Tissue Antioxidant Status Differs in Spontaneously Hypertensive Rats Fed Fish Protein or Casein

ABSTRACT The present study was designed to determine whether changes in dietary protein source are related to changes in antioxidant status determined by enzyme activities of catalase, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSH-Red) and lipid peroxidation levels in various tissues. Spontaneously hypertensive rats (SHR; 5 wk old) were fed diets containing 20% casein or fish protein for 2 mo. The fish protein diet lowered blood pressure and reduced plasma total cholesterol levels and SOD activity in all tissues except muscle compared with the casein diet. Feeding fish protein also enhanced GSH level and GSH-Px activity in liver and heart, accompanied by lower lipid peroxidation. In kidney, however, the lower catalase activity in rats fed fish protein was associated with an enhancement in lipid peroxidation. Plasma and VLDL + LDL lipid peroxidation was unaffected by dietary proteins. In conclusion, the fish protein diet did not play a relevant role in plasma antioxidative defense status but increased it in liver and heart compared with the casein diet. Fish protein attenuated the development of hypertension and also decreased plasma total cholesterol concentration. Thus, it enhances protection against cardiovascular diseases.

KEY WORDS: spontaneously hypertensive rats • fish protein • antioxidant enzymes • lipid peroxidation

Numerous reports have indicated that essential hypertension is associated with greater than normal lipoperoxidation and an imbalanced antioxidant status, suggesting that oxidative stress is important in essential hypertension (1,2). In spontaneously hypertensive rats (SHR), hypertension is associated with alterations in heart and RBC antioxidant enzymes accompanied by an increase in susceptibility to in vitro lipid peroxidation (3).

Nutritional antioxidants play an important role in cellular antioxidative defense mechanisms (4); vitamins E and C, β-carotenes, selenium, copper and zinc are the major dietary factors with the ability to act as antioxidants (5). In addition, antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSH-Red) act to protect tissues from oxidative injury generated by oxygen free radicals [e.g., superoxide anion (O₂⁻), hydroxyl radical (•OH) and hydrogen peroxide (H₂O₂)] (6,7).

Dietary protein may also influence lipid peroxidation and the activity of antioxidant enzymes. Chiang and Kimura (8) reported that the concentration of liver GSH, GSH-Px activity and liver and testis lipid peroxide concentrations are significantly lower in stroke-prone SHR fed soybean protein than in those fed casein, although soybean protein is not effective against hypertension. In contrast, Nevala et al. (9) reported diminished blood pressure by feeding soybean protein compared with casein in SHR. Interest in the role of fish and its constituents on hypertension and antioxidant status arose from the lower incidence of cardiovascular disease in populations consuming large amounts of fish. These effects have always been attributed to the presence of fish oils, because (n-3) fatty acids have been shown to be responsible for the cardiovascular protection. Only a few studies have directly addressed the role of fish protein in the regulation of blood pressure; however, no information is available regarding the effects of dietary fish protein on antioxidant status in SHR.

Therefore, in this study, the influence of a fish-protein diet on the blood pressure of SHR compared with a casein diet was evaluated from 6 to 14 wk. In this strain, blood pressure increases up to 3 mo of age and then remains stable. At the end of the 2-mo dietary experiment, lipid peroxidation, total glutathione contents and the activities of CAT, GSH-Px, GSSH Red and SOD in liver, heart, kidney, muscle and adipocyte tissue were studied.

MATERIALS AND METHODS

Animals and diets. Male SHR (n = 20; 4 wk old) rats were purchased from Ifa-Credo, (l’Arbresle, France). They were housed at 24°C, with constant humidity (60%) and a 12-h light:dark cycle. Rats had free access to a standard commercial rat diet for 6 d. They were then randomly divided into two groups of 10 rats fed for 2 mo a diet containing (g/kg diet): casein, 200 (95% purity) or fish protein [94% purity, provided by SEAH International (Vimille, France)] as protein source, combined with Isio 4 oil, 50; sucrose, 50; cornstarch, 590; cellulose, 50; vitamin, 20; mineral 40. The composition of mineral, vitamin and Isio 4 was previously reported by Frenoux et al. (10). Diets were isonenergetic (16.28 MJ/kg). Fish protein and casein were

peroxidase; GSSH-Red, glutathione reductase; HMG-CoA, hydroxy-3-methylglutaryl CoA; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances.
almost lipid free (0.05% fatty acids). The amino acid composition of both proteins was determined after separation by HPLC in the Biochemistry Laboratory from the Central Hospital of Nancy, France by F. Nabet and P. Nabet and is expressed as g/kg protein (fish protein vs. casein: Ala, 69 vs. 30; Arg, 63 vs. 36; Asp, 80 vs. 66; Gln, 130 vs.207; Gly, 123 vs. 18, His, 14 vs. 24; Ile, 28 vs. 42; Leu, 55 vs. 92; Lys, 70 vs. 74; Met + Cys, 37 vs. 26; Phe + Tyr, 63 vs. 62; Pro, 58 vs. 116; Ser, 53 vs. 55; Thr, 41 vs. 46; Val, 39 vs. 57; Lys/Arg, 1.07 vs. 2.05). Diets and water were freely available. Animals were weighed weekly. We followed the general guidelines for the care and use of laboratory animals as recommended by the Council of European Communities (11). Systolic blood pressure was measured weekly (10, 12).

**Blood and tissue isolation, and lipid determination.** After the 8-wk dietary period, rats were food deprived for 12 h and anesthetized with sodium pentobarbital. Blood was collected from the abdominal aorta (10) and plasma was prepared by low speed centrifugation (1000 x g for 20 min) (10). Liver, heart, kidney, gastrocnemius muscle and adipose tissues were removed immediately, rinsed with cold saline, weighed and then were cut into 50- to 100-mg portions, frozen in liquid nitrogen and stored at -80°C for later estimation of protein content, enzymatic antioxidant defense and lipid peroxidation. Liver lipids were extracted according to the method of Folch et al. (13). All samples were assayed within 20 d of collection. Total cholesterol in plasma and liver and plasma triglyceride and phospholipid concentrations were determined with Boehringer enzyme Kits (Boehringer, Meylan, France). Proteins were estimated according to the method of Schacterle and Pollack (14).

**Antioxidant enzymes.** CAT (EC 1.11.1.6) activity was measured according to Aebi (15); GSH-Px (EC 1.11.1.9) was assessed by the method of Paglia and Valentine (16); GSSG-Red (EC 1.6.4.2) activity was determined by the method of Goldberg & Spooner (17); and SOD (EC 1.15.1.1) activity was measured by the NADH oxidation procedure (18). Total glutathione was determined according to the procedure of Anderson (19). Free radical damage was determined by specifically measuring thiobarbituric acid-reactive substances (TBARS), as described by Quinlanilha et al. (20).

The total antioxidant status was defined as the capacity of RBC to withstand free radical–induced hemolysis (21) and was measured as described previously (22).

**Lipid peroxidation.** The VLDL + LDL fraction obtained by precipitation with dextran (23) was used for this study and subjected to Cu**2+** oxidation (24). TBARS production was measured as nmol TBARS produced/mg VLDL + LDL protein in 24 h.

**Statistics.** Data are reported as means ± SEM, n = 10. Statistical analysis of the data was conducted using STATISTICA (version 4.1, Statsoft, Tulsa, OK). The significance of differences in response to diet was analyzed statistically by ANOVA. A difference of P < 0.05 was considered significant.

**RESULTS**

**Weights, food intake, blood pressure and lipid concentrations.** SHR fed fish protein exhibited lower body weight and liver weights but similar food intake compared with SHR fed casein (Table 1). Heart and kidney weights were not significantly different between the two groups. Systolic blood pressure was significantly lower in the group fed fish protein compared with the group fed casein. Moreover, feeding fish protein significantly decreased plasma and liver total cholesterol concentrations, but did not affect plasma triacylglycerol and phospholipid levels.

**GSH and TBARS concentrations.** Compared with casein, TBARS concentrations in liver and heart were markedly decreased with the fish protein diet (~40% and ~48%), but were 54% greater in kidney (Table 2). There were no significant differences in TBARS concentrations in muscle and adipose tissue. Feeding fish protein compared with casein enhanced liver and heart GSH concentrations without affecting those of kidney, muscle and adipose tissue.

**Activities of antioxidant enzymes.** SOD activity was significantly lower in the group fed fish protein compared with casein, in all tissues studied except muscle (~39, ~59, ~70 and ~48% for liver, heart, kidney and adipose tissue, respectively) (Table 3). Feeding fish protein also diminished muscle GSH-Red activity and kidney CAT activity. Compared with casein, fish protein diminished GSH-Px activity in adipose tissue, but increased it in liver, heart and kidney.

**Plasma and VLDL + LDL TBARS.** Plasma TBARS concentrations, plasma total antioxidant status (expressed as T50% of hemolysis) and lipid peroxidation of VLDL + LDL (expressed as TBARS production for 24 h) did not differ among rats fed the fish protein and casein diets (44.6 ± 34.0 vs. 36.1 ± 17.0; 67.8 ± 1.0 vs. 65.3 ± 1.1; 1.92 ± 0.92 vs. 2.29 ± 1.75, respectively).

**DISCUSSION**

Clinical and epidemiologic studies (25, 26) have shown the cardiovascular protective effects of fish oils. These substances have been reported to lower blood pressure and prevent the development of hypertension (26, 27), which is one of the most critical factors involved in cardiovascular pathologies such as atherosclerosis or stroke. No information is available concerning the antioxidant or prooxidant effects of fish proteins. Thus, if these proteins are effective in lowering blood pressure, they might play an important role in preventing cardiovascular diseases. Indeed, the aim of the present study was to investigate on the one hand, the effects of fish protein and casein on blood pressure and on the other hand, their effects on antioxidant status. Our results demonstrate that the fish protein diet lowered blood pressure significantly (~14%) compared with the casein diet. This effect was reported previously by Yamori et al. (29, 30) in stroke-prone SHR. Lowered blood pressure was also observed when a high fish protein diet was fed to normotensive rats in which hypertension was induced by NG-nitro-L-arginine (31). This protein-dependent difference in blood pressure could be attributed in part to the difference in the amino acid composition. Fish protein contains more arginine than casein. Nitric oxide, the metabolic product of arginine by the enzyme nitric oxide synthase, was shown to play a pivotal role as vasorelaxant and lower blood pressure (32). Soybean protein also contains a higher arginine

**TABLE 1**

<table>
<thead>
<tr>
<th>Diets</th>
<th>Casein</th>
<th>Fish protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>332.8 ± 12.1</td>
<td>291.8 ± 26.4*</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>17.11 ± 1.25</td>
<td>16.33 ± 0.94</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>220.7 ± 8.7</td>
<td>189.8 ± 10.5*</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>10.08 ± 0.58</td>
<td>7.81 ± 1.19*</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.48 ± 0.13</td>
<td>1.42 ± 0.11</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>2.47 ± 0.18</td>
<td>2.13 ± 0.28</td>
</tr>
<tr>
<td>Liver cholesterol, mmol/g</td>
<td>21.66 ± 5.05</td>
<td>12.62 ± 3.42*</td>
</tr>
<tr>
<td>Plasma total cholesterol, mmol/L</td>
<td>1.56 ± 0.18</td>
<td>1.24 ± 0.42*</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/L</td>
<td>0.39 ± 0.12</td>
<td>0.39 ± 0.15</td>
</tr>
<tr>
<td>Plasma phospholipids, mmol/L</td>
<td>2.79 ± 0.46</td>
<td>2.57 ± 0.68</td>
</tr>
</tbody>
</table>

1 Values represent means ± SEM, n = 10. 2* Different from casein, *P < 0.05.
level than casein and has been shown to have an antihypertensive effect in SHR (9). Although food intake was similar, rats fed the fish protein diet exhibited significantly lower body weight and liver weights compared with those fed the casein diet. These results suggest the development of visceral obesity and peripheral insulin resistance in the rats fed the casein diet. An increased availability of free fatty acids was also present in obesity-associated insulin resistance, and therefore the beneficial effects of fish protein are likely to reflect better insulin sensitivity caused by a lower weight. Indeed, Lavigne et al. (33) reported in rats that feeding fish protein or soy protein, compared with casein, improves glucose tolerance and insulin sensitivity.

The fish protein diet produced significantly lower plasma cholesterol concentrations (−20%) than the casein diet. Similar findings were reported in normal Wistar rats (34). In rabbits, the fish protein diet, like the soybean protein diet, lowers plasma cholesterol level and causes fewer atherosclerotic lesions compared with rabbits fed the casein diet (35). The lowered plasma cholesterol concentration obtained in the fish protein–fed group could be related to the lowered cholesterol synthesis via diminished 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity, the rate-limiting enzyme in the biosynthesis of cholesterol. Indeed, rats consuming fish protein had lower cholesterol concentrations in their livers compared with rats fed the casein diet (Table 1). A similar finding was reported in normal Wistar rats (34). Fish protein contains almost twofold greater levels of arginine than casein. Feeding fish protein might raise plasma arginine level, and subsequently the glucagon level (36). Glucagon inhibits the activity of HMG-CoA reductase (37). Moreover, fish protein contains more sulfur amino acids (methionine and cystine) than casein. Diets supplemented in methionine and cystine have been shown to reduce plasma cholesterol concentrations in rats (38).

Several reports in experimental animals and humans have shown that oxygen radical production is enhanced by hyper-

tension (39–41). Little is known, however, regarding the influence of dietary fish protein on antioxidant status in SHR. Our findings show that rats fed fish protein exhibited lower SOD activity in all tissues, except for muscle. Free radicals are the source of lipid peroxidation derived from oxygen, and the first line of defense against them is SOD. Hence, the lower SOD activity in rats fed fish protein, compared with casein, may result from lower quantities of \( \text{O}_2^- \) present in tissues. Indeed, lipid peroxidation, expressed as nmol TBARS/g tissue, was significantly lower in liver and heart of rats fed fish protein compared with casein. Moreover, the GSH level, an important antioxidant factor (42), was increased in liver and heart of SHR fed the fish protein diet. This might account for the high GSH-Px activity observed in liver and heart and the low TBARS level found in this group because GSH acts as a substrate for GSH-Px (41). In kidney, however, enhanced lipid peroxidation in rats fed fish protein compared with those fed casein (Table 2), may be due to lower SOD and CAT activities (Table 3). Impairment in the ability to detoxify \( \text{H}_2\text{O}_2 \) via CAT in this tissue appears to be responsible for the enhanced TBARS concentrations. Whatever the mechanism involved, the effect is specific to fish protein because kidney showed a significant decrease only in CAT activity and this was associated with enhanced TBARS concentrations. Moreover, the total antioxidant status, plasma total antioxidant capability and VLDL + LDL resistance to copper-induced lipid peroxidation did not differ between diets. Enhanced oxidation of LDL is one of the critical mechanisms involved in the progression of atherosclerosis. Taken together, these find-

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>GSH, nmol/g tissue</th>
<th>TBARS, nmol/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>6.34 ± 1.8</td>
<td>7.96 ± 0.8*</td>
</tr>
<tr>
<td>Heart</td>
<td>0.79 ± 0.26</td>
<td>1.22 ± 0.34*</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.58 ± 0.28</td>
<td>0.72 ± 0.33</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.95 ± 0.38</td>
<td>3.01 ± 0.33*</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>0.56 ± 0.32</td>
<td>0.74 ± 0.56</td>
</tr>
</tbody>
</table>

1 Values represent means ± SEM for 10 rats per group.

2* \( P < 0.05 \), fish protein vs. casein.

3 Abbreviations used: TBARS, thiobarbituric acid reactive substances; GSH, glutathione.

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Catalase, U/mg protein</th>
<th>GSH-Px, U/mg protein</th>
<th>GSSH-Red, U/mg protein</th>
<th>SOD, U/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>3.64 ± 1.21</td>
<td>4.36 ± 2.03</td>
<td>2.92 ± 1.36</td>
<td>142.7 ± 37.0</td>
</tr>
<tr>
<td>Heart</td>
<td>6.75 ± 1.08</td>
<td>7.57 ± 1.33</td>
<td>4.19 ± 0.88</td>
<td>68.2 ± 14.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.68 ± 0.33</td>
<td>1.99 ± 0.28</td>
<td>1.36 ± 0.22</td>
<td>176.6 ± 21.3</td>
</tr>
</tbody>
</table>

1 Values represent means ± SEM for 10 rats per group.

2* \( P < 0.05 \); fish protein vs. casein.
ings suggest that fish protein does not play an important role in the antioxidative defense status of plasma, but increases it in liver and heart.

In conclusion, fish protein attenuates the development of hypertension and also decreases plasma total cholesterol concentrations compared with casein in SHR. Similar findings were reported for fish oil diets (10). Thus, extrapolating to humans would indicate that fish consumption might be beneficial for patients with hypertension. Nutritional recommendations for the prevention of cardiovascular diseases call for increased fish in the diet.

ACKNOWLEDGMENT
The authors thank Anne Magnet, an ESP linguist at the University of Burgundy, for editing the manuscript.

LITERATURE CITED