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Detraining Leads to Weight Gain and a Decrease in Hepatic Glycogen after 8 Weeks of Training

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Abstract

Many studies have evaluated the effects of physical training on several metabolic parameters, but few studies have been conducted to evaluate the effects of detraining on these variables. Female mice were distributed into three experimental groups: sedentary controls (C-SED, not trained), trained controls (TR, trained for 10 weeks) and a detraining group (DT, animals detrained for 2 weeks after 8 weeks of training). The exercise protocol was performed by swimming applied for 60 min/day on 5 days/week. The DT group showed an increase of body weight in the 10th week when compared to the 8th week (after training cessation) and the TR group. The groups did not show differences in the plasma levels of corticosterone, glucose, total cholesterol or triglycerides. The DT group showed decreased glycogen content when compared to the TR group. No significant differences were found in the gene expression of glycogen synthase or glycogen phosphorylase or in hepatic glycogen content between CT and TR or DT group. We verified that after a training period of 8 weeks, the animals had an increase in body weight after two weeks of detraining. After two weeks of detraining, animals showed a decrease in liver glycogen content, without an altered fasting glucose concentration in their plasma.

Keywords: Detraining; Glycogen; Liver; Physical exercise

Introduction

Physical exercise has been proposed as a therapeutic strategy to treat a variety of metabolic diseases. Many studies have evaluated the effect of physical training in several metabolic parameters, but few studies have been conducted to evaluate the effects of training interruption on these variables. Detraining has been defined as the partial or complete loss of training-induced anatomical, physiological and performance adaptations as a consequence of training reduction or cessation [1].

It has been shown that variables such as weight, carbohydrate and lipid metabolism and myocardial mechanics can be affected after a short period without training (detraining) [2-6].

Fats and carbohydrates are the main substrates for ATP resynthesis in tissues. Felig and Wahren [7] showed that glucose mobilization during exercise is associated with the metabolic demands of muscles during activity. The maintenance of steady levels of blood glucose during physical exercise is performed through very precise controls of the hepatic production of glucose, which involves hormonal feedback mechanisms [8,9]. Fatty acids are important oxidative substrates at rest and during exercise [10]. During exercise, fatty acid oxidation can increase 5–10 times above resting levels, with maximum oxidation rates observed at exercise intensities ~65% of maximal oxygen uptake (VO_2max) [10].

Endogenous carbohydrates are mainly stored as muscle and liver glycogen and represent less than 5% of total energy storage [11]. Most studies have investigated the role of muscle glycogen after exercise [reviewed in 12], but very few studies have focused on the potentially important role of substrates in the liver. Evidence from early studies in rodents suggests that when carbohydrates are available after exercise, liver glycogen resynthesis is the first priority and muscle glycogen synthesis is secondary [13]. Other authors showed that a single feeding of glucose or fructose was sufficient to initiate liver glycogen synthesis after exercise, without affecting muscle glycogen synthesis [14].

The size of pre-exercise glycogen stores has been recognized as one of the most important factors limiting the maintenance of moderate-to-high power output for extended periods of time [15]. A number of studies have shown that physical training increases intramuscular and hepatic glycogen storage [16-19].

Detraining of one week reduces the levels of muscle GLUT4 gene expression in rats [20]. Moreover, 48 hours of detraining decreases insulin binding, IRβ protein and IRβ tyrosine phosphorylation in muscle [21]. This evidences suggested that muscle glycogen content decrease after detraining. However, the effects of detraining on hepatic glycogen storage are not clear.

Discontinuation of exercise is common among the athletes and also in general population. Athletic individuals have detraining periods because of injuries, vacation, and overtraining, which leads to a rapid gain of body weight and excess fat while general population disrupt the training for a number of reasons, including work needs, family obligations, and social factors. It is crucial to investigate the physiological alterations during this period and their role in human health.

The aim of the present study was to evaluate the effects of detraining for 2 weeks on biometrical and biochemical parameters in female mice after an 8-week training period.

Methods

Ethical considerations

The institutional review board (Comissão de Ética em Pesquisa da Universidade Federal de São Paulo) approved this study and ensured that animal care and evaluations were performed according to ethical standards (CEP: 1894/09). The mice used in this study were treated in accordance with the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals [22].

Experimental groups

Three-month-old Swiss female mice from the CEDEME (Centro de Desenvolvimento de Modelos Experimentais) facility were housed in standard polypropylene cages in a temperature-controlled room (21 ± 2°C) with a 12:12-h light-dark cycle (lights on at 0700 h). Female mice were used in the present study to avoid the effect of exercise training on body weight in male mice. They were allowed free access to food and water. To determine the regularity of two estrous cycles, the female mice were subjected to a vaginal smear two weeks prior to the experimental procedures [23]. Animals were distributed into three experimental groups: a sedentary control group (C-SED), a Trained Group (TR) and a Detraining Group (DT): [see Figure 1].

Training protocol

The exercise protocol was performed according to the Resource Book for the Design of Animal Exercise Protocols of American Physiological Society [24]. Swimming training was performed individually in circular plastic receptacles (20 cm diameter) filled with water (35 cm deep) at 32–34°C. Water was maintained in continuous turbulence to provide continuous exercise. For adaptation, training was limited to 5 min on the first day and increased by 10 min each day. Mice swam for a total period of 10 weeks, 60 min/day and 5 days/week as previously described [25]. All the manipulations were conducted between 0700 h and 0900 h.

To minimize the stress effect among the trained animals, all the other groups (C-SED and DT - after training protocol interruption) were placed in the swimming tank for 5 min/day, 2 days/week.

Twenty-four hours after the last swimming training session, the animals in diestrus phase were fasted for four hours and euthanized by decapitation between 0800 h and 1100 h.

Biometric evaluation

During the entire protocol period, weight measurements were taken on a weekly basis.

Analytical procedures

The blood was collected in tubes containing Ethylenediaminetetra acetic acid (EDTA), heparin or no anticoagulant (Becton Dickinson, New England, UK). Immediately after collection, the total blood aliquots were used for the determination of glucose (Average intra-assay CV: 2.5%), total cholesterol (Average intra-assay CV: 2.3%) and triglycerides (Average intra-assay CV: 3.4%) using a photometric measurement of reflection by Accutrend Plus (Roche, Mannheim, Baden-Württemberg, Germany).

The tubes were centrifuged at 4°C for 10 min at 3,000 rpm to extract the plasma and serum aliquots. A set of serum aliquots was stored at −80°C for the corticosterone measurements (ICN-Biomedical, Orangeburg, NY, USA) using Radioimmunoassay kits (Average intra-assay CV: 4%). Perigonadal fat, liver, heart and gastrocnemius muscle were carefully removed. An analytical balance (Bioprecisa, Model-FA2104N, Curitiba, Paraná, Brazil), accurate to 0.001 g was used to evaluate the mass of the organ, and the calculation of the relative mass of tissue was performed based on the percentage of adipose tissue, taking into account the total weight of the animal.

The liver samples were rapidly excised, weighed and small pieces (0.5 g) were kept in a potassium hydroxide (30%) solution until the carbohydrate content (manly glycogen) was measured by the sulfuric acid-anthrone reaction on the day of euthanasia, as described elsewhere [26,27].

Real-time PCR

For the reaction, TRIzol reagent (Invitrogen, Carlsbad, CA) was added to approximately 100 mg of liver and the total RNA was isolated according to the manufacturer's protocol. An agarose gel (1%) electrophoresis was performed to evaluate the integrity of the molecules. After DNAse (Promega, Madison, USA) treatment, the total RNA was reverse-transcribed using ImProm-IITM Reverse Transcriptase (Promega, Madison, USA). The diluted cDNA was added to 2× SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) together with the respective primers for Glycogen Phosphorylase (GP) and Glycogen Synthase (GS). The expression of the target genes was normalized using Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the endogenous control. The quantitative real-time PCR assays were performed using a 7500 Real-Time PCR instrument (Applied Biosystems). The analysis of the relative expression was performed using a standard dilution curve-based method for relative real-time PCR data processing performed based on the 2(ΔΔ(CT)) method [28].

Statistical Analysis

A repeated measures Analysis of Variance (ANOVA) test was used to analyze weight gain; one-way ANOVA test was used to analyze all the other parameters. When necessary, an a posteriori Duncan's test was used. All the variables were checked for normality and transformed when necessary, using the Z-score, to normalize the data. The data were analyzed using the statistical software SAS System for Windows (Statistical Analysis System, V 8.02 SAS Institute Inc, 1999-2001, Cary, NC). The results are presented as the mean ± S.E.M., and the level of significance was set at $p<0.05$.

Results

Body weight

Figure 2 represents changes in the body weight of mice submitted to training or detraining in different periods of time. Significant differences between the groups were found ($F(6, 78) = 2.81$, $p=0.01$): the DT group showed an increase of body weight in the 10th week when compared to both the 8th week (after training cessation) ($p<0.001$) and the TR group ($p<0.001$).

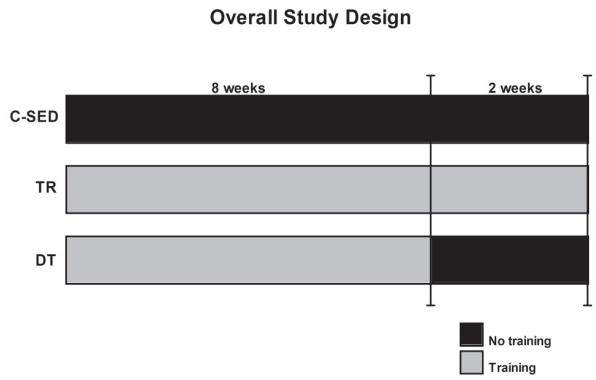

Figure 1. Overall study design. C-SED=sedentary controls, TR=trained and DT=detraining.

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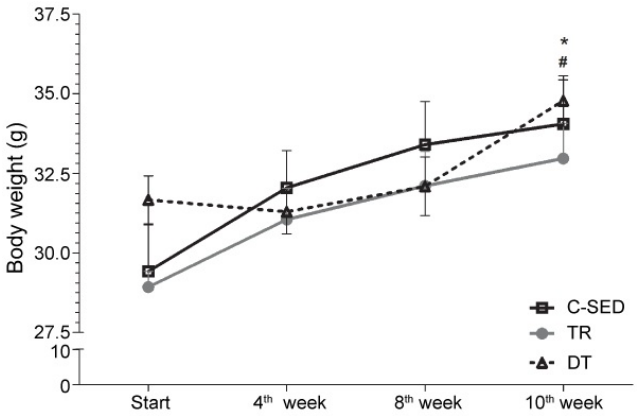

Figure 2: Body weight gain of female mice after the training and detraining periods. C-SED=sedentary controls (N=6), TR=trained (N=12) and DT=detraining (N=11). The values are presented as the mean ± S.E.M. * Different from the CT group; # Difference between the 8th and 10th weeks ($p<0.05$).

Organs

As demonstrated in Table 1, the animals did not show differences in organ weight (perigonadal fat ($F(2, 26)=2.15$, $p=0.14$); liver ($F(2, 21)=0.10$, $p=0.91$); heart ($F(2, 25)=1.48$, $p=0.25$); gastrocnemius muscle ($F(2, 26)=0.67$, $p=0.52$)). When expressed as a percentage of total body mass, a similar result was observed (perigonadal fat ($F(2, 26)=2.00$, $p=0.16$); liver ($F(2, 21)=0.70$, $p=0.51$); heart ($F(2, 26)=0.49$, $p=0.62$); gastrocnemius muscle ($F(2, 26)=0.57$, $p=0.57$) (Table 2).

Biochemical profile

The groups did not show differences in the plasma levels of corticosterone ($F(2, 26)=1.26$, $p=0.30$), glucose ($F(2, 31)=0.16$, $p=0.85$) total cholesterol ($F(2, 31)=0.34$, $p=0.71$), or triglycerides ($F(2, 31)=1.11$, $p=0.34$), as depicted in Table 3.

	C-SED	TR	DT
Heart (g)	0.15 ± 0.00	0.15 ± 0.01	0.14 ± 0.00
Liver (g)	1.46 ± 0.07	1.50 ± 0.08	1.4 ± 0.04
WAT (g)	0.78 ± 0.07	0.71 ± 0.11	1.03 ± 0.15
Gastrocnemius (g)	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
C-SED = sedentary controls (N=10), TR = trained (N=10), DT = detraining (N=9) and WAT=white adipose tissue. The values are presented as the mean ± S.E.M			

Table 1: Organ weights of female mice after the training and detraining periods

	C-SED	TR	DT
Heart (%)	0.41 ± 0.05	0.46 ± 0.03	0.42 ± 0.02
Liver (%)	4.34 ± 0.20	4.78 ± 0.54	4.36 ± 0.17
WAT (%)	2.32 ± 0.20	2.12 ± 0.30	3.06 ± 0.50
Gastrocnemius (%)	0.50 ± 0.03	0.55 ± 0.05	0.53 ± 0.03
C-SED=sedentary controls (N=10), TR = trained (N=10), DT = detraining (N=9) and WAT=white adipose tissue. The values are presented as the mean ± S.E.M			

Table 2: Relative organ weights (%) of female mice after the training and detraining periods

	C-SED	TR	DT
Glucose (mg/dL)	157.8 ± 6.95	162.8 ± 5.29	159.8 ± 7.03
Cholesterol (mg/dL)	159.6 ± 0.93	159.2 ± 1.50	158.1 ± 1.28
Triglycerides (mg/dL)	192.0 ± 29.29	210.0 ± 18.59	166.5 ± 15.80
Corticosterone (ng/mL)	59.0 ± 1.57	61.5 ± 1.56	58.7 ± 1.10
C-SED=sedentary controls (N=10), TR = trained (N=10), DT = detraining (N=9) and WAT=white adipose tissue. The values are presented as the mean ± S.E.M.			

Table 3: Biochemical profile of female mice after the training and detraining periods

Hepatic glycogen, GS and GF

To evaluate the hepatic profile, we analyzed the hepatic glycogen content and the expression of glycogen synthase and glycogen phosphorylase. Differences in the hepatic glycogen content were found among the groups ($F(2, 23)=5.13$, $p=0.01$) (Figure 3). The DT group showed decreased glycogen content when compared to the TR group ($p=0.01$). When the expression of genes involved in the control of the storage (glycogen synthase) and degradation (glycogen phosphorylase) of hepatic glycogen was evaluated, no significant differences were found ($F(2, 21)=2.16$, $p=0.14$ and $F(2, 14)=0.48$, $p=0.63$, respectively; Figure 4 and 5, respectively). These results suggest that a mechanism other than gene expression is involved in the alteration of glycogen content observed in this study.

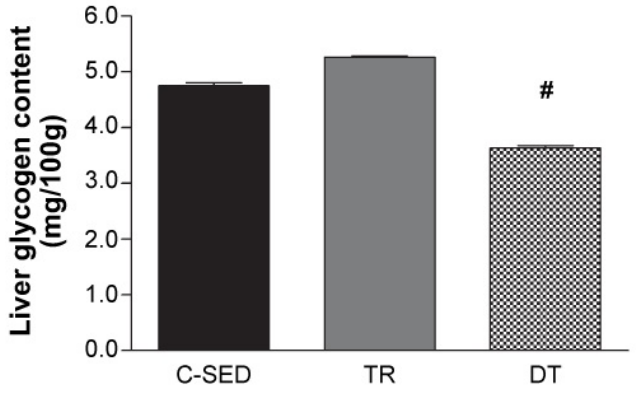

Figure 3: The liver glycogen content of female mice after the training and detraining periods. C-SED=sedentary controls (N= 6), TR=trained (N= 10) and DT=detraining (N= 10). The values are presented as the mean ± S.E.M. # Difference from the TR group ($p<0.05$)

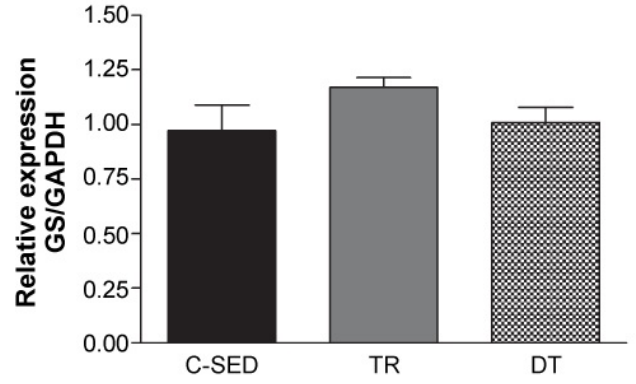

Figure 4: The relative expression of glycogen synthase in the livers of female mice after the training and detraining periods. C-SED=sedentary controls (N=6), TR=trained (N=5) and DT=detraining (N=6). The values are presented as the mean ± S.E.M

Discussion

Body and organ weight

We found that the exercise protocol was effective in maintaining the body mass of animals submitted to the 10-week training protocol (Figure 2). Other authors showed that swim training results in skeletal muscle adaptations consistent with increased resistance to fatigue, including increased mitochondrial enzyme activity [29], increased lipogenic enzyme expression and enhanced muscle capillarization [30,31]. Other studies have shown that regular exercise in humans and animals are effective in maintaining body mass [32-34]. Allen and co-workers [35] demonstrated that voluntary wheel running does not tend to change the body weight, which follows instead the weight gain of age-matched sedentary control mice.

Despite knowledge of the benefits of physical exercise, a current decrease of physical activity has been identified [36,37]. For years, detraining in animals has been associated with a rapid gain of fat mass [2,3]. Yasari and co-workers [38] showed that female mice maintained with a standard diet presented increased energy intake and reduced leptin concentration after 2 weeks of detraining. In humans, interruption of physical exercise combined with a high fat diet was a stimulus for obesity development [39,40]. These findings are in accordance with the weight gain observed in our work, which showed that exercise associated with a standard diet led to an increase in body weight after the cessation of exercise (Figure 2).

Despite the fact that a 2-week detraining increased body weight, no differences in organ weight or relative weight were observed in these animals (Table 1 and 2, respectively). Although no statistically significant differences in the organs were found, it is possible to observe a tendency toward an increase in white adipose tissue in the detraining group. In accordance with this finding, other studies have shown that an increase in body weight is linked to fat mass gain in animals and humans [2,3,41].

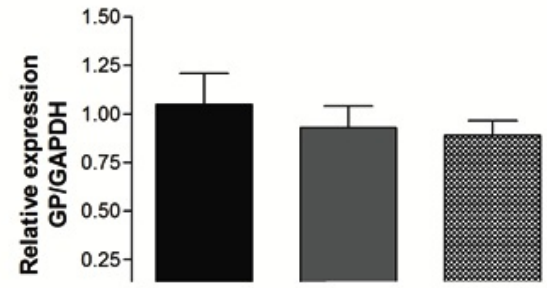

Figure 5: The relative expression of glycogen phosphorylase in the livers of female mice after the training and detraining periods. C-SED=sedentary controls (N=6), TR=trained (N= 5) and DT=detraining (N=6). The values are presented as the mean ± S.E.M

Biochemical profile

Despite the body weight gain in mice from the detraining group, we did not find alterations in the serum glucose, triglyceride and total cholesterol levels (Table 3).

Kawanaka and co-workers [4] showed that after physical training, animals have an increase in glucose transport type 4 (GLUT-4) expressions and improvement in insulin sensitivity in muscle. It was reversed after ninety hours of detraining. Others studies showed that insulin sensitivity decreased after a few days of detraining in physically active people [42,43]. Lehnen and co-workers [44] showed that, although a reduction in GLUT4 expression was observed, some metabolically beneficial effects of exercise were preserved, including an insulin response verified by the tolerance test. Yasari and co-workers [5] did not find differences in glucose concentration, which is in agreement with our work. They observed an increase in insulin levels after 4 weeks of detraining associated with a high fat diet. It is possible that the increase in insulin secretion is sufficient to maintain the

normal levels of glucose. Considering these data, we could suggest that the normal glucose concentrations observed in this study (Table 3) were maintained by the increase in insulin levels.

The effects of exercise on lipid metabolism were investigated many years ago [45,46]. It is well established that chronic exercise training has favorable effects on the plasma lipid profile [47,48]. Physical inactivity is related to excess plasma triglyceride concentration, which contributes, at least partially, to the increased risk of chronic disease that is observed in association with atherosclerosis, fatty liver, diabetes, and obesity [49]. Plasma triglyceride and total cholesterol levels were not affected by the detraining status in this present work (Table 3). These data are consistent with Sandretto and co-workers, who observed that, even in high fat diet hamsters, the triglyceride and total cholesterol levels were not affected by cessation of exercise, despite the weight gain. Some studies showed that after the training cessation, some metabolic variables are maintained including fat free acids and highest lipid oxidation in liver and adipose tissue [5,50]. Exercise led to an increase of lipoprotein lipase, even two weeks after training cessation and despite the increase in lipid oxidation due to physical training, the benefits of exercise are gradually lost [2,5,51].

Liver glycogen content and gene expression

The contribution of muscle and liver glycogen to energy metabolism during exercise varies according to its intensity and duration. Blood glucose plays a greater role during the low intensity exercise, whereas muscle glycogen is the main source of glucose in high intensity exercise (>80% of VO2max). In moderate exercise, the first metabolic pathways of carbohydrate metabolism to be involved are skeletal muscle glycogenolysis and glycolysis. Later circulating glucose, formed through activated gluconeogenesis, becomes an important energetic source and a crucial role in preventing hypoglycemia during exercise, and it is generally believed that strategies to enhance liver glycogen after exercise would increase the exercise capacity in a subsequent exercise bout [14,52]. Hypoglycemia has been described as a possible cause of fatigue during prolonged endurance exercise, and hepatic glycogenolysis contributes significantly to the prevention of hypoglycemia. Having higher liver glycogen stores could be beneficial [53,54]. Many studies have focused only on muscle glycogen, but evidence from early rodent studies suggests that when carbohydrates are available after exercise, liver glycogen resynthesis is the first priority and muscle glycogen synthesis is secondary [13].

We showed an increase in the liver glycogen content of mice trained after 10 weeks, however no statistical difference was observed. When compared the trained group with detraining group, we showed that this modest increase was lost after two weeks of detraining (Figure 3). A reduction in the liver glycogen content is associated with important metabolic consequences. Fatty acids become important substrates for energy production [55]. Moreover, when liver glycogen stores are reduced, the gluconeogenesis is activated to maintain the glucose needs for glucose-dependent tissues such as brain or erythrocytes. Amino acids are important substrates to gluconeogenesis, many of them originating from skeletal muscle. Loss of liver glycogen with subsequent accelerated gluconeogenesis may therefore represent a muscle wasting [56].

We hypothesized that when exercise activity is abruptly discontinued, the state of equilibrium is interrupted and some effects of exercise are lost in a short period. When the expression of genes involved in the storage and degradation of glycogen were evaluated, we did not find differences between the groups (Figure 4 and 5). Tsai and co-workers [57] showed that liver glucose-6-phosphate dehydrogenase activity was increased at 8 days after the discontinuation of running. It is possible that despite there being no alterations in gene expression, the activity of some enzymes such as glucose-6-phosphate dehydrogenase, glycogen synthase and glycogen phosphorylase may affect glycogen content in detraining animals. Marinho and co-workers [19] demonstrated that obese mice submitted to eight weeks of aerobic exercise training showed enhanced synthesis of hepatic glycogen by Akt phosphorylation activating glycogen synthase through the inhibition (phosphorylation) of glycogen synthase kinase 3 beta (GSK3b). This pathway was blocked and was less active in obese, sedentary mice. The control of Akt activity in the liver is important for the control of glucose homeostasis and also could explain the glycogen reduction after the detraining period.

Conclusion

We verified that, after a training period of 8 weeks, the animals had an increase in body weight after two weeks of detraining. After the two-week detraining period, the animals showed a decrease in liver glycogen content without alterations in the fasting glucose concentration in their plasma. It is possible that, during exercise, the decrease in hepatic glycogen can lead to a lower performance in this group when resubmitted to training, because the liver plays a crucial role in preventing hypoglycemia during exercise. These data suggest that a short period of detraining alters some metabolic parameters important to performance, and elite athletes must pay attention to this effect in their strategies.

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