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Unveiling the effects of exercise and protein diet on pancreatic structure in ovariectomized aged rats

Revelando os efeitos do exercício e da dieta proteica na estrutura pancreática em ratas idosas ovariectomizadas

Kemily Loren Barros Chucata,[1] Walkyria Villegas Magalhães,[1] Jessica Pedroso de Sousa,[1] Gabriela Bexiga Veloso,[1] Clever Gomes Cardoso,[2] Mara Rubia Marques,[2] Ricardo Aparecido Baptista Nucci,[1,3] Fernando Luiz Affonso Fonseca,[4] Laura Beatriz Mesiano Maifrino[4,5]

ABSTRACT

Objective: Evaluate structural alterations of the pancreas in ovariectomized elderly female rats that ingested different animal or vegetable protein sources and who underwent moderate resistance exercise. **Methods**: Female Wistar rats were divided into eight experimental groups: sedentary and non-ovariectomized animals plus vegetable protein diet (CVS) or animal protein diet (CAS); trained and non-ovariectomized rats plus vegetable protein diet (CVT) or animal protein diet (CAT); sedentary and ovariectomized groups plus vegetable protein diet (VOS) or animal protein diet (AOS); trained and ovariectomized animals plus vegetable protein diet (VOT) or animal protein diet (AOT). **Results**: Our results showed a decrease in pancreatic islet area in sedentary groups submitted to ovariectomy. On the other hand, we observed an increased frequency of minor and medium pancreatic islets in vegetable and animal diets, respectively. Additionally, all groups had a significant difference in volume density of acini, ducts, blood vessels, and pancreatic islets. Finally, immunohistochemistry demonstrated a decrease of glucagon in sedentary ovariectomized groups and a decrease of insulin in ovariectomized groups submitted to resistance training. **Conclusion**: Both resistance training and dietary intake contributed to pancreatic modifications in estrogen-deprived groups. Accordingly, further studies are needed regarding the structural and functional properties involving both interventions and menopause for a better understanding of our results.
Keywords: menopause; pancreas; resistance training; high-protein diet; ovariectomy; rats.

RESUMO

Objetivo: avaliar alterações estruturais no pâncreas de ratas idosas submetidas à ovariectomia que ingeriram diferentes fontes de proteína animal ou vegetal e foram submetidas a exercícios moderados de resistência. **Métodos**: ratas Wistar fêmeas foram divididas em oito grupos experimentais: animais sedentários e não ovariectomizados com dieta de proteína vegetal (CVS) ou animal (CAS); ratas treinadas e não ovariectomizadas com dieta de proteína vegetal (CVT) ou animal (CAT); grupos sedentários e ovariectomizados com dieta de proteína vegetal (VOS) ou animal (AOS); ratas treinadas e ovariectomizadas com dieta de proteína vegetal (VOT) ou animal (AOT). **Resultados**: nossos resultados mostraram uma diminuição na área de ilhotas pancreáticas em grupos sedentários submetidos à ovariectomia. Por outro lado, observamos um aumento na frequência de ilhotas pancreáticas menores e médias nas dietas vegetal e animal, respectivamente. Além disso, houve diferença significativa na densidade volumétrica de ácinos, ductos, vasos sanguíneos e ilhotas pancreáticas em todos os grupos. Finalmente, a imunohistoquímica demonstrou uma redução de glucagon em grupos sedentários ovariectomizados e uma diminuição de insulina em grupos ovariectomizados submetidos ao treinamento resistido. **Conclusão**: tanto o treinamento resistido quanto a ingestão dietética contribuíram para modificações no pâncreas em grupos privados de estrogênio. Portanto, mais estudos são necessários em relação às propriedades estruturais e funcionais envolvendo ambas as intervenções e a menopausa para uma melhor compreensão de nossos resultados.
Palavras-chave: menopausa; pâncreas; treinamento resistido; dieta rica em proteínas; ovariectomia; ratos.

[1] Laboratório de Estudos Morfológicos e Imunohistoquímicos. Universidade São Judas Tadeu – São Paulo (SP), Brasil.
[2] Universidade Federal de Goiás (UFG) – Goiânia (GO), Brasil.
[3] Laboratório de Neurociências. Hospital Sírio-Libanês – São Paulo (SP), Brasil.
[4] Faculdade de Medicina do ABC (FMABC) – Santo André (SP), Brasil.
[5] Instituto Dante Pazzanese de Cardiologia – São Paulo (SP), Brasil.
Autor correspondente: Ricardo Aparecido Baptista Nucci
E-mail: nucci.ricardo.ab@gmail.com
Laboratório de Neurociência, Hospital Sírio-Libanês, R. Prof. Daher Cutait, 69, Bela Vista, CEP.: 01308-060 – São Paulo (SP), Brasil.
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INTRODUCTION

Reduced estrogen production in women during the menopausal period, along with a sedentary lifestyle and obesity, can induce alterations in carbohydrate and lipid metabolism.[1] Estrogen deprivation correlates with diminished hepatic B/E cholesterol receptors and reduced cholesterol 7 alpha-hydroxylase activity, leading to decreased cholesterol excretion through bile.[2] These mechanisms contribute to an approximate 25% increase in total cholesterol, LDL-cholesterol, and VLDL levels.[3] Additionally, LDL-cholesterol particles become more susceptible to oxidation, a process associated with atherosclerosis formation. Factors associated with menopause are pivotal in the pathogenesis of cardiovascular diseases.[4]

In the context of menopause, a heightened susceptibility to weight gain increases the risk of developing diabetes mellitus. This weight gain induces glucose resistance and diminishes peripheral insulin sensitivity, impeding glucose absorption by the body's cells.[5,6] The ensuing glucose resistance burdens the pancreas, compelling it to elevate insulin production and secretion as a compensatory response. This pancreatic stress, in turn, adversely impacts β-cell function, potentially culminating in deleterious morphological alterations and apoptosis.[7,8] Moreover, estrogen deprivation induces lipogenesis, the accumulation of intermediary lipids, and apoptosis in β-cells.[9]

These factors are intricately linked to dietary patterns, exerting diverse impacts on the body's metabolism. Individuals adhering to a plant-based diet typically exhibit a lower body mass index.[10] This is attributed to reduced cholesterol, saturated fat intake, and a higher proportion of polyunsaturated fatty acids, directly influencing the lipid profile. Such dietary choices are associated with lower serum levels of total cholesterol and LDL, resulting in a diminished susceptibility to diseases linked to dyslipidemia.[11,12] Conversely, consuming animal proteins from sources like lean, unprocessed red meat, poultry, eggs, and dairy products is advocated to prevent cardiometabolic diseases.[13] While studies suggest the superiority of plant-based proteins over animal proteins,[14-17] a more comprehensive examination through additional research is warranted for a nuanced understanding of both dietary paradigms.

Resistance exercise in hypertensive, cardiac, diabetic, and obese individuals seeks to mitigate risk factors linked to metabolic and cardiovascular diseases. Across both animal and human studies, engagement in resistance exercises has shown enhancements in lipid profile, heightened β-cell functionality, elevated muscle glucose uptake and insulin sensitivity, and reductions in overall body fat and blood pressure levels among obese and hypertensive populations.[18-22] In this sense, limited information exists regarding the impacts of resistance exercise coupled with an animal or vegetable protein diet in experimental models experiencing estrogen deprivation. Consequently, this investigation aims to explore structural changes in the pancreas of ovariectomized elderly female rats subjected to distinct animal or vegetable protein sources and moderate resistance exercise.

MATERIALS AND METHODS

Division of Animals

The study was approved by the Ethics Committee in Research of the Universidade São Judas Tadeu, São Paulo, under protocol number A-00610. It used 40 female Wistar rats, 21 days old, weighing between 220 - 250 grams, obtained from the University of São Paulo - USP. Animals were kept in cages in a room with controlled temperature (22° C - 24° C) and a light/dark cycle of 12/12 hours until they were aged 14 months, with free access to water supply, and the standard chow offered for laboratory animals (NUVILAB CR1, produced by NUVITAL Nutrientes LTDA, Curitiba, PR), composed of protein exclusively from vegetable sources. The animals were randomly divided into eight groups (n = 5/group): non-ovariectomized, vegetable protein diet group kept sedentary (CVS) or submitted to resistance training (CVT); ovariectomized, vegetable protein diet group kept sedentary (VOS) or submitted to resistance training (VOT); non-ovariectomized, animal protein diet group kept sedentary (CAS), or submitted to resistance training (CAT); ovariectomized, vegetable animal diet group kept sedentary (AOS), or submitted to resistance training (AOT).

Ovariectomy Procedure

The animals were weighed at 6 months for the ovariectomy procedure.[12,20] The confirmation of the efficacy of ovariectomy was determined by analysis of vaginal secretions during four consecutive days, with a final analysis at the day of euthanasia of animals.

Resistance Training Protocol

Resistance Training (RT) started at 14 months old and was applied over a vertical ladder made of wood with iron steps. The height of the equipment (ladder) was 110 cm, with an inclination of 80°. On top, a plastic box was placed for the animals to be accommodated during climbing intervals. The overload was carried out with weights fixed to the base of the animal's tail using adhesive tape.

All groups were submitted to a prior period of adaptation to the training protocol for a week,[18] without any added load to reach the rest area at the top of the stairs. This procedure was repeated for six consecutive occasions with 60-second intervals in three sessions a week on alternate days without any overload.[12,18]

The RT program was based on the principle of overload, with a number of repetitions and rest closest to human RT models. The initial load was established as 75% of the body weight of each animal. The RT protocol was of moderate intensity, in which the trained groups (CVT, VOT, CAT, and AOT) climbed the ladder six times with 60-second intervals in three sessions a week on alternate days for 12 weeks.[12,18] In each week, the animals were weighed to correct the initial load in case of any weight change. The adjustment of increment of the initial loads to promote training progression was done biweekly, always maintaining 75% of the weight updated, with 10% increases every two weeks for a progressive increase in the training load.[12]

Sedentary groups (CVS, CAS, VOS, and AOS) performed the exercises once a week without overload until they were euthanized to trigger a similar stress to the trained groups.

Diet Protocol

A 12-week diet program (starting at 14 months) was performed.[12] From weaning until 14 months of age, animals were fed with specific chow for laboratory mice (following the recommendations of the National Research Council and National Institute of Health, USA, NRC, 2011) of the Nuvital brand (NUVILAB CR1, produced by Nuvital Nutrients LTDA, Curitiba, PR), which is composed of 52% carbohydrates, 21% protein, and 4% lipids. The chow ingredients are of vegetable origin: ground whole corn, soybean meal, wheat bran, calcium carbonate, dicalcium phosphate, sodium chloride, and vitamin-mineral premix.

At 14 months old, the groups submitted to a vegetable protein diet maintained the abovementioned chow, simultaneously with RT, until euthanasia.[12] On the other hand, at 14 months of age, groups submitted to an animal protein diet were fed with a chow specially formulated for this study by the Rhoster Laboratory (Rhosterlab, Rhoster Ltda.). The chow, made with milk protein (casein) in the same proportion as the initial chow, was introduced into the routine of animals simultaneously with the beginning of the RT program. The ingredients of this chow were: corn starch, casein (85% of the total protein source), soybean protein, sucrose, soy oil, calcium carbonate, dicalcium phosphate, sodium chloride, and vitamin-mineral premix. For technological reasons, to obtain an optimum pellet texture, it was not possible to provide 100% of the dietary protein from animal sources, including about 15% of vegetable protein. According to the National Research Council and the National Institute of Health (USA) recommendations, the animals had free access to the chow in hanging feeders.

Sample Collection

The euthanasia of the animals was performed at the age of 17 months, at the end of the 12-week period of physical training and specific diet (CVS, VOS, VOT, CAS, OSA, and AOT). The animals were weighed and subsequently decapitated. A laparotomy was performed where the pancreas was identified, withdrawn, and weighed.

Immunohistochemistry Technique

Pancreas samples fixed in 10% buffered formalin and embedded in paraffin were cut into 5 µm slices and mounted onto silanized slides. The sample was deparaffinized and hydrated. Assay procedures were performed according to the manufacturer's instructions. The epitopes were blocked with peroxidase and incubated with a specific first antibody. This study used the following primary antibodies for glucagon (Santa Cruz Biotechnology, sc-1309) and insulin (Santa Cruz Biotechnology, sc-7839). The sections were washed with PBS-Tween solution and incubated with biotinylated secondary antibody, avidin-chain enzyme, stained with DAB, and lastly with hematoxylin. The sections were dehydrated conventionally with ethanol, cleared with xylene, and mounted with synthetic resin for light microscopy analysis.

Quantitative Analysis

Pancreas images with 400x magnification were acquired and digitized for morphometric analysis (AxioVision software, version 4.8). Five slices per animal with four fields were obtained (20 images per animal, totalizing 100 images per group). Stereological analysis were performed using Image J software, version 1.47 (National Institute of Health, USA). We analyzed, through morphometric methods, the pancreatic islets area (µm²) and mean diameter (µm) between the groups, as well as the frequency (%) of pancreatic islets due to its area. Additionally, we analyzed, through stereological methods, the volume density (%) of (a) acini, (b) ducts, (c) blood vessels, (d) pancreatic islets, (e) α-cells stained with glucagon antibody, and (f) β-cells stained with insulin antibody.

Statistical Analysis

The results were presented as mean and standard error (SEM). Analysis of variance (ANOVA) and Newman-Keuls' *post-hoc* tests were properly applied in data analysis using GraphPad Prism 5.0 software (GraphPad Prism, Inc., San Diego, CA, USA). The significance level for all tests was $p < 0.05$.

RESULTS

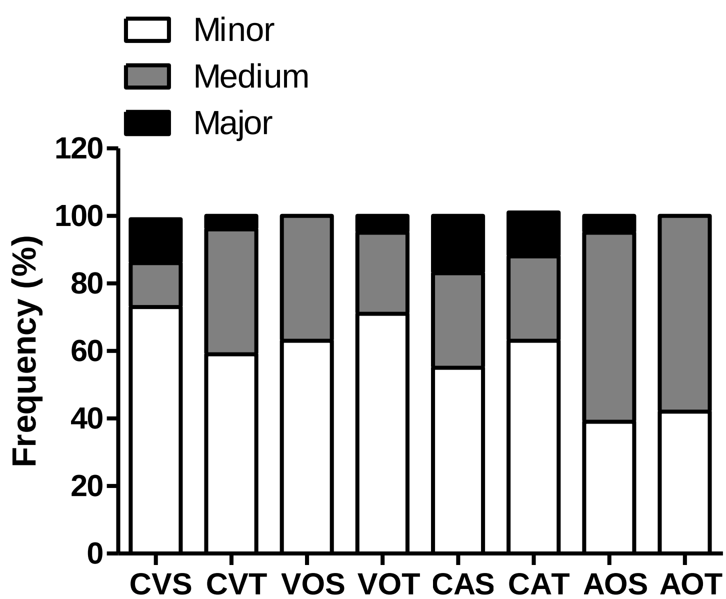
We observed a decrease in pancreatic islet area in sedentary groups submitted to ovariectomy (VOS and AOS, respectively) groups when compared to CVT (Table 1). Additionally, we observed an increased frequency of minor and medium pancreatic islets in vegetable diet and animal diet, respectively (Figure 1). However, there was no significant difference between pancreatic islets' mean diameter between the groups (Table 1).

Table 1. Pancreatic islets area (µm²) and mean diameter (µm) between the groups.

	Groups									
Variables	CVS	CVT	VOS	VOT	CAS	CAT	AOS	AOT	*F*	*P* value
Area (µm²)	13940 ± 1224	17980 ± 2362	9098 ± 1335^{b}	10270 ± 2102	12770 ± 1927	12200 ± 1106	9808 ± 866^{b}	10750 ± 1366	2.874	0.006
Mean diamenter (µm)	134.2 ± 7.39	154.4 ± 10.20	107.7 ± 8.83	115.5 ± 14.46	118 ± 8.92	129.2 ± 6.92	194.3 ± 80.73	114.2 ± 7.25	1.043	0.400

Values are mean ± SEM. ^{b}p < 0.05 versus CVT group.

Figure 1. Pancreatic islet frequency due to its area (µm²). We classified the size in minor pancreatic islets (< 13.333 µm²), medium pancreatic islets (13.333 – 26.666 µm²), and major pancreatic islets (> 26.666 µm²).



In Table 2, we observed a significant difference in all parameters analyzed. We observed that sedentary ovariectomized animals had an increase in acini followed by a decrease in pancreatic ducts when compared to sedentary non-ovariectomized rats. In addition, the VOS group had an increase in blood vessels when compared to animal diet groups. Additionally, the group with animal protein intake (AOS) showed an increase in the volume density of blood vessels when compared to the CAS group. We also observed a sharp decrease in animal diet groups, as well as ovariectomized rats in the vegetable diet groups in pancreatic islets (%) when compared to both CVS and CVT groups.

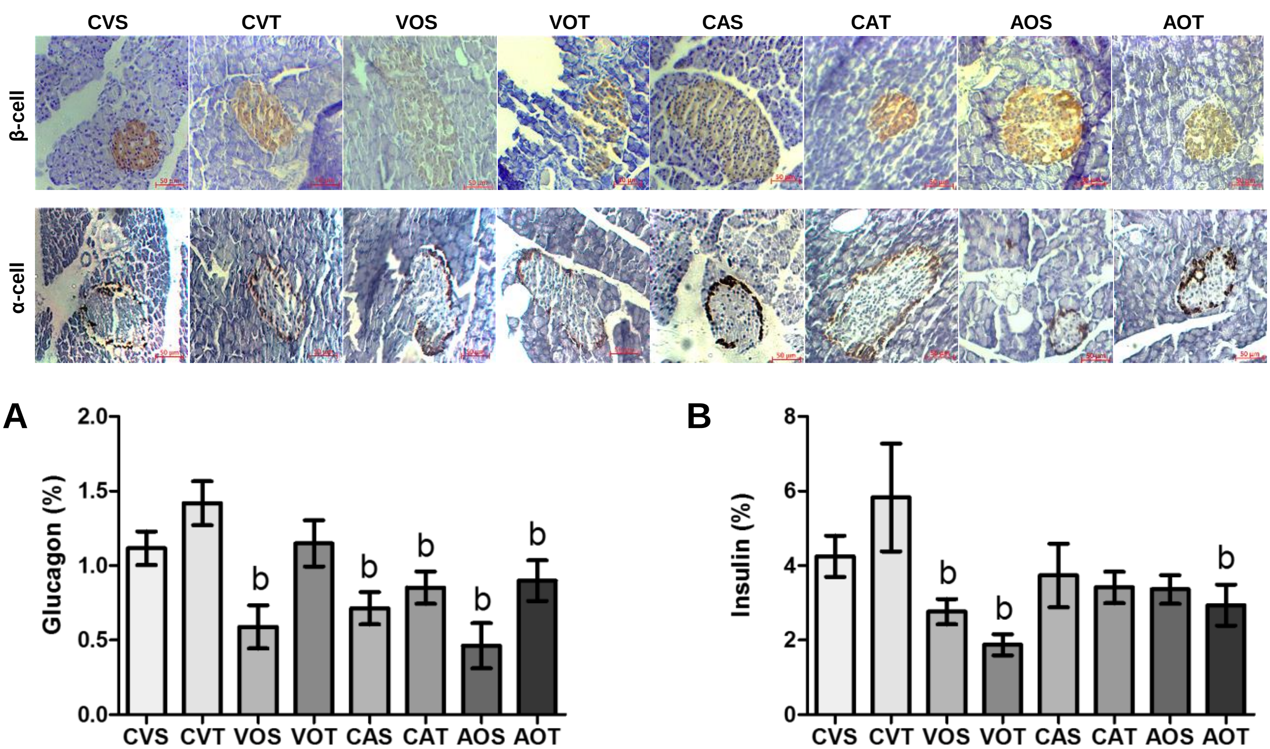
Figure 2 summarizes our immunohistochemical analysis. We observed a decrease of glucagon in sedentary ovariectomized groups regardless of diet protocol. Finally, our results showed a decrease in insulin in ovariectomized groups submitted to resistance training when compared to non-ovariectomized rats submitted to exercise protocol.

Table 2. Volume density (%) of acini, ducts, blood vessels, and pancreatic islets between the groups.

	Groups									
Variables	CVS	CVT	VOS	VOT	CAS	CAT	AOS	AOT	*F*	*P* value
Acini (%)	66.6 ± 1.80	75.38 ± 1.90^{a}	79.22 ± 2.81^{a}	75.01 ± 3.95	70.79 ± 1.73	76.78 ± 2.54^{a}	80.20 ± 2.47^{ae}	78.66 ± 1.81^{a}	4.867	0.000
Ducts (%)	19.82 ± 1.54	10.51 ± 1.43^{a}	12.48 ± 1.75^{a}	11.36 ± 2.15^{a}	20.1 3 ± 1.36^{bcd}	10.17 ± 1.33^{ae}	8.95 ± 1.32^{ace}	11.63 ± 1.12^{ae}	10.08	0.000
Blood vessels (%)	3.65 ± 0.52	2.37 ± 0.42	5.32 ± 0.98^{b}	4.45 ± 1.76	1.55 ± 0.30^{c}	2.27 ± 0.46^{c}	4.61 ± 0.99^{e}	2.23 ± 0.32^{cg}	4.518	0.000
Pancreatic islets (%)	9.40 ± 0.62	9.69 ± 1.03	3.71 ± 0.52^{ab}	5.73 ± 1.10^{ab}	5.87 ± 0.76^{ab}	5.14 ± 0.52^{ab}	4.49 ± 0.37^{ab}	5.71 ± 0.59^{ab}	8.760	0.000

Values are mean ± SEM. $^{a}p < 0.05$ versus CVS group; $^{b}p < 0.05$ versus CVT group; $^{c}p < 0.05$ versus VOS group; $^{d}p < 0.05$ versus VOT group; $^{e}p < 0.05$ versus CAS group; $^{g}p < 0.05$ versus AOS group.

Figure 2. Summarizes our immunohistochemical analysis. We observed a decrease of glucagon in sedentary ovariectomized groups regardless of diet protocol. Finally, our results showed a decrease in insulin in ovariectomized groups submitted to resistance training when compared to non-ovariectomized rats submitted to exercise protocol.



Immunohistochemistry (IHC). Quantitative analysis of (A) glucagon (%) and (B) insulin (%). Representative immunohistochemical images for α-cells (glucagon) and β-cells (insulin) are showed above. Values represent mean ± SEM. $^{b}p < 0.05$ vs CVT.

DISCUSSION

Our preliminary study analyzed the effects of resistance training associated with different diet intakes on female rats' pancreas components in an experimental estrogen deprivation model. Menopause is related to the onset of metabolic changes, diabetes, and cardiovascular disease.[23,24] These changes favor a marked prevalence of cardiovascular diseases as causes of death and functional disability in the female population in this age group.[1] Estrogen regulates various aspects of glucose and lipid metabolism, and in pancreatic β-cells, this hormone regulates insulin and glucagon secretion.[9]

The role of estrogen and its receptor in the functioning and protection of β-cells has been evidenced in animal studies. In rodents, estrogen treatment protects pancreatic cells against various types of injuries associated with type 1 and type 2 diabetes, such as oxidative stress, amyloid polypeptide toxicity, and apoptosis.[25] The absence of estrogen leads to a decrease in β-cell stimuli, consequently resulting in changes in cellular metabolism and survival. These effects on the pancreas associated with alterations in other organs produce systemic manifestations such as metabolic syndrome, diabetes, hypertension, and cardiovascular problems.[23,24] Although we hypothesized that ovariectomy should play a major factor in pancreatic structure, our results demonstrated that dietary intake and exercise training played key factors in pancreatic structure and function regarding estrogen deprivation.

Diet and exercise interventions are known to improve insulin sensitivity in aged populations.[16,21,22,26,27] Long-term adherence to high-protein diets may increase pancreatic function, leading to structure modifications because compared to vegetable protein, animal protein can promote greater muscle mass gain and hypertrophy in animals subjected to resistance training, which improves both glucagon and insulin sensitivity.[16,27-29]

Experimental studies suggested that low to moderate intensity aerobic exercises may directly affect the pancreas.[21,22,27] Although exercise training is known as a therapeutic intervention for postmenopausal women,[30-32] there is still unclear evidence about the effects of resistance training on pancreas structure and function during estrogen deprivation. Even though there were a few significant differences from a statistical point of view, the groups submitted to moderate resistance training in both vegetable and animal protein diets showed a tendency to positively modify the pancreas structure when compared to the sedentary groups.

Despite studies demonstrating an improvement in the function of β-cells in the pancreas,[19-22] our study demonstrated a reduction in β-cell immunostaining. However, we highlight a significant reduction of α-cells in animals submitted to an exercise routine with a diet rich in animal protein. A study of obese rats found that large islets (> 0.45 mm) produce more insulin than small islets (< 0.12 mm), whereas small islets stimulated by arginine from vegetable diets release more glucagon than large islets.[33] In humans, large islets, although smaller in number, play most of the endocrine functions.[34] We suggest that during resistance exercise, there must be an increase in serum glucagon concentration and a reduction in serum insulin concentration to maintain homeostasis.[35,36] However, with menopause and animal protein intake, this balance may be affected.

CONCLUSION

We conclude that both resistance training and dietary intake contributed to pancreatic modifications in estrogen-deprived groups. Accordingly, further studies are needed regarding the structural and functional properties involving both interventions and menopause for a better understanding of our results.

Acknowledgment

None.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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