Physical training during nutritional recovery does not affect the muscular glucose metabolism in rats

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ABSTRACT

This study had the main aim to assess the food intake, the weight gain, and the muscular glucose metabolism in rats submitted to aerobic training while recovering of protean malnutrition. For this, 60 male Wistar rats were separated in two groups: normoprotean (NP) and hypoprotean (HP), according to the diet: NP (17% protein), or HP (6% protein), respectively, which was received after the ablactation (21 days) on their 90th day of life. Then, very animal received the NP diet, and they were submitted or not (trained – TRA; sedentary – SED) to physical training that consisted in running on a treadmill for 25 m/min., 50 min/day for 5 days a week for 30 days, compounding the NP-SED, NP-TRA, HP/NP-SED, and HP/NP-TRA groups. The glucose metabolism was assessed in slices of the soleum muscle incubated in the presence of insulin (100 µU/L), and glucose (5.5 mM containing glucose [C^{14}], and 2-deoxyglycose [H^3]). The daily food intake (g/100 g of the body weight) for the HP/NP-TRA group (24.39 ± 4.07) was larger than the HP/NP-SED group (21.62 ± 4.69). The weight gain (g) was similar in both groups HP/NP-TRA (203.80 ± 34.03) and HP/NP-SED (214.43 ± 30.54). There was no difference between both groups in relation to the following parameters: glucose uptake and oxidation, and glycogen synthesis by the soleum muscle. Thus, it can be concluded that the aerobic training did not have any impact on the nutritional recovery, as there was no metabolic or somatic differences among recovered animals in the presence or absence of the training.

Keywords: Training. Protean malnutrition. Nutritional recovery.

INTRODUCTION

Despite several studies have demonstrated that it is possible to produce sufficient food for the whole world population, 826 million people continue suffering chronic malnutrition. Every year, 36 million people die as consequence of the starvation in the world[1].

The protean malnutrition implies in several metabolic changes. Several studies on underfed children indicate a decrease in the glycemic levels and low hepatic glycogen content[2]. The reestablishment of the metabolic variables during the nutritional recovery is slow. According to the literature, the physical exercise can achieve benefits to the nutritional recovery process. Upon the comparison of the growth rhythm in children recovering from malnutrition in hospitals, it was verified that the mild active children who participated in activities involving low energetic expenditure presented more slim mass and had a faster growth than those performing the hospitals' standard procedure[3]. Furthermore, it is widely known that the physical training causes an increase in the tissular glycogen reserves[4], and this can help to re-establish the underfed glycidic metabolism.

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For obvious reasons, a great number of researches involving the protean malnutrition have been conducted in lab animals, especially rats, once they present metabolic similarities to the human beings. An experimental model of early chronic malnutrition that is highly accepted and which, in many aspects is similar to what happens among the Brazilian childish population is the food protean restriction[5]. Nevertheless, as to the importance of the problem, there are few studies in the literature involving biochemical factors in underfed rats associated to the chronic physical exercise.

Gobatto *et al.*[4] performed a three steps study the metabolic aspects in rats: protean restriction for 60 days followed by a 6 weeks nutritional recovery, and the subsequent physical training constituted by 1 hour of swimming sessions a day five days a week for seven weeks. The authors verified that animals treated with the hypoprotean diet presented similar performance to the rats that were not submitted to the malnutrition, and the tissular glycogen stocks together with the blood lactate response to the acute exercise, as well as the seric substrate concentrations presented similar values between trained groups, regardless their previous nutritional state.

Galdino *et al.*[2] assessed the effects of the intra-uterine and postnatal protean malnutrition up to the adult age (10 days) in the adaptations of the muscular glucose metabolism on the swimming training. The authors verified an increase in the glucose uptake and in the content of muscular glycogen in trained animals. The glycogen synthesis has been reduced in sedentary underfed animals, but it was restored in the exercised animals. The authors have concluded that the prolonged protean malnutrition did not damage the metabolic adaptations on training.

Papoti *et al.*[6] assessed the kinetics of the blood lactate in adult rats recovered from protean malnutrition during a swimming exercise, through the determination of the maximal steady lactate phase. For this, each animal performed four swimming tests for 20 minutes bearing overloads between 4.5 and 7.5% of their body weight. The maximal steady lactate phase was attained in the 5.5% load of the body weight at the 5.5 and 4.7 nmol/l blood concentration, respectively, to the control and recovered of underfeeding rats. These results led the authors to conclude that the early malnutrition can affect the lactate kinetics during the exercises.

As there are reports suggesting that the physical training can be benefic to the nutritional recovery process, the present study was planned to assess the muscular glycidic metabolism in rats submitted to early food protean restriction and later nutritional recovery associated or not to the physical training.

MATERIALS AND METHODS

Animals and diets

The experimental sampling was composed by 60 male Wistar rats which initiated the trial in their 21st day of life. All rats were

kept in individual polyethylene cages under controlled clare-obscure cycle conditions (12/12 h), with free access to water and food (normoprotean and hypoprotean diets) manipulated at the Laboratory of Biodynamics of the Paulista Júlio de Mesquita Filho State University, Rio Claro *campus*. The diet composition is described on table 1. Every trial with animals was performed according to the guidelines provided by the European Convention for Protection of Vertebrate Animals used for Experimental and other Scientific Purposes[7].

TABLE 1
Diet composition

Components (g/Kg)	Normoprotean* (17% protein)	Hypoprotean** (6% protein)
Casein (84% protein)	202	71.5
Corn starch	397	480
Corn dextrin	130.5	159
Sucrose	100	121
L-cystine	3	1
Soybean oil	70	70
Salt mix (AIN 93-G)	35	35
Vitamin mix (AIN 93-G)	10	10
Microcellulose	50	50
Chlorine hydrochloride	2.5	2.5

* Diet to the growth, pregnancy, and lactation phases in rodents – AIN – 93G[33].
** Diet to induce a malnutrition picture[33].

Planning and experimental groups

The study was composed of two steps. In the first step, the animals were separated in two groups according to the diet: normoprotean – fed on a normoprotean diet from their ablactation in the 60th day of life, and hypoprotean – fed on a hypoprotean diet from their ablactation in the 60th of life.

Next, it started the second step, when the animals from both groups received a normoprotean portion, thus compounding 4 groups according to their nutritional state and the presence or absence of the physical training: Sedentary Normoprotean (NP-SED), Trained Normoprotean (NP-TRA), Sedentary Hypo/Normoprotean (HP/NP-SED), and Trained Hypo/Normoprotean (HP/NP-TRA). In case of animals initially submitted to the protean malnutrition, the normoprotean portion nutrition had as purpose the nutritional recovery.

During the whole experiment, the animals had their weight gain and amount of food ingestion recorded once a week. Also, they had their muzzle-anus length measured at the end of the first and second steps.

Physical training protocol

Before beginning the training period, the rats assigned to the NP-TRA and HP/NP-TRA groups performed a 10 days adaptation on the treadmill, where they ran for 5 minutes at a 10 m/min velocity for 3 days, 6 minutes at the 15 m/min velocity for 3 days, and 7 minutes at a 20 m/min velocity for 3 days.

After the adaptation, the training period initiated, and it was composed by daily running sessions 5 days a week for 4 weeks. The time of the exercising and the treadmill velocity were gradually incremented up to the animals ran for 50 minutes at a 25 m/min velocity. Such training protocol was chosen because these are aerobic exercises prescribed for rats[8].

Strength test

Before and after the training period, the NP-TRA and HP/NP-TRA groups were submitted to a running session on the treadmill at constant 25 m/min velocity for 20 minutes. On rest and every 5 minutes running, every rat was withdrawn from the treadmill for a few minutes, to collect 25 µl blood from the tail in order to determine the lactate. The blood samplings were immediately transferred to Eppendorf tubes with 50 µl hemolyzing solution (1% sodium fluoride) for later blood analysis in an electrochemical analyzer (YSI 1500 Sport Lactate Analyzer).

Sacrifice of animals and obtention of biological material

48 hours after the last strength test, the animals were sacrificed on rest through decapitation. The trunk's blood was collected in glass tubes, where it was separated from the serum, in order to determine the amount of glucose, free fatty acids (FFA), albumin, and protein. After the blood collection through a median laparotomy, a portion of the liver was withdrawn for later determination of the glycogen and fat. The two soleum muscles were extracted from the back foot for the incubation procedures, aiming to assess the glycidic metabolism. 48 hours after the first strength test, the blood sampling was collected from the cut located on the distal end of the animals' tail, in order to determine the glucose concentrations, the FFA, the amount of protein and the albumin[9].

Incubation of the soleum muscle

The slices of the soleum muscle were placed in siliconized 20 mL scintillation vials containing 1.5 mL of Krebs-Ringer bicarbonate buffer.

The vials were closed with rubber lids, sealed with plastic rings and submitted to 30 minute pre-incubation under agitation in Dubinoff bath at 60 rpm and continuing gas injection with O_2/CO_2 (95%/5%). After that period, the muscle slices were transferred to new scintillation vials (external vial), and inside the vial it was installed small shell-shaped tubes (internal vial) with an approximately 3 cm straight stick inserted through the rubber lid of the external vial. Each external vial contained 1.5 mL of the Krebs-Ringer buffer, and each internal vial had 700 mL of 10x hyamine. After 60 minute incubation in that system with gas injection for the first 15 minutes, it was added 100 µL of 25% trichloroacetic acid (TCA) in the external vial, with the purpose to release the CO_2. The preparation was kept in that system for more than three hours. After that period, 200 µL of the liquid contained within the internal vial was withdrawn, in order to determine the produced CO_2. The acidified incubation media contained in the external vial was stored to determine the lactate, and the muscle slice was immediately digested in 0.5 mL KOH, in order to assess the muscular glycogen content[11]. The pre-incubation and incubation temperature was 37º.

The Krebs-Ringer buffer, which was the basis to the pre-incubation and incubation medias consisted in 6% NaCl, 6.64 mM HEPES, 0.032% KCL, 1.12 nM $CaCL_2$, 0.015% KH_2PO_4, 0.19% $NaCO_3$, 0.03% $MgSO_4$. That solution was gas injected for 30 minutes in O_2/CO_2 (95%/5%), and the pH was adjusted at 7.4. It was added 20 volumes of fat free seric albumin to that solution, and sodium pyruvate was added to the pre-incubation media in the 5 mM concentration, and (5.5 mM) glucose was added to the incubation media containing (0.25 µCi/mL) glucose [U_{-14C}], (2-DG = 0.5 µCi/mL) 2-deoxyglucose [^3H], and (10025 µUL/mL) insulin.

After these additions, the pH was adjusted at 7.4, and the media was transferred to the vials that were sealed and balanced into a 37ºC bath under O_2/CO_2 gas injection for at least 15 minutes. Slices of the same muscle of similar weight than the incubated ones were used to determine the controlling glycogen concentration. It was assessed the glucose uptake using the 2-DG as marker, incorporating the ^{14}C to the glycogen (synthesis), and measuring the radioactivity of the glycogen's ^{14}C and the 2-DG's ^3H contained respectively in the precipitate and in the alcoholic phase of the glycogen extraction using the counter of the beta particles. The lactate released into the incubation media was determined separating the metabolites in the ionic exchange column (Dowex-2, Sigma), and this represents an index of the glucose transportation under these conditions. Besides the radioactive lactate, the total lactate was also determined into the incubation media using the enzymatic

method. In order to assess the oxidant glucose (CO_2 production), the ^{14}C radioactivity present in the liquid (hyamine) collected in the internal vial of the incubation system was determined.

Biochemical determinations

The seric glucose was determined using the enzymatic method of the oxidase glucose, the total proteins was determined by the biuret reagent, the albumin was determined by the bromokresol green, and the FFA was determined by the carbamate diethyl. The tissular glycogen was extracted according to the Sjorgren *et al.*[10] method, and the color of the precipitate was performed using the phenol, following Dubois *et al.*[11].

Statistical analysis

The statistical procedures included the unpaired *t* Student or the two-entry ANOVA test, and whenever necessary, this was followed by the *post-hoc* Bonferroni test. In every case, it was adopted $p < 0.05$ as significance level.

RESULTS

In the first phase of the study, the animals that received the hypoprotean diet had a significantly lower weight gain, and a significantly higher food intake compared to the normoprotean groups after the 8 week training period with the diets (table 2). The animals kept in the hypoprotean diet presented significantly higher seric FFA concentrations, and significantly lower total proteins, albumin, and glucose related to the normoprotean group (table 2).

TABLE 2
Weight gain (g) and daily food intake (g/100 g of body weight), seric contents of free fatty acids (FFA IN µEq/L), total proteins (g/dL), albumin (g/dL) and glucose (mg/dL) at the end of the first step of the study

	Control	Underfed
Weight gain	232.18 ± 21.11	76.16 ± 21.01*
Food intake	17.4 ± 4.11	22.1 ± 4.09*
FFA	260.0 ± 12.0	322.0 ± 45.0*
Total protein	6.5 ± 0.2	5.5 ± 0.1*
Albumin	5.9 ± 0.1	4.7 ± 0.1*
Glucose	122.1 ± 5.1	100.0 ± 4.2*

Results expressed as mean and standard deviation in 20 animals per group.
* Significant difference ($p < 0.05$, test t) related to the control.

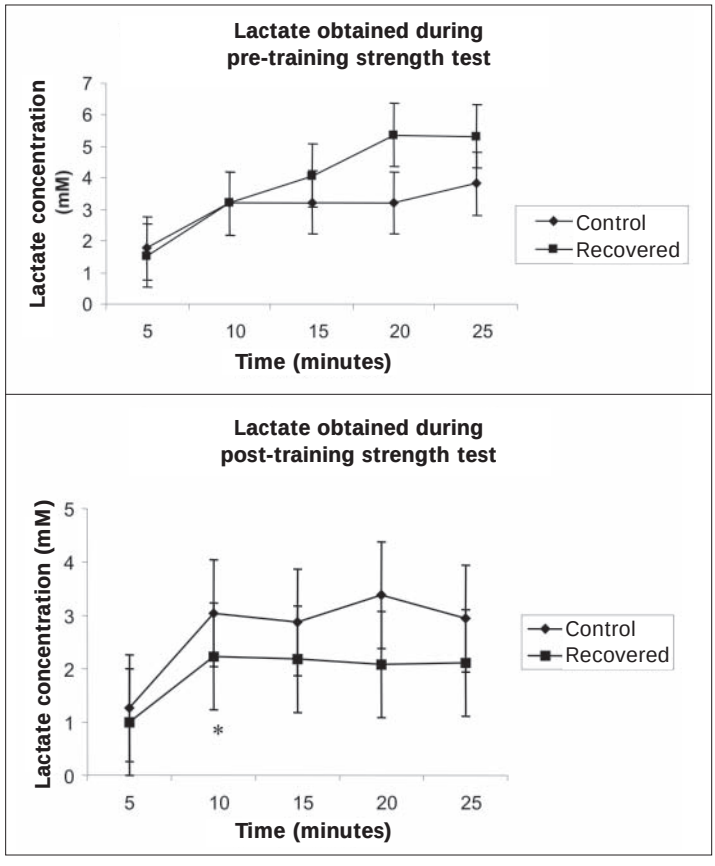

Figure 1 *– Blood lactate of groups CT and RT during the strength tests performed before (upper panel) and after (lower panel) the physical training. Before the training, the blood lactate stabilized after 5 minutes of the exercising in the recovered group (3.72 ± 1.06 mmol/L), and after 10 minutes in the control group (4.60 ± 1.44 mmol/L). After the training, the blood lactate stabilized 5 minutes after the exercise in both groups (control, 2.98 ± 1.02, and recovered 2.15 ± 0.97* mmol/L). As stabilization criterion, it was considered the difference lower than 1.0 mmol/L between groups. The recovered group presented a significant decrease in the lactate concentration or a significant decrease in the lactate concentration after the 30 days training on treadmill. Resultados expressos como média e desvio padrão de 8 amostras teciduais em cada grupo.*

* Significant difference $p < 0.05$, related to the exercise performed before the 30 day training in the same group.

TABLE 3
Daily rats' food intake (g), weight gain (g), length of the rats (cm), hepatic glycogen (mg/110 mg), hepatic lipids (mg/100 mg) and muscular glycogen (mg/110 mg), seric content of free fatty acids (FFA in µEq/L), total proteins (g/L), albumin (g/L) and glucose (mg/dL), glycogen concentration (mg/100 mg), glucose uptake, oxidation and incorporation (synthesis) in glycogen (mmol/g.hour) by the isolate soleum muscle in the end of the second step of the study

	Sedentary control (12)	Trained control (8)	Sedentary recovered (12)	Trained recovered (8)
Daily intake	17.84 ± 4.47	21.58 ± 4.91*	21.62 ± 4.69	24.39 ± 4.07£
Weight gain	341.35 ± 33.47	319.68 ± 23.04	214.43 ± 30.54*	203.80 ± 34.03#
Length	9.86 ± 0.91	9.79 ± 0.87	8.06 ± 0.66*	8.03 ± 0.67
FFA	273.3 ± 43.96	260.1 ± 31.84	278.1 ± 42.07	272.8 ± 40.75
Total proteins	6.43 ± 0.57	6.66 ± 0.19	6.62 ± 0.29	6.59 ± 0.17
Albumin	4.12 ± 0.32	4.08 ± 0.30	4.09 ± 0.34	4.24 ± 0.19
Glucose	124.0 ± 23.32	134.1 ± 17.18	130.4 ± 17.54	132.2 ± 14.85
Hepatic glycogen	5.74 ± 1.04	7.36 ± 1.40*	4.39 ± 1.40*#	5.77 ± 1.40#
Hepatic lipids	3.8 ± 0.35	4.1 ± 0.53	4.14 ± 0.56	4.25 ± 0.36
Muscular glycogen	1.44 ± 0.48	0.85 ± 0.20*	1.44 ± 0.53#	0.97 ± 0.17*£
Glucose uptake	5.78 ± 1.34	5.75 ± 1.60	5.28 ± 1.21	6.78 ± 1.65
Glucose oxidation	3.93 ± 0.60	4.11 ± 0.77	4.49 ± 0.66	3.96 ± 0.90
Glycogen synthesis	0.86 ± 0.60	0.65 ± 0.60	0.34 ± 0.08	0.56 ± 0.52

Results expressed as mean ± standard deviation, with the amount of animals are parenthesized.
* Significant difference ($p < 0.05$, *two-way Anova*) related to the sedentary control.
£ Significant difference ($p < 0.05$, *two-way Anova*) related to the recovered sedentary.
\# Significant difference ($p < 0.05$, *two-way Anova*) related to the trained control.

In the second step of the study, the trained groups (NP-TRA, and HP/NP-TRA) showed a daily food intake higher than the respective sedentary groups (NP-SED, and HP/NP-SED) (table 2). Related to the weight gain, the trained recovered groups (HP/NP-TRA) had a significantly lower gain compared to the remaining groups (HP/NP-SED, NP-TRA, and NP-SED).

Figure 1 shows the blood lactate values of the trained groups (NP-TRA, and HP/NP-TRA) on the strength tests performed before (upper panel) and after (lower panel) the physical training. In the test performed before the training, the blood lactate was stabilized from 5 minutes of the exercise in the recovered group (HP/NP-TRA), and after 10 minutes in the control group (NP-TRA) with similar values (3.72 ± 1.06, and 4.60 ± 1.44 mmol/L to the NP-TRA, and the HP/NP-TRA, respectively).

In the test performed after the 4 week training period, the blood lactate stabilized after 5 minutes of the exercise in both groups, presenting lower values than those observed in the first test (2.98 ± 1.02, and 2.15 ± 0.97 mmol/L, to the HP-TRA and HP/NP-TRA, respectively). The reduction was significant only to the recovered group (HP/NP-TRA).

The trained control group (NP-TRA) presented a significantly higher hepatic glycogen concentration compared to the remaining groups (NP-SED, HP/NP-SED, and NP-TRA). As to the hepatic lipids, it was verified no significant differences between groups. The muscular glycogen presented significant lower values in trained animals (NP-TRA, and HP/NP-TRA) than the sedentary ones (NP-SED, and HP/NP-SED (table 3).

There was no significant difference between the values of the glucose uptake, the glucose oxidation and incorporation (synthesis) in the glycogen by the isolate soleum muscle in rats (table 3).

DISCUSSION

The results related to the gain in the body weight, food intake and the length in rats in the first step of the study indicated that the 6% protein diet was effective in inducing a malnutrition state in animals. Likewise, the blood glucose concentrations, the total protein, low albumin and high seric FFA content are commonly observed features in underfed human beings[12] and in underfed animal models[2,13,14].

The reduction in the body weight gain in rats submitted to protean restriction is a widely reported phenomenon in the literature[15], and this has been associated to functional and morphological changes.

Rats kept in a hypoprotean diet showed a similar picture than the childish Kwashiorkor-type malnutrition that has as basic characteristics: low weight, hypoproteinia, hypoalbuminemia, high seric FFA contents and hepatic steatosis or fatty liver[1,2].

An important aspect is that the alterations observed were consequence of the protean instead of the caloric deficiency, as both were isocaloric diets and the underfed rats of such model presented a higher food intake than the control group. Several prior studies within our group have shown a high food intake in rats kept in a hypoprotean diet[4,8,16].

Among other factors, such alteration may be due to modifications in the mediators such as the ghrelin and the leptin. Leptin is a protein produced by the adipose tissue that has an important role through the increase in the calories burning and in the reduction in the food intake[17-19]. Ghrelin is a gastrointestinal hormone recently identified in the rats' stomach[20]. Studies in animal models indicate that the ghrelin has an important role in the signalization of the hypothalamic centers that regulate the food intake and the energetic balance[21-25].

Still, it is known that the seric ghrelin concentrations are influenced by the acute and chronic changes in the nutritional state, and they rise in the presence of nervous anorexia, and reduced in the obesity, unlike the leptin that is high in obese rats[24,26-28]. Thus, it is necessary to assess the protean malnutrition model, in order to precisely interfere in the contribution of those mediators as to the food intake alterations observed in the present study.

In the second step of the study, it was verified that the amounts of seric protein, glucose, albumin, and FFA, as well as the hepatic and muscular glycogen and the hepatic lipids presented similar concentrations in the recovered animals than the amounts found in the control animals. The body mass remained reduced. These data are in accordance to the previously found results[1,6], and they suggest the partial nutritional recovery in previously underfed rats.

The glucose uptake, oxidation, and incorporation (synthesis) in glycogen by the isolate soleum muscle in rats at the end of the second phase of the study present no significant differences between the recovered and control groups both in trained and sedentary animals, suggesting that the early food protean restriction does not interfere in the muscular metabolism of the glucose after the recovery.

Crace *et al.*[29] reported an increase in the glycogen synthesis in rats submitted to protean restriction, and also a higher glucose uptake by the isolate soleum muscle in rats submitted to hypoprotean diet compared to rats fed with a normoprotean diet. On the other hand, Nolte *et al.*[30] found no difference in the glycogen synthesis between the control and protein recovered animals, and they studied muscular slices incubated upon the absence and presence of FFA.

The results in the strength tests showed that the training treadmill running protocol led to a reduction in the blood lactate accumulation during the exercise, suggesting an improvement in the aerobic conditioning in these animals. Normally, the physical training reduces the lactate accumulation during the exercising at the same submaximal intensity of the strength due to a higher removal capability of the circulating substrates[31].

On the other hand, the reduction in the blood lactate during the exercises in the trained groups compared to the sedentary groups may be due simply to the reduction in the muscular reserves of the glycogen, since in the present study the animals submitted to the physical training, regardless their nutritional state, presented a reduction in the muscular glycogen concentrations. These results are opposed to the classical reports found in the literature, indicating the presence of high muscular glycogen stocks in human beings and animals submitted to the physical training[32].

Considering that trained and sedentary animals have shown similar values for the glucose uptake and *in vitro* glycogen synthesis by the isolate soleum muscle, the low muscular glycogen concentrations in the trained groups assessed in the resting state may be consequence of the incomplete recovery after each exercising session.

Summarizing, it can be concluded that the hypoprotean diet in the first step was efficient in inducing the protean malnutrition state in rats. In the second step, there was a return of the biochemical parameters values analyzed in the normal state.

As to the training, it had no impact on the nutritional recovery, as there were no metabolic or somatic changes between the recovered animals in the presence or absence of the training. It was detected only an increase in the food intake among the trained groups, suggesting a reduction in the alimentary efficiency, in function of the increasing energetic demand that is consequence of the training.

Probably, the frequency of the exercise was inadequate to observe the desired effects. Further studies are in progress in our laboratories aiming to enlarge the animals' training period, as well as to assess the oxidant adaptation of the skeletal muscle through the enzymatic activities.

All the authors declared there is not any potential conflict of interests regarding this article.

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