Serum Aminopeptidase A Activity of Mice Is Related to Dietary Fat Saturation

Garbine Arechaga; Jose M Martinez; Isabel Prieto; Maria J Ramirez; et al
The Journal of Nutrition; Apr 2001; 131, 4; Health & Medical Complete
pg. 1177

Biochemical and Molecular Action of Nutrients
Research Communication

Serum Aminopeptidase A Activity of Mice is Related to Dietary Fat Saturation

Garbine Arechaga, José M. Martínez, Isabel Prieto, María J. Ramírez, María J. Sánchez, Francisco Alba,* Marc De Gasparo† and Manuel Ramírez†

Unit of Physiology, University of Jaén, 23071 Jaén, Spain; *Department of Biochemistry and Molecular Biology, Medical School, University of Granada, Granada, Spain; and †Novartis Pharma, CH-4002, Basel, Switzerland

ABSTRACT A high intake of monounsaturated fat has been proposed to be a dietary factor that can decrease the incidence of cardiovascular disease and hypertension. In addition, increasing dietary fat saturation has been shown to increase plasma total cholesterol and elevate systolic and diastolic blood pressures. We demonstrated previously that cholesterol selectively increases in vitro aminopeptidase A activity, which is related to angiotensin metabolism. In this study, we investigated the effect of different degrees of dietary fatty acid saturation on serum aminopeptidase activities in vivo. Serum total cholesterol concentrations were also measured. Five groups of male Balb/C mice were fed for 10 wk diets containing 2.4 g/100 g of sunflower oil, fish oil, olive oil, lard or coconut oil. We measured alanil-, arginyl-, cysteinyl-, pyroglutamyl-, aspartyl- and glutamyl-specific aminopeptidase activities using arylamides as substrates. Serum total cholesterol levels were higher in mice fed diets containing saturated oils (lard and coconut) than in those consuming sunflower oil, which is unsaturated. Two of the serum aminopeptidase A activities (aspartyl and glutamyl aminopeptidase) increased progressively with the degree of saturation of the dietary fatty acids; activities were significantly greater in mice fed coconut oil than in those fed sunflower or fish oil. Therefore, the substrates hydrolyzed by this activity as well as their functions may be similarly affected. These results may have some implication for the treatment of cardiovascular disease. J. Nutr. 131: 1177–1179, 2001.

KEY WORDS: aminopeptidases • dietary fat saturation • cholesterol • mice

Aminopeptidases (AP)2 are generally zinc-metalloenzymes, which have been used in clinical chemistry as serum markers for several diseases and which also are involved in the regulation of circulating hormones and biologically active peptides in tissues (1). Hormonal changes in serum may thus be reflected in this enzymatic activity. Significant changes in serum AP activities during the estrous cycle of rats have been reported (2). Orchietomy and ovariectomy modified AP activities significantly (3) and cholesterol and steroids affected AP activities in vitro (3,4). These results have suggested a role for these substances in the regulation of serum AP activities.

A high intake of monounsaturated fat has been proposed to decrease the incidence of cardiovascular disease and hypertension (5). However, the mechanisms that underlie this effect are unknown. In addition, increasing saturation of dietary fat resulted in an increase of plasma total cholesterol concentration (6) and systolic and diastolic blood pressures (7).

Aminopeptidases also play a major role in the regulation of the renin-angiotensin system (1). In this system, angiotensin (Ang) III is produced from Ang II by AP A or A-like activity. Ang III is also produced from Ang 1 through the production of des-Asp1-Ang I, which is further converted to Ang III by the action of angiotensin-converting enzyme. Because GluAP has been ruled out as the particulate enzyme that degrades Ang I to des-Asp1-Ang I, another enzyme (AspAP) with higher affinity for Asp-2-naphthylamide than Glu-2-naphthylamide must be responsible (8). Ang III is further converted to Ang IV by AP B (ArgAP) or AP M (AlaAP) (9).

We demonstrated previously that cholesterol selectively increased in vitro the activity of GluAP and AspAP (aminopeptidase A activity), two aminopeptidases directly related to Ang I and Ang II metabolism. In contrast, there was no change in activity of other AP such as AlaAP and ArgAP, which are involved in Ang III metabolism, CysAP, described as vasopressin-degrading activity and pGluAP, described as tirotrpin-releasing hormone (TRH) or gonadotropin-releasing hormone (GnRH)-degrading activity (3, 4). In vitro cholesterol selectivity prompted us to investigate the effect in vivo of different degrees of dietary fatty acid saturation on serum aminopeptidase activities, including angiotensinase activities and others that are not related to angiotensin metabolism.

MATERIALS AND METHODS

Male Balb/C mice, 1–2 wk old, supplied by Harlan Ibérica (Barcelona, Spain), were used in this study. Mean body weight was 12.4 g at the start of the study. The mice were housed at constant temperature (25°C) and with a constant day length (12 h). The experimental procedures for animal use and care were in accordance with

---

2 Abbreviations used: asNNap, aminooacetyl-2-naphthylamide; ADG, average daily gain; Ang, angiotensin; AP, aminopeptidases; BSA, bovine serum albumin; CO, coconut oil; DTT, dithiothreitol; FO, fish oil; GnRH, gonadotropin-releasing hormone; L, lard; OO, olive oil; pGlu, pyroglutamyl; SFO, sunflower oil; TRH, thyrotropin-releasing hormone.

---

0022-3166/01 $3.00 © 2001 American Society for Nutritional Sciences.
### TABLE 1
Composition of different diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.9</td>
</tr>
<tr>
<td>Saccharose</td>
<td>26.0</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>26.0</td>
</tr>
<tr>
<td>Denatured wheat starch</td>
<td>15.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
</tr>
<tr>
<td>Sunflower oil, fish oil, olive oil, lard or coconut oil</td>
<td>2.4</td>
</tr>
<tr>
<td>Corn oil</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral and vitamin mix2</td>
<td>3.0</td>
</tr>
<tr>
<td>dl-methionine</td>
<td>0.5</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.4</td>
</tr>
</tbody>
</table>

1 Ingredients purchased from Harlan Ibérica, Barcelona, Spain.
2 Mineral and vitamin mixture (μg/g mixture): calcium phosphate (83.3 x 10^3), sodium chloride (12 x 10^3), potassium citrate (36 x 10^3), potassium sulfate (8.6 x 10^4), magnesium oxide (4 x 10^4), manganese carbonate (580), ferrum citrate (1000), zinc carbonate (260), cupric carbonate (50), potassium iodide (1.6), sodium selenite (1.6), potassium bromide (91), thiamine (100), riboflavin (100), pyridoxine (110), nicotinic acid (500), calcium pantothenate (260), folic acid (33), cyanocobalamin (0.16), retinyl acetate (85.67), all-rac-a-tocopherol (0.83), cholecalciferol (16.67), menadione (0.83).

RESULTS AND DISCUSSION

In the present study, increasing fat saturation in the diet tended to decrease weight gain and increase the energy required per unit of weight gain (P = 0.056; data not shown). However, there is evidence that unsaturated fat results in lower body fat and body weight (15), and conjugated linoleic acid has been found to lower body fat and weight in mice (16). The contrast with the present results could be due to differences in age or strain of mice studied. Although we used male BALB/c mice and started with the defined diets at 1–2 wk, West et al. (16) used male inbred AKR/J mice and started the study at 5 wk.

Serum total cholesterol levels were 46% higher (P < 0.05) in mice fed diets containing saturated oils (L and CO) than in those fed diets containing SFO (Fig. 1). AspAP (P < 0.01) and GluAP (P < 0.01) were three- and fourfold greater, respectively, in mice fed CO than in those fed SFO (Fig. 2).

The two serum aminopeptidase A activities increased progressively with the degree of saturation of the fatty acid used in the diet. Mice fed the diet containing CO differed significantly (P < 0.01) from those fed diets containing SFO and FO in AspAP and GluAP activities (Fig. 2). The other activities measured in this study (AlaAP, ArgAP, CysAP, and pGluAP) did not differ among groups (data not shown). Interestingly, the relatively low amounts of the various fats (2.4 g/100 g) added to the diets in this study exerted notable effects on cholesterol and aminopeptidase. This suggests that BALB/c mice, in particular, may be an excellent model for these fat studies.

Previous results have suggested that cholesterol and steroids influence serum AP activities (3,4), raising the possibility that these substances create a biochemical environment that regulates the activity of these enzymes. These earlier studies demonstrated that, in vitro, cholesterol increased serum AspAP and GluAP activities. Therefore, the present results may be due in part or indirectly to an increase in serum total cholesterol, as a result of increasing saturation of dietary fat. However, it should be taken into account that the origin of...
FIGURE 2  Serum aspartyl aminopeptidase (A) and glutamyl aminopeptidase (B) activities in Balb/C mice fed diets containing sunflower oil (SFO), fish oil (FO), olive oil (OO), lard (L) or coconut oil (CO). Values are means ± SEM, n = 8–10. Groups are ordered from left to right according to the degree of saturation of the fat used in the diet. Values without a common letter differ, P < 0.01.

In accordance with the present results, an increase in aminopeptidase A activity suggests a heightened metabolism of Ang II, which leads to an increase in Ang III formation. Therefore, if aminopeptidase A activity is modified by the degree of dietary fat saturation, its substrates, such as Ang I and Ang II, and its metabolic products, such as Ang III and des-Asp^1^-Ang I, may also be modified. Consequently, their actions in the control of blood pressure and other physiologic functions may be similarly affected. It was demonstrated recently that the fat saturation of the diet also influences other enzymes such as esterases (21). Taken together, these results suggest that dietary fat saturation has a wide range of effects on various enzyme systems.

LITERATURE CITED