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Exercise at anaerobic threshold intensity and insulin secretion by isolated pancreatic islets of rats

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To evaluate the effect of acute exercise and exercise training at the anaerobic threshold (AT) intensity on aerobic conditioning and insulin secretion by pancreatic islets, adult male Wistar rats were submitted to the lactate minimum test (LMT) for AT determination. Half of the animals were submitted to swimming exercise training (trained), 1 h/day, 5 days/week during 8 weeks, with an overload equivalent to the AT. The other half was kept sedentary. At the end of the experimental period, the rats were submitted to an oral glucose tolerance test and to another LMT. Then, the animals were sacrificed at rest or immediately after 20 minutes of swimming exercise at the AT intensity for pancreatic islets isolation. At the end of the experiment mean workload (% bw) at AT was higher and blood lactate concentration (mmol/L) was lower in the trained than in the control group. Rats trained at the AT intensity showed no alteration in the areas under blood glucose and insulin during OGTT test. Islet insulin content of trained rats was higher than in the sedentary rats while islet glucose uptake did not differ among the groups. The static insulin secretion in response to the high glucose concentration (16.7 mM) of the sedentary group at rest was lower than the sedentary group submitted to the acute exercise and the inverse was observed in relation to the trained groups. Physical training at the AT intensity improved the aerobic condition and altered insulin secretory pattern by pancreatic islets.

Introduction

As there are obvious limitations in research with human beings, especially in clinical studies, animal models have supplied important information related to the origin and consequences of certain diseases that involve alteration of insulin secretion/action, including obesity,[1,2] diabetes mellitus[3-5] and malnutrition.[6,7] On the other hand, physical exercise has been considered an important tool in the treatment of obesity as well as of diabetes[8,9] and malnutrition.[10]

In this sense, the study of the effects of exercise on insulin secretion in animal models can contribute to the development of more effective treatment procedures of several diseases. Zawalich et al.[11,12] reported that aerobic physical training in animals reduced insulin secretion in response to glucose at different concentrations. Peres[13] found reduced insulin release after acute effort test in trained rats.

On the other hand Fluckey et al.[14] reported that four days of resistance exercise increased pancreatic islet insulin secretion. This indicated that physical training may alter insulin secretory pattern in different ways, depending of exercise intensity.

The study of the metabolic effects of exercise in rats is frequently questioned by the lack of information regarding the intensity of effort performed by the animal. Recently in our laboratories, a protocol for determination of the anaerobic threshold (AT), based on the lactate minimum test adapted for the conditions of the rat was developed, allowing the estimation of the individual effort intensity for these animals during swimming exercise.[15,16] In this context, the present study was designed to evaluate the effect of exercise training at the intensity equivalent to the AT on the aerobic conditioning and insulin secretion by pancreatic islets of rats.

Results

Figure 2 shows mean values of workload equivalent to the AT calculated for the rats of the sedentary and the trained groups while **Figure 3** shows mean of the blood lactate concentrations at the beginning and end of the experiment. At the end of the experiment, the mean values of workload equivalent to the AT were higher ($p < 0.05$) (sedentary 4.86 ± 0.03; trained 5.53 ± 0.03% b.w.), whereas the blood lactate concentrations were lower ($p < 0.05$) (Sedentary 7.14 ± 0.53; Trained 4.85 ± 0.19 mmol/L) in the Trained group than in the Sedentary one.

Table 1 shows results referring to the areas under body weight (ΔW) registered once a week during the eight weeks of

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Table 1. Areas under body weight (ΔW, g x 8 weeks) during the experimental period and blood glucose (ΔG, mg/dL x 120 min) and insulin (ΔI, ΔU/ml x 120 min) during the glucose tolerance test of the animals at the end of the experiment period

	ΔW	ΔG	ΔI
Sedentary (16)	6463.4 ± 185.2	11500.30 ± 440.30	81.22 ± 10.61
Trained (16)	6455.1 ± 192.2	11162.10 ± 552.20	71.07 ± 22.51

Results are mean ± standard deviation, *n* is between parentheses.

the experimental period and serum glucose (ΔG) and insulin (ΔI) concentrations during the OGTT at the end the of experiment. No difference between the groups was observed in these paremeters.

Figure 4 contains the results referring to the insulin content in the pancreatic islets. The insulin content of both trained groups (Trained Rest and Trained Acute) were significantly higher ($p < 0.05$) than in the sedentary groups (Sed Rest and Sed Acute).

Figure 5 contains the results referring to the glucose uptake by isolated islets in response to different glucose concentrations after 8 weeks of experiment. There was an increase in the glucose uptake by islets when the glucose concentration in the incubation medium changed from 2.8 mM to 16.7 mM, as expected. No difference among the groups has been evidenced.

Results referring to the glucose oxidation by isolated islets in response to different glucose concentrations after 8 weeks of experiment are shown in **Figure 6**. There was an increase in the rate of glucose oxidation when the glucose concentration was raised from 2.8 mM to 16.7 mM, but no difference among the groups was observed.

The static insulin secretion by isolated pancreatic islets of animals from the four groups are showed in **Figure 7**, stimulated by low (2.8 mM) and high (16.7 mM) glucose concentrations. As occurred with glucose uptake and oxidation, insulin secretion was significantly higher in response to the high glucose concentration. No significant difference among the groups was observed in the low glucose concentration. On the other hand, the response to the high glucose concentration was quite irregular. The secretion of the sedentary group at rest (Sed Rest) was lower ($p < 0.05$) than the sedentary group that was submitted to the acute exercise (Sed Acute). The inverse was observed in relation to the trained groups.

Discussion

There are reports in the literature that, in human beings and experimental animals, the insulin response to some secretagogues is reduced as consequence of aerobic resistance exercise training[11,17,18] and increased after strength exercise in rats.[14] However, the studies carried out with experimental animals are questioned, since, in many cases, the intensity of the effort experienced by the animals during the exercise was not evaluated. Therefore, the aim of this study was to evaluate aerobic capacity and insulin secretion by isolated pancreatic islets of rats exercised at the intensity equivalent to the AT, estimated by the LMT, adapted to rats.[15]

In the present study, physical training increased the work load equivalent to the AT and reduced the blood lactate concentration at this work load, as demonstrated the results of the LMT carried out at the end of the experiment. During exercise, blood lactate concentration depends on the balance between its production by the active muscles and its removal from blood stream.[19,20] The LMT carried out at the end of the experimental period demonstrated that the swimming training at the intensity of the AT reduced the accumulation of blood lactate during exercise. This result indicates that the protocol was efficient in improving the aerobic conditioning of the animals, since there are evidences that blood lactate concentration during exercise displays a similar pattern in rats and human beings.[21]

No difference between Trained and Sedentary groups was detected with respect to the areas under serum insulin and glucose curves during the OGTT. These results contrast with other studies that described higher glucose tolerance[22] and increased glucose uptake by the skeletal muscle[23,24] in individuals submitted to physical training in relation sedentary individuals.

In the present study, to evaluate pancreatic islet function of animals submitted to training in intensity equivalent to the AT, we evaluated insulin content as well as static insulin secretion, glucose uptake and glucose oxidation by this tissue in response to different glucose concentrations.

Trained animals presented higher insulin content in the islets than the sedentary ones, both when evaluated in rest, and immediately after an exercise session, at the AT intensity. Insulin release in response to the low glucose concentration (2.8 mM) did not suffer influence neither from acute exercise nor from exercise training in the intensity of the anaerobic threshold. In response to high glucose concentrations (16.7 mM), insulin secretion by isolated islets of the trained rats evaluated at rest was significantly higher in comparison to their sedentary counterparts. Also acute exercise at the AT intensity significantly increased glucose stimulated insulin release in sedentary rats in comparison to the resting situation. This suggests that the overall glucose-induced insulin secretion may be larger in the exercise trained group than in the sedentary group. As the glucose-stimulated insulin release experiments were performed "in vitro" with isolated islets, neural and hormonal effects of exercise were eliminated.

Therefore, the mechanisms responsible for the alterations in the pattern of insulin secretion observed as consequence as acute exercise and exercise training are intrinsic to the β-cell. This hypothesis is supported by previous reports.[25]

Aiming at clarifying some of the possible mechanisms involved in the alteration of insulin release in function of exercise at the AT intensity, glucose uptake and oxidation by isolated islets have been evaluated. However, no difference among the groups for these measures was found. Previous studies showed contrasting results.[11,17,18,25-27]

King et al.[26] showed reduced plasma insulin response to glucose in trained subject and suggested that regular exercise at 65–85% of maximal oxygen uptake (Vo2max) produces either several adaptations within the β-cell or a single alteration of the β-cell that results in an attenuation of the insulin secretory response to glucose. Engdahl et al.[18] demonstrated that endurance

training reduced insulin secretion per burst without alterations in the periodicity of insulin bursts and nonpulsatile basal insulin, similar results were reported in studies with rats submitted to aerobic physical training.[11,12]

The response of the β-cells to glucose after aerobic training was investigated at the molecular level by Koranyi et al.[17] These authors studied rats submitted to aerobic training during three weeks and evaluated the expression of genes particularly related to the glucose induced insulin synthesis and release, evidencing reduction of pro-insulin mRNA and of glucokinase mRNA content, but they did not register alteration in the glucose transporter GLUT2 mRNA content. They had also evidenced a positive correlation between the decrease of mRNA of glucokinase and the decrease of mRNA of pro-insulin, suggesting that the regulation of these genes occurs in parallel.

On the other hand, different results were found with other types and intensity of exercise. Fluckey et al.[14] showed that four days of resistance exercise increased insulin secretory ability of pancreatic islets from rats. In a recent study, Moreira et al.[27] demonstrated that insulin secretion by the islets isolated from rats submitted to moderate aerobic exercise training during nutritional recovery from protein malnutrition was improved when compared with sedentary animals in same conditions. Taken together, our results and those from Korany et al.[17] Fluckey et al.[14] and Moreira et al.[27] indicate that the exercise intensity and the nutritional status interfere in the effects of training on insulin secretion pattern.

In conclusion, physical training at the AT intensity improved the aerobic condition and altered insulin secretory pattern of the rats.

Materials and Methods

Animals. All experiments involving animals followed the specific Brazilian Bioethics of Experiments with Animals resolutions (law No 11.794 of November 8th 2008, National Council for the Control of Animal Experimentation-CONCEA). Young male Wistar rats (60 days old at the beginning of the experiment), from the Central Bioterium—UNESP—São Paulo State University/Botucatu were used. The animals remained in collective cages (5 rats per cage), being kept under periodic 12 hours light-dark cycle at mean temperature of 25 ± 2°C, with free access to standard rat chow and water. Body weight was registered once a week. The results were analyzed by the area under the curve of body weight evolution during the experiment, calculated by the trapezoidal method[28] using the software Origin 6.0®-1999.

Adaptation to the liquid environment. The adaptation consisted of maintaining the animals in shallow water at temperature of 32 ± 2°C during 3 weeks, 5 days a week for 45 minutes. At the 2nd and 3rd adaptation weeks, the animals supported sinker overloads inserted into cotton "knapsacks" fastened with Velcro® and attached to the thorax with the aid of rubber band. The objective of such adaptation was to reduce the stress of animals in face of the exercise performed in the water. After adaptation, the animals were submitted to swimming LMT for determination of the Anaerobic Threshold (AT), with the results shown as overload (% of body weight—% b.w.) and blood lactate concentration (in mmol/L) corresponding to the AT.

Experimental groups. The rats were allocated into two groups, with 16 rats in each:

Sedentary: rats submitted only to the protocol for adaptation to the liquid environment;

Trained: rats that besides the protocol for the adaptation to the liquid environment performed exercise training consisting of swimming 1 hour/day, 5 days/week, during 8 weeks (without interruption), with an overload equivalent to AT, in a swimming pool filled with water at 32 ± 1°C.

At sacrifice, the same animals were allocated into the following sub-groups, with 8 rats in each:

Sed Rest: sedentary rats kept at rest;

Trained Rest: trained rats kept at rest 48 h before sacrifice;

Sed Acute: sedentary rats that exercised at the AT intensity for 20 minutes immediately before being sacrificed.

Trained Acute: trained rats that exercises at the AT intensity for 20 minutes immediately before being sacrificed.

Lactate minimum test (LMT). Initially, the animals were placed in a tank full of water, supporting an overload equivalent to 50% of the body weight, performing high intensity swimming exercise during 6 minutes (30 seconds of exercises interrupted by 30 seconds of rest) for the elevation of blood lactate concentration. After 9 minutes of rest, the animals started swimming with intensities progressively higher (4.5; 5.0; 5.5; 6.0; 6.5 and 7.0% b.w.), 5 minutes for each load. Before the beginning of the test (rest) and at each load change, blood samples were collected (25 μl) for determination of lactate concentration. The minimum values of blood lactate (mmol/L) and workload (% b.w.) were obtained by Microsoft Excel (polynomial function of degree 2) and indicate the AT.[15] This test was performed at the beginning and repeated at the end of the experimental period. **Figure 1** shows blood lactate concentrations and workloads during the determination of aerobic threshold by the lactate minimum test of one rat, as an example.

Oral glucose tolerance test (OGTT). After a 15 hour fasting and 48 hours after the last lactate minimum test, a glucose solution (80%) was administered into the stomach of the rats through a gastric catheter at the final dose of 2.0 g Kg^{-1} b.w. Blood samples for determination of serum glucose (25 μl) and insulin (75 μl) levels were obtained, from a small cut in the tip of the tail, immediately before (0 min) and 30, 60 and 120 min after the glucose administration. Blood glucose was determined by the glucose-oxidase method (*CAT n° 02200, Kit Laborlab®, Brazil*) and insulin by radioimmunoassay (*RIA Kit Coat-A-Count, USA*).[29] Results are shown as areas under blood glucose [mM $(90\ min)^{-1}$] and insulin [nM $(90\ min)^{-1}$] curves during the OGTT, calculated by the trapezoidal method,[28] using the software Origin 6.0®-1999.

Pancreatic islets isolation. For islets isolation (collagenase method[30,31]) all rats were killed by decapitation and a pool of islets from the eight rats of each subgroup was made. From these pools, islets were collected for insulin concentration, insulin secretion, glucose uptake and glucose oxidation evaluations.

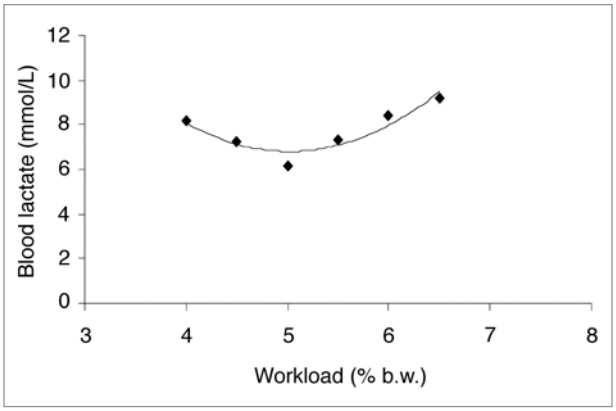

Figure 1. Determination of the Anaerobic Threshold (AT) of a single rat of the control group during lactate minimum test at the beginning of the experiment. Each point indicates blood lactate concentration (mmol/L) on the ordinate after 5 min exercise bout with the animal supporting each load indicated on the abscissa. The blood lactate concentration (6.81 mmol/L) and the respective workload (5.02% b.w.) were calculated using the degree 2 polynomial function ($y = 1.2214x^2 - 12.267x + 37.607$) that defined the curve. This minimum value of blood lactate concentration indicated the workload equivalent to AT.

Insulin extraction from pancreatic isolated islets. For the analysis of insulin content, five pancreatic islets were placed in polypropylene tubes containing 200 µL of distilled water and centrifuged during 20 minutes at 4,000 rpm at 15 to 18°C. The supernatant was discarded and 200 µL of distilled water was added. The tubes were sonicated for 40 seconds. The homogenate (50 µL) was transferred to new polypropylene tube containing 150 µL of extractor solution (0.18 M HCl in ethanol 96%). The mixture was shaken and kept at 4°C for 24 hours for insulin extraction. The extract was then centrifuged during 10 minutes at 4,000 rpm, at 15 to 18°C. The supernatant (50 µL) was transferred to new polypropylene microtubes and diluted in 950 µL of phosphate buffer, pH 7.4. The insulin content of the extracted material was measured by RIA.[29]

Static insulin secretion by isolated islets. To measure static insulin secretion, groups of five islets were incubated for 30 min at 37°C, in Krebs bicarbonate medium containing glucose (5.6 mM), supplemented with bovine serum albumin (3 g l^{-1}) and balanced with a mixture 95% O_2–5% CO_2; pH 7.4. The solution was then replaced by fresh buffer, and the islets were incubated for a further hour with different glucose concentrations (2.8 and 16.7 mM). The insulin content of the medium at the end of incubation period was determined by RIA.[29]

Glucose uptake by isolated islets. For the measurement of the glucose uptake, groups of 10 islets were incubated in small flasks (inner vials) placed inside bigger flasks (outer vials). The incubation was performed in Krebs-Ringer bicarbonate buffer, pH 7.4, containing non-radioactive D-glucose and 2-Deoxy-^{3}H glucose, specific activity 0.5 mCi/mmol, to the final concentration of 16.7 mM, in a Dubnoff metabolic shaker (60 cycles/min), at 36°C. During the first 20 seconds of incubation, the content of the flasks was gassed with a high flux of 95% O_2–5% CO_2 and then the outer flasks were sealed with rubber caps. After 90 minutes of incubation, the islets were transferred from the inner vials to scintillation flasks containing 200 µL of KOH 30%. Five mL of scintillation liquid was added and the radioactivity contained in the islets was measured in a liquid-scintillation counter (Tri Carb 2100 TR-Packard).

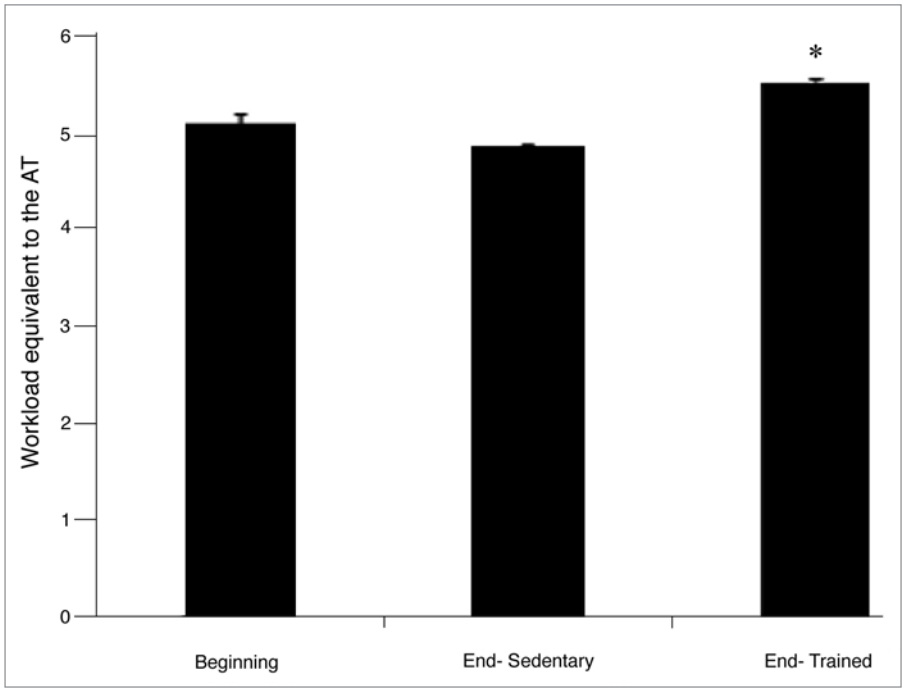

Figure 2. Mean values of workload (% of body weight) equivalent to the anaerobic threshold (AT) determined by the lactate minimum test for the rats assigned to sedentary and trained groups at the beginning and the end of the experimental period. *Significantly different ($p < 0.05$, ANOVA two-way) in relation to equivalent sedentary group.

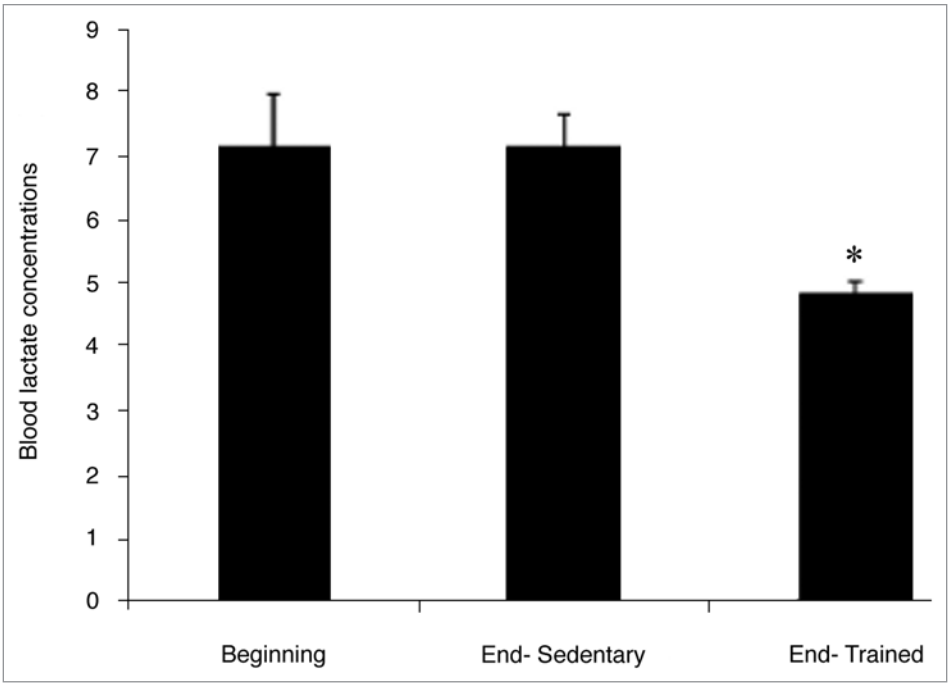

Figure 3. Mean values blood lactate concentration (mmol/L) equivalent to the anaerobic threshold (AT) determined by the lactate minimum test for the rats assigned to sedentary and trained groups at the beginning and the end of the experimental period. *Significantly different ($p < 0.05$, ANOVA two-way) in relation to equivalent sedentary group.

Glucose oxidation by isolated islets. For the measurement of the glucose oxidation rates, groups of 10 islets were incubated in small flasks (inner vials) placed inside bigger flasks (outer vials). The incubation was performed in Krebs-Ringer bicarbonate buffer, pH 7.4, containing non-radioactive D-glucose and [U-^{14}C] glucose, specific activity 0.5 mCi/mmol, to the final concentration of 16.7 mM, in a Dubnoff metabolic shaker (60 cycles/min), at 36°C. During the first 20 seconds of incubation, the content of the flasks was gassed with a high flux of 95% O_2–5% CO_2 and then the outer flasks were sealed with rubber caps. After one hour incubation, HCl was added to the inner vials to stop the oxidation, and hyamine was added to the outer vials, to trap $^{14}CO_2$. The flasks were shaken for a further 30 min at 36°C. Then, the inner vials, containing the islets, were discarded and the radioactivity contained in the outer vials was measured in a liquid-scintillation counter (Tri Carb 2100 TR-Packard).[32]

Statistical analysis. Data were expressed as Mean ± Standard Deviation and analyzed by Student's unpaired t-test (serum glucose and insulin during GTT) and two-way ANOVA (other comparisons). A $p < 0.05$ was taken as the level of statistical significance. When necessary, the Bonferoni test was used for post-hoc comparisons. The software employed was SPSS 10.0 for Windows.

Acknowledgements

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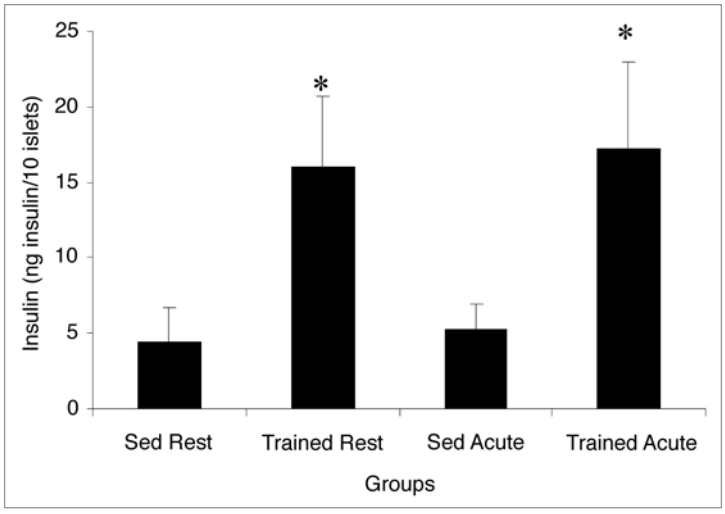

Figure 4. Insulin concentration in pancreatic isolated islets of the animals at the end of 8 weeks of the experimental period. Results are mean ± standard deviation of 6 experiments. *Significantly different ($p < 0.05$, ANOVA two-way) in relation to equivalent sedentary group.

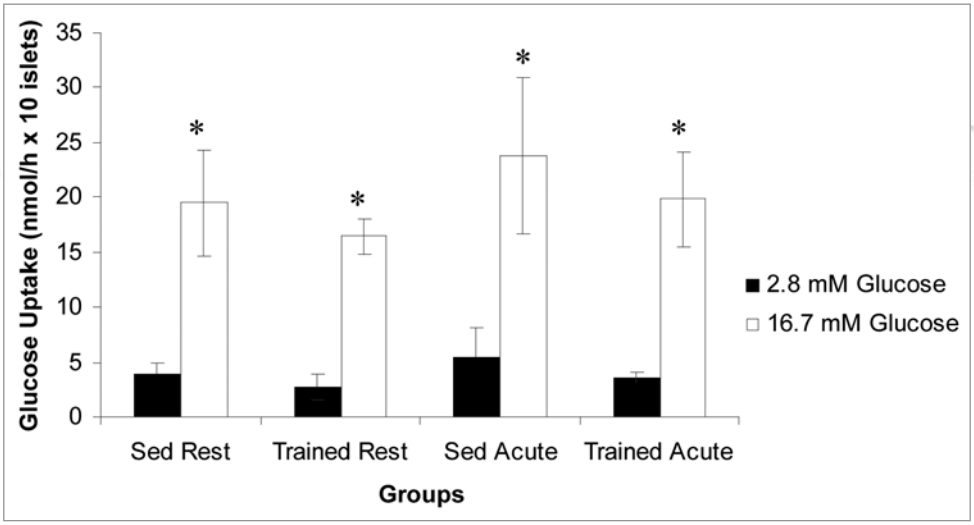

Figure 5. Glucose uptake by pancreatic isolated islets in response to different glucose concentrations at the end of the experimental period. Results are mean ± standard deviation of 8 experiments. *Significantly different ($p < 0.05$, ANOVA two-way) from glucose concentration at 2.8 mM.

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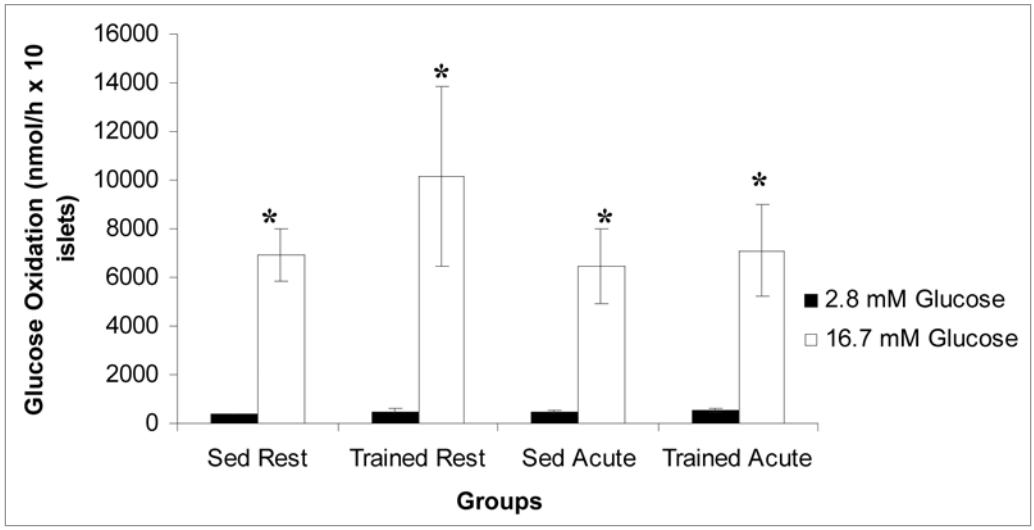

Figure 6. Glucose oxidation by pancreatic isolated islets in response to different glucose concentrations at the end of the experimental period. Results are mean ± standard deviation of 8 experiments. *Significantly different ($p < 0.05$, ANOVA two-way) from glucose concentration of 2.8 mM.

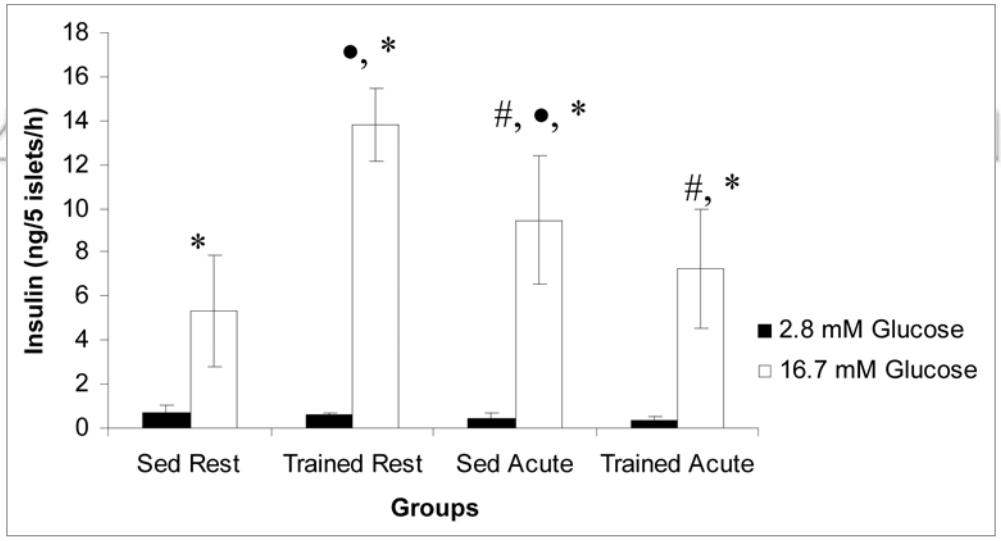

Figure 7. Static insulin secretion by pancreatic isolated islets in response to different glucose concentrations at the end of the experimental period. Results are mean ± standard deviation of 8 experiments. *Significantly different ($p < 0.05$, ANOVA two-way) from glucose concentration at 2.8 mM; ● significantly different ($p < 0.05$, ANOVA two-way) from sedentary group at rest (Sed Rest); # significantly different ($p < 0.05$, ANOVA two-way) from trained group at rest (Trained Rest).