Previous physical exercise slows down the complications from experimental diabetes in the calcaneal tendon

Márcio Almeida Bezerra[1]
Cybelle da Silva Nery[2]
Patrícia Verçoza de Castro Silveira[3]
Gabriel Nunes de Mesquita[2]
Thainá de Gomes Figueiredo[3]
Magno Felipe Holanda Barboza Inácio Teixeira[2]
Silvia Regina Arruda de Moraes[2]

[1] Department of Physiotherapy, Federal University of Ceará, Fortaleza, Brazil
[2] Federal University of Pernambuco, Brazil
[3] Physiotherapist

Corresponding author:
Márcio Almeida Bezerra
Department of Physiotherapy, Federal University of Ceará, Fortaleza
Luiza Miranda Coelho
60811110 Fortaleza, Brazil
E-mail: malmeidab.ufc@gmail.com

Summary

Background: the complications caused by diabetes increase fragility in the muscle-tendon system, resulting in degeneration and easier rupture. To avoid this issue, therapies that increase the metabolism of glucose by the body, with physical activity, have been used after the confirmation of diabetes. We evaluate the biomechanical behavior of the calcaneal tendon and the metabolic parameters in rats induced to experimental diabetes and submitted to pre- and post-induction exercise.
Methods: 54-male-Wistar rats were randomly divided into four groups: Control Group (CG), Swimming Group (SG), Diabetic Group (DG), and Diabetic Swimming Group (DSG). The trained groups were submitted to swimming exercise, while unexercised groups remained restricted to the cages. Metabolic and biomechanical parameters were assessed.
Results: the clinical parameters of DSG showed no change due to exercise protocol. The tendon analysis of the DSG showed increased values for the elastic modulus (p<0.01) and maximum tension (p<0.001) and lowest value for transverse area (p<0.001) when compared to the SG, however it showed no difference when compared to DG.

Conclusion: the homogeneous values presented by the tendons of the DG and DSG show that physical exercise applied in the pre- and post-induction wasn't enough to promote a protective effect against the tendinopathy process, but prevent the progress of degeneration.

KEY WORDS: diabetes mellitus, biomechanics, tendinopathy, animals.

Introduction

Despite proven correlation between diabetes and the emergence of changes in tendon structure promoting degenerative injuries that impair biomechanical function[1,2], as yet little attention has been paid to the study of interventions that can prevent such disturbances.

Diabetes may involve the development of physio-pathological complications in tissues rich in collagen due to the reduction of sugars in the proteins through a process called non-enzymatic glycosylation. This reaction promotes structural changes in collagen that resemble the natural aging process of the tendon matrix, resulting in biomechanical impairment[3].

According to the American Diabetes Association[4] planned and structured physical exercise should be carried out as a form of diabetes management in order to prevent the consequences of chronic hyperglycemia. This intervention raises the energy needed by the body and promotes changes in cellular metabolism and endocrine control[5] and may, in some cases, increase the secretion of insulin[6] and provide glycemic control[7], avoiding chronicity and containing some cardiovascular risks[8].

An improvement has been demonstrated experimentally in the biomechanical parameters of the tendon of animals in the process of developing diabetes, as a result of the training on the treadmill[9]. On the other hand, the use of physical exercise in an aquatic environment, which is less physically aggressive than the treadmill, may be a more appropriate method for this analysis. Changes in tendon plasticity have been documented in animals subjected to physical exercise in an aquatic environment, in which an increase was observed in the load supported by the tendons when submitted to the mechanical test of maximum traction[10]. In addition, previous studies have suggested that the mechanisms linked to muscular development with increased transport of glucose by glucose me-

diators (GLUT-4)[11] may serve as part of the solution to chronic diabetic hyperglycemia.

Our study hypothesis was that the muscle work developed by series of contractions imposed by aquatic activity promotes an increase in the consumption of corporal glucose, lowering blood glucose levels and generating a protective effect on the tendon in rats induced to diabetes when compared to sedentary groups. Thus, the objective of this study was to evaluate the biomechanical and structural behavior of the calcaneal tendon of rats induced to experimental diabetes and submitted to pre- and post-induction physical exercise in an aquatic environment.

Methods

Animals

In the study 54 male, *Wistar* rats (*Rattus Novergicus Albinus*) were used (70 days old; 314.57±36.54 g), kept in polypropylene cages of size 41x34x16 cm (four animals per cage), in an air-conditioned environment (22±1° C), with an inverted light/dark light cycle of 12 h and free access to ration (Labina®-Purina) and water (study approved by the Committee of Ethics in Animal Experimentation – EAEC - at the Federal University of Pernambuco – UFPE- protocol # 23076.028377/2010-25). This research was conduct ethically according to international standards and as required by principles and recommendations in clinical and field Science[12].

The animals were randomly divided into four experimental groups: a) the Control Group (CG, n=15), in which animals did not undergo any kind of exercise, restricted only to free movement inside the cage; b) Diabetic Group (DG, n=15), in which animals were not subjected to any kind of physical exercise, restricted only to the activity inside the cages and induced to diabetes; c) Swimming Group (SG, n=12), submitted to physical exercise with swimming for eight weeks; d) Diabetic Swimming Group (DSG, n=12), subjected to physical exercise for eight weeks and induced to diabetes.

Physical exercise in the aquatic environment

At 70 days of life the animals of the SG and DSG groups started the exercise period for four weeks (Fig. 1). The first week of activity consisted of the adaptation of the animals to the aquatic environment, in which they were placed in anasbestos tank, 60 cm long, 50 cm wide and 40 cm deep, where the training was held, with the water temperature at 31±1° C. The first day of adaptation consisted of swim, without overload, during 10 minutes, increased by 10 minutes on the remaining days of the week. From the second to fourth experimental week, animals were exercised for 60 minutes, five days a week and once a day without overload. In the fifth week, physical exercise was interrupted for the realization of induction to diabetes. In the following week, the animals began the post-induction experimental period that consisted of four weeks of physical exercise for 60 minutes, five days a week without overload[13,14].

During the period of physical exercise the animals in groups CG and DG were kept in their respective cages.

Table 1. Physical exercise protocol.

Week	Day	Time (minute)	Physical exercise
First week (adaptation)	1	10	Swim
	2	20	Swim
	3	30	Swim
	4	40	Swim
	5	50	Swim
Second week	1 - 5	60	Swim
Third week	1 - 5	60	Swim
Fourth week	1 - 5	60	Swim
Fifth week	1 - 5	Diabetes induction	-
Sixth week	1 - 5	60	Swim
Seventh week	1 - 5	60	Swim
Eighth week	1 - 5	60	Swim
Ninth week	1 - 5	60	Swim

Induction to diabetes

Upon completing 96±1 day of life, with an average weight of 364.84±45.48 g and after a fast of 14 h, the animals were induced to experimental diabetes (type 1) through injection of Streptozotocin (Sigma Chemical Co., USA) by intraperitoneal injection, diluted in 10 mM sodium citrate buffer at pH 4.5, using a single dose (60 mg/kg of animal weight). The animals of the control groups received the same dose only of sodium citrate buffer solution to reproduce the same stress of induction. Thirty minutes after the induction, all animals had free access to ration and water[15].

All the animals who obtained blood glucose above 200 mg/dl were considered diabetic[16] and one week post-induction resumed swimming exercise.

Metabolic analysis and glycemic control

The analysis of water consumption, food intake and diuresis was accomplished using the metabolic cages (MC) (TECNIPLAST model 3701M081) during a period of 72 hours, in a random sample of five animals in each group, at two moments: 1) at 99 days (±1 day), after diabetes or stress induction; 2) at 130 days (±1 day), the week before collection of material.

To reduce the influence of the difference of weight found in each group on the collections, the findings are presented and discussed with the correction of the animal's weight to each 100 g adopting the values found after 48 hours within the metabolic cage.

Checking blood glucose was done after a 12-h fast, once a week, from 60 days of life for animals and three and seven days after the induction of diabetes using reagent strips (Accu-Chek Activ) for determination of blood glucose from a drop of blood taken from the animal's tail.

Material collection

After anesthesia of animals with a xylazine (Rompum®-Bayer) (10 mg/kg) and ketamine hydrochloride (Ketalar®) (25 mg/kg) solution, 0.10 ml for each 100 g weight of the animal, followed by euthanasia with exsanguination, the left hind leg of the animal was removed and a dissection performed for the removal of the calcaneal tendon. The material was moistened with physiological saline and forwarded under refrigeration to the Laboratory of Chemical Engineering-UFPE for realization of the mechanical test.

Mechanical test

For the realization of the mechanical test, the tendon was outfitted with two metal connectors (2.5 × 3.5 cm), one at each end. The proximal end, composed of the calcaneal tendon and myo-tendonous joint, was positioned superiorly and the distal end, composed of the tendon and its junction with the calcaneum, was positioned inferiorly in anatomical position. To improve anatomical specimen fixation to the connectors, a drop of monoacrylategel (super glue) was used. Next, the ellipse formula was used to measure the transverse area of the tendon in his medial third[17]. Subsequently, the set (connector and tendon) was connected to a conventional EMIC mechanical testing machine (model DL 500, Brazil) through the self-locking grippers, with the length of the sample for testing then being gauged.

Tendinous samples were tractioned to the point of failure, at a speed of 0.1 mm/s and 500 N load cell. The load-deformation curve were obtained from the tests, which made it possible to analyze the structural properties: maximum strength (N), maximum load supported by the piece; maximum deformation (mm), maximum deformation reached by testing, as well as parameters such as transverse area (mm^2) and initial length of the tendon (mm). After normalizing force by the transverse area and deformation by the initial length, the stress × strain curve was obtained, from which the following tendinous biomechanical characteristics were assessed: elastic modulus (MPa), represented by the tangent of the angle formed by the more linear, positive-sloping portion of the graph; tension at maximum strength (MPa), calculated by the ratio of the maximum load supported by the anatomical specimen by its transverse area; and specific deformation (%), represented by the ratio between the base length and maximum deformation multiplied by 100.

Statistical analysis

The statistical analysis of the results was carried out with the software SPSS, version 17. The Kolmogorov-Smirnov test for normality was applied in the groups and the one-way ANOVA test was used to study the variables, with post hoc Bonferroni, at a significance level of 5%.

Results

During the experiment there were sample losses in the following groups: CG, three samples due to technical problems with the mechanical testing machine (one sample lost in a power outage and two lost at the time of the test when the tendon slipped into the connector); SG, two animals died due to unidentified causes; DSG, a sample was lost at the time of the test (the tendon slid into the connector); and DG, two samples did not reach optimal levels to be considered diabetic. As a result, 10 samples were evaluated in the CG, 10 samples in the SG, 11 in the DSG and 13 in the DG.

Metabolic analysis and glycemic control

The data from the two moments of analysis in the metabolic cages are shown in Table 1. A decrease in the weight of the animals was observed 96 hours after the induction of experimental diabetes, as well as increased fluid and ration intake and increased elimi-

Table 1. Metabolic monitoring of animals in metabolic cages (MC) with the values represented by means and standard deviation.

GROUPS MC 1	WEIGHT (grams)	FOOD INTAKE (grams)*	WATER (milliliters)*	URINE (milliliters)*
CG	397.2 ± 49.16	7.49 ± 0.47	14.64 ± 4.43	4.06 ± 0.35
DG	350.4 ± 35.31	11.3 ± 1.62[c]	54.61 ± 7.88[c]	41.63 ± 8.40[c]
SG	399.2 ± 31.41	6.76 ± 0.7[d]	10.02 ± 1.8	3.92 ± 1.31
DSG	328 ± 21.58[a,b]	11.47 ± 0.99[d,e]	49.44 ± 6.94[d,e]	37.43 ± 5.76[d,e]
GROUPS MC 2	WEIGHT (grams)	FOOD INTAKE (grams)*	WATER (milliliters)*	URINE (milliliters)*
CG	450.8 ± 49.10	5.76 ± 0.87	9.70 ± 2.32	3.17 ± 0.78
DG	297.2 ± 28.51[c]	16.89 ± 1.58[c]	88.06 ± 13.01[c]	68.8 ± 10.14[c]
SG	451.2 ± 32.01	6.42 ± 0.48	11.17 ± 2.37	3.08 ± 0.39
DSG	272.4 ± 44.39[d,e]	18.55 ± 2.73[d,e]	95.28 ± 19.56[d,e]	74.59 ± 19.96[d,e]

*metabolic values in reference to 100 grams body weight of the animal;
(a) CG→DSG $p<0.05$; (b) SG→DSG $p<0.05$; (c) CG→DG $p<0.0001$;
(d) SG→DSG $p<0.0001$; (e) CG→DSG $p<0.0001$

nation of urine in MC1. The physical exercise protocol before and after the induction of diabetes was not able to normalize the metabolism of the animal, and thus increases in the values analyzed and a decrease in body weight persisted quite evidently in diabetic animals.
As for glycemic control, the average values for blood glucose of the animals during the trial period are shown in Figure 2. The blood glucose of diabetic animals subjected to physical exercise showed a decrease of 24.7% ($p<0.01$) compared with the values of the diabetic group without exercise on the day of euthanasia. However, these values remained greatly above normal.

Structural analysis and biomechanics of the tendon

Structural and biomechanical behaviors of the tendon presented some modifications (Figs. 3, 4). An increase in the value of elastic modulus was observed in the tendons induced to diabetes, becoming a more rigid material. In reference to DG and DSG, there were increases of 202 and 198%, respectively.
The area of the transversal section presented a decrease of 44% in the DG and 47% in the DSG when compared with the value of the control group, in addition to an increased tension of the DG and the DSG of 123 and 182%, respectively.
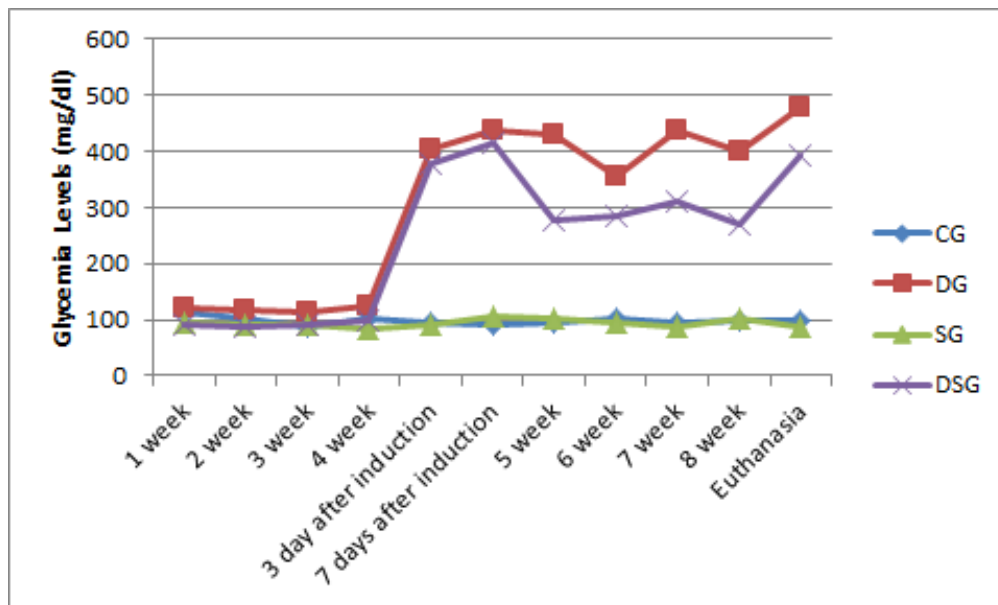

Figure 2. Weekly evaluation of the glycemia levels of the experimental groups.
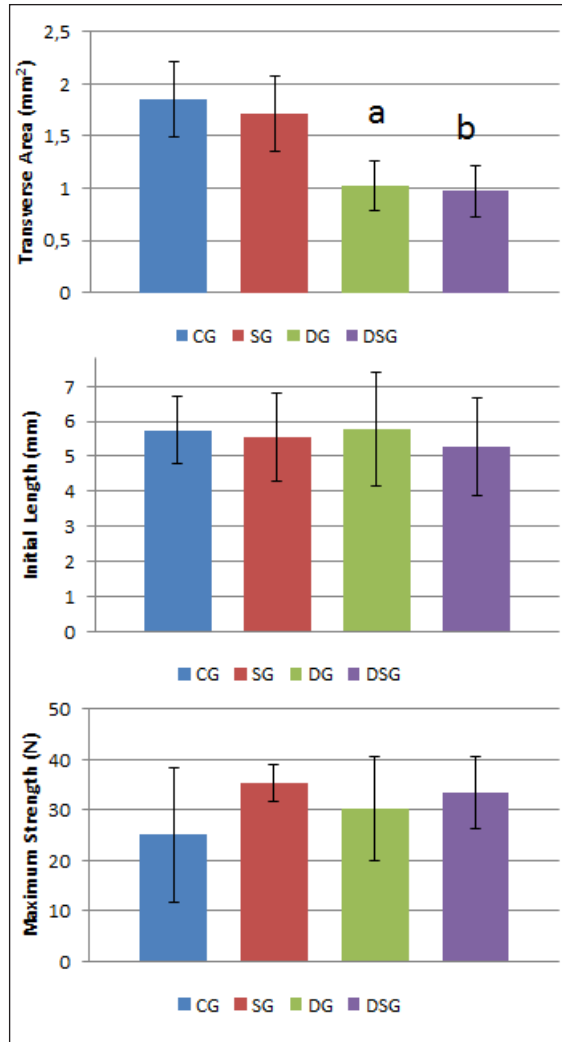
Figure 3. Results of the structural properties of tendons in the experimental groups. CG – control group; SG – swimming group; DG – diabetic group; DSG – diabetic swimming group; (mm) – millimeters; (%) – percentage; (N) – Newton; (MPa) – mega-pascal; (a) CG→DG p<0.001; (b) SG→DSG p<0.001.
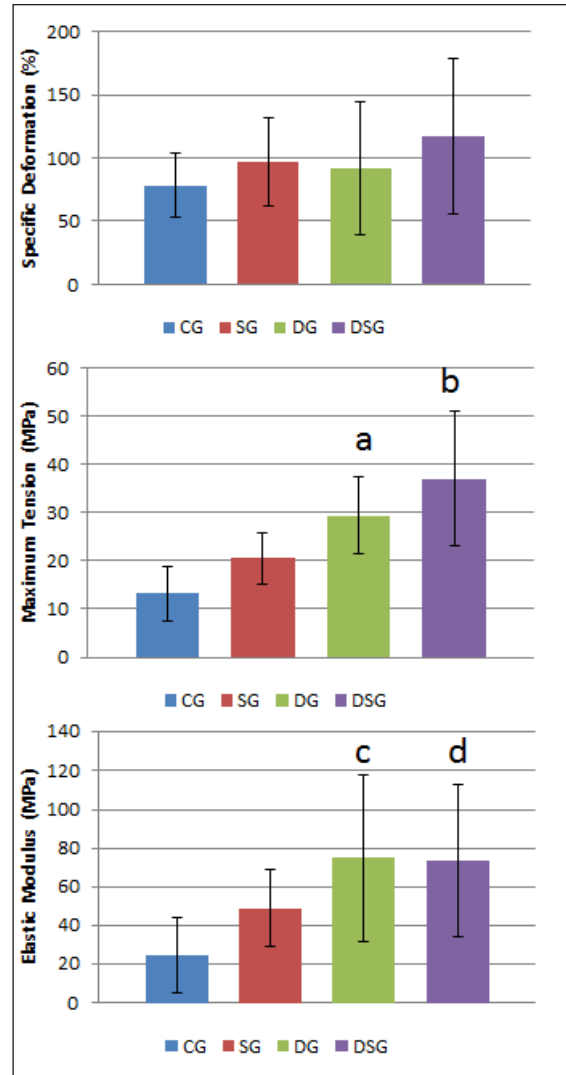

Figure 4. Results of the biomechanical properties of the tendons of the experimental groups. CG – control group; SG – swimming group; DG – diabetic group; DSG – diabetic swimming group; (mm) - millimeters; (%) – percentage; (N) – Newton; (MPa) – mega-pascal; (a) CG→DG p<0.001; (b) SG→DSG p<0.001; (c) CG→DG p<0.005; (d) CG→DSG p<0.01

Discussion

The results of this study corroborates previous results from our research group[9] which demonstrated changes in structural (transverse area) and biomechanical properties (elastic modulus and maximum stress) of tendons stemming from diabetic induction. However, the novelty behind the use of an exercise protocol before and after the induction has not been enough to reduce the tendon degenerative process.

Swimming exercise, used in this study, was characterized by means of a pilot experiment as being moderate physical exercise without the imposition of overload to the animals. The use of this modality had the aim of avoiding possible injury to the animals that would prevent them from completing the trial period while at the same time evaluating its use in the prevention of myo-tendinous comorbidities in diabetic animals.

The physical exercise protocol applied pre- and post-induction was not enough to revert changes in the biomechanics of the tendon nor in the metabolic parameters evaluated. However, the same protocol seems to have contributed to slowing the development of these changes, avoiding the progression of degeneretive process.

We have previously demonstrated that physical exercise promotes changes in tendinous stiffness, causing an increase in the elastic modulus in animals that have been subjected to physical exercise in an aquatic environment[18]. This change may facilitate the transmission of energy from the muscles to the bone

framework. In contrast, in animals induced to experimental diabetes, the significant increase in this parameter may be related to disorganization of the collagen fibers which, in their normal state are arranged in parallel[19], thereby making them prone to breakage. In addition, the accumulation of glucose in the body without concurrent increased metabolism can promote a series of associations and secondary connections that become set among the collagen fibers. These successive connections resulting in the non-enzymatic glycation of connective tissues, tend to increase over the course of weeks in diabetic animals[20], which may also influence the disorganization of collagen and consequently its rigidity.

On the other hand, when comparing the findings in animals of the DG and DSG, a difference was observed between the tendon stiffness values. Theoretically, the overlap of effects of training and diabetes would promote a more increase in elastic modulus, however such an outcome did not happen. This result suggests an interruption in the progress of tendinopathy, promoted by the exercise protocol applied, despite this parameter not having returned to values compatible to the SG.

The smallest value of the transverse area of the tendons in the diabetic animals corroborate with earlier findings[2], suggesting that the decrease in transverse area cannot be related solely to the changes of collagen tissue, but also to the low weight of the animals and to increased diuresis, thus reducing the proportion of water in the composition of the tendon.

On the other hand, the increase in maximum tension supported by the tendon may give a false impression of improvement biomechanics. Tension is calculated from the quotient between the load supported by the tendon and the transverse area. As there was no change among the groups in the maximum load supported by the tendon, the reduction in transverse area became the determining factor in the increase in this biomechanical parameter. The substantial increase observed in our study corroborates with those found by others researchers[2,3,19].

However, studies with genetically modified diabetic animals have increased cross-sectional area and still maximun stress decreased[21]. Thus probably the stress of inducing diabetes by streptozotocin can cause greater damage than the diabetes itself due to its toxicity.

Physical exercise results in the development of muscle mass and, concomitantly, promotes an increase in circulating glucose uptake by Glut-4 transporters, thus lowering blood glucose levels[11] in addition to improving insulin pathways in the body. Through this mechanism, muscle simulates endocrine function, helping control blood glucose levels as has been demonstrated in some studies[9,22]. In our study, the reduction of these levels was noticeable in the animals of the DSG, but not enough to be considered normal (Fig. 2). Probably the burden of the physical exercise was not enough to help the endocrine control, softening the hormonal imbalance that leads to reduction of the structural homogeneity of the tendons and biomechanical changes[23].

In relation to the clinical signs evaluated, such as polyuria, polydipsia, and polyphagia (striking features in diabetic patients), which were observed three days after diabetes induction, showed no return to normal at the end of the experiment in this preventive and continuous protocol of exercise. It is believed that the model of induction to type 1 diabetes, with massive destruction of pancreatic beta cells, requires other stimuli beyond the application of moderate physical exercise to mobilize a greater amount of glucose in the body[24]. Furthermore, the fact that the animals had free access to ration intake may have contributed to the maintenance of above-normal glucose levels and, thus, contributed to the maintenance of the evaluated clinical signs. A balanced diet, using appropriate proportions of carbohydrates and proteins, can promote an improvement in glucose metabolism[25] and applied concomitant with exercise, can assist in improvement of the diabetic condition.

In Summary, swimming exercise, applied preventively and continuously after the induction of experimental diabetes, was not enough to completely reverse the metabolic parameters and tendinous complications arising from diabetes, keeping the tendon prone to injury. On the other hand, the exercise protocol shown to be possible to prevent the progression of diabetic tendon stiffness and reduce blood glucose levels in some animal during the experimental period of DSG.

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Conflict of interests

The Authors declare that they have no conflict of interests regarding the publication of this paper.

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