Blood Pressure in Spontaneously Hypertensive Rats Fed Butterfat, Corn Oil, or Fish Oil

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Dietary fats have been shown to influence blood pressure in humans and animal models of hypertension. The ability of a particular fat to modulate arterial pressure appears to depend on its fatty acid profile rather than its degree of saturation or unsaturation. Little is known about the effects of specific dietary fats of animal origin on blood pressure. We tested the concurrent effects of both calcium and dietary fat on blood pressure development in the spontaneously hypertensive rat. Sixty animals were fed diets containing butterfat, fish oil, or corn oil from 3–26 weeks of age. Each diet among the three oils was further modified to contain either 0.25% or 2.0% of the diet as calcium. All six diets provided 18% of the diet (36% of the calories) as fat. The polyunsaturated-to-saturated fat ratio was 0.07, 0.84, and 4.54 for butterfat, fish oil, and corn oil, respectively. Fish oil consumption resulted in lower blood pressures compared with butterfat (p < 0.036) or corn oil (p < 0.0009). Similarly, butterfat feeding resulted in lower blood pressures when compared with corn oil (p < 0.054). Supplementing the diet with calcium decreased blood pressure in both the butterfat and corn oil diets. When butterfat diets were supplemented with calcium, the resulting blood pressures did not differ significantly from those obtained with the two fish oil diets. It is concluded that butterfat, though highly saturated, is associated with less of an increase in the spontaneously hypertensive rat’s blood pressure than is corn oil, which is highly unsaturated. More importantly, when calcium is supplemented, butterfat appears as efficacious as fish oil in lowering blood pressure in the spontaneously hypertensive rat. (Hypertension 1989;14:674–679)

Populations that subsist on vegetarian diets have blood pressures (BPs) that are lower than the general population.1,2 Increased consumption of fiber, other complex carbohydrates, calcium, magnesium, vitamins A and C, along with lower total and saturated fat intake typify a vegetarian diet. These same dietary factors account for 83% of the variation in BP when non-vegetarians are placed on vegetarian diets.3 The prominence of lower dietary fat as one factor that distinguishes the vegetarian from the nonvegetarian diet suggests that a causal relation exists between fat intake and the control of arterial pressure.

This hypothesis has been tested in humans4–10 and in animal models of hypertension.11–22 Studies in humans have suggested that the reduction of total fat intake or increasing the polyunsaturated-to-saturated fat (P/S) ratio decreases blood pressure in normotensive4–6 and hypertensive6,7 subjects. Subsequent clinical trials have failed to confirm these findings in either normotensive6,8 or hypertensive10 humans.

The data gathered in animal experiments indicate that a hypotensive effect of corn oil or safflower oil can be demonstrated in some, but not all, animal models of hypertension. Normotensive rodents made hypertensive with salt loading20–22 or partial nephrectomy19 experience a significant fall in BP with linoleate feeding, as do Dahl salt-sensitive rats.19 Spontaneously hypertensive rats (SHR), on the other hand, show either no reduction in BP11 or experience an acceleration of hypertension12,14,16,17 when linoleic acid is supplied as either corn or safflower oil. These findings strongly suggest that the antihypertensive actions of polyunsaturated fatty acids (PUFA) of vegetable origin are predicated on the type of experimental hypertension. Accordingly, animal models in which the initiation (but not necessarily the maintenance) of hypertension depends on a primary dysfunction of the kidney appear more responsive than SHR to PUFA of vegetable origin. Fatty acids originating from marine oils, in contrast,
show a very consistent depressor effect in both animals23-28 and humans29-35 irrespective of the type of hypertension.

Few studies have directly compared the effects of specific fats on BP. In animal studies, PUFA of vegetable origin are generally compared with saturated fats also derived from plants (hydrogenated coconut or palm oil). Consequently, the BP effects of saturated fats derived from animal sources are largely unknown. Data from our laboratory indicate that in the SHR a diet that contains 40% of the energy as fish oil lowers BP when compared with a similar quantity of corn oil. 17 In these experiments, an additive antihypertensive effect was also observed when additional calcium was provided in these diets.

The purpose of the current study was to compare the effect of corn oil, butterfat, and fish oil on BP development in the SHR. Furthermore, concurrent effects of modifying dietary calcium intake were assessed since this nutrient is known to influence BP development in the SHR. In addition, concurrent effects of modifying dietary calcium intake were assessed since this nutrient is known to influence BP development in the SHR. In all, 15 BP measurements were obtained throughout the study.

Materials and Methods

Sixty male weanling SHR were obtained from Charles River Breeding Laboratories (Boston, Massachusetts) at 21 days of age. On arrival, the rats were randomly assigned to diets of the composition shown in Table 1. Rats were housed in groups of 2-3 in stainless steel wire cages kept under constant conditions of humidity (50-55%), temperature (25-27 °C), and lighting (11:13-hour light/dark cycle).

Powdered diets were prepared as needed and fed ad libitum for a period of 23 weeks. All diets contained 18 g total fat, an amount that supplies 36% of the energy as fat. The P/S ratios were 0.07, 0.84, and 4.54 for butterfat, fish oil, and corn oil, respectively. The fatty acid profile of the three fats used in the study are shown in Table 2. Menhaden fish oil was donated by Dr. A. Bimbo (Zapata Hynie Co., Reedsville, Virginia). The oil was stored frozen, as were the fish oil diets, to prevent autoxidation. Unsalted, nonfortified butter used in the butterfat diets was purchased from a local dairy, and corn oil was purchased from a local supermarket. The butterfat and corn oil diets were refrigerated after mixing. Each diet containing the three oils was further modified to contain either a low (↓) concentration of Ca2+ at a level of 0.25% or a high (↑) Ca2+ concentration of 2.0%, provided as calcium carbonate for a total of six diets.

Indirect systolic BPs were measured by a pneumatic pulse transducer (Narco Biosystems, Houston, Texas) in prewarmed restrained rats. Five readings were obtained from each rat and averaged. If the coefficient of variation exceeded 10% for any one set of measurements, those pressures were discarded and new measures were obtained on the following day. BP was evaluated on a weekly basis for the first 12 weeks of age, and then once every 2 weeks from 14–26 weeks of age. In all, 15 BP measurements were obtained throughout the study.

Data were analyzed with repeated-measures analysis of variance (ANOVA) for body weight and BP. When the results of ANOVA were significant, Bonferroni-adjusted pairwise contrasts were used to compare the diets with one another. Data are presented as mean±SD.

Results

Table 3 displays patterns of growth for each of the six diets. Repeated-measures ANOVA indi-
The effect of the three fats (averaged over the calcium levels) on BP is shown in Figure 2. Multivariate analysis revealed that fish oil yielded arterial pressures that were significantly lower than those of either butterfat ($p<0.036$) or corn oil ($p<0.0009$). Similarly, butterfat displayed BPs that were significantly lower than corn oil ($p<0.054$). Overall then, fish oil was associated with the lowest pressor response and corn oil was associated with the highest pressor response of the three dietary fats.

Table 3 represents BP development as a function of dietary calcium (averaged over the three dietary fats). Feeding rats a 2.0% Ca$^{2+}$ diet significantly ($p<0.0009$) attenuated the development of hypertension compared with feeding 0.25% of the diet as calcium irrespective of the type of dietary fat. Supplemental calcium was associated with a reduction in both BP and weight between 3 and 12 weeks of age. To determine the extent to which weight rather than dietary calcium contributed to this attenuation in BP between 3 and 12 weeks of age, actual BPs were regressed against weight residuals after factoring out the effects of diet on weight. All correlations obtained in this manner revealed no significant relation between BP and weight responses. Thus, the attenuation in BP with additional calcium was independent of its effect on weight.

To gain insight into how each of the six diets compared with one another with regard to their effects on BP, absolute mean differences were calculated using Tukey's pairwise comparisons. Matrices of comparison probabilities indicated that the three least hypertensive diets were FO $\uparrow$Ca$^{2+}$,

| Table 3. Growth Patterns Among the Six Diets |
|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age (wk)          | BF $\uparrow$Ca$^{2+}$ | BF $\uparrow$Ca$^{2+}$ | CO $\uparrow$Ca$^{2+}$ | CO $\uparrow$Ca$^{2+}$ | FO $\uparrow$Ca$^{2+}$ | FO $\uparrow$Ca$^{2+}$ |
| 3                | 38±9             | 38±8             | 46±17             | 47±13             | 44±6             | 41±7             |
| 5                | 96±14            | 92±15            | 92±10             | 88±7              | 92±13            | 83±11            |
| 7                | 159±19           | 149±24           | 154±13            | 143±15            | 157±22           | 142±15           |
| 9                | 223±20           | 210±22           | 206±27            | 197±29            | 218±24           | 198±18           |
| 11               | 270±16           | 242±22           | 252±19            | 250±13            | 257±23           | 244±13           |
| 13               | 296±29           | 270±41           | 292±36            | 276±10            | 283±30           | 278±36           |
| 15               | 317±29           | 287±40           | 300±27            | 303±20            | 299±30           | 298±23           |
| 17               | 337±32           | 322±30           | 315±28            | 327±17            | 315±28           | 316±33           |
| 19               | 348±24           | 327±22           | 335±29            | 342±17            | 328±16           | 331±24           |
| 21               | 346±27           | 333±25           | 341±33            | 347±19            | 340±18           | 348±36           |
| 23               | 372±30           | 361±25           | 354±29            | 369±13            | 358±19           | 365±34           |
| 25               | 380±33           | 357±22           | 366±25            | 372±12            | 358±16           | 370±35           |

Values are weight in grams±SD for 8-10 rats/diet. BF, butterfat; $\uparrow$Ca$^{2+}$, 0.25%; $\uparrow$Ca$^{2+}$, 2.0%; CO, corn oil; FO, fish oil.

| Table 4. Blood Pressure Development for Each Diet |
|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age (wk)          | BF $\uparrow$Ca$^{2+}$ | BF $\uparrow$Ca$^{2+}$ | CO $\uparrow$Ca$^{2+}$ | CO $\uparrow$Ca$^{2+}$ | FO $\uparrow$Ca$^{2+}$ | FO $\uparrow$Ca$^{2+}$ |
| 6                | 131±16           | 122±11           | 136±12           | 122±11           | 126±14           | 123±18           |
| 8                | 167±23           | 170±16           | 167±18           | 160±18           | 158±20           | 147±24           |
| 10               | 173±9            | 169±10           | 181±14           | 178±10           | 171±10           | 164±13           |
| 12               | 193±18           | 177±19           | 198±12           | 186±10           | 182±17           | 173±25           |
| 14               | 192±13           | 174±11           | 204±21           | 191±11           | 176±14           | 163±11           |
| 16               | 194±13           | 177±11           | 211±11           | 188±14           | 185±16           | 173±21           |
| 18               | 201±15           | 176±19           | 221±14           | 193±19           | 181±13           | 180±23           |
| 20               | 196±13           | 180±19           | 212±11           | 196±15           | 188±12           | 165±10           |
| 22               | 193±9            | 179±11           | 218±6            | 196±13           | 190±8            | 168±13           |
| 24               | 195±15           | 181±18           | 217±16           | 199±10           | 191±15           | 171±11           |
| 26               | 198±15           | 182±11           | 218±19           | 202±19           | 191±16           | 174±19           |

Values are systolic blood pressure (mm Hg)±SD for 8-10 rats/diet. BF, butterfat; $\uparrow$Ca$^{2+}$, 0.25%; $\uparrow$Ca$^{2+}$, 2.0%; CO, corn oil; FO, fish oil.
FIGURE 1. Line graph showing time course of blood pressure (BP) development for each of the six diets. Δ, CO/0.25% Ca^{2+}; ○, BF/0.25% Ca^{2+}; △, FO/0.25% Ca^{2+}; ▲, CO/2.0% Ca^{2+}; ●, BF/2.0% Ca^{2+}; ■, FO/2.0% Ca^{2+}. CO, corn oil; BF, butterfat; FO, fish oil.

**Discussion**

The present study compares the effect of milkfat on BP development to that of corn oil and menhaden oil. To our knowledge, this study constitutes one of the first reports comparing the effects of a saturated fat of animal origin on BP in the SHR with that of other fats. Based on our findings, the efficacy of attenuating BP development in the SHR appears to be: fish oil > butterfat > corn oil.

The finding that fish oil diets are associated with lower BP is consistent with other reports in the literature gathered in laboratory models of hypertension and in humans. Similarly, a number of studies have shown that in the SHR, linoleate supplied as corn or safflower oil either has no effect on BP or produces an outright pressor response when compared with other vegetable fats, a finding that is confirmed in this study.

The finding that a diet whose fat source is milkfat produces BPs that are lower than those of a diet containing a highly unsaturated fat such as corn oil was not expected. This outcome is in direct contrast to the view that diets that have a low P/S ratio are associated with higher BP in humans. It also contradicts data gathered in sodium chloride-sensitive or renal models of hypertension that indicate that saturated fats of vegetable origin such as palm and coconut oil are hypertensive.

The difference in BP responses to butterfat compared with other saturated fats is not immediately clear. It may be that unlike the salt or renal models the SHR responds differently to these fats because of its specific type of hypertension. Alternatively, the difference may lie in the differing composition of milkfat compared with other saturated fats. As can be seen in Table 2, approximately 23% of all the saturated fat in milkfat is in the form of short chain fatty acids (12 carbons or less). This differs from saturated fats of vegetable origin that contain mainly C12–C18 saturated fatty acids. In addition, butterfat contains a small amount (≈2%) of linolenic acid that is an n-3 fatty acid also found in fish oil but not in saturated fatty acids from vegetable sources. Whether these differences explain the variations in BP responses reported here remains unclear. Nevertheless, it is clear that all saturated fats do not behave equally with respect to BP regulation in the SHR. This is also true of polyunsaturated fats.

Sacks et al compared the short-term effects of safflower oil with butterfat (81 g cream/day) in hypertensive subjects. They reported no difference in BP between the two sources of fat in their subjects after 6 weeks of supplementation. Those findings combined with the findings reported here for the SHR appear to suggest that butterfat, a
saturated fat of animal origin, is no more deleterious in terms of BP control than a polyunsaturated fat.

The antihypertensive effect of supplemental dietary calcium is now well documented in the literature.\(^6\) It has also been demonstrated that supplemental calcium can reduce the severity of hypertension associated with a corn oil–rich diet in SHR.\(^7\) Both these findings were confirmed in this study. In addition, supplemental calcium decreased BP in butterfat-fed rats to a level that did not differ significantly from that observed in rats fed fish oil (Figure 1 and Table 4). This synergy is noteworthy since foods that contain milkfat are also the principal sources of calcium in the human diet.

In summary, the effect of butterfat on BP was compared with a highly unsaturated fat of vegetable origin and to a marine oil of intermediate saturation. Fish oil–enriched diets were associated with lower BP, whereas vegetable oil–rich diets were associated with higher BP. The effect of butterfat on BP was intermediate between corn and fish oil. When additional calcium was provided in the diets, the high calcium/butterfat diet did not differ from either of the fish oil diets in terms of BP. Additional dietary calcium also attenuated the BP increase observed with the corn oil diets. These results indicate that the action of dietary fats on BP is not wholly dependent on the P/S ratio. Thus, although the P/S ratio may still be important, the overall fatty acid profile of a particular fat including carbon chain length and the positioning of the initial double bond collectively determines pressor responses to a specific fat. Modification of the human diet for purposes of reducing cardiovascular risk usually includes recommendations to increase the P/S ratio. In light of the current findings, this recommendation may ultimately need to be reexamined if future research demonstrates that the BP responses in humans to various sources of dietary fat are the same as those reported here for the SHR. Finally, our results have implications for laboratory studies involving experimental models of hypertension. They suggest that fat composition of the diet needs to be defined and controlled to properly interpret BP responses in dietary experiments.

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References


**Key Words** • dietary fats • calcium • blood pressure • spontaneously hypertensive rats
Blood pressure in spontaneously hypertensive rats fed butterfat, corn oil, or fish oil.

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