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Effect of safflower oil (*Carthamus tinctorius* L.) supplementation in the abdominal adipose tissues and body weight of male Wistar rats undergoing exercise training

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ABSTRACT

This study aimed to evaluate the effects of safflower oil supplementation on the metabolic parameters, body weight, and abdominal adiposity in male Wistar rats fed with a high-fat diet (HFD) while undergoing exercise training. The rats were assigned to four groups: standard diet and sedentary (SDS), high-fat diet and sedentary (HFDS), high-fat diet and training (HFDT), and high-fat diet, training, and safflower oil (HFDTSO) groups. HFD significantly increased the abdominal adiposity in male Wistar rats. The safflower oil had no effect on the body weight and levels of blood glucose, TG, and TC, but it significantly reduced abdominal adiposity in male Wistar rats fed with an HFD while undergoing exercise training. Safflower oil supplementation reduced the abdominal fat in rats undergoing swimming training.

1. Introduction

Obesity and overweight are chronic diseases characterized by abnormal or excessive accumulation of body fat and have reached epidemic proportions worldwide (Dai et al., 2020). According to the World Health Organization, in 2016, more than 1.9 billion adults aged 18 years and older were classified as overweight and over 650 million were classified as obese (WHO, 2021). Abdominal obesity is a major risk factor for diabetes, systemic inflammation, hypertension, and other chronic diseases (Paley & Johnson, 2018). The management and treatment of obesity aim to promote weight loss, reduce the risk of other diseases, and improve general health. These goals can be achieved more effectively through the modification of lifestyle, incorporation of a healthy diet, and performance of regular physical activity (Aktar, Qureshi, & Ferdous, 2017).

Sufficient levels of physical activity can significantly reduce abdominal obesity, enhance long-term weight loss, minimize weight regain, and improve the health-related risk factors for various chronic diseases (Kay & Fiatarone Singh, 2006; Cox, 2017; Paley & Johnson, 2018). Interestingly, some studies showed that regular and consistent exercise can reduce the amount of total abdominal fat without changes in body weight (Kay & Fiatarone Singh, 2006; Paley & Johnson, 2018). In addition to regular physical activity, total control of calorie ingestion and consumption of healthy fat, especially a diet rich in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), improves the metabolic profile associated with obesity, promotes body weight control, and results in favorable changes in body composition (Philippou, Chryssanthopoulos, Maridaki, & Koutsilieris, 2019; Wang et al., 2017).

Safflower (*Carthamus tinctorius* L.) is an oilseed that belongs to the Compositae family. It is an annual herbaceous plant native to East Asia, Egypt, and the western coasts of North America (Baümler, Fernández,

Abbreviations: HFD, High-fat diet; HFDS, High-fat diet and sedentary; HFDT, High-fat diet and training; HFDTSO, High-fat diet, training and safflower oil; L, lard-based diet; LA, Linoleic acid; LDL, Low-density lipoprotein; MUFAs, Monounsaturated fatty acids; PUFAs, Polyunsaturated fatty acids; S, safflower-linseed oil-based diet; SD, Standard diet; SDS, Standard diet and sedentary; TC, Total cholesterol; TG, Triglycerides.

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Nolasco, & Pérez, 2014). Safflower oil contains 90% of the total unsaturated fatty acids, of which 14% are MUFAs and 76% are PUFAs. Among the typical n-6 PUFAs present in the oil, the main constituent is linoleic acid (LA), which accounts for 74% of the total lipid content (Katkade, Syed, Andhale, & Sontakke, 2018). Although safflower oil is rich in PUFAs, its effects on obesity and metabolic parameters remain controversial. Studies have shown that safflower oil is effective in reducing TC levels in exogenously hypercholesterolemic rats (Sato, Yoshida, Nagao, & Imaizumi, 2000) and in altering the adipocytic adiposity-related gene expression, resulting in the effective amelioration of diet-induced obesity in mice (Zhang, Li, Liu, Sun, & Zhang, 2010). However, other studies suggest that safflower oil supplementation can have possible deleterious effects on the lipid profile as it increased the levels of TC and low-density lipoprotein (LDL)-cholesterol in male Wistar rats but had no effects on the abdominal adipose tissue weight (Takeuchi, Matsuo, Tokuyama, Shimomura, & Suzuki, 1995; Santana et al., 2017). Moreover, studies on the effects of safflower oil in combination with aerobic exercise on obesity is limited. Therefore, this study aimed to investigate the effects of safflower oil supplementation in association with aerobic exercise training on the metabolic parameters that are normally impaired in the obesity.

2. Material and methods

2.1. Safflower oil

Cold-pressed extra virgin safflower seed oil (Bio Nutrition Inc. Oceanside, NY, USA) was obtained from a local market in Fortaleza. Its oil contained 75% conjugated LA, 14% oleic acid, 6% palmitic acid, 2% stearic acid, and 3% other fatty acids.

2.2. Preparation of diet

The high-fat diet (HFD) prepared for the study was a modified version of the HFD used in Estadella et al. (2004) and comprised the following: 10 g of standard diet (SD) (Evialis do Brasil Nutrição Animal LTDA, SP, Brazil), 5 g of milk chocolate (Mondelez Lacta Alimentos LTDA, PA, Brazil), 5 g of milk white chocolate (Mondelez Lacta Alimentos LTDA, PA, Brazil), 10 g of roasted ground nut (Santa Helena Industria de Alimentos S/A, Ribeirão Preto, SP, Brazil), 0.3 g of butter (Itambe Alimentos LTDA, SP, Brazil), and 5 g of sweet wheat biscuit (Fortaleza®). All components were powderized and mixed. These ingredients were ground and prepared as pellets. The diets were freshly prepared weekly and stored at 4 °C.

2.3. Diet analysis

The proximate proportions of moisture, ash, protein, fat, and carbohydrates were determined using the methods adopted by the Association of Official Analytical Chemists (AOAC, 2000). Chemical analyses were performed in triplicate. Moisture was analyzed by gravimetric analysis after oven drying (SP Labor, Presidente Prudente, SP, Brazil), lipids by gravimetry after ethyl ether extraction using Soxhlet, ash by gravimetry after burning in a muffle furnace Q318A24 (Quimis, Diadema, SP, Brazil), and proteins by the Kjeldahl method in a Kjeldahl distillation apparatus (TE-036/1, Tecnal Equipamentos para Laboratórios, Piracicaba, SP, Brazil). The total carbohydrate content was calculated as the difference in proteins, lipids, ash, and moisture. The ATWATER coefficient was used to determine the caloric value (Atwater & Woods, 1986). All the reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Animals

Male Wistar rats (159.41 ± 11.59 g, n = 18) were supplied by the Ceará State University Animal Center. During the experiment, the animals were housed in collective polypropylene cages (41 × 34 × 30 cm) at a constant room temperature of 24 ± 2 °C with a 12-h light and 12-h dark cycle. Rat chow and filtered water were provided ad libitum. The study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Ceara State University.

2.5. In vivo experimental design

The rats were assigned to four groups according to the protocol of exercise training and the type of diet offered: the first group fed with an SD and was sedentary (SDS), the second group that was fed with an HFD and was also sedentary (HFDS), and the third and fourth groups that were fed with an HFD and underwent exercise training (HFDT) for 21 weeks. Endurance swimming was used as a model for exercise intervention and was adapted from Pejon, Gobatto, Fabrício, & Beck (2020). Exercise was performed three times a week at the same time of the day (9:00–11:00 AM) for 4 weeks. On the first day, the rats swam for 10 min. The duration of exercise training was then increased by 10 min daily until each rat could swim continuously for 30 min. In subsequent weeks, the rats were allowed to swim for 30 min per day for 4 weeks. Safflower oil (40 mg/kg) was administered by gavage once a day during the last 2 weeks of the experimental procedure in the fourth group (HFDTSO). Body weight and food intake were measured every week.

2.6. Abdominal adiposity

Abdominal adiposity (adiposity index) was calculated as the sum of weights of white adipose tissues divided by the final body weight × 100 and expressed as adiposity percent (Jeyakumar et al., 2009; White et al., 2015).

2.7. Total cholesterol, triglyceride, and blood glucose levels

At the end of the experiment, the animals were fasted overnight, and blood samples were collected from the retrobulbar venous plexus using a heparin-pre-soaked capillary glass tube for various estimations. The rats were anesthetized during blood collection (Meyer et al., 2020). Subsequently, the rats were sacrificed by injecting with sodium pentothal (Cristália, São Paulo, SP, Brazil) (Zatroch, Knight, Reimer, & Pang, 2016). The serum was separated from the blood by centrifugation at 3,500 rpm for 10 min at room temperature. The TC and TG concentrations were measured using commercial kits from Labtest® Diagnostica S/A (Lagoa Santa, MG, Brazil) (Azuma et al., 2018). The blood glucose concentration was measured in blood samples obtained from the tip of the tail vein using a glucometer (Accu-Chek Active ®, Roche Diagnostics). All the reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.8. Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM) and analyzed using Prism version 9.3.1 for Windows (GraphPad Software, CA, USA). One-way ANOVA followed by Tukey's test and student's *t*-test were used to assess whether the mean values of the groups were significant; a p value of < 0.05 was considered significant.

3. Results

3.1. Diet analysis

The SD and HFD compositions are listed in Table 1. The HFD had a total lipid content of 22.52%, whereas the SD had a total lipid content of 4.73%. This increase in lipid content of HFD contributed to the total increase its calories compared to SD. The HFD prepared in the present study had a lower protein content and higher lipid content than the HFD prepared by Estadella et al. (2004). However, the carbohydrate and

Table 1
General compositions of diets.

	Standard diet (%)	High-fat diet (%)	High-fat diet (%) *
Protein	18.49	16.15	20
Fat	4.73	22.52	20
Moisture	8.23	9.10	-
Ash	9.16	3.42	-
Carbohydrates	59.39	48.81	48
Calories (Kcal/100 g)	354.09	452.52	21.40 Kj/g

*Estadella et al., 2004.

calorie contents were similar.

3.2. Weight of rats

The dietary intake did not vary significantly among the groups (data not shown). The changes in animal body weight are shown in Fig. 1, while the mean body weight and weight gain are shown in Table 2. At the beginning of the study, no differences were found in the body weight between the two groups. At weeks 7–14, the rats in the HFD group showed greater weight gain than those in the SDS group (Fig. 1). At weeks 10–12, weight gain was significantly higher in the HFD groups than in the SDS group; at week, weight gain was significantly higher in the SDS group than in the HFDSO group (Fig. 1). At weeks 18–20, no differences were observed in weight gain between the groups (Table 2).

3.3. Abdominal adiposity

The percentage of abdominal adiposity in Wistar rats is shown in Table 2 and Fig. 2A. The rats in the HFD groups (HFD, HFDT, and HFDTSO) showed a significant increase in abdominal adiposity compared with that in the SDS group. The rats supplemented with safflower oil (HFDTSO) showed a reduction in abdominal adiposity compared with that in the HFD and HFDT groups.

3.4. Triglyceride, total cholesterol, and blood glucose values

The mean TG, TC, and blood glucose levels of the Wistar rats are shown in Table 2 and Fig. 2B–D. No significant differences were observed in the mean levels of TC, TG, and glucose between the groups.

Table 2
General physical and plasma biochemical parameters of rats.

Variable	SDS	HFDS	HFDT	HFDTSO
Initial body weight (g)	151.40 ± 4.84[a]	166.25 ± 5.10[a]	155.25 ± 2.29[a]	166.75 ± 6.54[a]
Body weight at week 18	288.20 ± 8.81[a]	305.75 ± 2.69[a]	312.00 ± 8.78[a]	300.00 ± 15.76[a]
Body weight at week 19	293.20 ± 9.97[a]	321.50 ± 5.04[a]	318.50 ± 7.33[a]	305.25 ± 17.28[a]
Final body weight (g)	288.00 ± 22.41[a]	309.75 ± 20.14[a]	321.75 ± 14.50[a]	307.25 ± 32.26[a]
BWG (g) at weeks 18–19	5.00 ± 1.18[a]	15.75 ± 7.07[a]	6.50 ± 2.72[a]	5.25 ± 2.49[a]
BWG (g) at weeks 19–20	0 ± 0[a]	1.00 ± 0.71[a]	4.00 ± 1.96[a]	2.75 ± 1.38[a]
Total BWG (g)	136.60 ± 12.98[a]	143.50 ± 8.99[a]	166.50 ± 9.36[a]	140.50 ± 11.97[a]
Abdominal adiposity (%)	0.88 ± 0.32[a]	2.43 ± 0.69[b]	2.45 ± 0.64[b]	1.61 ± 0.27[a, b]
TC (mg/dL)	105.64 ± 12.25[a]	126.24 ± 14.09[a]	105.26 ± 19.12[a]	112.86 ± 13.95[a]
TG (mg/dL)	77.49 ± 19.80[a]	119.41 ± 37.95[a]	97.02 ± 33.92[a]	114.03 ± 22.31[a]
Glucose (mg/dL)	98.60 ± 6.54[a]	103.40 ± 5.08[a]	97.20 ± 6.22[a]	97.00 ± 8.67[a]

HFDS, high-fat diet and sedentary; HFDT, high-fat diet plus exercise training; HFDTSO, high-fat diet plus exercise training plus safflower oil supplementation; SDS, standard diet and sedentary; TC, total cholesterol; TG, triglycerides; BWG, body weight gain. The values are presented as the mean ± standard error of the mean (SEM), n = 4–5. The mean values on the same line with different letters differ significantly from each other, as observed using Tukey's test ($P < 0.05$).

4. Discussion

In the present study, the effect of safflower oil, administered daily in a single dose, within a short period of time (2 weeks), associated with swimming training, on the weight and biochemical parameters of animals fed with an HFD was verified. Thus, this study differs from other studies, which evaluated the effect of safflower oil included in the diet for a different period of time in the absence of physical activity (Sato et al., 2000; Crescenzo et al., 2015; Shen et al., 2014). The HFD in the present study, despite having been prepared in accordance with the study by Estadella et al. (2004), presented a difference in protein and fat content, which could be due to the use of different types of products. The concentration of safflower oil administered by gavage was 40 mg/kg, which was equivalent to a dose of 2.800 mg for a 70-kg human.

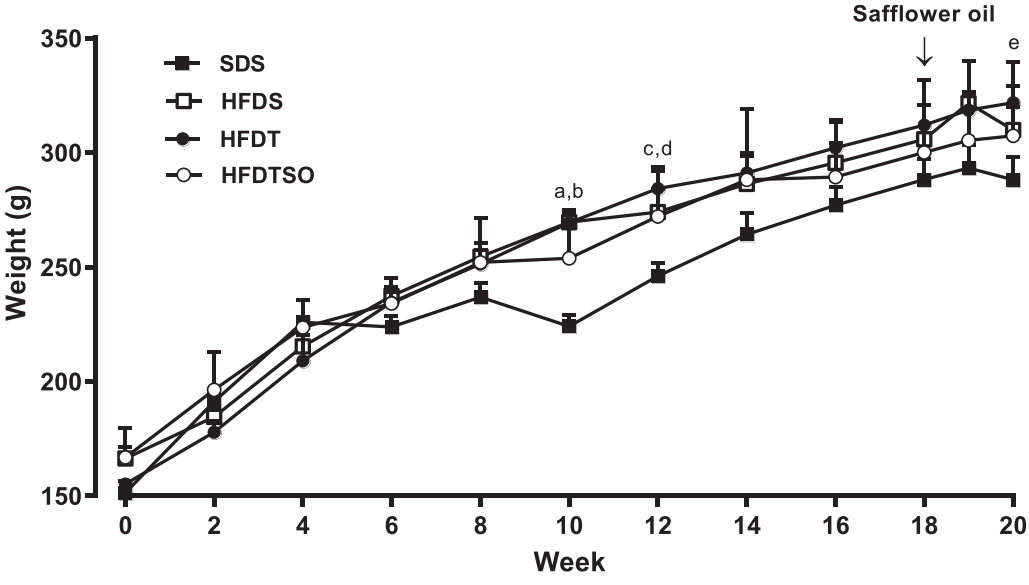

Fig. 1. Weight of rats fed with a standard diet and sedentary (SDS), fed with a high-fat diet (HFD), fed with a high-fat diet plus exercise training (HFDT), and fed with a high-fat diet plus exercise training plus safflower oil supplementation (HFDTSO). The values are presented as mean ± standard error of mean (SEM), n = 4–5. The letters show significant differences: [a] $P < 0.001$ SDS *versus* HFD and HFDT; [b] $P < 0.05$ SDS *versus* HFDTSO; [c] $P < 0.05$ SDS *versus* HFD; [d] $P < 0.01$ SDS *versus* HFDT; [e] $P < 0.05$ SDS *versus* HFDT.

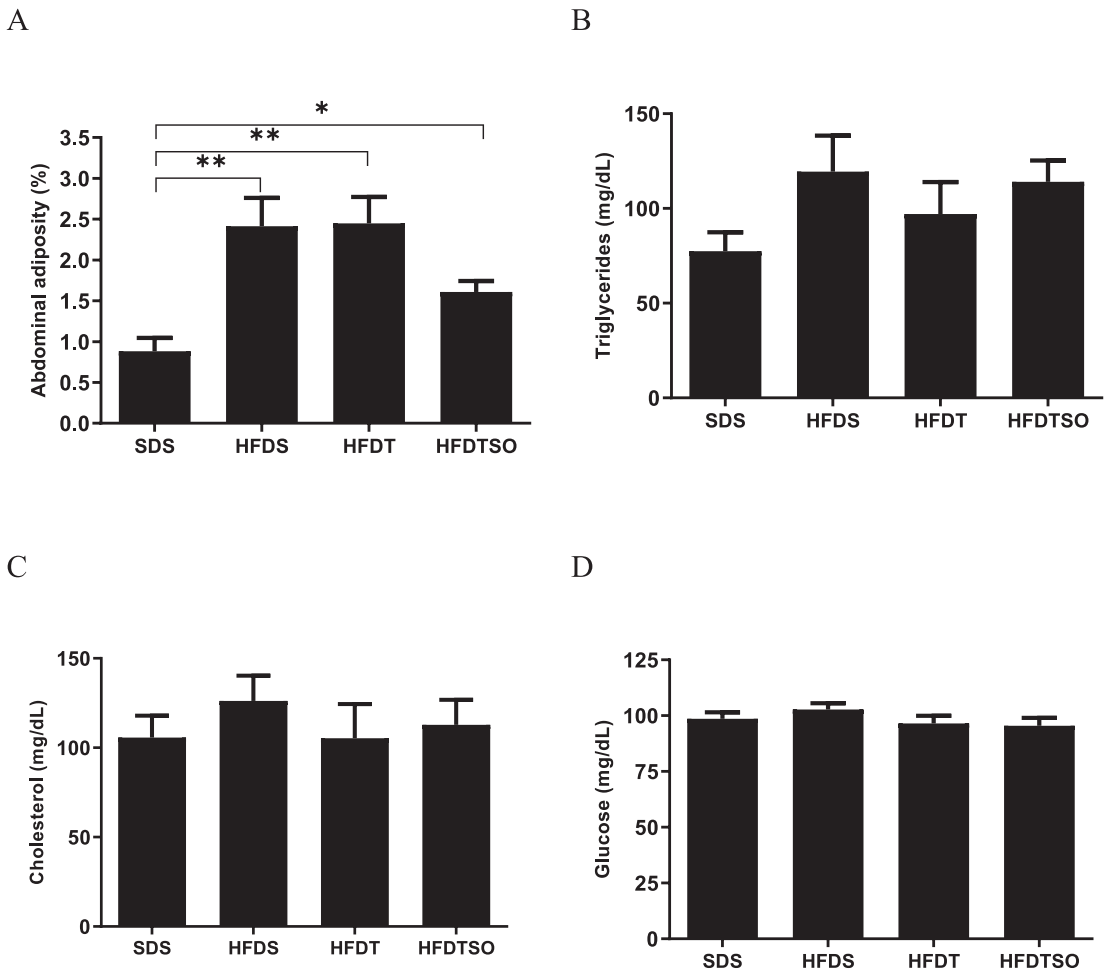

Fig. 2. Abdominal adiposity (a), triglyceride (b), total cholesterol (c), and glucose (d) levels in rats fed with a standard diet and sedentary (SDS), fed with a high-fat diet (HFD), fed with a high-fat diet plus exercise training (HFDT), and fed with a high-fat diet plus exercise training plus safflower oil supplementation (HFDTSO). The values are presented as mean ± standard error of mean (SEM), n = 4–5. Asterisks show significant differences: * $P < 0.05$; ** $P < 0.01$.

The HFD was effective in significantly increasing the abdominal adiposity in rats. These results were expected, since HFD is known to contribute to a positive fat balance and, consequently, to adipose mass accumulation (Coelho et al., 2011; Madkhali et al., 2021). Safflower oil supplementation decreased the abdominal adiposity associated with physical activity, but it did not reduce the final body weight. These findings are in agreement with those of previous studies, which demonstrated that 2 weeks of safflower-linseed oil (17.3% safflower oil and 8.7% linseed oil) diet (S) had no effect on the final body weight in Sprague-Dawley rats, but significantly decreased the epididymal and visceral fat weight compared with that in the rats on a lard (26% lard content) die (L) (Crescenzo et al., 2015). In the same study, Crescenzo et al. (2015) showed that S rats exhibited higher protein gain than L rats. Thus, protein gain may have compensated for fat loss when analyzing the final body weight, which was also observed in our study. Another study showed that 12 weeks of consuming a safflower oil-based diet had no effect on final body weight and abdominal adipose tissue weight in Sprague-Dawley male rats, compared with a lard diet, but a safflower oil diet significantly reduced the interscapular brown adipose tissue weight and increased the thermogenesis (Takeuchi, Matsuo, Tokuyama, Shimomura, & Suzuki, 1995). Similarly, the abdominal fat weight and final body weight of nude mice fed with 20% safflower oil diet for 18 weeks and 3 days were not significantly reduced compared with that in the group fed with a low-fat diet (5% corn oil diet) (Shen et al., 2014).

Safflower oil had no effect on the TC and TG levels. Results of studies that evaluated the effect of safflower oil on biochemical parameters remained controversial, which may be due to the differences in experimental protocols. Santana et al. (2017) evaluated the therapeutic effects of safflower oil supplementation administered by gavage (1.0 ml/1,000 g of animal weight, for 10 weeks) in an experimental model of metabolic syndrome, and their results showed an increase in TC and LDL cholesterol levels. Crescenzo et al. (2015) demonstrated that after 2 weeks of consuming a safflower-linseed oil-based diet (L), the male Sprague-Dawley rats exhibited no differences in TG levels but presented a significant reduction in TC levels compared with rats fed with a lard-based diet. Sato et al. (2000) demonstrated that a 10% safflower oil diet caused a significant reduction in TC levels in male exogenously hypercholesterolemic rats compared with the 10% olive oil and 10% coconut oil diets. In another study, male mice fed with a high-lard diet (45% lard + 5% safflower oil) for 20 weeks showed marked obesity (body weight and adiposity index), hyperinsulinemia, and hyperglycemia with abnormal lipid profiles (TG, LDL-cholesterol, and TC levels) compared with rats fed with a high-safflower oil diet (45% safflower oil + 5% lard) (Zhang et al., 2010). Therefore, further research is required to clarify the role of safflower oil in blood lipid metabolism.

The effects of safflower oil on blood glucose levels were measured as a previous study indicated that a high safflower oil diet (45% safflower oil + 5% lard) may improve glucose tolerance in male mice (Zhang et al., 2010). However, our results showed that safflower oil had no effect on the blood glucose levels. Similar results were demonstrated by Santana et al. (2017), who found no effect on glucose levels in rats that received safflower oil supplementation by gavage (1.0 ml/1,000 g of animal

weight, for 10 weeks). These differences in the results could be explained by the different methods of oil administration, since one study demonstrated its effects after treatment by gavage (Santana et al., 2017) and the other after including it in the diet (Zhang et al., 2010).

The differences between the studies could be explained by the differences in the time of administration, safflower oil concentration, and method of administration. Because controversy exists regarding the effect of safflower oil on blood metabolism, body weight, and abdominal adiposity, physical exercise could be recommended more often along with its supplementation in diets rich in ω-6 PUFAs. Therefore, our study supports the finding that physical activity and the intake of unsaturated oils (n-6 PUFAs) as a fat source reduces fat adiposity.

5. Conclusion

In conclusion, safflower oil supplementation reduced the abdominal fat in rats undergoing swimming training. In our study, the finding that safflower oil had no significant effect on lipid biochemical parameters and blood glucose is possibly due to the shorter time that the oil was administered.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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