Differences in the Influence of AIN-Purified and Non-purified Diets on the Development of Hypertension in SHR and DOCA-salt Hypertensive Rats

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Summary The influence of non-purified and AIN purified diets on the development of hypertension was examined in deoxycorticosterone acetate (DOCA)-salt hypertensive rats and SHRs. For DOCA-salt hypertensive rats, the development of hypertension was slower in rats fed the AIN 76-purified diet than in those fed the non-purified diet throughout the experimental period of about five weeks. In an experiment using spontaneously hypertensive rats (SHRs), 4-week-old rats were fed either a non-purified diet, a AIN76-purified diet or a AIN93G-purified diet for about nine weeks. In the first 1−2 weeks, the blood pressure was lower in the SHRs fed the AIN93G-purified diet than in those fed the non-purified diet. However, no significant difference in blood pressure was observed within the SHR group thereafter.

Key Words AIN-purified diet, non-purified diet, hypertension, spontaneously hypertensive rats (SHR), DOCA-salt hypertensive rats, blood pressure

Hypertension is an important risk factor in cerebral and cardiovascular diseases. It would therefore be of interest to clarify the dietary factors involved in the regulation of blood pressure. To evaluate factors involved in regulating blood pressure, many experiments have been performed using hypertensive rats such as spontaneously hypertensive rats (SHRs) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats (1−5). Generally, the effects of dietary factors on blood pressure is mild in comparison with those of drugs. Therefore, in designing blood pressure experiments dealing with dietary factors, it is important to select a suitable experimental diet.

Two types of rodent diets, non-purified commercial diet and American Insti-
stitute of Nutrition (AIN)-purified diet, have generally been used. In the case of the non-purified commercial diet, the composition of the ingredients has not been disclosed, and the nutrient composition may fluctuate with the components. This variation in composition may lead to inconsistent results. In addition, it is not easy to add a certain substance to such a commercial diet without changing the percentage of other substances such as protein, lipids, vitamins and minerals. Based on these considerations, it may be better to use the AIN-purified diet in experiments using rats. However, the AIN-purified diet has mainly been designed to promote the growth and reproductive rates in rats (6). It is not actually known whether the AIN-purified diet is suitable for blood pressure experiments or not. For example, the protein source of the AIN-purified diet is casein, which has been shown to have antihypertensive effects (2). In fact, we have observed that the development of hypertension was markedly slower in DOCA-salt hypertensive rats fed an AIN76-purified diet than in rats fed a non-purified diet (3). These findings suggest that the detection of the hypotensive effect of a substance may be more difficult in rats fed the AIN-purified diet than in those fed the non-purified diet, because the AIN-purified diet itself may superimpose its hypotensive effect on that of the substance. Therefore, it might be important to clarify the effects of non-purified and AIN-purified diets on the blood pressure of rats. However, such an experiment has not yet been reported. Recently, the AIN council has improved the AIN76 diet and reported it as AIN93G (for experiments of growth) and AIN93M (for maintenance) diets (7). It has been recommended that the AIN76 diet be replaced by the AIN93 diet. Sucrose and fatty acids at the ratio of n-6/n-3, which have been shown to enhance blood pressure (8, 9), have been decreased in content in the AIN93-purified diet. Therefore, in this study, we examined the influence of the AIN-purified diets and a non-purified diet on the development of hypertension in two types of hypertensive rats (DOCA-salt hypertensive rats and SHRs).

Method

**Diet and animals.** Non-purified commercial rodent diet (CE-2) was supplied by Nippon Formula Feed Mfg. Co., Ltd. (Ibaragi, Japan). AIN76 and AIN93G diets were prepared as recommended by the AIN (7, 10). The diets were in the form of a powder. Table 1 shows the general nutrient composition of the non-purified diet. Table 2 shows the amino acid and mineral contents, in the diets that might influence blood pressure.

In Experiment 1, male Sprague-Dawley rats (about 160 g) purchased from Japan Clea (Tokyo) were uninephrectomized, injected with DOCA (15 mg/kg, s.c., twice a week) and given NaCl to induce DOCA-salt hypertension. The NaCl loading was done by supplementation of 6% NaCl with 2.7% KCl in diets as reported by Bond et al. (4). In Experiment 2, male SHRs (4 weeks old) purchased from Japan Charles River (Kanagawa) were used.

The rats had free access to one of the diets and water throughout the experimental period. Mean blood pressure was measured by the tail-cuff method in
Table 1. General nutrients found in non-purified diets (CE-2).

| Nutrient                         | (g/kg diet) |
|---------------------------------|-------------|
| Water                           | 88          |
| Crude protein                   | 252         |
| Crude fat                       | 44          |
| Crude fiber                     | 44          |
| Total ash                       | 70          |
| Soluble non-nitrogenous matter  | 502         |

Table 2. Analytical values of several elements in experimental diets.

| Mineral            | Purified diet | Non-purified diet |
|--------------------|---------------|-------------------|
|                    | AIN-93G       | AIN-76            | CE-2  |
| Sodium (%)         | 0.114         | 0.113             | 0.295 |
| Calcium (%)        | 0.50          | 0.43              | 1.16  |
| Potassium (%)      | 0.367         | 0.357             | 0.650 |
| Magnesium (%)      | 0.049         | 0.044             | 0.217 |
| Sodium/Potassium   | 0.310         | 0.317             | 0.454 |
| Amino acid         |               |                   |       |
| Cystine (%)        | 0.457         | 0.132             | 0.461 |
| Methionine (%)     | 0.534         | 0.727             | 0.489 |
| Arginine (%)       | 0.629         | 0.599             | 1.401 |

unanesthetized rats.

Statistical analysis. Comparisons between groups were made by the Aspin-Welch test in Experiment 1 and by one-way ANOVA followed by Duncan’s multiple-range test in Experiment 2.

Results and discussion

In Experiment 1, using DOCA-salt hypertensive rats, the development of hypertension in the AIN76-purified diet group was significantly slower than that in the non-purified diet group (Fig. 1). Food intake was not different between the groups, indicating that similar NaCl loading had taken place in the groups. Body weight and relative heart weight did not differ between the groups, but relative kidney weight tended to be higher ($p=0.10$) in the rats fed the non-purified diet (data not shown). The results were consistent with a previous study (3), and indicated that the development of hypertension was markedly attenuated in DOCA-salt hypertensive rats fed the AIN-purified diet.

In Experiment 2, the dietary influence on blood pressure was also examined in SHRs fed either the non-purified diet or one of the purified diets (i.e., AIN76 or AIN93G). As Fig. 2 shows, the blood pressure in the non-purified diet group was significantly higher than that in the AIN93G diet group within the first 1–2 weeks.
Fig. 1. Changes in blood pressure in DOCA-salt hypertensive rats fed either a non-purified diet or AIN76-purified diet (Experiment 1). DOCA-salt hypertension was induced in uninephrectomized rats by injecting DOCA and giving salt (6% NaCl and 2.7% KCl) with the experimental diet. Each point and each vertical bar indicates the M±SEM for 5–7 rats. *Significance p<0.05.

Fig. 2. Changes in blood pressure in SHRs fed either a non-purified diet, AIN76-purified diet or AIN93G-purified diet (Experiment 2). Each point and each vertical bar indicates the M±SEM for 5–7 rats. At a given time point, values not sharing a letter are significantly different (p<0.05).

However, no significant difference in blood pressure was observed among the groups thereafter. The food intake was significantly different among the SHR groups, and was the highest in the non-purified diet group and the lowest in the AIN93G diet group. On the contrary, groups differed in body weight in the order of AIN76 diet group > AIN93G diet group > non-purified diet group. This might
be associated with the lower bioavailability of the nutrients in the non-purified diet \((6)\). Relative heart weight did not differ among the groups. The relative kidney weight in the non-purified diet group was significantly higher than that in the AIN-purified diet groups (data not shown). This indicated that none of the diets (i.e., non-purified diet, AIN76 and AIN93G-purified diets) markedly influenced the development of hypertension in SHRs.

Several differences exist between the non-purified diet and the AIN-purified diets in terms of minerals, proteins, amino acids and lipids which have been reported to influence blood pressure \((1, 2, 8, 9)\). Therefore, in this study, it was difficult to clarify which element in the diet was responsible for the differences in the blood pressure of DOCA-salt hypertensive rats, and the lack of differences in that of SHRs. However, these results may reflect the differences in the mechanisms of the hypertension in DOCA-salt hypertensive rats and SHRs.

In DOCA-salt hypertensive rats, salt loading is absolutely necessary to increase the blood pressure \((11)\). By contrast, SHRs develop high blood pressure without salt loading, although salt loading accelerates the development of hypertension \((12)\). Low protein intake or protein restriction has been reported to attenuate the development of hypertension in DOCA-salt hypertensive rats \((5)\). On the contrary, high protein intake has been shown to attenuate the development of hypertension in SHRs \((13)\). The protein content in the non-purified diet was 25.2\% and that in the AIN-purified diets was 20\%, although the quality of protein between the diets is not comparable. An increase in protein intake in rats has been shown to increase kidney weight \((14)\). The increase in kidney weight observed in DOCA-salt hypertensive rats and SHRs fed the non-purified diet may be related to the high protein intake. These factors may be responsible for the difference in the dietary influence on blood pressure in DOCA-salt hypertensive rats and SHRs.

In conclusion, the present study suggests that the AIN-purified diet attenuates the development of hypertension in DOCA-salt hypertensive rats but not in SHRs, and that there is no marked differences between the influence of AIN76 and AIN93G diets on the development of hypertension in SHRs. These data will hopefully be useful in the elucidation of hypotensive or hypertensive factors in the diet using two types of hypertensive rats.

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