BASIC SCIENCE

Evaluating the Effects of Low Carbohydrate and High Protein Diet on Erectile Function in Rats

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ABSTRACT

Introduction: Carbohydrate restriction in diet is becoming a popular means of losing weight nowadays, although it has been reported that excessive intake of low-carbohydrate and high-protein (LCHP) diet causes an adverse effect on cardiovascular function.

Aim: To investigate the influence of LCHP on erectile function in rats.

Methods: A total of 48, 12-week-old rats were divided into 2 groups and either fed a LCHP diet (LCHP group) or a normal diet (Control group). Hematological examination, blood pressure evaluation, erectile function assessments as well as evaluations of the relaxation and contractile responses of corpus cavernosum were carried out in these rats by using standardized methods. Statistical analysis using 2-way ANOVA and Welch's *t*-test was conducted to examine the obtained data.

Main Outcome Measure: At the end of the study period, the evaluated outcomes to assess erectile function were intracavernosal pressure , mean arterial pressure , endothelial functions, nitric oxide (NO)-operated nerve functions and the expressions of endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and sphingosine-1-phosphate receptor 1 (S1P1).

Results: The intracavernosal pressure / mean arterial pressure ratio was significantly lower in the LCHP group (*P* < .05) at 4 weeks. Compared to the Control group, the LCHP group exhibited significantly lower responses to ACh and EFS and a decreased nNOS mRNA expression. The results based on this animal model indicate that extreme carbohydrate restricted diet may affect erectile function. Our study identified that LCHP decreased erectile function in rats. A major limitation of this study is, due to the extreme condition of completely replacing carbohydrates with protein, that carbohydrate intake will be gradually increased in the future.

Conclusion: Extreme carbohydrate restriction and high protein in diet may cause ED with vascular endothelial dysfunction and a decrease in the relaxation response of the corpus cavernosum smooth muscle via NO-operated nerves. **Kataoka T, Hidaka J, Suzuki J, et al. Evaluating the Effects of Low Carbohydrate and High Protein Diet on Erectile Function in Rats. Sex Med 2021;10:100500.**

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Key Words: Carbohydrate; Diet; Protein; Erectile Dysfunction; Endothelial Dysfunction; NO-Operated nerves

INTRODUCTION

Carbohydrate restriction has lately emerged as a commonly followed method of dieting.[1,2] Calorie restriction has been reported to have health benefits, including an extended lifespan.[3,4] On the other hand, the Diabetes Foundation said that extremely limiting carbohydrates to reduce weight is not only effective, but also lacks evidence such as long-term dietary compliance and safety, so further research should be carried out. Carbohydrate restriction in diet has been reported to be associated with certain adverse effects and the most noteworthy of

these are cardiovascular diseases, such as angina and myocardial infarction. Studies in Sweden have shown that the incidence of cardiovascular disease increases with a low-carbohydrate and high-protein diet (LCHP).[5] It has also been reported that a LCHP diet is involved in the formation of arterial plaques.[6] Furthermore, it has been reported in animal models that a LCHP diet causes bodily dysfunctions such as left ventricular hypertrophy in aged mice, decreased aortic extensibility, decreased endothelial function of the mesenteric artery, and atherosclerosis.[7-8] However, the influence of LCHP on erectile function has not been explored yet and remains unclear.

We hypothesized that LCHP may have negatively influence on the erectile function. Therefore, in this study, we investigated whether a carbohydrate restricted, high protein diet affects erectile function, by feeding rats with a diet in which carbohydrate components were replaced with protein components of the same caloric value. Furthermore, we evaluated vasculature related parameters such as blood pressure and contraction-relaxation reaction of corpus cavernosum (CC) smooth muscle.

MATERIAL AND METHODS

Animals and Treatment Protocols

A total of 48, 12-week-old male Wistar/ST rats were purchased from Japan SLC, Inc. (Hamamatsu, Japan). All experimental protocols were approved by the ethics review board of Nagoya City University and conducted in accordance with our institutional standards for the care and use of animals (H25-P-09). The rats were kept in a temperature- and humidity-controlled room, with a 12-h/12-h light/dark cycle and free access to normal water.

The rats were divided into 2 groups based on the diet: a control group and a LCHP group (Figure 1). The control group rats were fed a normal diet (CLEA Rodent Diet CE-2; CLEA Japan, Inc., Tokyo, Japan). The LCHP group rats were administered a diet in which the sugars (sucrose and cornstarch) of CLEA Rodent Diet CE-2 (protein energy ratio 24.8%, lipid energy ratio 21.6%, and sugar energy ratio 53.6%) were replaced with proteins (milk casein). The same proportion of vitamins and minerals were used. In addition, mix so that the total energy amount per feed weight is the same feed (protein energy ratio 76.7%, lipid energy ratio 21.6%, sugar energy ratio 1.7%), and cover each feed with a powder feeder cover (Natsume Seisakusho Co, Ltd, Tokyo, Japan).

Blood Samples and Measurement of Biological Parameters

At the end of the treatment period (4 weeks), blood samples were obtained from all the rats via the vena cava under 3−4% isoflurane anesthesia (Mylan, Canonsburg, PA, USA) with the use of inhalation anesthesia apparatus (Nakazawa Seisaku-sho, Funabashi, Japan) before the isometric tension measurement. These blood samples were taken from a rat different from the rat used for erectile functional measurement (Figure 1). After coagulation and centrifugal separation at 800 g for 20 minutes at 4°C, serum samples were stored at -80°C until analysis. Thereafter, the penis was removed and the urethra, dorsal artery, and the dorsal vein were removed for the isometric tension measurement.

Measurement of Blood Pressure

Blood pressure was measured as per methods previously reported.[9-10] Systemic and diastolic arterial blood pressures of the rats were measured at 0, 2, and 4 weeks, using a noninvasive automatic device (BP-98A-L, Softron, Tokyo, Japan). Mean arterial pressure was automatically calculated by the device. The rats (n = 8) were restrained in an attached cloth product and maintained at 37°C during the measurements by means of a warmer (THC-31, Softron). After acclimatizing the rats for 30 minutes or more, blood pressure was measured on the tail using
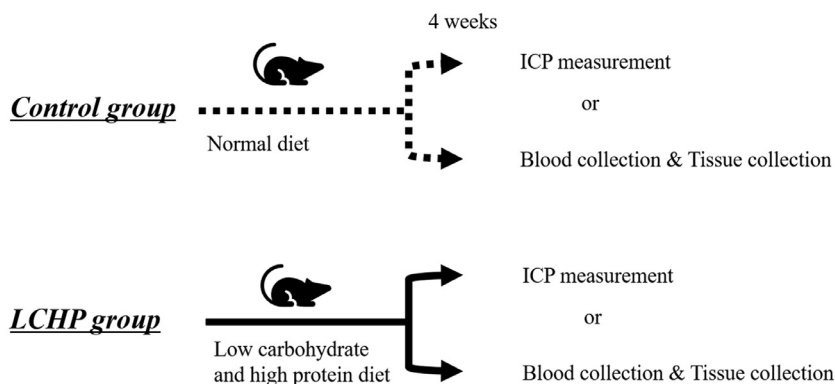

Figure 1. Schematic describing the experimental design of this study. Each rat was assigned to one of the following groups: control and low-carbohydrate and high-protein diet (LCHP) group. The control group rats were fed a normal diet. The LCHP group rats were administered a diet in which the sugars (sucrose and cornstarch) of CLEA Rodent Diet CE-2. At the end of the treatment period (4 weeks), one group of rats had intracavernous pressure (ICP) measurement and the other had blood sample and then the isometric tension measurements of corpus cavernosal tissue.

a cuff. Each measurement was performed 3 times per rat (while awake), and the mean value was used for the analysis.

Examination of Erectile Function

Intracavernous pressure (ICP) was measured by electrical stimulation as previously reported.[9-13] Briefly, rats from each group (n = 8) were anaesthetized with isoflurane using inhalation anesthesia apparatus. The carotid artery was cannulated for continuous monitoring of the mean arterial pressure (MAP) and the left crus of the CC was cannulated using a 23-G needle for continuous ICP monitoring. The pressure transducer was connected through an amplifier to a data acquisition board (PowerLab 2/26, ADInstruments Pty. Ltd., New South Wales, Australia). Stainless steel bipolar wire electrodes (Unique Medical, Osaka, Japan) and a pulse generator (Nihon Kohden, Tokyo, Japan) were used for the penile stimulations with the following parameters: 1 min at 5 V, 1 −16 Hz, and a square wave duration of 5 microseconds. Erectile function was evaluated using the maximum ICP/MAP ratio.

Relaxation and Contractile Responses of Corpus Cavernosum Analysis

Relaxation and contractile responses of CC was measured using isometric tension measurement, as previously reported (n = 11).[9,11-13] The harvested CC strips (n = 16) were equilibrated for at least 60 minutes in an aerated organ bath containing Kreb's solution (119 mM NaCl, 4.6 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 15 mM $NaHCO_3$, 11 mM D-glucose, and 1.2 mM NaH_2PO_4) at 37°C with 5% CO_2 and 95% O_2. The resting force for tissue was set at 600 mg, and changes in isometric tension were recorded using a force transducer (Nihon Kohden) that was connected to a data acquisition board (PowerLab 4/26). Relaxation experiments were conducted using CC strips that were pretreated with 10 μM noradrenaline (NA; Sigma Aldrich, MO, USA); the muscle relaxant effect was induced by acetylcholine (ACh; Wako Pure Chemical Industries) and sodium nitroprusside (SNP; Sigma Aldrich). The contractile capacity of the strips was tested by exposing them to 80 mM of KCl Krebs' solution (36.7 mM NaCl, 80 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 14 mM D-glucose, and 1.2 mM KH_2PO_4). The contractile effect was induced by NA. Cumulative dose (10^{-10} − 10^{-4} M) response curves were obtained for ACh, SNP, and NA using different tissue specimens.

Electrical field stimulation (EFS) was performed in CC strips placed between 2 platinum plates (distance: 5 mm, length: 15 mm) using an electronic stimulator (Nihon Kohden, Tokyo). Stimulus conditions were set as follows: voltage -16 V, duration - 5 microseconds, frequencies - 1, 2, 4, 8, 16, 32, 64, and 128 Hz, and time - 5 seconds. To assess the relaxation response induced by the nitrergic nerve, strips were precontracted with NA and underwent EFS after preincubation with a beta-receptor blocker (propranolol, 10 μM), a muscarinic receptor blocker (atropine, 10 μM), and an ATP receptor blocker (suramin, 10 μM).[14]

Table 1. Primer sequences for real-time quantitative polymerase chain reaction (qRT-PCR)

mRNA		Sequence
eNOS	Forward	5'-GCTGGCCTTACTGGAGTGGTC-3'
	Reverse	5'- CAGTGCCACGGATGGAAATT -3'
nNOS	Forward	5'- TCAGCAGATCCAACCCAATG -3'
	Reverse	5'- TGCTGACCCGTTCCTTCAC -3'
S1P1	Forward	5'- TTCTGCGGGAAGGAAGTATG -3'
	Reverse	5'- TGCTGCCGTTGTGTAGTTTC -3'
NF-κB	Forward	5'-AGAGAAGCACAGATACCACTAAG-3'
	Reverse	5'-CAGCCTCATAGAAGCCATCC-3'
IL-6	Forward	5'-CAAGAGACTTCCAGCCAGTTGC-3'
	Reverse	5'-TGTTGTGGGTGGTATCCTCTGTG-3'
β-actin	Forward	5'- TGTGTGGSTTGGTGGCTATC -3'
	Reverse	5'- CATCGTACTCCTGCTTGCTGATC -3'

eNOS = endothelial nitric oxide synthase; nNOS = neuronal nitric oxide synthase; S1P1 = sphingosine-1-phosphate receptor 1; NF-κB = nuclear factor-kappa B; IL-6 = interleukin-6.

Real-time Quantitative Polymerase Chain Reaction

Real-time quantitative polymerase chain reaction (qRT-PCR) analysis was performed as previously reported.[11-13] Total RNA was extracted from CC samples using TriPure Isolation Reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. Using a ReverTra Ace-α kit (Toyobo, Osaka, Japan) and 1 μg of total RNA was reverse transcribed into complementary DNA, which served as the template for real-time PCR using the Power SYBR PCR Master Mix (Applied Biosystems, Tokyo, Japan). The primer sequences are shown in Table 1. Amplification and detection were performed using the ABI 7300 system (Applied Biosystems). Target gene expression was quantified relative to β-actin expression using the comparative CT method. All measurements were performed in triplicate.

Statistical Analyses

Obtained data were expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using Welch's *t*-test and 2-way analysis of variance (ANOVA), 2-way repeated measures ANOVA in conjunction with post-hoc Tukey-Kramer analysis, using in EZR on R commander ver 1.41.[15] *P*-values < .05 were considered statistically significant.

RESULTS

Biological Parameters

Figure 2A shows the comparison of the mean body weight between the control group and LCHP group as a function of time. The mean body weight in LCHP group was significantly lower than that in the control group after the first week of the experiment initiation (*P* < .01). On the other hand, when the change in body weight compared to the first week was evaluated (Figure 2B), no significant change was observed between the Control group and the LCHP group (*P* > .05) Figure 2C shows the weekly food intake of
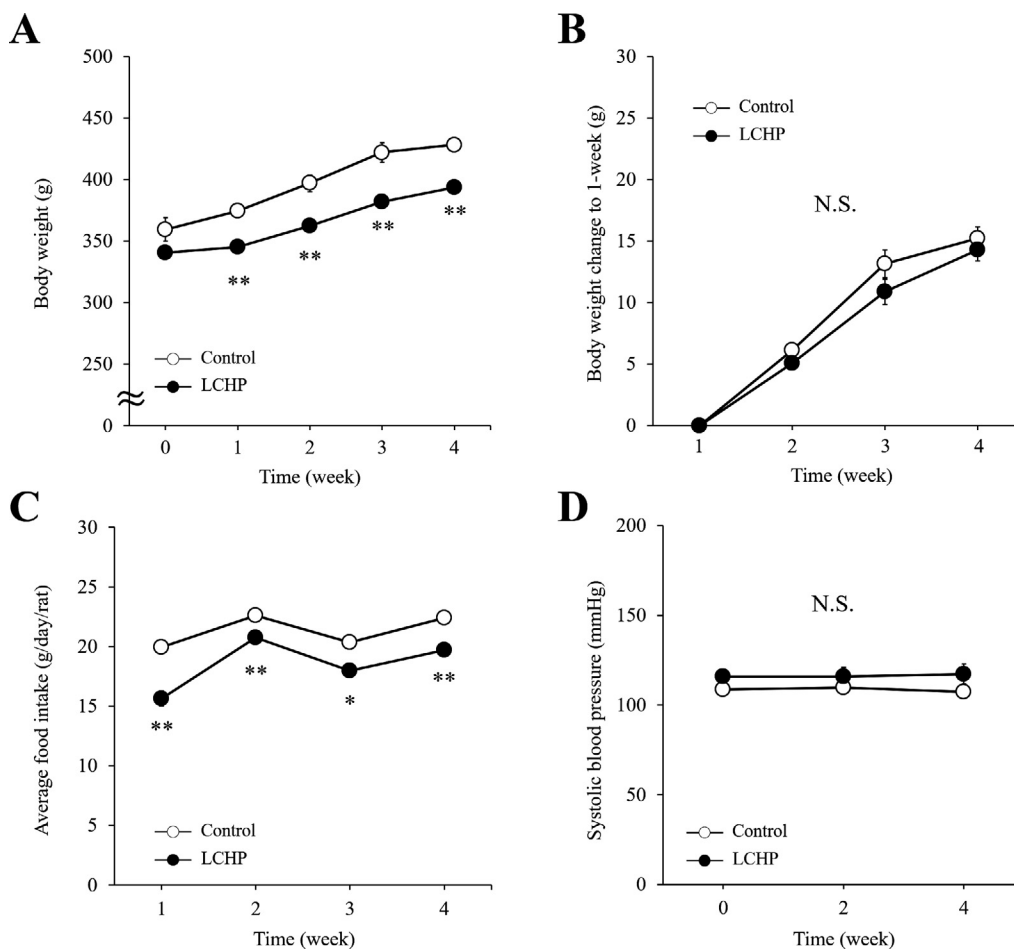
Figure 2. Biological parameters. (A) Body weight. (B) Body weight changes with respect to the first week. (C) Average food intake. (D) Systolic blood pressure. Data are reported as mean ± standard error of the mean (n = 8–16). *$P < .05$, **$P < .01$ vs each group using analysis of Welch's *t*-testing.

each group. The amount of food consumed in the LCHP group was significantly lower than that in the Control group ($P < .05$) Figure 2D shows changes in systemic blood pressure (SBP) of the rats in the 2 groups. There was no significant difference in SBP, mean blood pressure (MBP, data not shown), diastolic arterial blood pressure (DBP, data not shown) between the 2 groups at any point in time ($P > .05$).

Table 2 shows data pertaining to the various biological parameters which were evaluated in the study rats. The electrolyte levels and glucose levels did not significantly differ between the 2 groups ($P > .05$). Liver function parameters (aspartate transaminase [AST] and alanine aminotransferase [ALT]), renal function parameter (blood urea nitrogen [BUN]), and lipid levels (total cholesterol) in the LCHP group were higher than that in the Control group ($P < .05$).

Erectile Function

Figure 3A shows representative tracings of the ICP and the mean arterial pressure changes during electrical stimulation of the cavernous nerve. ICPs in the LCHP group at 4 weeks appeared to be significantly lower than that in the Control group

Figure 3B shows the ICP/MAP ratios for different stimulation frequencies. The ICP/MAP ratio in LCHP group was significantly lower than that in Control group at stimulation frequencies of 4, 8, and 16 Hz ($P < .05$).

Relaxation and Contractile Responses of Corpus Cavernosum

The contractile responses of the CC strips to increasing concentrations of NA are shown in Figure 4A. The responses to NA at 10^{-10}-10^{-4} M did not significantly differ between the 2 groups ($P > .05$). The relaxation responses of the NA-pretreated rat CC strips to increasing concentrations of ACh are shown in Figure 4B. The LCHP group exhibited significantly lower responses to ACh than the Control group ($P < .01$). The relaxant responses of the CC strips to increasing concentrations of SNP are shown in Figure 4C. The SNP responses at $10^{-10}-10^{-4}$ M did not significantly differ between the 2 groups ($P > .05$). The relaxant responses of the CC strips to increasing frequency of EFS are shown in Figure 4D. The LCHP group exhibited significantly lower responses to EFS than the Control group ($P < .05$).

Table 2. Biological parameter data of the study rats

Biological parameter	Unit	Control	LCHP	P value
Na	μEq/l	141.3 ± 0.68	143.1 ± 0.70	.0823
K	μEq/l	4.63 ± 0.15	4.53 ± 0.11	.6037
Ca	mg/dL	10.0 ± 0.12	9.9 ± 0.12	.4214
Glucose	mg/dL	248.4 ± 19.9	241.6 ± 11.4	.7709
Total protein	g/dL	5.0 ± 0.12	5.2 ± 0.10	.2925
Albumin	g/dL	3.6 ± 0.08	3.8 ± 0.04	.0385*
GOT (AST)	IU/l	86.0 ± 8.18	117.0 ± 7.00	.0141*
GPT (ALT)	IU/l	40.9 ± 1.20	58.0 ± 2.32	.0001**
LDH	IU/l	492.4 ± 188.8	587.7 ± 125.4	.6827
Amylase	IU/l	1300 ± 56.0	1262 ± 89.4	.7241
Total bile acid	μmol/l	0.037 ± 0.004	0.037 ± 0.004	1.0000
BUN	mg/dL	19.5 ± 0.92	36.1 ± 1.71	.00001**
Creatinine	mg/dL	0.38 ± 0.02	0.26 ± 0.01	.0001**
Total cholesterol	mg/dL	64.3 ± 2.63	78.6 ± 4.65	.0243*
Triglyceride	mg/dL	24.9 ± 4.44	46.1 ± 8.89	.0615

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; LDH = lactate dehydrogenase. Data have been reported in terms of mean ± standard error (n = 7 per group). P values were evaluated for each group by using Welch's t-test.

mRNA Expression Analysis

Figure 5 shows the mRNA expression levels in the CC. The expression of endothelial nitric oxide synthase (eNOS) did not significantly differ between the 2 groups ($P > .05$). The expression of neuronal nitric oxide synthase (nNOS) and sphingosine-1-phosphate receptor 1 (S1P1) were significantly lower in the LCHP group as compared to the Control group ($P < .05$). The expression of nuclear factor-kappa B (NF-κB) and interleukin-6 (IL-6) did not differ significantly between the 2 groups ($P > .05$).

DISCUSSION

The results of this study demonstrated that the administration of LCHP diet to rats for a duration of 4 weeks decreased the ICP/MAP ratio and reduced relaxation response of CC to Ach and EFS, which are indicative of the development of erectile dysfunction (ED). Thus, it was suggested that excessive dietary restrictions may reduce the erectile function in rats.

In this study, carbohydrate-restricted and high protein diet significantly reduced body weight and food intake in the study rats. Previously, it has been reported that a carbohydrate-restricted diet causes weight loss.[16] However, it is believed that this weight loss might be associated with a decreased caloric intake rather than decrease in carbohydrate content of the diet. In this study, the increase in body weight and food intake decreased in the LCHP group, but the weight gain after the second week was similar to that in the control group. Therefore, when the change in body weight was calculated in comparison to the first week, no significant difference was found. Previous studies on carbohydrate restriction and high-protein diets using rodent models have reported impaired glucose tolerance,[17] but the blood glucose levels did not change in the LCHP group in this study. In addition, studies using rodents have reported that changes in dietary components such as carbohydrates, proteins,
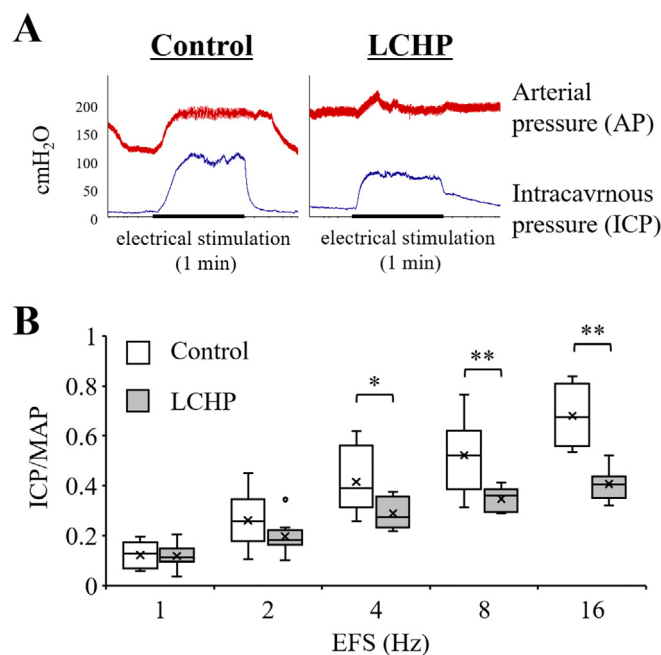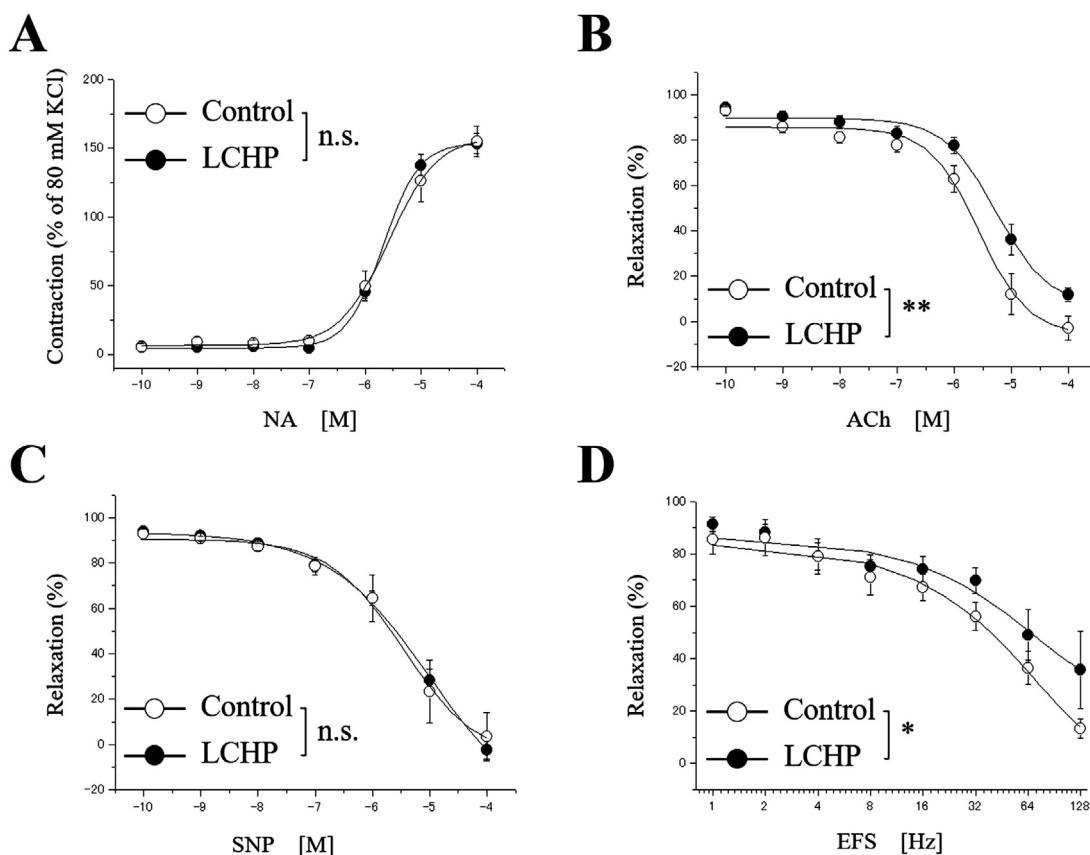

Figure 3. (A) Representative tracings of intracavernous pressure (ICP) and arterial pressure changes during electrical stimulation (16 Hz) of the cavernous nerve in Control and LCHPrats. (B) Erectile function according to the ICP/mean arterial pressure (MAP) ratio. Data are presented as box-and-whisker plot (n = 4−8). *$P < .05$, **$P < .01$ vs each group using analysis of 2-way repeated measures ANOVA in conjunction with post-hoc Tukey-Kramer analysis.

Figure 4. The changes of contractile or relaxation response. (A) The changes of the contractile response by the cumulative administration of NA. (B) The changes of the relaxation response by the cumulative administration of ACh. (C) The changes of the relaxation response by the cumulative administration of SNP. (D) The changes of the relaxation response by EFS. Data are reported as mean $\pm$ standard error of the mean (n = 4−10). *$P < .05$, **$P < .01$, N.S.; not significant, vs each group using analysis of 2-way ANOVA.

and lipids affect survival.[18] Furthermore, it has been reported in studies on rodent models that an LCHP diet causes bodily dysfunctions such as endothelial dysfunction.[7,8]

Biochemical serological tests showed no change in glucose levels between the 2 groups, despite restricted carbohydrate consumption in the LCHP group. In this study, as blood glucose level was measured using the blood collected from the vena cava under anesthesia, so it is possible that the value was extremely high. In the future study, it may be necessary to measure blood glucose level using peripheral blood sample. On the other hands, since AST and ALT levels were increased in this study, LCHP may impair liver function. It has been reported that escalated gluconeogenesis in the liver in turn leads to the induction of AST and ALT production.[19-21] Excessive diet may reduce liver function, so we should take care of our diet.

It has been previously reported that BUN/Cr ratio increases due to increased intake of protein and promotion of protein catabolism.[22] In addition, it is also known that the amount of albumin in plasma depends on the amount of protein in the diet.[23] Therefore, it was suggested that the increase in BUN/Cr ratio and albumin value in the LCHP group may be due to the high protein content of the diet. Based on the above information

it can be stated that caution must be exercised while following a low-carbohydrate/high-protein diet, as it might cause renal and hepatic dysfunction.

In a previous study, where an increased proportion of carbohydrates were administered in the diet, reported a decrease in the total cholesterol level and this effect was ascribed to the increase in bile excretion and intestinal viscosity due to soluble dietary fiber in carbohydrates.[24] Therefore, it was suggested that the increase in total cholesterol level in the LCHP group in our study may be due to the decrease in soluble dietary fiber in the LCHP diet.

Regarding evaluation of erectile function by ICP measurement, it was suggested that the ICP/MAP ratio decreased in the LCHP group, showing a decrease in erectile function of the rats. It has been reported that the incidence of cardiovascular disease increases with a low-carbohydrate, high-protein diet.[5] It has also been reported that ED can be an important marker of cardiovascular disease.[25] Therefore, according to the results of this study, it can be conjectured that development of ED in humans due to excessive carbohydrate restriction may lead to development of heart diseases.

We evaluated the contractile-relaxation reaction of the CC smooth muscle by isometric tension measurement, in order to
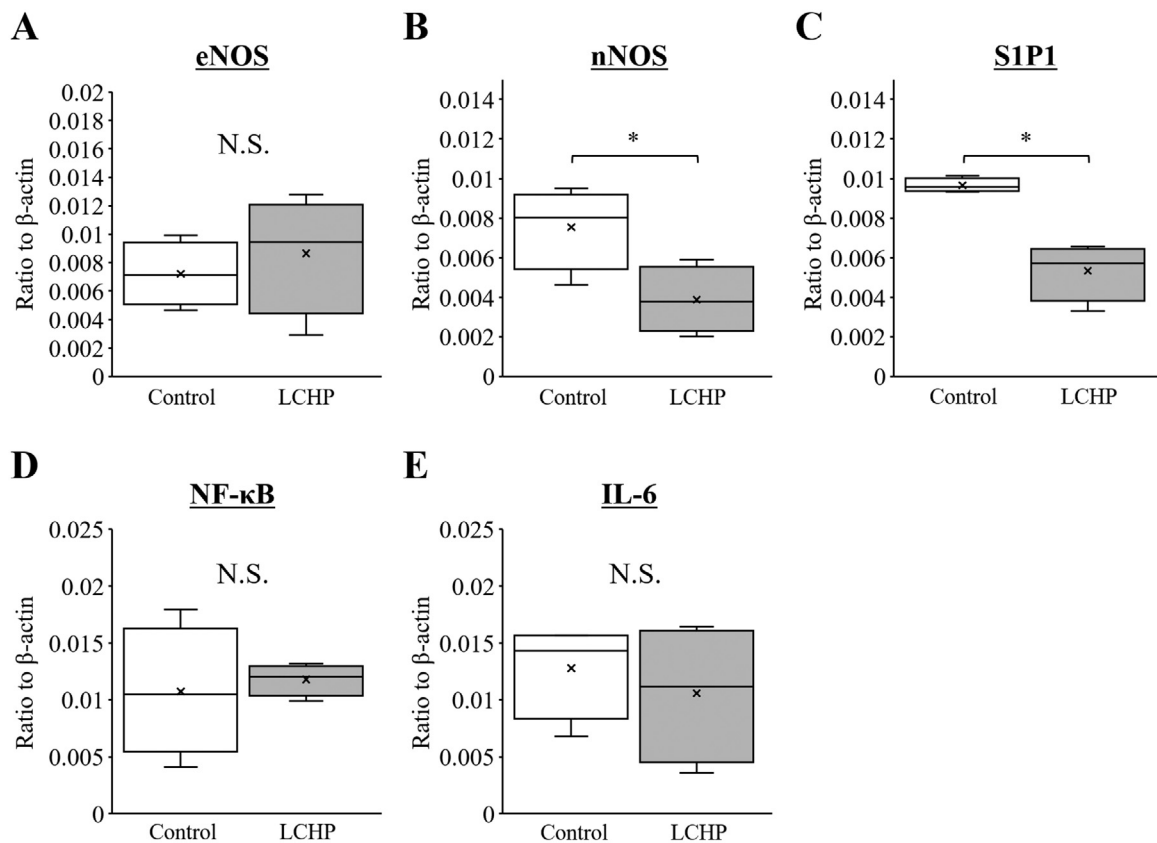
A

eNOS

N.S.

B

nNOS

*

C

S1P1

*

D

NF-κB

N.S.

E

IL-6

N.S.

Figure 5. Expression of mRNA in the corpus cavernosum of rats. Target gene expression was quantified relative to the expression of β-actin using the comparative CT method. Data are presented as box-and-whisker plot (n = 4 per group). *$P < .05$ vs each group using analysis of Welch's t-testing.

clarify the cause of decline in erectile function. Our findings revealed no change in the contractile response due to NA between the 2 groups, suggesting that the cause of the decline in erectile function was not the enhancement of the contractile response of the CC. On the other hand, the relaxation response to ACh was significantly reduced in the LCHP group, while the relaxation response by SNP did not show any difference between the 2 groups, suggesting endothelial dysfunction in CC of rats. A previous study reported that LCHP reduced the responsiveness to ACh in the aorta of mice and caused vascular endothelial dysfunction.[7] Further, it has been reported that an endothelial thioredoxin-interacting protein is involved in vascular dysfunction caused by LCHP.[26] Endothelial thioredoxin-interacting protein has also been reported to be associated with ischemia and diabetes related vascular dysfunction and may be useful as a therapeutic and prophylactic target for ED associated with LCHP.[27-28] In addition, an impairment in the nitrergic relaxation response under the conditions of suramine, atropine, and propranolol incubation indicated a decrease in the relaxation response via Nitric oxide (NO)-operated nerves. From the above results, it became evident that the relaxation response of the CC smooth muscle is not reduced by extreme carbohydrate restriction and high protein in diet, but the reduction in the relaxation response via the endothelial dysfunction and the relaxation response via the NO-operated nerves.

Furthermore, in order to clarify the mechanism of decrease in the CC relaxation response, the mRNA expression level of eNOS and nNOS was measured by real-time PCR. The eNOS mRNA expression level did not show a change between the 2 groups, but the nNOS mRNA expression level decreased in the LCHP group, indicating that the relaxation response mediated by NO-operated nerves decreased. Next, the mRNA expression level of the factor related to S1P (sphingosine-1-phosphate) was measured. The decrease in S1P1 mRNA expression level in the LCHP group suggested that extreme carbohydrate restriction may have reduced S1P1 and suppressed Akt activation, thus damaging nerve cells.[29-31] However, since there is insufficient information to describe the relationship between S1P1 and Akt in this study, it is necessary to conduct genetic studies on Akt such as phosphorylation of Akt protein in the future.

Soy protein is also used in other experiments as a protein source.[32] However, isoflavones contained in soybean are known to have an estrogen-like action.[33] Estrogen itself has been reported to cause ED [34−36]; furthermore, isoflavone administration has been reported to cause ED.[37,38] Therefore, milk casein was used as a protein, which is reported to have pro-inflammatory effect.[39] In this study, pro-inflammatory factors (NF-κB and IL-6) did not change in the LCHP group. It is possible that the amount of milk casein used in this study was not sufficient to

cause an inflammatory reaction in the corpus cavernosum. Further studies on the use of other proteins may be necessary.

The present study suggests that extreme carbohydrate restriction and high protein diet reduces erectile function, but due to the extreme condition of completely replacing carbohydrates with protein, carbohydrate intake will be gradually increased in the future. It is also necessary to consider if returning to a normal diet after a decrease in erectile function due to a carbohydrate-restricted and high protein diet can restore erectile function. This study shows an extreme experimental result of replacing carbohydrate components with proteins. Therefore, it is considered difficult to express the results of this study as they are to be implemented in humans. However, we should be aware that extreme diet habits may affect sexual function.

CONCLUSION

It can be stated on the basis of the findings of the present study that extreme carbohydrate restriction in diet causes reduction in erectile function. The possible causes of this erectile dysfunction may be vascular endothelial dysfunction and a decrease in the relaxation response of the CC smooth muscle via NO-operated nerves.

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Conflict of Interest: The authors report no conflicts of interest.

STATEMENT OF AUTHORSHIP

Conceptualization, T.K. and K.K.; Methodology, T.K. and Y.H.; Investigation, T.K., H.J., J.S., T.M., D.N. and Y.H.; Writing − Original Draft, T.K. and K.K.; Writing − Review & Editing, T.K. and K.K.; Funding Acquisition, T.K. and K.K.; Resources, T.K. and K.K.; Supervision, A.S., Y.M., and Y.F-H.

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