THE EFFECT OF EXERCISE TRAINING ON GLUCOSE TOLERANCE AND SKELETAL MUSCLE TRIACYLGLYCEROL CONTENT IN RATS FED WITH A HIGH-FAT DIET

M. STRACZKOWSKI (1), I. KOWALSKA (1), S. DZIENIS-STRACZKOWSKA (1), M. KINALSKI (1), J. GÓRSKI (2), I. KINALSKA (1)

SUMMARY - The aim of the present study was to evaluate the effect of exercise training on glucose tolerance and glycogen and triacylglycerol (TG) content in different types of skeletal muscles and in the liver of rats fed with a high-fat diet. From 8 to 11 weeks of age male Wistar rats were fed with isocaloric standard (control) or high-fat diet (HFD – 59% calories as fat) and were additionally assigned to a sedentary or trained group (4 weeks of training on a treadmill). An intravenous glucose tolerance test (IVGTT) with the determination of basal and post load insulin was performed before the final tissue sampling. HFD rats developed marked hyperinsulinemia. Exercise training improved glucose tolerance and insulin response in the control group only (AUC for glucose in control sedentary vs control trained, p < 0.05; AUC for insulin: control sedentary vs control trained, p < 0.005). Liver glycogen was significantly lower in the HFD group (p < 0.05 vs control sedentary) and did not increase after exercise training. Muscle and liver TG content was markedly higher in the HFD group in comparison to control (p < 0.0001 in all cases). Exercise training increased TG content in the control group in all examined tissues except white gastrocnemius (p < 0.001 in all cases compared to sedentary controls), and did not affect tissue TG in the HFD group. After exercise training there was still markedly higher tissue TG content in the HFD group vs control (p < 0.0001 in all cases). We conclude that beneficial metabolic effects of training are impaired in high-fat fed rats and that training does not completely reverse metabolic disturbances in this group of animals.

Key-words: high-fat diet, training, muscle TG.

RÉSUMÉ - Effets de l'exercice physique sur la tolérance au glucose et le contenu du muscle squelettique en triacylglycérol de rats sous régime hyperlipidique.

L'objectif de cette étude était d'évaluer l'effet de l'exercice physique sur la tolérance au glucose et le contenu en glycérogène et en triacylglycérol (TG) de différents muscles squelettiques et du foie de rats soumis à un régime hyperlipidique. Des rats Wistar mâles âgés de 8 à 11 semaines ont été soumis à un régime isocalorique standard (contrôle) ou hyperlipidique (HFD – 59 % lipides) et ont été assignés à un groupe sédentaire ou entraîné (4 semaines d'exercice sur tapis roulant). Un test de tolérance IV au glucose (IVGTT) avec détermination de l'insulinémie basale et post-chargé a été réalisé avant l'analyse tissulaire finale. Les rats HFD ont développé un hyperinsulinisme franc. L'entraînement physique a amélioré la tolérance au glucose et la réponse insulinique dans le groupe contrôle seulement (aire sous la courbe du glucose chez les contrôles sédentaires vs contrôles entraînés, p < 0.05 ; AUC de l'insuline : contrôles sédentaires vs contrôles entraînés, p < 0.005). Le glycérogène hépatique était significativement plus bas dans le groupe HFD (p < 0.05 vs contrôles sédentaires) et n'a pas augmenté après entraînement. Le contenu TG musculaire et hépatique était beaucoup plus important dans le groupe HFD que chez les contrôles (p < 0.0001 dans tous les cas). L'exercice physique a augmenté le contenu TG dans le groupe contrôle dans tous les tissus examinés sauf le gastrocnemius blanc (p < 0.001 dans tous les cas comparés aux contrôles sédentaires), et n'a pas affecté le contenu TG du groupe HFD. Après exercice physique, le contenu TG était encore beaucoup plus élevé dans le groupe HFD que dans le groupe contrôle (p < 0.0001 dans chaque cas). En conclusion, les effets métaboliques bénéfiques de l'activité physique sont atténués chez les rats sous régime hyperlipidique, et l'entraînement ne peut pas complètement corriger les anomalies métaboliques de ce groupe.

Mots-clés : régime hyperlipidique, entraînement physique, triacylglycérol musculaire.
Skeletal muscles are the major site of insulin action and decreased muscle glucose utilization contributes to the development of insulin resistance, an important pathophysiological factor of type 2 diabetes mellitus [1]. Precise mechanisms of muscle insulin resistance are at present unknown, however it is supposed that lipids may suppress glucose utilization through a glucose-fatty acids cycle [2]. Especially skeletal muscle triacylglycerols (TG) seem to be an important lipid pool. Increased muscle TG was reported in diabetic subjects [3]. A negative correlation was found between intramuscular TG content and insulin sensitivity in rats [4] and in humans [5].

Physical exercise, especially prolonged exercise training, enhances insulin sensitivity. The amount of physical activity is positively related to insulin action [6] and it is supposed that a sedentary lifestyle is responsible for at least 25% of the incidences of type 2 diabetes mellitus [7]. Therefore, the role of exercise training in the prevention of insulin resistance and type 2 diabetes is a problem of major interest. The beneficial effects of training on insulin sensitivity have also been demonstrated in normal rats [8], but data obtained from rat models of insulin resistance are much more conflicting.

A rat model of insulin resistance may be obtained by feeding with a high-fat diet [9]. Recently we reported that tissue substrate utilization is altered during a single bout of exhaustive exercise in that group of rats [10], however little is known about the actions of prolonged exercise training in that animal model. Therefore the aim of the present study was to estimate the effect of exercise training on glucose tolerance and glycogen and TG content in different types of skeletal muscles and in the liver of high-fat fed rats.

### MATERIALS AND METHODS

All the experiments were approved by the Ethics Committee, Medical School, Białystok, Poland.

**Animals, diet and training protocol** – Studies were carried out on male Wistar rats, kept at a temperature 20 ± 1 °C, on a 12 h light-dark cycle. At 5 weeks of age the animals were randomly allocated to a sedentary or exercise trained group. Rats were adapted to the age the animals were randomly allocated to a sedentary or exercise trained group. Rats were adapted to a 20 °C environment and a 12 h light-dark cycle. At 5 weeks of age the animals were randomly allocated to a sedentary or exercise trained group. The incline of the treadmill was +10° and the speed was 20 m/min.

At 8 weeks of age, all the rats were additionally randomly divided into a group fed for 3 weeks standard (control) or high-fat diet (HFD – 59% of calories as fat). The composition of the HFD was described previously [9] (Safflower oil – 339g, Cornstarch – 254g, Casein – 254g, Methionine – 3g, Gelatin – 19g, Wheat bran – 51g, Vitamin mix – 13g, Mineral mix – 67g/kg). Control rats were fed a commercial pellet diet for rodents (“Bacutil”, Poland). Animals in both diet groups were fed isocalorically – 311 kJ per day. Each subgroup included 12 rats.

4 days before the final experiments an intravenous glucose tolerance test (IVGTT) was performed on all the rats. 40% solution of glucose (500 mg/kg body weight) was injected into a tail vein. Blood samples were collected from the tail vein at 0, 2, 5 and 10 minutes into the test to determine plasma glucose and insulin concentrations.

At 11 weeks of age, after the completing the exercise training protocol and/or high-fat feeding period, samples of the white gastrocnemius (WG), red gastrocnemius (RG) and soleus muscle and of the liver were collected to assess glycogen and TG content. Plasma glucose, insulin, TG and non-esterified fatty acids (NEFA) were also measured. Before sampling, rats were anaesthetized with pentobarbital sodium given intraperitoneally (80 mg/kg body weight). In the trained rats sampling was performed 48 hours after the training cessation.

**Biochemical analyses** – Plasma glucose was assessed by enzymatic method using a commercially available kit (GOD-PAP Cormay GS-120L). Plasma insulin was measured in duplicate by radioimmunoassay (Rat Insulin DLR-RI-13K). The area under the curve (AUC) for plasma glucose and insulin during IVGTT were calculated using linear interpolation. Plasma TG concentrations were determined by enzymatic method using Cormay TG Kit and plasma NEFA were measured by colorimetric method [11].

Tissue glycogen content was determined by the method described by Carroll [12]. In that method glycogen is precipitated from trichloroacetic acid filtrate of tissues by ethanol and determined by the use of an anthrone reagent. To determine tissue TG content, muscle and liver samples were dissected from fat and total lipid extraction was performed as described previously by Bligh and Dyer [13] using chloroform : methanol (2 : 1 vol/vol). Thin-layer chromatography was made with Pre-coated TIC plates silica gel 60 (Merck, Darmstadt) to avoid other lipid fraction contamination. TG was quantified spectrophotometrically at 520 nm (DU 640 Beckmann) according to a method proposed by Galetti [14].

**Statistical analysis** – All the data are presented as mean ± SEM. All the statistics were made using the STATISTICA 5.0 program (StatSoft, Krakow, Poland). To evaluate differences between groups unpaired Student’s t-test was used. Relationships between variables were analyzed with Pearson’s product-moment correlation and with partial correlation matrix. P values less than 0.05 were considered as statistically significant.
RESULTS

The basal characteristics of the examined groups is given in Table I. Body weight was significantly higher in the HFD group (p < 0.01) in the sedentary rats, but not in the trained animals. Both groups of trained rats had markedly lower body weight than their sedentary counterparts (p < 0.01 for the control and p < 0.001 for the HFD group).

There was no effect of diet or training on fasting plasma glucose levels. Fasting plasma insulin was markedly higher in the HFD rats both in the sedentary and trained groups (p < 0.001 vs respective controls in both cases). Trained rats had slightly lower plasma insulin levels than the respective sedentary animals but the observed differences were not significant.

In the sedentary rats, the HFD group had markedly higher AUC for glucose during IVGTT (p < 0.05). After exercise training the significant decrease in the AUC for glucose was observed only in the control (−7.35 %, p < 0.05) and not the HFD group (−6.68 %, p < 0.09, NS). In the HFD trained rats we still observed markedly higher AUC for glucose (p < 0.05).

Sedentary HFD rats had markedly higher AUC for insulin (+40.16 %, p < 0.0001). Training resulted in a decrease in the AUC for insulin by 16.73 % in the control rats (p < 0.005). In the HFD rats the decrease in the AUC for insulin was not significant (−9.41 %, p < 0.15 ; NS). Trained HFD rats had still greater AUC for insulin (+52.48 %, p < 0.001).

Plasma TG did not differ between the groups studied. Plasma NEFA were markedly higher in the sedentary HFD vs control rats (p < 0.001). Although training did not result in significant changes in plasma NEFA, such a difference was not observed in the trained rats.

There was no significant effect of diet or training on muscle glycogen content (Table II). Liver glycogen was markedly lower in the HFD group both in the sedentary and trained animals (p < 0.05 in both cases compared to the respective controls) (Table II). When all the sedentary animals were analyzed together, plasma NEFA were inversely related to glycogen content in the soleus muscle (r = −0.70 ; p < 0.05) and in the liver (r = −0.74 ; p < 0.02).

### Table I. Basal characteristics of the examined groups. All the data are presented as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Contr. sedentary</th>
<th>Control trained</th>
<th>HFD sedentary</th>
<th>HFD trained</th>
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</thead>
<tbody>
<tr>
<td>Initial body weight (g)*</td>
<td>174.22 ± 19.71</td>
<td>181.40 ± 16.82</td>
<td>169.40 ± 16.98</td>
<td>171.45 ± 19.75</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>323.08 ± 35.76</td>
<td>268.17 ± 17.89b</td>
<td>367.83 ± 24.51a</td>
<td>276.42 ± 29.61b</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>8.49 ± 0.69</td>
<td>8.06 ± 0.42</td>
<td>8.55 ± 0.52</td>
<td>8.29 ± 0.63</td>
</tr>
<tr>
<td>Plasma insulin (ng/ml)</td>
<td>1.47 ± 0.39</td>
<td>1.26 ± 0.28</td>
<td>2.44 ± 0.36a</td>
<td>2.23 ± 0.39a</td>
</tr>
<tr>
<td>AUC glucose (mmol × min/L)</td>
<td>107.01 ± 7.55</td>
<td>99.15 ± 4.89b</td>
<td>117.85 ± 13.45a</td>
<td>109.97 ± 10.11a</td>
</tr>
<tr>
<td>AUC insulin (ng × min/L)</td>
<td>43.93 ± 5.28</td>
<td>36.58 ± 5.06b</td>
<td>61.58 ± 9.53a</td>
<td>55.78 ± 8.24a</td>
</tr>
<tr>
<td>Plasma NEFA (µEq/L)</td>
<td>187.33 ± 37.54</td>
<td>240.25 ± 71.56</td>
<td>319.75 ± 50.65a</td>
<td>305.83 ± 44.25</td>
</tr>
<tr>
<td>Plasma TG (mmol/L)</td>
<td>0.48 ± 0.07</td>
<td>0.43 ± 0.09</td>
<td>0.54 ± 0.25</td>
<td>0.47 ± 0.07</td>
</tr>
</tbody>
</table>

* 7 weeks of age ; a p < 0.05 between respective control and HFD animals, b p < 0.05 between respective sedentary and trained animals.

### Table II. Tissue glycogen (µmol of glucose units per g of tissue weight). All the data are presented as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Contr. sedentary</th>
<th>Control trained</th>
<th>HFD sedentary</th>
<th>HFD trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>White gastrocnemius</td>
<td>6.41 ± 1.49</td>
<td>6.77 ± 1.36</td>
<td>5.87 ± 0.64</td>
<td>6.97 ± 1.95</td>
</tr>
<tr>
<td>Red gastrocnemius</td>
<td>6.09 ± 1.43</td>
<td>6.39 ± 1.35</td>
<td>5.28 ± 0.95</td>
<td>6.05 ± 2.39</td>
</tr>
<tr>
<td>Soleus</td>
<td>5.57 ± 0.75</td>
<td>6.08 ± 0.50</td>
<td>4.74 ± 0.76</td>
<td>5.28 ± 1.22</td>
</tr>
<tr>
<td>Liver</td>
<td>48.38 ± 16.11</td>
<td>57.46 ± 14.81</td>
<td>17.22 ± 5.02a</td>
<td>22.01 ± 9.27b</td>
</tr>
</tbody>
</table>

*p < 0.05 between respective control and HFD animals ;

*p < 0.05 between respective sedentary and trained animals.
High-fat feeding resulted in a significant TG accumulation in all the examined tissues (HFD sedentary: p < 0.0001 in all cases vs control sedentary) (Table III). Training induced an increase in TG content in the control group in the RG, the soleus muscle and in the liver (p < 0.001 in all cases compared to the sedentary controls) and did not cause any changes in tissue TG in the HFD group. In spite of these different effects, the trained HFD rats still had markedly higher tissue TG compared to the trained controls (p < 0.0001 in all cases).

In the sedentary rats we found significant correlation between the AUC for insulin and TG content in the RG (r = 0.87), soleus (r = 0.93) and in the liver (r = 0.90; p < 0.0001 in all cases). Plasma NEFA were also positively related to the AUC for insulin in the sedentary rats (r = 0.74; p < 0.005). Such relationships were no longer persistent in the trained rats.

### DISCUSSION

In the present study, in agreement with previous data, the HFD rats developed hyperinsulinemia, accompanied by slightly increased glucose response to an intravenous challenge. Although some beneficial actions of training could be detected both on glucose tolerance and hyperinsulinemia in this group of rats, these effects are less expressed in comparison to the control group in the RG, the soleus muscle and in the liver (p < 0.001 in all cases compared to the sedentary controls), and did not cause any changes in tissue TG in the HFD group. In spite of these different effects, the trained HFD rats still had markedly higher tissue TG compared to the trained controls (p < 0.0001 in all cases).

In the sedentary rats we found significant correlation between the AUC for insulin and TG content in the RG (r = 0.87), soleus (r = 0.93) and in the liver (r = 0.90; p < 0.0001 in all cases). Plasma NEFA were also positively related to the AUC for insulin in the sedentary rats (r = 0.74; p < 0.005). Such relationships were no longer persistent in the trained rats.

To evaluate possible factors responsible for those differences we measured tissue glycogen and TG content, as they are major sources of energy used by muscles. Results obtained from the sedentary animals were similar to the data described previously [10]. Muscle glycogen content was not affected by training. Both unchanged [15] and increased [16] muscle glycogen in response to training was observed. We detected decreased glycogen content in the liver of HFD rats. It was caused by fat excess, as liver glycogen content was inversely related to plasma NEFA. Exercise training was not sufficient to restore liver glycogen in the HFD rats to the values observed in the control animals.

Another finding of our study is the marked TG accumulation in all the examined tissues after high-fat feeding. A significant correlation between muscle TG and insulin resistance was reported previously [4, 5]. We found marked correlation between tissue TG and the AUC for insulin. In the present study we did not measure insulin action, however AUC for insulin during the IVGTT may serve as a crude index of insulin sensitivity, when beta-cell function is not impaired. As was observed previously by other authors [3] and by ourselves [10] increased muscle TG leads to the greater lipid utilization and decreased glucose oxidation during exercise. The strongest correlation between TG content and hyperinsulinemia was present in the soleus muscle, which is mainly slow-twitch oxidative and accounts for the highest rates of glucose metabolism [17].

Exercise training increased the TG content in the control rats in all the examined tissues except the white gastrocnemius and did not change muscle and liver TG in the HFD group. Increase in the muscle TG observed in the control group is probably an adaptive mechanism for the increased TG utilization [18]. In the trained HFD animals tissue TG did not change in comparison to the sedentary rats. However, it was still higher than in the control trained rats, i.e. greater than is necessary for the adaptation to training. It is unlikely to suppose that at a content at baseline approximately two-threefold higher than control values any further adaptations are needed. Therefore, the regulation of the muscle TG during training seems to depend on their initial levels.

In the control rats training improved glucose tolerance and insulin response and simultaneously increased tissue TG content. However, in the trained rats no correlation was found between the AUC for insulin and muscle TG. Therefore we can suppose that exercise training decreases the negative effect of muscle TG on hyperinsulinemia and insulin sensitivity. Such an effect may be due to the increased number and volume of mitochondria [19] and location of muscle

<table>
<thead>
<tr>
<th>Tissue</th>
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</tr>
</thead>
<tbody>
<tr>
<td>White gastrocnemius</td>
<td>2.08 ± 0.37</td>
<td>2.22 ± 0.43</td>
<td>3.84 ± 0.63a</td>
<td>4.07 ± 0.68a</td>
</tr>
<tr>
<td>Red gastrocnemius</td>
<td>2.73 ± 0.38</td>
<td>4.49 ± 0.62b</td>
<td>5.38 ± 0.68a</td>
<td>5.70 ± 0.47a</td>
</tr>
<tr>
<td>Soleus</td>
<td>4.14 ± 0.57</td>
<td>5.23 ± 1.21b</td>
<td>9.07 ± 0.63a</td>
<td>8.85 ± 2.52a</td>
</tr>
<tr>
<td>Liver</td>
<td>3.44 ± 0.37</td>
<td>6.78 ± 1.02b</td>
<td>9.94 ± 1.15a</td>
<td>9.01 ± 1.01a</td>
</tr>
</tbody>
</table>

*p < 0.05 between respective control and HFD animals;
*p < 0.05 between respective sedentary and trained animals.

### Table III. Tissue TG (µmol per g of tissue weight). All the data are presented as mean ± SEM.
TG just near the mitochondria [5]. Probably such distribution does not affect glucose metabolism, for instance probably does not decrease GLUT4 translocation. The explanation of that problem is at present unknown and our study does not answer that question, however it indicates that the physiological significance of the muscle TG increase after exercise training or high-fat diet is different.

We conclude that beneficial metabolic effects of training are impaired in high-fat fed rats and that training does not completely reverse metabolic disturbances in this group of animals. Altered regulation of tissue glycogen and TG may be one of the factors responsible for such impairment.

RÉféRences


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