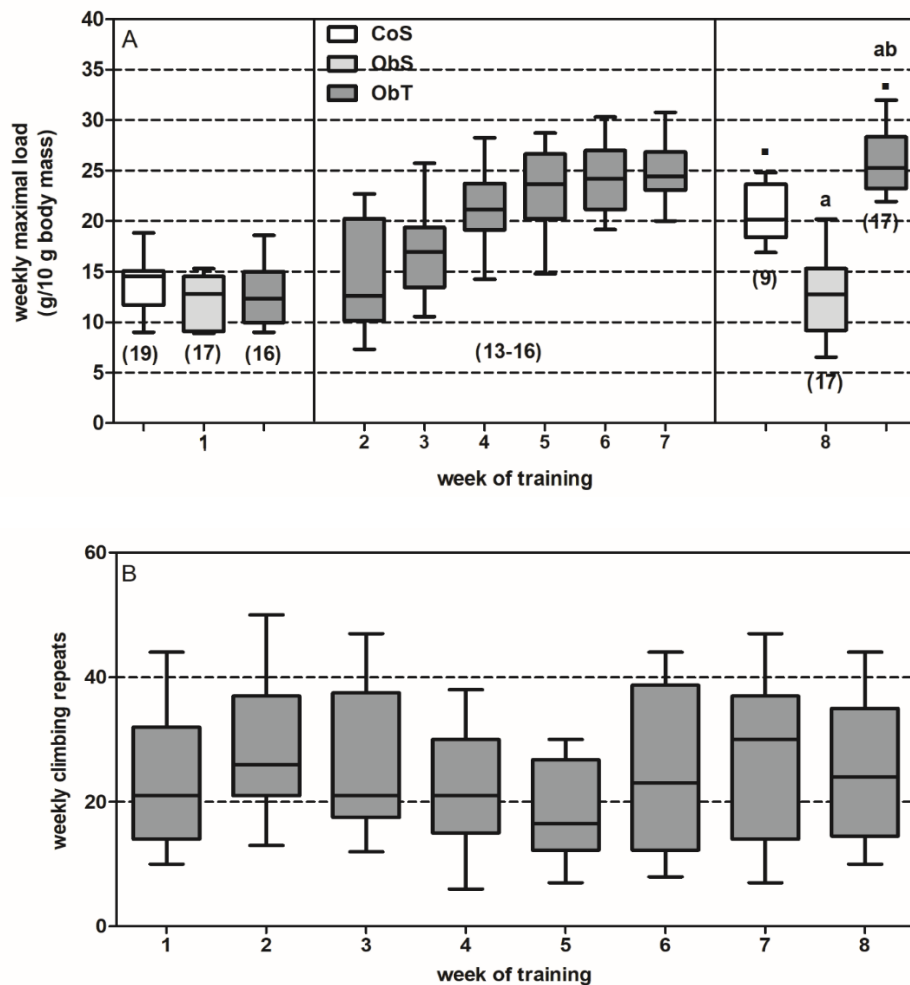
Organ Weight (g/10 g)	CoS (n=10)	ObS (n=8)	ObT (n=8)
Periepididymal WAT	0.222±0.012	0.376±0.021 a	0.387±0.021 a
Retroperitoneal WAT	0.079±0.006	0.154±0.015 a	0.180±0.005 a
Mesenteric WAT	0.149±0.007	0.227±0.012 a	0.214±0.007 a
Inguinal WAT	0.098±0.009	0.181±0.016 a	0.143±0.012 a
Liver	0.470±0.009	0.495±0.014	0.476±0.012
Kidneys	0.156±0.006	0.141±0.005	0.148±0.006
Heart	0.056±0.002	0.054±0.003	0.051±0.002
Gastrocnemius	0.079±0.001	0.073±0.003	0.073±0.002

Periepididymal, retroperitoneal and mesenteric WAT $p<0.0001$, inguinal WAT $p<0.001$, vs CoS (**a**), ANOVA/Tukey. Data shown as mean±SEM.

HCD Did Not Impair the HIIRT Performance of the Mice

The weekly ML is shown in Figure 4A. At the first week, there was no difference across the groups. At the 8th week, the ML/10 g b.m. of group CoS was increased by about 7 g. That of group ObS was not significantly altered relative to week 1, while in group ObT the increase was of about 13 g/10 g b.m. Consequently, at the 8th week the ML/10 g b.m. of group ObT was higher than the other groups, and that of ObS was lower than CoS.

Total climbing repeats of group ObT are presented in Figure 4B. There were no differences between any of the training weeks. The mean number of climbing repeats per week ranged from 18 to 28.

**Figure 4.** Training recordings of groups CoS, ObS and ObT.

(A) Relative maximal load of groups CoS, ObS and ObT; $p<0.0001$ vs week 1 of the group (▪), paired t test; $p<0.0001$ vs CoS (**a**) and vs ObS (**b**), ANOVA/Tukey; n is shown in (). (B) Total climbing repeats per week of group ObT; n=15-20. Data shown as box and whiskers.

HCD Caused Systemic Glucose Alterations That Were Partially Prevented by HIIRT

Blood glucose changes at the ML test at week 8 is in Figure 5. At all moments, groups ObS and ObT had higher blood glucose than group CoS. Blood glucose was not different between groups ObS and ObT at any moment.

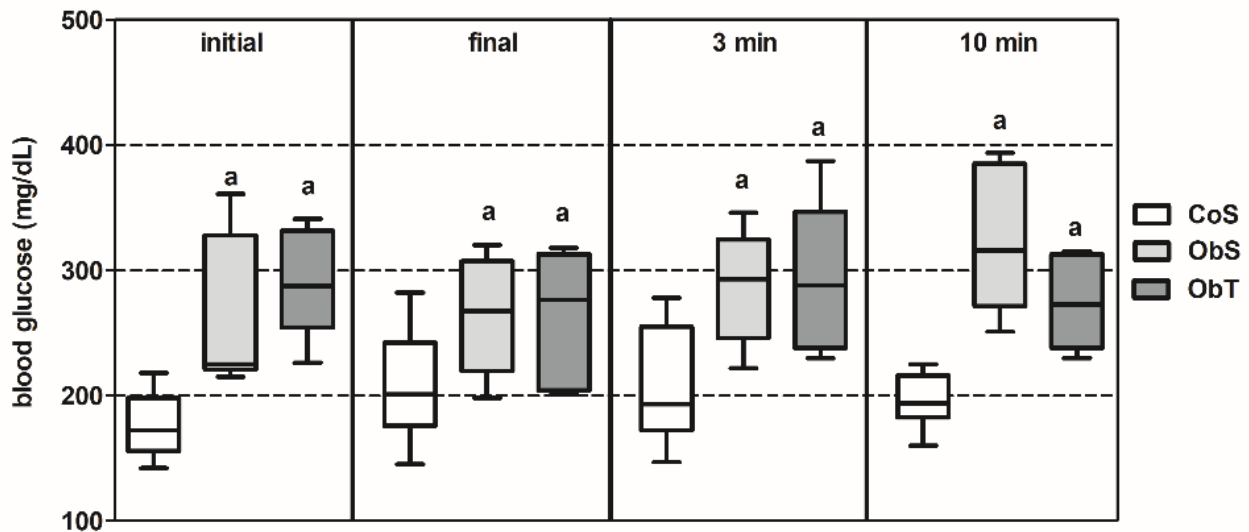
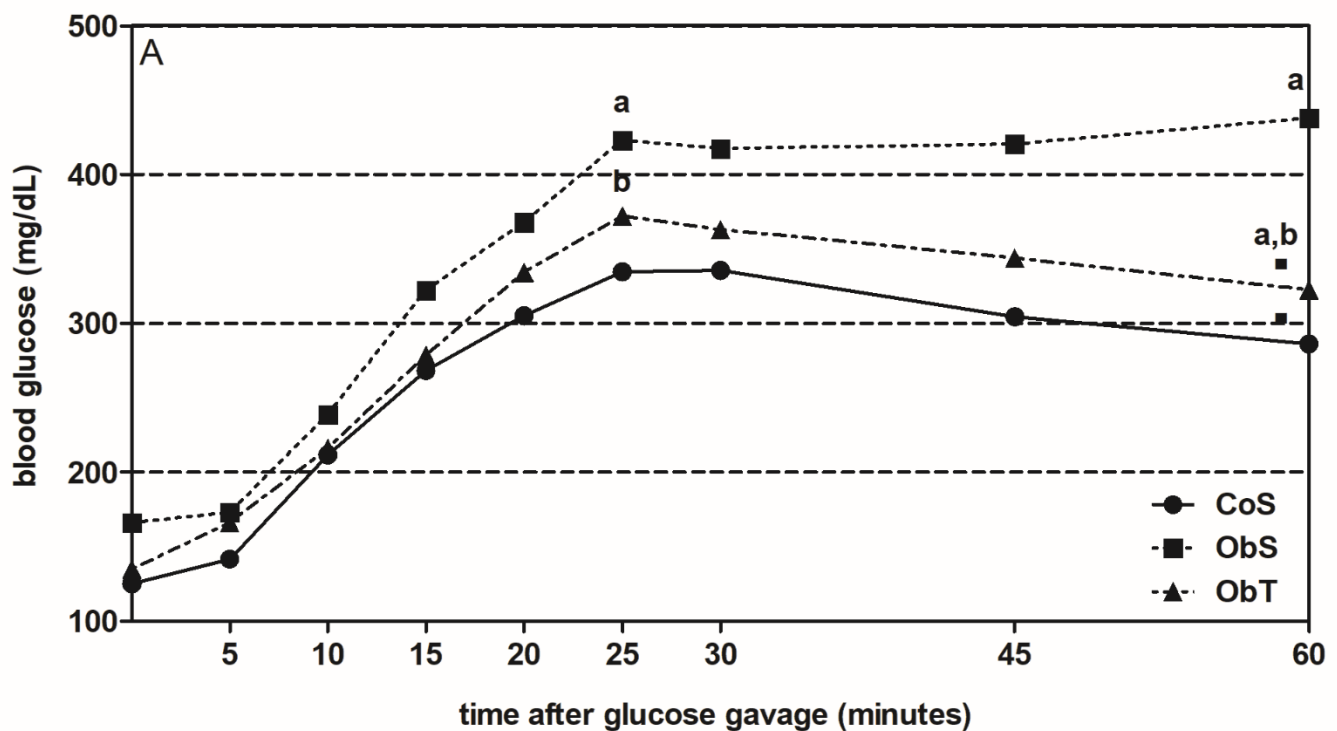


Figure 5. Blood glucose at an ML test of groups CoS, ObS and ObT

$p < 0.0001$ initial, $p < 0.05$ final, $p < 0.001$ 3 minutes and 10 minutes, vs CoS (a), ANOVA/Tukey. Data shown as box and whiskers; $n = 9-11$ /group.

Figure 6 illustrates the GTT. From zero to 25 minutes after glucose gavage, there was an increment of blood glucose, followed by stabilization (group ObS) or decrease (groups CoS and ObT) until the end of the test (Figure 6A). At 25 minutes, blood glucose of group ObS was higher than CoS; that of group ObT was lower than ObS and similar to CoS. At 60 minutes, group ObS exhibited the highest blood glucose, while ObT was intermediate and different from both other groups (Figure 6A).



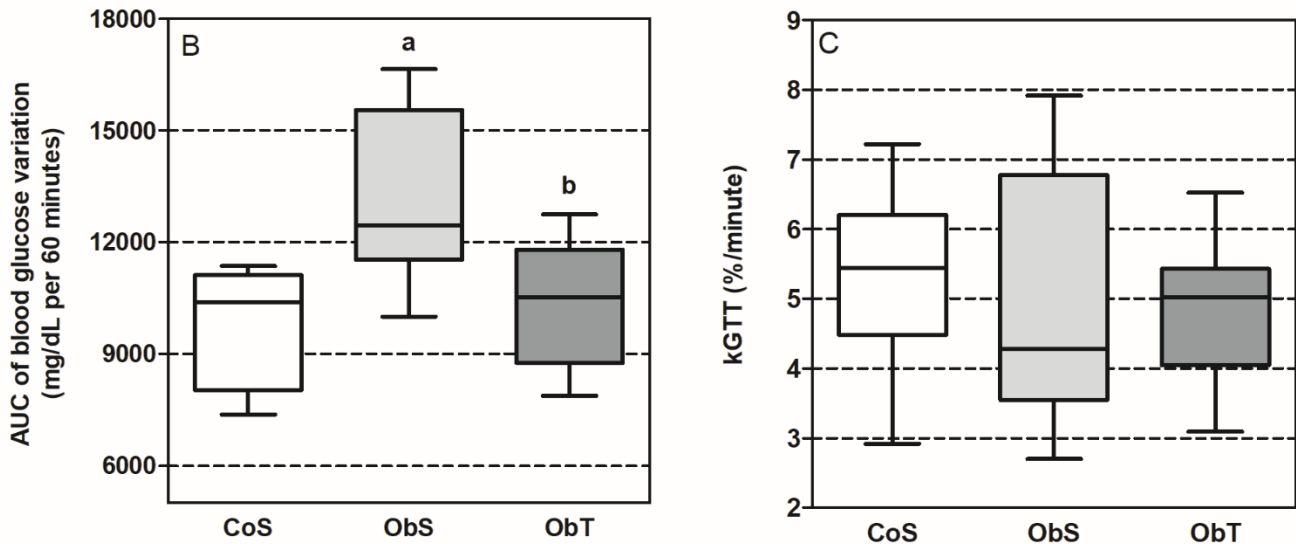
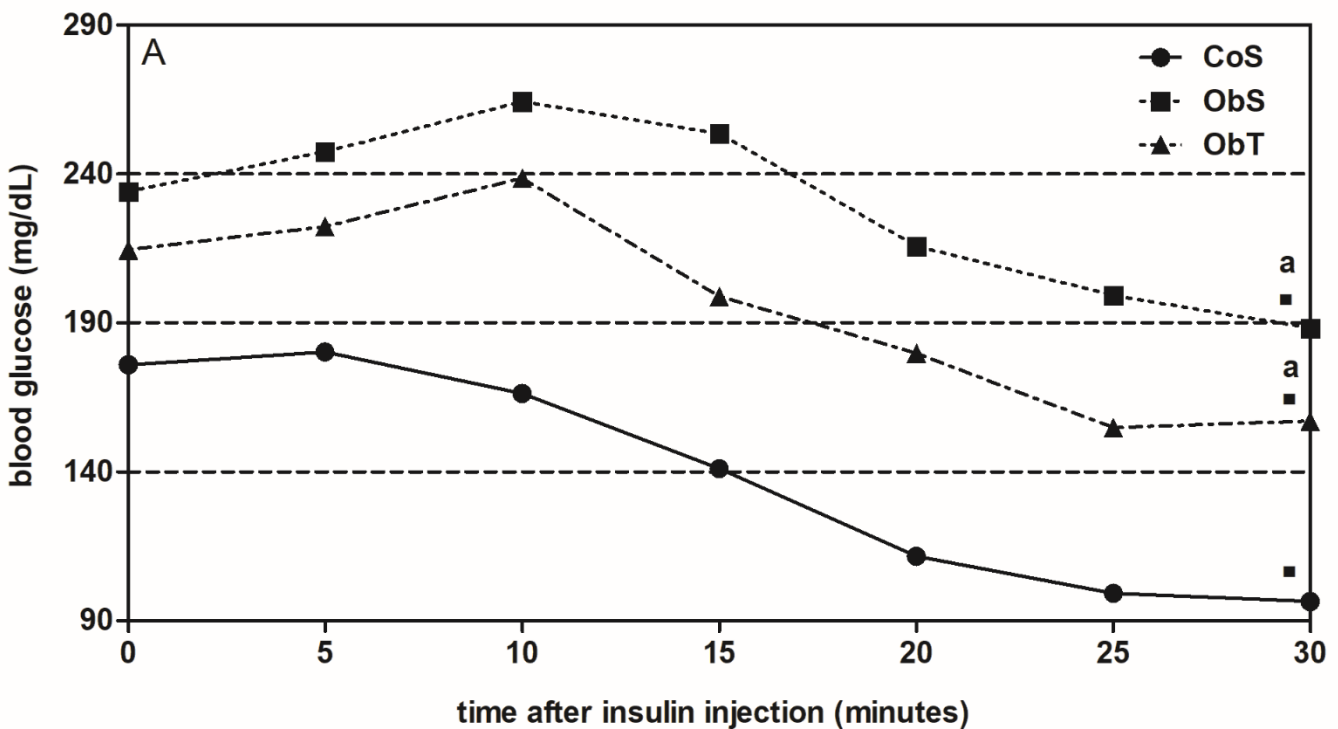


Figure 6. GTT of groups CoS, ObS and ObT.

(A) Time course of blood glucose; $p < 0.01$ for CoS, $p < 0.05$ for ObT, vs time 25 minutes of the group (■), paired t test; $p < 0.0001$ vs CoS (a) and vs ObS (b), ANOVA/Tukey. (B) Area under curve of blood glucose variation; $p < 0.01$ vs CoS (a) and vs ObS (b), ANOVA/Tukey. (C) Rate of blood glucose increase. Data shown as mean (A) and box and whiskers (B and C); $n = 7-9$ /group.

The AUC of blood glucose variation (Figure 6B) during the GTT of group ObS was 37.5% higher than that of CoS, while the value of ObT did not differ significantly from group CoS and was lower than ObS. The rate of blood glucose increase (kGTT, Figure 6C) for the first 15 minutes of the test did not detect significant differences across the groups.

Figure 7A shows the blood glucose profile after insulin administration, with a progressive decrease from 10 minutes onwards until the end of the test. Both HCD-fed groups ended the ITT with higher blood glucose than the CoS but did not differ from one another. As for blood glucose variation (AUC of the ITT, Figure 7B) and the rate of blood glucose decay after the 10th minute (kITT, Figure 7C), there were no significant differences across the three groups.



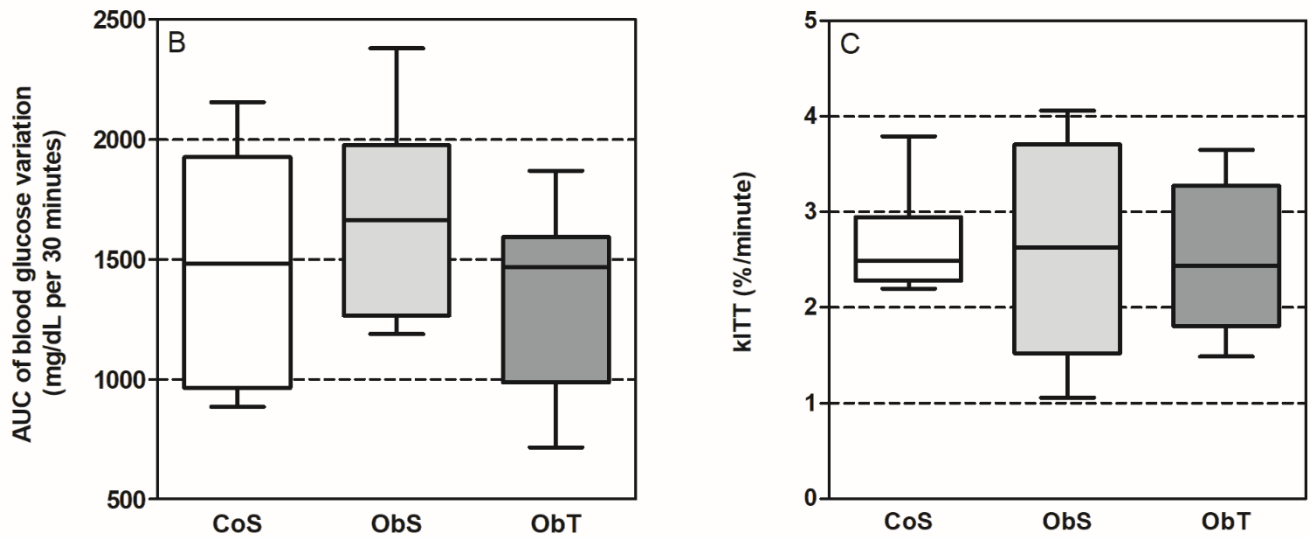
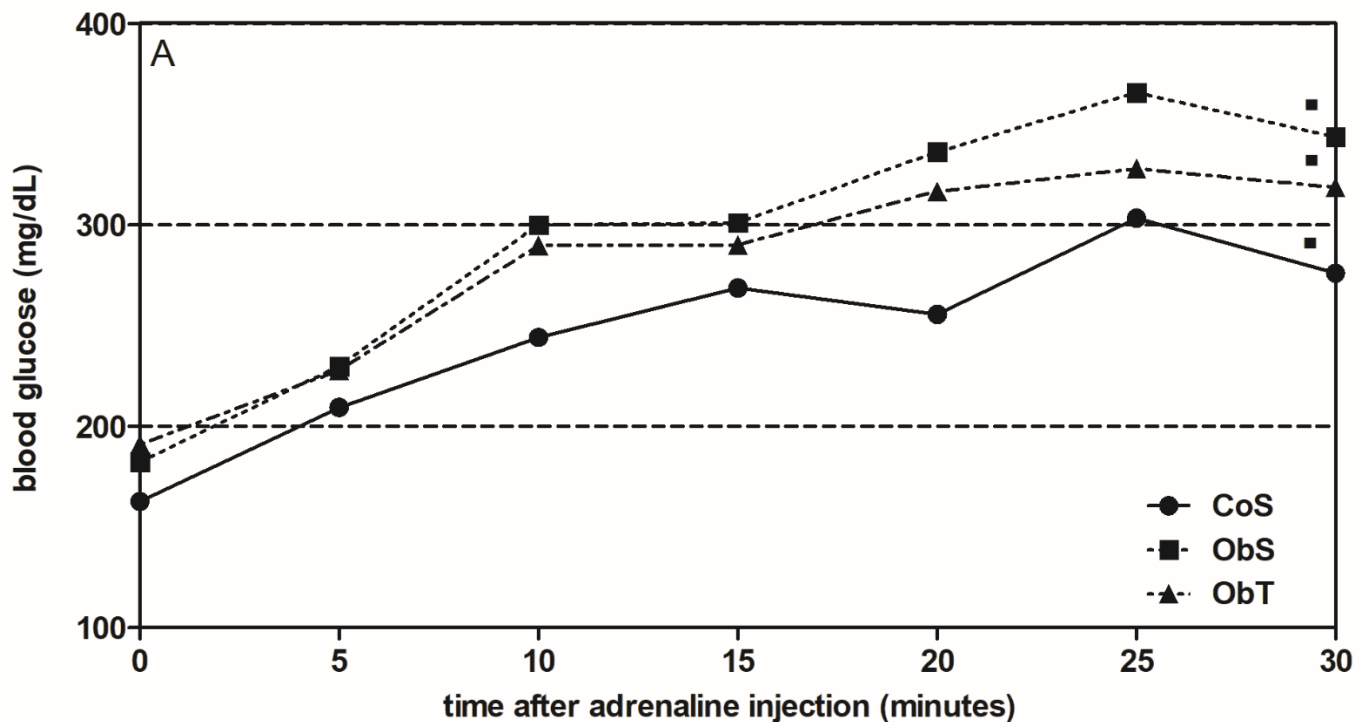


Figure 7. ITT of groups CoS, ObS and ObT.

(A) Time course of blood glucose; $p < 0.0001$ for CoS, $p < 0.05$ for ObS and ObT, vs time 10 minutes of the group (■), paired t test; $p < 0.05$ to $p < 0.0001$ vs CoS (▲), ANOVA/Tukey. (B) Area under curve of blood glucose variation. (C) Rate of blood glucose decrease. Data shown as mean (A) and box and whiskers (B and C); $n = 8-10$ /group.

The adrenaline tolerance test (ATT) is illustrated in Figure 8. All the animals responded to adrenaline injection with a progressive increase of blood glucose for 25 minutes (Figure 8A). The final blood glucose and the AUC of the ATT (Figure 8B) did not differ across the groups.



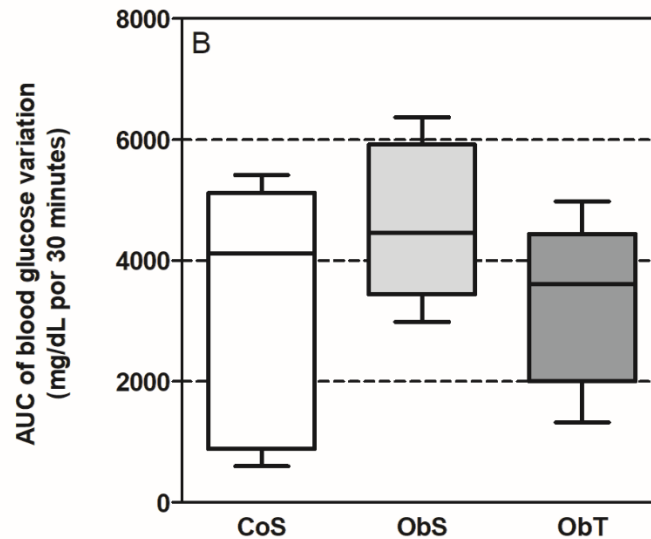


Figure 8. ATT of groups CoS, ObS and ObT.

(A) Time course of blood glucose; $p < 0.0001$ for CoS, $p < 0.001$ for ObS, $p < 0.01$ for ObT, vs time zero of the group (■), paired t test. (B) Area under curve of blood glucose variation. Data shown as mean (A) and box and whiskers (B); $n = 8-10$ /group.

HIIRT Partially Counteracted the Higher Fasting Blood Glucose, Plasma Lipids and TyG Index Induced by HCD, But Did Not Change the Liver Lipids

Plasmatic parameters and liver lipids of the groups are shown in Table 3. Fasting blood glucose was from time zero of ATT. The HCD in sedentary mice (group ObS) increased fasting blood glucose, triglycerides, VLDL and TyG index, all decreased by HIIRT (group ObT) to values intermediate between the two sedentary groups (CoS and ObS). In group ObT the cholesterol level was higher than in CoS. The other plasmatic parameters did not differ significantly across the three groups.

Liver total lipids and TG were higher in both HCD-fed groups, without significant effects of HIIRT.

Table 3. Plasma biochemistry and liver lipids of groups CoS, ObS and ObT.

Parameter	CoS	ObS	ObT
Cholesterol (mg/dL)	214.30±8.99 (9)	244.90±13.57 (9)	265.60±14.37 (8) a
Triglycerides (mg/dL)	120.00±16.79 (9)	177.90±10.08 (8) a	147.20±18.32 (9)
Glucose (mg/dL)	155.30±6.31 (9)	186.90±9.11 (7) a	171.30±7.95 (8)
HDL (mg/dL)	59.80±9.79 (8)	98.83±10.07 (9) a	92.40±5.96 (7)
VLDL (mg/dL)	24.00±3.36 (10)	35.57±2.02 (9) a	29.44±3.66 (10)
LDL (mg/dL)	121.50±18.49 (7)	128.70±18.97 (8)	115.10±14.17 (6)
AST (U/L)	141.70±21.52 (9)	204.80±53.42 (6)	102.90±20.89 (8)
ALT (U/L)	36.55±5.52 (8)	43.56±8.81 (9)	36.88±6.79 (9)
TyG index	4.866±0.069 (9)	5.163±0.066 (7) a	5.150±0.098 (8)
Atherogenic index	2.566±0.363 (6)	2.735±0.328 (8)	2.424±0.303 (6)
Liver lipids (mg/g liver)	25.60±1.46 (11)	43.64±3.19 (9) a	52.71±5.47 (9) a
Liver triglycerides (mg/g lipid)	104.30±16.37 (11)	263.00±19.35 (9) a	241.7±19.17 (9) a

Cholesterol, glucose, VLDL $p < 0.05$ vs CoS (**a**), ANOVA/Tukey; triglycerides, TyG index $p < 0.05$ vs CoS (**a**), KW/Dunns; liver lipids, liver triglycerides $p < 0.001$ vs CoS (**a**), ANOVA/Tukey. Data shown as mean±SEM (n).

HCD Decreased the Output of Compounds by the Perfused Liver, and the Preventive Effects of HIIRT Were Small

Figure 9 illustrates the glucose output of the experimental groups during 120 minutes of *in situ* liver perfusion.

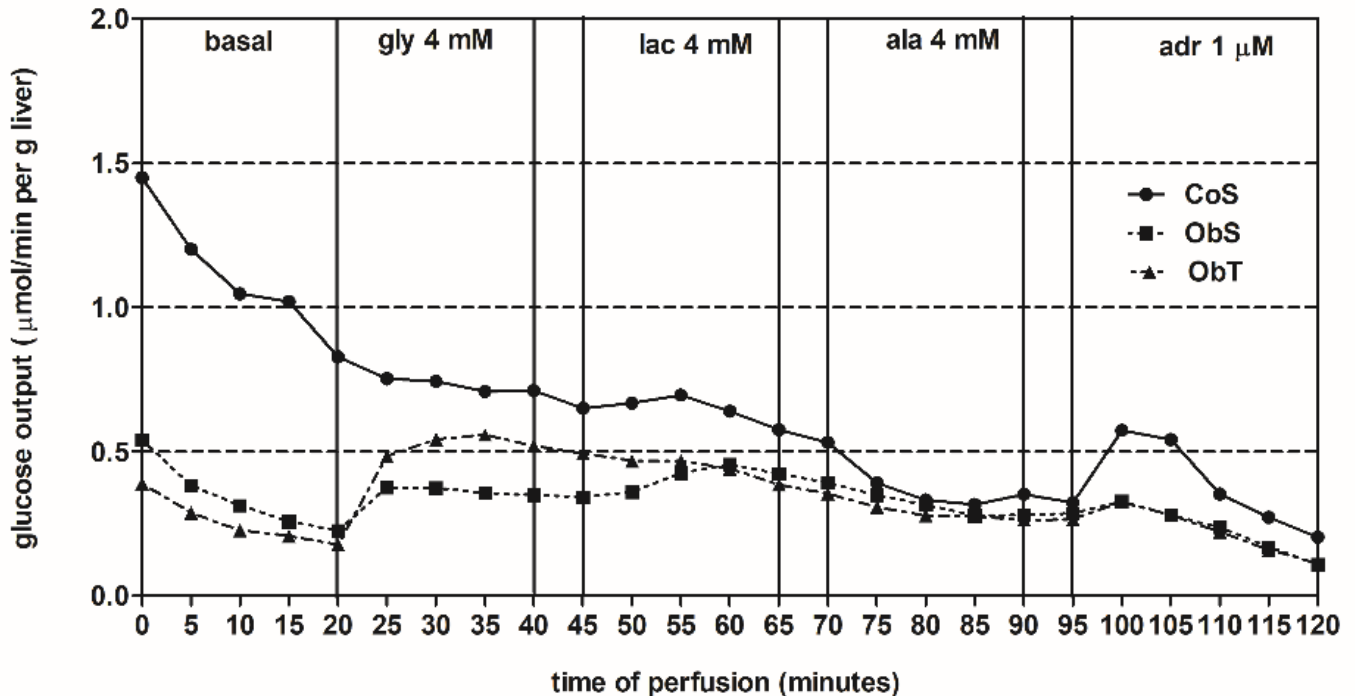


Figure 9. Glucose output during 120 minutes of *in situ* liver perfusion of groups CoS, ObS and ObT.

Data shown as mean. n=6-8 (CoS), n=4-7 (ObS and ObT). gly: glycerol; lac: lactate; ala: alanine; adr: adrenaline.

Group ObS had peak glucose outputs lower than group CoS except during alanine perfusion (Figure 10). The lower peak output of glucose in group ObT compared with group CoS occurred during basal perfusion and with alanine and adrenaline. During perfusion with glycerol and lactate, peak glucose output of group ObT did not differ from either CoS or ObS.

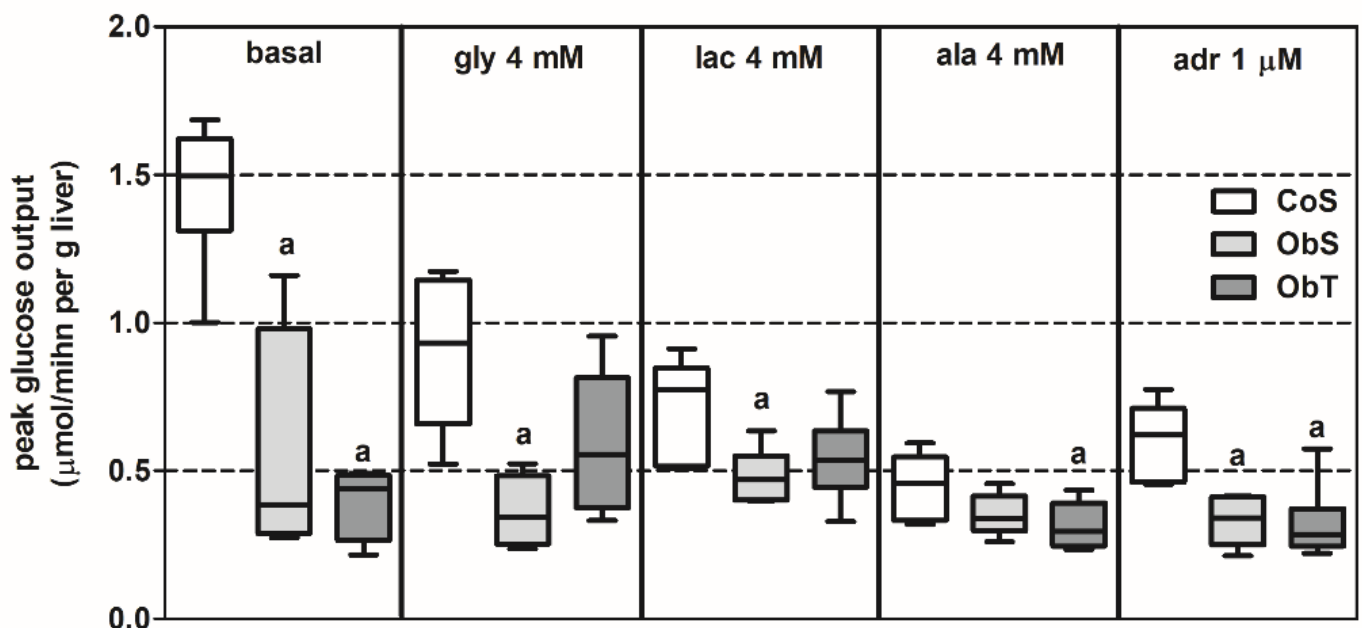


Figure 10. Peak glucose output during *in situ* liver perfusion of groups CoS, ObS and ObT.

$p < 0.001$ for basal and adrenaline perfusions, $p < 0.01$ for glycerol perfusion, $p < 0.05$ for lactate and alanine perfusion, vs CoS (a), ANOVA/Tukey. Data shown as box and whiskers; $n = 6-8$ (CoS), $n = 4-7$ (ObS and ObT).

The peak outputs of lactate (Figures 11A and 11B), pyruvate (Figures 11C and 11D) and nitrogen (Figure 11E) of all the groups were higher with alanine than at the basal period (not shown for nitrogen, which was null).

Peak lactate output in group ObS was lower than CoS both during basal perfusion and with alanine (Figures 11A and 11B). The slight increase in peak lactate output of group ObT at both periods of perfusion abolished the statistical difference with the sedentary groups. Peak pyruvate outputs during basal perfusion (Figure 11C) and with alanine (Figure 11D) were lower in groups ObS and ObT compared with CoS, but higher in group ObT than in group ObS.

The peak nitrogen output (Figure 11E) was 28-30% lower in both HCD-fed groups compared with the CoS and was similar between groups ObS and ObT.

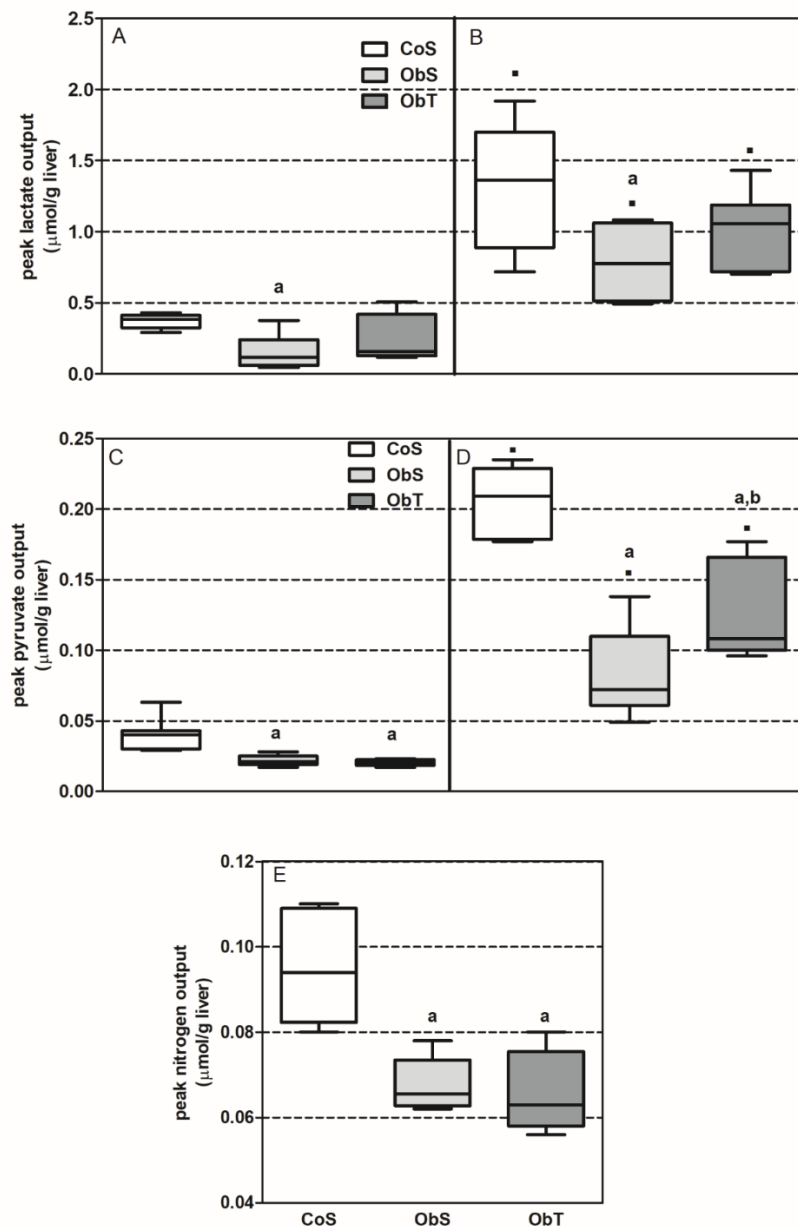


Figure 11. Peak outputs of lactate, pyruvate and nitrogen during *in situ* liver perfusion of groups CoS, ObS and ObT.

Peak lactate output during basal perfusion (A) and with alanine (B) $p < 0.001$ for CoS, $p < 0.0001$ for ObS, $p < 0.01$ for ObT, vs basal perfusion of the group (▪), paired t test; $p < 0.05$ vs CoS (a), ANOVA/Tukey. Peak pyruvate output during basal perfusion (C) and with alanine (D) $p < 0.0001$ for CoS and ObT, $p < 0.001$ for ObS, vs basal perfusion of the group (▪), paired t test; $p < 0.0001$ vs CoS (a) and vs ObS (b), ANOVA/Tukey. Peak nitrogen output during alanine perfusion (E) $p < 0.001$ vs CoS (a), ANOVA/Tukey. Data shown as box and whiskers. $n = 5-8$ (CoS), $n = 6-7$ (ObS), $n = 4-7$ (ObT).

The rates of glycolysis and glycogenolysis during basal perfusion are in Figure 12. The lower glycolytic rates (calculated from the output of pyruvate and lactate, Figure 12A) of groups ObS (2 times lower) and ObT (4.5 times lower) compared with CoS attained significance for group ObT. As for the basal rate of glycogenolysis, estimated by the output of glucose, lactate and pyruvate (Figure 12B), the values of groups ObS and ObT were much lower (7.6 and 5.8 times, respectively) than group CoS. The HCD-fed groups were similar in their basal rates of glycogenolysis.

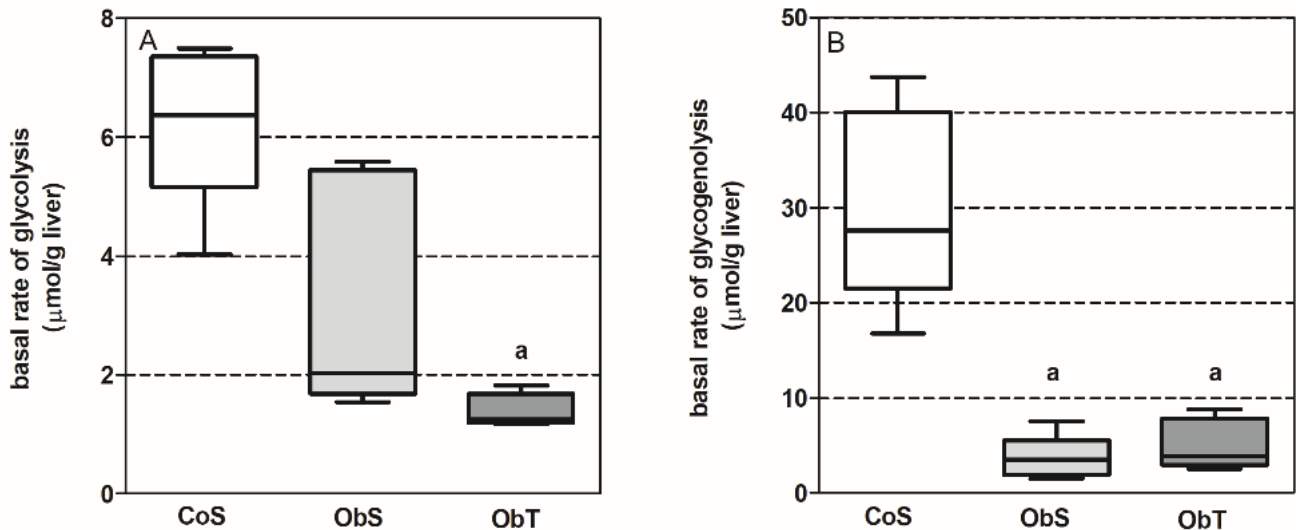


Figure 12. Basal rates of glycolysis and glycogenolysis during *in situ* liver perfusion of groups CoS, ObS and ObT.

(A) Rate of glycolysis; $p < 0.01$ vs CoS (a), KW/Dunns. (B) Rate of glycogenolysis; $p < 0.0001$ vs CoS (a), ANOVA/Tukey. Data shown as box and whiskers. $n = 6-8$ (CoS), $n = 6$ (ObS), $n = 4-5$ (ObT).

DISCUSSION

High-carbohydrate diet (HCD) and high-intensity interval resistance training (HIIRT) are customarily employed by the research group [23,27,28] and were well characterized under the existing experimental conditions, but this is the first time that they have been tested together. The experimental model using high carbohydrate diet (HCD) to induce the obese phenotype in the male Swiss mice of this investigation was shown to be effective and in agreement with the literature [21,23,24,29,37]: increased body mass, BMI and Lee index, higher body fat, elevated blood glucose, higher levels of plasma lipids, glucose intolerance and lipid accumulation in the liver. The reduced outputs of glucose, lactate, pyruvate and nitrogen in group ObS during *in situ* liver perfusion in a metabolic condition (post-absorptive state) that favors glycogenolysis and gluconeogenesis [2,6] were unexpected results.

The protocol of high intensity interval resistance training employed in this study (referred to as HIIRT) combined with HCD (group ObT), surprisingly, was incapable of affecting total body mass, adiposity and hepatic lipid content. In addition, the preventive effects of HIIRT against the changes of systemic and hepatic glucose metabolism were, at most, modest, and did not preserve glucose metabolism at its control levels.

HCD Caused an Obese Phenotype

Many studies [23,29,37,38] use condensed milk and crystallized sugar to increase the carbohydrate content of the diet to 66-68%; one study [31] had two types of HCD, one of them rich in highly digestible starch (corn starch) and being the most similar to the condensed milk/sugar HCD in carbohydrate content and digestibility.

The mice fed with HCD for eight weeks acquired features in accordance with the obesogenic nature of the diet. These features, no doubt, were the result of both diet composition (rich in simple carbohydrates and more caloric) and increased ingestion [31]. However, neither an increased food ingestion nor a higher body mass are universal observations in rodents fed high-carbohydrate or high-fat obesogenic diets [22,23,29,37,38].

There was a considerable increase of WAT (about 70% for the visceral WAT) and of liver lipid content (twice or more for total lipids and triglycerides) because of the HCD. The similar relative mass of the organs

across the three groups confirmed that the increased body mass, BMI and Lee index of the HCD-fed groups was due to the higher WAT mass, in accordance with other studies in rodents given obesogenic diets [13,18,22,23,29,37,39-41]. Increased visceral WAT is a major issue in overweight or obese individuals and is tied to the emergence and worsening of many health conditions, especially those of metabolic, cardiovascular or hormonal nature [1,15,17,42,43].

Excessive adiposity is frequently accompanied by lipid accumulation in the liver, or hepatic steatosis, which predisposes to metabolic syndrome, NAFLD/MAFLD and hepatic cirrhosis [2,8,12,19,44]. Diets rich in carbohydrates seem to be particularly prone to cause this condition. *De novo* lipogenesis, which turns excess acetyl-CoA from dietary carbohydrates into fatty acids, is stimulated by HCD and seems to remain insulin-responsive even when other metabolic actions of insulin in the liver or elsewhere are compromised [8,19]. Compared with lipid-rich diets (another common type of obesogenic diet for rodents), HCD results in higher hepatic levels of saturated and mono-unsaturated fatty acids [21,44].

HCD Disturbed Systemic and Liver Glucose Metabolism

Disturbances in plasmatic concentrations of lipid compounds are frequently reported in conditions of increased adiposity and derive from insulin resistance in adipose tissue (favoring lipolysis and decreasing lipid uptake) and liver (favoring *de novo* lipogenesis and VLDL release) [1-3,6,12,17-20,22,23,29,35,37,41,43,45-47]. In accordance with those reports, increased levels of triglycerides, VLDL and TyG index (an indirect marker of insulin resistance) [34,35] were recorded in the sedentary HCD-fed mice.

Fasting hyperglycemia is indicative of glucose intolerance and/or insulin resistance [3,5,7,8,39]. They are typical of the metabolic and hormonal impairment caused by excess fat, both in experimental animals and in humans, and are a red flag for the appearance or escalation of several obesity-related disorders collectively known as metabolic syndrome [14-17,31,42,45].

In order to access dynamic features of systemic and hepatic glucose metabolism, *in vivo* experiments and *in situ* liver perfusion were carried out. *In vivo* tests do not determine the specific mechanisms or cell types involved in the response; however, they provide an overview of the response of an organism to these challenges [5].

The research group to which the authors belong investigates how different interventions affect systemic and hepatic glucose homeostasis in rats and mice. A central experimental procedure in this research is *in situ* liver perfusion. The view of glucose metabolism obtained with the perfused liver can be analyzed in an integrative manner with classical *in vivo* tests, such as GTT and ITT. The preservation of liver viability during *in situ* perfusion was attested by the higher peaks of lactate, pyruvate and nitrogen during alanine perfusion (70-90 minutes) than at the basal period (0-20 minutes) in all the groups. The assessment of blood glucose during an ML test was designed to see the possible impact of diet and training on glycemic homeostasis during a session of incremental exercise. Both HCD-fed groups remained at higher blood glucose levels than the CoS at all the moments of the ML test. This test was performed in fed animals, that is, in post-prandial metabolic state, in which the carbohydrates are still being absorbed. The cells take up the absorbed glucose through insulin action (insulin-dependent cells, such as adipocytes and skeletal muscle fibers at rest) or through insulin-independent facilitated diffusion (for instance, nervous cells, hepatocytes and skeletal muscle fibers during or soon after exercise) [3,4,6,7]. It is possible to suggest that the kinetics of glucose removal from the blood to the cells was compromised after eight weeks of HCD, occurring at lower rates and resulting in higher post-prandial blood glucose in groups ObS and ObT than in CoS even during or soon after the ML exercise. This could be caused by either a deficient uptake of glucose by muscle cells or an altered glucose absorption by enterocytes [5].

The higher blood glucose at times 25 and 60 minutes, as well as the higher blood glucose variation (AUC) in group ObS than in CoS during the GTT, suggests an impaired efficiency of the cell mechanisms of glucose uptake. This condition is named glucose intolerance and is a frequent outcome in obesity [7,18,23,39,46], as already mentioned.

Nevertheless, glucose intolerance (during GTT) was not accompanied by impaired insulin action during ITT, as both the AUC and the kITT of this test did not differ across the groups. Yet, blood glucose of the HCD-fed groups at 30 minutes of the ITT was higher than that of CoS. In addition, as already noted, TyG index was higher in ObS. These observations may indicate that some insulin resistance was present, although its usual ITT-related markers – AUC and kITT – did not reveal it.

The pathway of *de novo* lipogenesis in the liver converts the excess acetyl-CoA from dietary carbohydrates into fatty acids. It is stimulated by HCD and seems to remain responsive to insulin (which stimulates it) even when other metabolic functions of the liver become resistant to this hormone [7,8,19]. *De*

de novo lipogenesis may use other substrates in addition to carbohydrates, such as the carbon skeletons of alanine, lactate and glutamine, to synthesize fatty acids, while glycerol is needed for the synthesis of triglycerides [19]. Based on that, it is possible to suggest that the glycerol and lactate supplied to the liver in the perfusion fluid, rather than being preferentially converted to glucose, may have been used in *de novo* lipogenesis in groups ObS and (at least partially) ObT.

During alanine perfusion, in addition to glucose, the peak outputs of lactate, pyruvate and nitrogen, compounds related to liver alanine metabolism [48,49], were measured. All of them, as well as glucose output, were higher in group CoS, and lower in groups ObS and/or ObT, suggesting that HCD reduced the flux through these metabolic routes (maybe because of a shift to lipogenesis).

The similarity of the groups during the ATT is suggestive that the systemic action of this hormone was preserved after eight weeks of HCD. However, the glycogenolytic effect of adrenaline during *in situ* liver perfusion was more marked in group CoS. Considering that the CoS mice also had higher peak glucose output and basal rates of glycolysis and glycogenolysis, they might have had a larger store of glycogen despite the six hours of food deprivation that preceded the experiment [50].

HIIRT Performance Increased, but it Did Not Prevent Against the Changes Induced by HCD

The vertical ladder is an alternative of easy implementation to assess the acute and chronic effects and responses that take place because of physical exercise in healthy animals as well as in disease models – such as diabetes mellitus and diet-induced obesity [13,22,27,28,51,52]. This device is ideal because training is individualized, monitored and adjusted according to the performance of each animal, number of sessions per week, number of training weeks and training format [13,22,27,28,51].

High intensity interval training (HIIT) has high-intensity moments intercalated with periods of rest or low-intensity activity. It is reported that it has many metabolic, cardiovascular and operational advantages [14,22,41,53-57]. The training designed for group ObT of this study was the high intensity interval resistance training (HIIRT) in vertical ladder, already shown to be successful for strength gain [28,29]. Each week the load was corrected so that the animals were always performing the sessions at high intensity (90% ML).

Group ObT improved its performance during the training weeks, as demonstrated by the gradual increase of the relative ML without a decrease in the number of climbing repeats, resulting in a progressive increase of the training volume. On the other hand, the sedentary groups (CoS and ObS) climbed the ladder with a relative ML markedly lower than group ObT after eight weeks. These findings show that the performance of group ObT was not compromised by the obese phenotype (because groups ObS and ObT were similar in body composition and adiposity) or favored by aging (because all the mice had the same age when the tests were performed).

However, HIIRT had a very mild effect on the alterations caused by HCD. First, the obese phenotype of both HCD-fed groups was similar: body and fat masses, BMI, Lee index, liver lipids, fed and fasting blood glucose were as high in group ObT as they were in group ObS. In contrast, experimental studies and reviews reporting decreased body weight and/or body and liver fat upon exercise training are numerous [13,14,22,41,55].

Second, HIIRT in group ObT only partially prevented the following changes caused by HCD (group ObS): final blood glucose and AUC of the GTT; glucose output with glycerol and lactate; and pyruvate and lactate outputs by the perfused liver. Even so, although the ObT values were statistically intermediate to those of groups CoS and ObS (either different from or similar to both), the mean values were closer to those of group ObS.

These were quite surprising results in spite of the many reports of the beneficial effects of regular exercise (irrespective of the exercise characteristics) on the obese phenotype and on systemic and hepatic metabolism of glucose (and fatty acids for that matter) [13,14,22,41,53-55]. One possible explanation concerns the actual energy expenditure of the protocol used in this investigation. Its characterization as “high intensity” was made based on the measurable variables of load and climbing repeats. More specific measurements were not available. Importantly, mice performed the HIIRT protocol twice a week (Wednesdays and Fridays), with the ML test being performed on Mondays. Therefore, although performance did improve progressively during the eight weeks of training and exercise intensity within the sessions was high, total energy expenditure or training volume might not have been enough to avoid fat accumulation and the impairments of glucose metabolism caused by HCD.

CONCLUSION

The obese phenotype induced by HCD was accompanied by changes in systemic and hepatic glucose metabolism, against which the HIIRT protocol, as designed in this study, demonstrated modest or no preventive effect. It seems that, at least in Swiss mice, the actions of HIIRT against the effects of HCD may demand higher energy expenditures by adjustments of training volume, intensity or frequency. Nevertheless, it should be noticed that exercise performance improved in the obese trained mice, suggesting that excess body mass and adiposity do not interfere negatively with this training protocol in mice.

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