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Chitosan combined with swimming promotes health in rats

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ABSTRACT

Purpose: Chitosan is an easily accessible and biocompatible natural molecule which facilitate the immune system. In recent studies, chitosan is being applied to the drug nanosphere to deliver drugs. However, whether chitosan could promote health under exercising condition remains yet to be elucidated. Hence, we designed to investigate the effect of chitosan on swimming rats.
Methods: Sprague-Dawley (SD) rats were divided into four groups, exercise with chitosan, exercise with water, sedentary with chitosan, and sedentary with water. After four weeks of exercise and chitosan/water gavage, the blood was collected, and its biochemical index, complete blood count, and related parameters, and cytokines were detected and analyzed.
Results: The level of blood urea nitrogen ($p = 0.0380$), total cholesterol ($p = 0.048$), and low-density lipoprotein ($p = 0.0338$) were decreased, while the number of red blood cells ($p = 0.001$), hematocrit ($p = 0.01$), and mean corpuscular volume ($p = 0.039$) were increased in chitosan group. Furthermore, the combination of chitosan and swimming decreased the red blood cells distribution width.
Conclusions: Our study support that chitosan could facilitate the health during exercise.

1. Introduction

Chitosan, a deacetylated chitin, is naturally derived fro1m partial chitin *N*-deacetylation and is synthesized by many living organisms, including insects and crustaceans [1, 2]. Chitosan is a linear polysaccharide composed of randomly distributed β-1-4-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). Chitosan is an easily accessible molecule which can be decomposed into the diverse semi-solid and solid substances [3]. Chitosan is used in various applications due to its distinct biological properties such as biocompatibility, non-toxicity, biodegradability, bacteriostaticity, and strong adhesion [4–6]. Literature reports indicate that the deacetylated chitosan could exhibit anticarcinogenic [7] and/or antimicrobial [8, 9] activity. Chitosan and its water-soluble derivatives could against lead-induced oxidative stress [10]. Moreover, the chitosan has been heavily studied due to its drug carrying property [11–13]. Hence, because of its natural and easy accessibility and biocompatibility, more aspects for its application were studied [14–16].

The immune system provides protection againts pathogens or hazardous foreign invaders, and maintains internal homeostasis. Literature indicates that chitosan act as an adjuvant for the immune system [17]. Gudmundsdottir [18] documented that chitosan can downregulate the activation of the inflammasome in human macrophages. Furthermore, chitosan also exhibits antioncogenic properties. Highton AJ demonstrated that vaccination with chitosan hydrogel was as effective as dendritic cell vaccination in melanoma prevention and also has more readily detectable protective immune correlates [19]. Moreover, Folate-conjugated chitosan nanoparticles can exert antitumorogenic effects in hepatocellular cancer [20]. Alongside, chitosan nanoparticles also modulate innate immune responses by inducing and augmenting immune responses in plants [21]. Although chitosan plays a significant role in the immune responses, the correlation of chitosan and exercise are barely addressed so far.

Exercise is a complex phenomenon which modulates various indices of body, these indicators influence the immune system and also depicts the body condition [22–24]. Blood urea nitrogen (BUN) [25], lactate dehydrogenase (LDH) [26], blood lactic acid (LA) [27], and hemoglobin (GHB) [28] are key molecules to reflect the status of exercising body. BUN is usually used to evaluate protein metabolic process, and under physiological conditions, the generation and excretion of the BUN is under a dynamic balance called nitrogen balance. When increase in protein metabolism or decline in renal function, the concentration of BUN alters [29]. Serum BUN is also positively related to the exercise stress and fatigue level, which makes BUN a vital index to evaluate athletes' state. Blood LA, another critical index, is used to assess the exercise

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Table 1
Exercise monitoring indicators (mean ± SD) (n = 9, each).

	ALB (g/L)	BUN (mmol/L)	CR (μmol/L)	LDH (U/L)	LA (mmol/L)
EC	24.78 ± 0.93	6.34 ± 0.76	19.47 ± 7.11	756.84 ± 311.37	4.87 ± 1.55
SC	25.12 ± 1.36	5.97 ± 0.70	19.07 ± 3.54	782.6 ± 270.78	5.81 ± 1.94
EW	25.43 ± 1.20	6.71 ± 0.46	19.83 ± 3.88	849.38 ± 381.25	4.75 ± 0.88
SW	25.6 ± 1	6.76 ± 1.01	24.37 ± 6.31	973.6 ± 491.61	6.01 ± 1.74

	ALB (g/L)		BUN (mmol/L)		CR (μmol/L)		LDH (U/L)		LA (mmol/L)	
	F	P	F	P	F	P	F	P	F	P
C	1.951	0.172	4.678	0.038	2.178	0.15	1.154	0.291	0.006	0.937
E	0.387	0.538	0.348	0.559	1.159	0.29	0.323	0.574	3.888	0.057
E + C	0.043	0.837	0.609	0.441	1.165	0.208	0.139	0.712	0.078	0.782

All the data are expressed as the mean ± SD. The two-way ANOVA test was applied for synergism effect. A value of $p < 0.05$ was considered statistically significant. EC, exercise and chitosan gavage; SC, sedentary and chitosan gavage; EW; exercise and water gavage; SW, sedentary and water gavage. ALB, albumin; BUN, blood urea nitrogen; CR, serum creatinine; LDH, lactate dehydrogenase; LA, lactic acid.

load. The alteration in LA concentration depends on the frequency of exercise, duration, and other factors, such as age and amount of stored muscle glycogen [30]. LDH exists in nearly all the tissues and is released into circulation with tissue damage. Strenuous exercise leads to a significant amount of LDH escape from the muscle cells, which results from insufficient LA metabolism [31]. The characteristic property of hemoglobin is to bind and supply oxygen to all the tissues, this makes HB as a crucial indicator of body status. Exercise up to an optimal level can increase HB level, while strenuous exercise will decrease the HB and consequently decline in the exercising potential [32].

Proper nutrition keeps the body healthy and improve the overall immune status. Meanwhile, chitosan is an easily accessible, natural, and nutritious compound which can boost the immune system. However, the impact of combined exercise and chitosan on body remains unknown. Thus, we designed this study to explore the effects of chitosan and swimming on the immune system.

2. Methods

2.1. Animals and drugs

Thirty-six male Sprague-Dawley (SD) rats (100 ± 10 g) were purchased from Wuhan Centre for Disease Prevention and Control (WCDPC) and maintained in a light (12 h light/12 h dark), and temperature (20 ± 2 °C) controlled environment. They were given free access to food and water. The rats were randomly separated into four groups (nine animals per group): sedentary and water gavage administration (SW), sedentary and chitosan gavage administration (SC), exercise and water gavage administration (EW), and exercise and chitosan gavage administration (EC). All animal experiments were complied with the ARRIVE guidelines and carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals.

Chitosan used in our study was one kind of chitosan oligosaccharide. This chitosan was prepared in our laboratory with Mw approximately $2.3 * 10^3$ and with a degree of deacetylation of 91%.

2.2. Exercise and drug treatment protocol

Exercising rats swam for 30 min in the first week, 1 h in the second week, and 2 h in the third and fourth weeks. Rats performed the exercise six times a week, followed by a day off. Training was timed between 9:00 a.m. to 12:00 a.m. in water at the temperature of 30 ± 2 °C. Subsequently, rats were towel-dried and returned to their respective cages.

Chitosan was diluted in double distilled water at 80 g per 100 mL and dose was adjusted according to the weight of rats (200 mg/kg). Subsequently, rats were alimented with chitosan for four weeks, six days a week, followed by a day off. To take as a control, another group of rats was gavaged with the water only. All the rats were weighed every other day during the entire experiment.

2.3. Blood collection

After a careful follow-up of exercise and gavage protocol, rats were anesthetized with 10% chloral hydrate at a dose of 0.3 g/kg via intraperitoneal injection. The blood samples were collected by heart puncture.

Table 2
Blood lipoprotein parameters (mean ± SD) (n = 9, each).

	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
EC	1.99 ± 0.24	1.81 ± 0.51	0.66 ± 0.08	0.23 ± 0.05
SC	2.18 ± 0.22	1.94 ± 0.65	0.66 ± 0.11	0.24 ± 0.11
EW	2.14 ± 0.34	1.97 ± 0.64	0.68 ± 0.12	0.25 ± 0.04
SW	2.34 ± 0.26	1.17 ± 0.46	0.75 ± 0.12	0.30 ± 0.05

	TC (mmol/L)		TG (mmol/L)		HDL (mmol/L)		LDL (mmol/L)	
	F	P	F	P	F	P	F	P
C	2.469	0.111	2.322	0.137	2.254	0.143	3.726	0.062
E	4.243	0.048	2.725	0.109	0.822	0.371	1.299	0.263
E + C	0.002	0.963	5.377	0.027	0.675	0.417	0.338	0.565

All the data are expressed as the mean ± SD. The two-way ANOVA test was applied for synergism effect. A value of $p < 0.05$ was considered statistically significant. EC, exercise and chitosan gavage; SC, sedentary and chitosan gavage; EW; exercise and water gavage; SW, sedentary and water gavage. TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

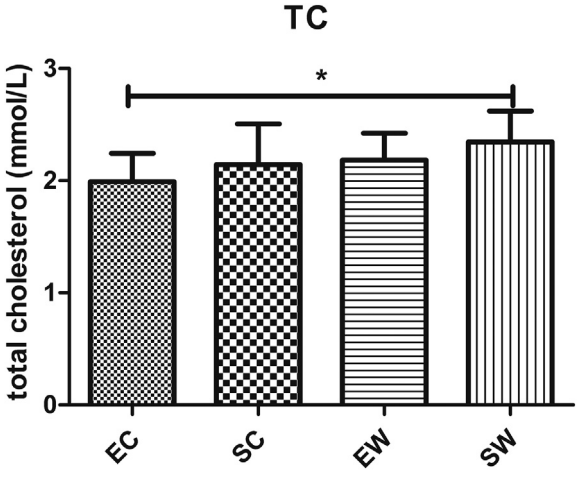

Fig. 1. Total blood cholesterol levels in EC, SC, EW, SW group.