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The Effects of a Raw Diet on Plasma Fasting Glucose Concentration and Immune Function in Streptozotocin-induced Diabetic Rats

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This study was performed to investigate the effect of a raw diet (RD) on blood glucose and immune function in non-diabetic (normal) and streptozotocin (STZ)-induced diabetic rats. Male Sprague-Dawley rats were assigned to four groups (normal control, normal RD, diabetic control and diabetic RD). The control groups and the RD groups were fed an AIN-diet and RD for four weeks, respectively. Weight gain was statistically lower in the RD groups than in the controls. Fasting plasma glucose was significantly lower in the diabetic RD group than in the diabetic control group. The $CD4^+$ T-cell population was higher along with the $CD4^+/CD8^+$ ratio of the mesenteric lymph nodes in the normal RD group compared to the other groups. It can be concluded that RD may reduce the plasma fasting glucose concentration in diabetic rats and improve mesenteric lymph node immune function in normal rats.

Key Words : Raw diet, Plasma glucose, Immune, Diabetes, Rats

INTRODUCTION

The dictionary definition of living on a raw diet is "eating food that is uncooked, which is the opposite of eating a diet of cooked food".[1] A raw diet can also be defined as "a diet consisting of vegetables and excluding animal food which is consumed without heating; a meal prepared without any process thereof and addition thereto that can contain various nutrients and vital substances, such as vitamins, minerals, enzymes, chlorophylls and so on, just as they are, and that has further natural vitality by preventing proteins, fat and the like from being transformed".[2]

In studies on the nutritive conditions of people on a raw diet or vegetable diet, it has been shown that a raw diet is superior to a diet of cooked food in many respects.[1] It has also been shown that if a raw diet is practically and properly followed, it represents the ideal dietary plan. The following useful studies are available: Study on the changes in circulatory function, blood elements and weight of people on a raw diet before and after engaging in exercise,[3] study on the differences in dietary behavior, nutritive conditions and health conditions of people on raw diets versus those that are not,[4] study on the raw diet's potential for preventing cancer,[2] and so on.

In this study, in order to verify the clinical effects of a raw diet, diabetes was induced through an animal experiment and then its effect on improving blood glucose was measured. In addition, this study was conducted to examine whether the intake of a raw diet has a physiological effect on the enhancement of immunity. $CD4^+$ T cells, $CD8^+$ T cells and a ratio of $CD4^+/CD8^+$ in the mesenteric lymph nodes were analyzed and compared.

METHODS

Experimental Animals

The experimental animals were 60 male Sprague-Dawley rats whose weight was 150±10g. Solid feed was supplied to them during a two-week adaptation period. They were divided into 4 groups (15 rats per group) and appropriate feed was supplied to each group (Group 1: Normal control group, Group 2: Normal raw diet (RD) administration group, Group 3: Diabetic control group, Group 4: Diabetic RD group). The chow diet fed to the animals in this experiment was the AIN-93 diet[5] and its ingredients are as shown in Table 1. In order to keep both the food and the powder feed for the raw diet in the same condition, the solid feed for groups 1 and 3 was ground to powder and supplied. For the experimental period, the living conditions for the experimental animals were as follows: temperature: 25±1℃, humidity: 50% or so, lighting: light and darkness was adjusted in a cycle of 12 hours (light 8:00~20:00), an unlimited supply of food and drinking water was available.

Experimental Diet

As described in detail elsewhere,[6] according to a reference,[7] the following grains, fruits, mushrooms, seaweeds, green leaves, herbs, and sugars were selected as

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Table 1. Composition of diets (g/kg)

Ingredients	
Corn Starch	529.486
Casein	200.000
Sucrose	100.000
Soybean oil	70.000
Cellulose	50.000
Mineral mixture[1)]	35.000
Vitamin mixture[2)]	10.000
L-Cystine	3.000
Choline bitartrate	2.500
T-butylhydroquinone	0.014

1) Mineral mixture(per 1kg): Calcium carbonate, 357g; Monopotassium phosphate, 196g; Potassium citrate, 70.78g; Sodium chloride, 74g; Magnesium oxide, 24g; Ferric citrate, 6.06g; Zinc carbonate, 1.65g; Manganous carbonate, 0.63g; Cupric carbonate, 0.30g; Potassium iodate, 0.01g; Ammonium paramolybdate, 0.00785g

2) Vitamin mixture(per 1kg): Nicotinic acid, 3.0g; Ca Pantothenate, 1.6g; Pyridoxine HCl 0.7g; Thiamin HCl, 0.6g; Riboflavin 0.6g; Folic acid, 0.2g; D-Biotin, 0.02g; Vitamin B_{12}, 2.5g; Vitamin E, 15.0g; Vitamin A, 0.8g; Vitamin D_3, 0.25g; Vitamin K, 0.075g; Powdered sucrose, 974.655g

ingredients of the raw diet. Brown rice, barley, green peas, bean, glutinous brown rice, wheat, corn, glutinous millet, sorghum, dried barley sprouts, red beans, sesame, adlay (Job's tears), black bean, black sesame, apple, sea tangle, sweet potato, sweet pumpkin, shiitake mushroom, kale, yam, pine needle, jujube, radish leaves, sea mustard, chestnut, spinach, green tea, Angelica keiskei, bonnet bellflower, rose of Sharon, burdock, carrot, mulberry leaves, laver, arrowroot, Houttuynlae Herba, Saururus chinensis, suki mushroom, licorice root, banana, maesil, Pleuropterus multflorus, Hardy ruber, agaricus, raspberry, and gasiogapi were either freeze-dried or dried for 12 hours at under 50℃. Oligosaccharides, salt, xylitol, spirulina, aloegel, dong-chunghacho, royal jelly, lactic acid bacteria, and propolis were mixed and ground with the ingredients listed above.

Inducement of Diabetes and Identification

Diabetes was induced in group 3 and 4 as follows: The rats bred under the above conditions were left to fast for 24 hours, and then, streptozotocin (STZ) was abdominally injected at 50 mg per 1 kg of weight to achieve a total injection amount of 0.5 ml. STZ was dissolved in the citrate buffer (pH 4.0). It was used within 10 minutes after removal from cold storage because it grows inert rapidly at neutral pH and room temperature. Inducement of diabetes was identified as follows: Blood was collected from a vein when the rats had an empty stomach, 24 hours after STZ had been injected. Then, the blood glucose was measured with a blood glucose measuring kit (Youngdong Diagnostics®) and urine sugar was analyzed and measured with a strip. As a result, when a rat showed 300mg/dl or more, diabetes was considered to have been induced and that rat was used in the experiment. In order to maintain the same conditions in terms of the abdominal administration, a sham injection using saline was given to groups 1 and 2.

Preprocessing for Experimental Groups

After 4 weeks had passed in the experiment, each rat in the experimental groups was supplied with water but no feed for a period of 12 hours. Then, it was lightly anesthetized with ethyl ether and upon cutting open the abdomen, blood was collected from the heart. The collected blood was left at room temperature for about 30 minutes, then plasma was separated from it by centrifuging for 15 minutes at 3,000 rpm.

Measurement of Body Weight, Dietary Intake and Food Efficiency Ratio

Each rat used in the experiment was weighed once per week. The intake of food per day was measured by weighing it at a set time every day. The food efficiency ratio (FER) was calculated by dividing the increased amount of body weight by the intake of food.

$$\text{Food Efficiency Ratio (FER)} = \frac{\text{Weight Gained/Lost (g)}}{\text{The Amount of Food Consumed (g)}}$$

Blood Glucose

The blood glucose, which is used most frequently as an indicator of diabetes, was measured using the glucose oxidase method.

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard Solution}} \times 200$$

Measurement of Immune Cell Population

The mesenteric lymph nodes extracted from a white rat were put into an RPMI 1640 culture solution containing 10% fetal calf serum and homogenized using a 40μm nylon cell strainer (BD Bioscience, USA).[8)] The cell count was then adjusted to 4×10^6 cells/mℓ. The cytosol (250μℓ) was divided and put into Eppendorf tubes so that the final cell count was 1×10^6 cells/mℓ. It was centrifuged at 1000×g for 5 minutes. The cell precipitate was rendered turbid by adding 500μℓ of PBS (pH 7.2) containing 0.1% sodium azide and then centrifuged once again at 100×g for 5 minutes to get the cell precipitate. A total of 50μℓ of the solution prepared by mixing PBS solution containing 0.1% sodium azide 2% FBS with 10μg/mℓ PE (phycoerythrin) labeled anti-rat CD8 antibody (BD Pharmingen, USA) and 10μℓ/mℓ FITC (fluorescein isothiocyanate) labeled anti-rat CD4 antibody (BD Pharmingen, USA) was added to each cell precipitate and pipetted. It was cultured on ice for 30 minutes. 500μℓ of ice cold PBS (pH 7.2) containing 0.1% sodium azide was added and the resulting solution was mixed well. Then, it was centrifuged at 1000×g for 5 minutes. 300μℓ of PBS solution containing 0.1% sodium azide and 1% paraformaldehyde, as a fixing buffer was added to the cell precipitate obtained by removing the supernatant. The solution was mixed well before the immune cell count was measured by observing fluorescence by means of a fluorescence-activated cell sorter

Table 2. Initial and final body weights (g), dietary intake (g/day) and food efficiency ratio (FER)

Group	Initial body weight(g)	Final body weight(g)	Dietary intake(g/day)	FER
Normal control	$205.4\pm8.3^{N.S.}$	337.5 ± 18.4^{a}	17.8 ± 2.4^{a}	0.27 ± 0.19^{a}
Normal RD	203.1 ± 7.3	276.6 ± 15.6^{b}	15.3 ± 1.9^{a}	0.17 ± 0.09^{ab}
Diabetic control	203.8 ± 8.9	187.6 ± 26.7^{c}	28.9 ± 4.2^{b}	-0.02 ± 0.03^{c}
Diabetic RD	204.5 ± 9.1	206.5 ± 23.5^{c}	25.4 ± 3.4^{b}	0.003 ± 0.001^{d}

N.S. : not significant
Values with different superscript within the same column are significantly different at 5% level.

FACScan, Bector Dickinson Co., USA).

RESULTS AND DISCUSSION

Body Weight, Dietary Intake and Food Efficiency Ratio

The weight change of the rats in each group over 4 weeks is shown in Table 2. Over the entire experimental period, the normal control group showed the greatest weight change, while the diabetic control group showed a weight reduction. In terms of final weight, the normal RD group showed a significant difference in comparison with the normal control group. It is thought that in the case of the group in which diabetes was induced, the intake of a raw diet allowed the initial weight to be maintained by alleviating any extreme reductions in weight to some extent.

The results of many past studies show that if diabetes is induced due to hyposecretion of insulin caused by the administration of STZ, an anomaly occurs in the energy metabolism and weight is reduced.[9-13] Furuse et al.[14] and Fisher et al.[15] reported that both growth and weight were reduced in rats in which diabetes was induced by STZ.[14,15] Also, pertinent studies[16,17] reported that any reduction in the weight of rats in which diabetes was induced by STZ was caused by atrophy of the skeletal muscles. This was due to a relative reduction in the maximum surface area of the capillaries where the exchange of matter with solute takes place. Moreover, in the case of reductions in the weight of rats in which diabetes was induced by STZ, the weight was not recovered easily, unlike in cases of reductions in the weight of rats in which diabetes was induced by alloxan.

Dietary intake and food efficiency ratio are also shown in Table 2. Compared with the normal control group, the diabetic groups showed a substantial increase in food intake. It is thought that this was due to polyphagia, a major symptom of diabetes. Meanwhile, in the case of the diabetic control groups, the food efficiency ratio was shown to be a negative numerical value. There was a significant difference in the food efficiency ratio between the normal control group and the diabetic groups. Even though food intake was greater in the diabetic groups than in the normal control group, a continuous reduction in weight was seen. It is thought that this was due to degenerative changes in the internal metabolism caused by diabetes.[18] Thus, there was a significant difference in the food efficiency ratio between the diabetic control group and the diabetic RD group.

Plasma Fasting Blood Glucose

As shown in Figure 1, there was no significant difference in fasting blood glucose between the normal control group and the normal RD group. But, compared with the blood glucose of the normal control group, the blood glucose of the two groups in which diabetes was induced by STZ increased 3.5-fold and 2.6-fold, respectively. In the case of the diabetic groups, administration of the raw diet had a significant effect in reducing blood glucose.

Any increase in blood glucose that can be observed while diabetes is present may be caused by the resistance of internal insulin.[19] It was found that the new synthesis of glucose increased and the utilization of glucose decreased through the inhibited action of pyruvate dehydrogenase.[20-23]

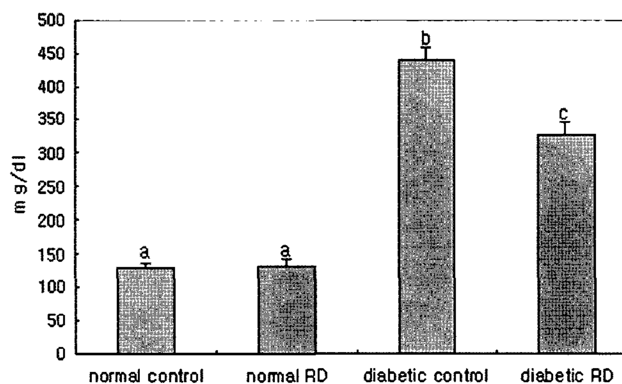

Fig 1. Plasma fasting glucose level of rats fed experimental diets
Results are presented as mean ± standard error.
Different letters above the bar indicate significant differences at $p < 0.05$.

Immune Cell Population

$CD4^{+}$ T-cells of the mesenteric lymph nodes had a tendency to be significantly high in the normal RD group in comparison with those of the other experimental groups, but any effect resulting from the intake of a raw diet was not found in the diabetes-induced group. There was no significant difference in the distribution of $CD8^{+}$ T-cells among all of the experimental groups ($p < 0.05$) (Fig. 2).

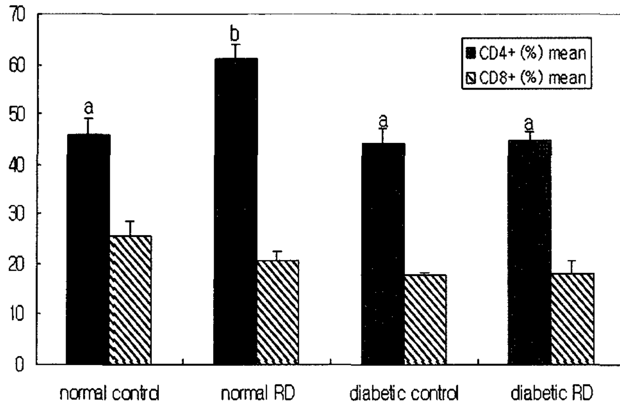

Fig. 2. CD 4⁺ and CD 8⁺ T-cell population in mesenteric lymph nodes of rats fed experimental diets.

Different letters above the bar indicate significant differences at $p < 0.05$. Results are presented as mean ± standard error.

The ratio of $CD4^+/CD8^+$ of the mesenteric lymph nodes showed a significant difference in normal RD in comparison with that of the other experimental groups, but any experimental effect resulting from the intake of a raw diet was not found in white rats suffering from diabetes ($p < 0.05$) (Fig. 3).

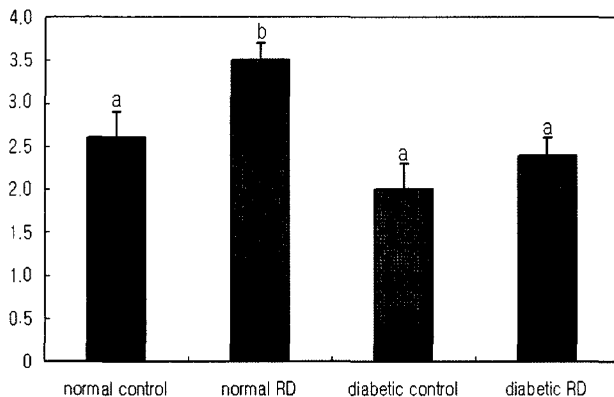

Fig. 3. $CD4^+/CD8^+$ ratio in mesenteric lymph nodes of rats fed experimental diets

Results are presented as mean ± standard error.
Different letters above the bar indicate significant differences at $p < 0.05$

Among T cells in charge of cellular immunologic function, the $CD4^+$ T-cell is a marker of a helper T cell. As a result of the fact that the distribution of $CD4^+$ T-cells increased in a stable condition, it can be interpreted that immunologic function was enhanced. Meanwhile, $CD8^+$ T-cell, as a marker of cytotic T-cells, plays the role of decomposing virus-infected cells and cancer cells. As a result of the fact that distribution of $CD8^+$ T-cells increased in a stable condition, it can be interpreted that immunologic function deteriorated.[24-25] A ratio of $CD4^+/CD8^+$ is used as an important indicator of immunologic function. It is known that $CD8^+$ T-cells promote the growth of T-helper type 1 cells of $CD4^+$ T-cells, while inhibiting the growth of T-helper type 2 cells. Any increase in the ratio of $CD4^+/CD8^+$ means that overall immunologic function was enhanced since there was an increase in the total count of T-helper type 1 cells and T-helper type 2 cells, which are a $CD4^+$ T-cell subset.[26]

The need for $CD4^+$ T-cell help in the $CD8^+$ T-cell response to pathogens and the nature of the help have intrigued immunologists for years. To this day, the question of whether $CD8^+$ T-cell response to many pathogens is dependent or independent of $CD4^+$ T-cell help is a source of controversy. Specifically, in a study,[27] the role of $CD4^+$ T-cell help in primary $CD8^+$ T-cell response was examined, including expansion, contraction and generation of memory.

It has been suggested that expanded antigen-specific $CD8^+$ T-cells may be programmed to subsequently contract, and factors such as a primed $CD4^+$ T-cell help, inflammation and cytokine production may differentially contribute to the regulation of antigen specific $CD8^+$ T-cell contraction.[28] The results of the related study showed the importance of $CD4^+$ T-cell help in regulating the contraction of activated $CD8^+$ T-cells.[27]

Immunological protection depends on both the quality and quantity of memory cells. Specifically, proliferative response of memory $CD8^+$ T-cells to both antigen and homeostatis signals determines the quality of immune protection against many infectious diseases and malignancies. Signals from $CD4^+$ T-cell help, perhaps both directly and indirectly, to mold the functional responsiveness of memory $CD8^+$ T-cells and, in particular, their ability to acquire a high proliferative potential against infection by pathogens. Therefore, it is necessary to implement further studies on the effect of a mixed raw diet on immunological function.

CONCLUSION

In short, it was found that when a raw diet was administered after inducement of diabetes, it had a significant effect in terms of reducing fasting blood glucose concentration in diabetic rats. An examination of the immunologic activity of the mesenteric lymph nodes showed that $CD4^+$ T-cells of the mesenteric lymph nodes had a tendency to be significantly high in the normal RD group compared with the other experimental groups. But, there was no significant difference in distribution of $CD8^+$ T-cells among the experimental groups. The ratio of $CD4^+/CD8^+$ of the mesenteric lymph nodes showed a significant difference in the normal RD group compared to the other experimental groups. Based on the results of this study, it is thought that intake of a raw diet may improve mesenteric lymph node immune function in normal rats.

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