Effect of fatty Amazon fish consumption on lipid metabolism

Efeito do consumo de peixes amazônicos gordurosos sobre o metabolismo lipídico

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ABSTRACT

Objective
The present study aimed to evaluate the effect of feeding diets enriched with fatty fish from the Amazon basin on lipid metabolism.

Methods
Male Wistar rats were divided into four groups: control group treated with commercial chow; Mapará group was fed diet enriched with Hypophthalmus edentatus; Matrixnã group was fed diet enriched with Brycon spp.; and, Tambaqui group was fed diet enriched with Colossoma macropomum. Rats with approximately 240±60 g of body weight were fed ad libitum for 30 days, and then were sacrificed for collection of whole blood and tissues.

Results
The groups treated with enriched diets showed a significant reduction in body mass and lipogenesis in the epididymal and retroperitoneal adipose tissues and carcass when compared with the control group. However, lipogenesis in the liver showed an increase in Matrixnã group compared with the others groups. The levels of serum triglycerides in the treated groups with Amazonian fish were significantly lower than those of the control group. Moreover, total cholesterol concentration only decreased in the group Mapará. High Density Lipoprotein cholesterol levels increased significantly in the Mapará and Tambaqui compared with control group and Matrixnã group. The insulin and leptin levels increased significantly in all treatment groups.

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Conclusion
This study demonstrated that diets enriched with fatty fish from the Amazon basin changed the lipid metabolism by reducing serum triglycerides and increasing high density lipoprotein-cholesterol in rats fed with diets enriched with Mapará, Matrinxã, and Tambaqui.


INTRODUCTION

Obesity is considered a global public health problem in developed and developing countries. Obesity pathophysiology involves complex neuroendocrine and metabolic mechanisms. The main processes involved in cardiac diseases are atheroma, thrombogenetic, and dietary, and factors that affect lifestyle. High intake of saturated fats, cholesterol, and calories lead to obesity, and therefore, are factors that affect lipid metabolism. The quantity and nature of the fat ingested daily influence the concentration of plasma cholesterol. High cholesterol levels in the blood are related to the incidence of atherosclerotic vascular diseases, especially coronary diseases. On the other hand, high density lipid cholesterol plays an important role in maintaining the plasma cholesterol levels within a compatible range, since it removes free cholesterol from plasma. The main function of High Density Lipoprotein (HDL) is to build temporary reserves for total lipids, cholesterol, and apoproteins, protecting against atherosclerosis. Estadella et al., showed that a high-fat or mixed diet promotes a smaller rate of lipogenesis in retroperitoneal adipose and epididymal rat tissues. The accumulation of fat in the tissues and insulin resistance has been associated with dyslipidemia,
higher plasma total cholesterol level, Low Density Lipoprotein-cholesterol (LDL-c), Very Low Density Lipoprotein-cholesterol (VLDL-c), triglycerides; and lower HDL-c.

The ability of some foods to reduce cholesterol levels has been investigated in the last decades. Fish lipids are among these components. Since the 1970s, studies have confirmed the role of omega-3 fatty acids, especially Eicosapentaenoic Acid (EPA), in controlling cholesterol and its fractions, and consequently, in preventing cardiovascular diseases\(^8\). Researchers have found that the daily intake of omega-3 fatty acids can reduce the levels of triglycerides from 25% to 30%\(^9\). Xin \textit{et al.}\(^10\) state that short-term fish oil supplementation can have a favorable influence on the frequency domain of the heart rate variability.

The nature and amount of dietary lipids protect against and/or promote cardiovascular problems, and considering the high seafood intake in the Brazilian North region, this study aimed to examine whether diets containing fatty fish from the Amazon region, such as: mapará (\textit{Hypophthalmus edentatus}), matrinxã (\textit{Brycon spp.}), and tambaqui (\textit{Colossomoa macropomum}) affect lipid metabolism in Wistar rats.

**METHODS**

**Animals**

Forty adult male Wistar (\textit{Rattus norvegicus}) rats weighing approximately 240±0.60 grams, from the animal facility of the Universidade Federal do Amazonas (UFAM) were divided into four groups according to diet: Control Group (CG), Mapará Group (GMAP), Matrixxã Group (GMAT), and Tambaqui Group (GTAB). All animals were kept in individual cages under a temperature of 24°C to 28°C and 12/12-hour light-dark cycle. The animals had free access to food and water. The experimental period lasted 30 days. The study was approved by the Animal Ethics Committee of the UFAM under Protocol 00014/12.

**Preparation of the diets**

The fish were transported to the Coordenação de Pesquisas em Tecnologia do Alimento/Instituto Nacional de Pesquisas da Amazônia (CPTA-INPA) where they were filleted and submitted to mechanical muscle separation in a German Baader model 694 separator. The resultant meat was distributed in rectangular metal trays and frozen to -30°C. The frozen blocks weighting roughly 7kg and having a width of 5cm each were cut with an electric band saw in portions of roughly 500g and wrapped in polyethylene film. The frozen blocks were placed in isothermal boxes containing ice, transported to the Physiology Laboratory of UFAM and stored at -18±1°C. The Amazon fish-based diet consisted of Labina chow (72.5%), casein (12.5%), and mechanically separated meat of each species (15.0%). All components were ground and mixed. The final composition of each chow was approximately 22.0% proteins, 10.5% fats, 40.0% carbohydrates, and 16.0% fibers (Table 1). The caloric density determined by an adiabatic calorimeter (IKA-C400) was approximately 251.40kJ/g±0.30kJ/g (35.0% calories as fats) for each palatable high-fat diet, and 17.03kJ/g for the standard diet.

**Experimental Procedure**

**Body weight and food intake**

The rats’ food intake and body mass were assessed daily for 30 days. Energy intake was determined by multiplying food intake by energy density. At the end of 30 days, the forty rats were sacrificed by decapitation and the total blood extracted. Next, this material was centrifuged at 7500rpm for 2 minutes in an Eppendorf Model 5415 centrifuge. After one hour, an intraperitoneal injection of 3mCi \(^3\)H\(_2\)O in a volume of 0.3mL was given for determining the rate of lipogenesis \textit{in vivo}. Total blood was collected. The carcass and the tissues liver, Retroperitoneal Adipose (RET),
and Epididymal (EPI) were immediately weighed. *In vivo* lipogenesis was determined by the incorporation of $^3$H$_2$O in saponified lipids according to the Robinson and Williamson method\(^{11}\). The tissue samples were digested in 3.0mL of 30% KOH and 3.0mL of ethanol during 2h at 70°C. After cooling, 2.0mL of $\text{H}_2\text{SO}_4$ 12N was added and the lipids were extracted with 10.0mL of ether in petroleum\(^{12}\). This extract was washed with 2.0mL of distilled water and evaporated until dry. The radioactivity of 20µL of the serum in the samples was used to determine specific activity. The rate of lipogenesis was calculated as micromoles $^3$H$_2$O of lipids incorporated by gram by hour. The lipid content of the tissue was determined by the gravimetric method\(^{13}\). The glycogen\(^{14}\) content of the liver was determined by the anthrone method - $\text{H}_2\text{SO}_4$. Plasma was obtained by centrifugation and aliquots were used for measuring glucose, triglycerides, total lipids, cholesterol, high-density lipoprotein, insulin, and leptin. For these measurements, Doles (Brazil) kits were used. The concentration of insulin (Coat-A- Count DPC MedLab, CA, USA) and leptin (Linco Research, INC, MO, USA) were determined by radioimmunoassay kits.

### Statistical analysis

The statistical tests were one-way Analysis of Variance (Anova) followed by the Tukey test. The differences were considered significant when \(p<0.05\).

### Diet composition

The percent macronutrient composition and fatty acids of the total lipid content of the CG, GMAP, GMAT, and GTAB diets are shown in Table 1; we see that the total protein content did not differ significantly between the groups. The percentage of carbohydrates in the CG group was statistically smaller than that of the groups fed Amazon fish, except for the group GMAP. Regarding fibers present in the percent composition of the diets, the CG had the lowest percentage. The GMAP group had higher fiber percentage than the groups GMAT and GTAB. The GMAP group had a higher lipid percentage than groups CG, GMAT, and GTAB. The CF fatty acids have a higher percentage of linolenic and linoleic acids. The GMAP was the only group with Docosahexaenoic (DHA) and EPA.

<table>
<thead>
<tr>
<th>Macronutrients</th>
<th>GC</th>
<th>GMAP</th>
<th>GMAT</th>
<th>GMAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>22.4(^a)</td>
<td>22.3(^a)</td>
<td>22.6(^a)</td>
<td>23.7(^a)</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>39.1(^a)</td>
<td>38.2(^a)</td>
<td>41.3(^c)</td>
<td>40.3(^d)</td>
</tr>
<tr>
<td>Fibers</td>
<td>11.4(^a)</td>
<td>16.7(^b)</td>
<td>15.0(^c)</td>
<td>16.0(^d)</td>
</tr>
<tr>
<td>Lipids</td>
<td>4.8(^a)</td>
<td>11.8(^b)</td>
<td>10.0(^c)</td>
<td>9.9(^c)</td>
</tr>
<tr>
<td>Palmitic (16:9)</td>
<td>20.4(^a)</td>
<td>23.93(^b)</td>
<td>24.58(^c)</td>
<td>24.8(^d)</td>
</tr>
<tr>
<td>Oleic (18:1n-9)</td>
<td>27.4(^a)</td>
<td>24.55(^b)</td>
<td>27.9(^a)</td>
<td>38.85(^d)</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>5.12(^a)</td>
<td>7.65(^b)</td>
<td>6.92(^a)</td>
<td>9.84(^c)</td>
</tr>
<tr>
<td>Palmitoleic (16:1n-7)</td>
<td>-</td>
<td>3.56(^b)</td>
<td>7.5(^a)</td>
<td>1.72(^d)</td>
</tr>
<tr>
<td>Linolenic (18:3n-3)</td>
<td>9.91(^a)</td>
<td>3.76(^b)</td>
<td>2.35(^c)</td>
<td>2.66(^c)</td>
</tr>
<tr>
<td>Linoleic (18:2n-6)</td>
<td>41.0(^a)</td>
<td>11.11(^b)</td>
<td>18.52(^a)</td>
<td>18.62(^c)</td>
</tr>
<tr>
<td>Myristic (14:0)</td>
<td>2.07(^a)</td>
<td>0.56(^b)</td>
<td>2.46(^a)</td>
<td>0.66(^b)</td>
</tr>
<tr>
<td>Docosahexaenoic (22:6n-3)</td>
<td>-</td>
<td>2.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eicosapentaenoic (20:5n-3)</td>
<td>-</td>
<td>2.4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: The values are expressed as means ± standard error mean. Values followed by the same letter do not differ according to the Tukey test \((p<0.05)\).
**Weight gain, energy value, metabolic efficiency, and fat content**

Table 2 shows that the fat content of the carcass in the groups fed diets with 15% meat was smaller than that of the control. However, the fat content of the carcass of groups GMAT and GTAB did not differ from each other, but both were higher than GMAP. The measure of the masses of the adipose epididymal and retroperitoneal tissues and liver of the GMAT, GMAP, and GTAB groups were greater than those of the control group. The CG consumed less energy than the other groups. On the other hand, the GMAP group had greater total energy intake than the GTAB and GMAT. GTAB total energy intake was significantly higher than that of GMAT. The control groups had more weight than the groups GTAB and GMAP. The metabolic efficiency of the control group was lower than that of the other groups. On the other hand, the GTAB group had a metabolic efficiency greater than the GMAP and GMAT groups. The GMAT had a lower metabolic efficiency than the group GMAP.

**Lipogenesis**

In the CG's, GMAP's, and GTAB's livers, the rate of lipogenesis was similar (Table 3). However, the GMAT group had a higher rate of liver lipogenesis than the GMAP, GTAB, and CG groups. The lipogenesis rates of the GMAP, GMAT, and GTAB were lower than those of the CG group. The animals in the GMAP, GMAT, and GTAB groups had lower RET and EPI adipose tissue lipogenesis than the CG. However, the lipogenesis rate of the EPI adipose tissue of the GMAT group was higher than that of the groups GTAB and GMAP.

### Table 2. Body weight gain (f), total energy intake (kJ), metabolic efficiency (kJ/g of body weight gain), fat content of the carcass (g/100g), Retroperitoneal (RET) adipose tissue mass, Epididymal (EPI) adipose tissue mass, and liver mass of rats fed control diet and diets enriched with Ground Mapará (GMAP); Ground Matrinxã (GMAT) or Ground Tambaqui (GTAB). Manaus (AM), Brazil, 2012.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CG</th>
<th>GMAP</th>
<th>GMAT</th>
<th>GTAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain</td>
<td>80.30</td>
<td>6.78*</td>
<td>66.98b</td>
<td>3.34b</td>
</tr>
<tr>
<td>Total energy intake</td>
<td>6840.4</td>
<td>153.9*</td>
<td>7910.6</td>
<td>183.2*</td>
</tr>
<tr>
<td>Metabolic efficiency</td>
<td>96.44</td>
<td>7.07*</td>
<td>119.72</td>
<td>4.43b</td>
</tr>
<tr>
<td>Fat content of the carcass</td>
<td>8.2</td>
<td>0.025*</td>
<td>3.78</td>
<td>1.56*</td>
</tr>
<tr>
<td>EPI mass</td>
<td>0.86</td>
<td>0.025*</td>
<td>1.04</td>
<td>0.0006*</td>
</tr>
<tr>
<td>RET mass</td>
<td>0.65</td>
<td>0.06*</td>
<td>1.05b</td>
<td>0.007b</td>
</tr>
<tr>
<td>Liver mass</td>
<td>9.08</td>
<td>0.300a</td>
<td>11.59</td>
<td>0.007b</td>
</tr>
</tbody>
</table>

Note: The values are expressed as means ± standard error mean. Values followed by the same letter do not differ according to the Tukey test (p<0.05).

### Table 3. In vivo lipogenesis rate (µmol ³H₂O incorporated in lipids/g of tissues.h) in the Epididymal (EPI) and Retroperitoneal (RET) adipose tissues, liver, and carcass of the rats fed a control diet and diets enriched with Ground Mapará (GMAP); Ground Matrinxã (GMAT) or Ground Tambaqui (GTAB). Manaus (AM), Brazil, 2012.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>CG</th>
<th>GMAP</th>
<th>GMAT</th>
<th>GTAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPI</td>
<td>34.46</td>
<td>39.88*</td>
<td>3.42</td>
<td>0.79b</td>
</tr>
<tr>
<td>RET</td>
<td>12.92</td>
<td>9.9*</td>
<td>3.73</td>
<td>1.47b</td>
</tr>
<tr>
<td>Liver</td>
<td>8.24</td>
<td>2.22*</td>
<td>7.63</td>
<td>2.64*</td>
</tr>
<tr>
<td>Carcass</td>
<td>7.71</td>
<td>4.83*</td>
<td>1.30</td>
<td>0.43b</td>
</tr>
</tbody>
</table>

Note: The values are expressed as means ± standard error mean. Values followed by the same letter do not differ according to the Tukey test (p<0.05).
Table 4 shows that the total cholesterol and total protein contents did not change in the groups treated with the enriched diets with respect to the Control group. The groups GMAP, GMAT, and GTAB presented a significant reduction in plasma Triglyceride (TG) levels compared with the Control group. The groups GMAP and GTAB presented a significant increase in HDL-c when compared with the control and GMAT groups. Additionally, the plasma glucose of group GTAB decreased significantly when compared with the groups GMAT, GMAP, and CG. The increase in the glucose levels of the group GMAP was greater than that of the other groups. However, plasma insulin did not change significantly in any of the groups. Leptin in the CG was significantly lower than in the treated groups (Table 4).

**Plasma metabolites**

Table 4 shows that the total cholesterol and total protein contents did not change in the groups treated with the enriched diets with respect to the Control group. The groups GMAP, GMAT, and GTAB presented a significant reduction in plasma Triglyceride (TG) levels compared with the Control group. The groups GMAP and GTAB presented a significant increase in HDL-c when compared with the control and GMAT groups. Additionally, the plasma glucose of group GTAB decreased significantly when compared with the groups GMAT, GMAP, and CG. The increase in the glucose levels of the group GMAP was greater than that of the other groups. However, plasma insulin did not change significantly in any of the groups. Leptin in the CG was significantly lower than in the treated groups (Table 4).

**DISCUSSION**

In the literature the effect of high-fat diet on body weight is very controversial\textsuperscript{15,16}, especially of rats fed diets high in saturated fats and omega-6 polyunsaturated fats\textsuperscript{17}. The degree of satiety promoted by high-fat diet depends on their physical and chemical properties (chain length, saturation, and conjugation), its carbohydrates, and its palatability\textsuperscript{18}. These factors may influence the release of inhibitory gastrointestinal peptides such as cholecystokinin, enterostatin, and apoprotein A-IV\textsuperscript{19,20} and also the digestion, absorption, and metabolic rates. Himaya et al.\textsuperscript{21} reported that the time of satiety in high-fat diets is longer in diets high in carbohydrates because of the higher levels of plasma metabolite substrates such as glucose, TG, and fatty acids. These results may partly explain the fact that Wistar rats present lower intake of diets enriched with mapará (Hypophthalmus edentatus), the fish with the highest lipid content. On the other hand, the rats fed matrixá (Brycon spp.) consumed as much food as the controls.

Other studies have reported that rats kept with diets enriched with polysaturated and saturated fish oil did not vary their food intake\textsuperscript{22}. Interestingly, in past decades, this dietary practice of feeding high-fat diet was used as strategy to control obesity, but with little success, since in general this type of diet has high energy content leading to body weight gain\textsuperscript{21}. This is in agreement with the group that was fed the diet enriched with matrixá, which gained more weight than the other groups. The intake of each macronutrient seems to be under strict control to maintain oxidation and the balance status. In case of fats,
the adjustment is much less precise and higher intake does not stimulate proportional oxidation. The oxidation rate of fatty acids may also be another factor that controls food intake. The accumulation of fats in tissues and the presence of insulin resistance has been associated with dyslipidemias, increase in the plasma level of total cholesterol, LDL-c, VLDL-c, IDL-c, TG, and low HDL-c. Hence, many studies have reported that obesity, dyslipidemia, diabetes type II, high blood pressure, and cardiovascular diseases lead to interrelated metabolic changes known as plurimetabolic syndrome. Nieves et al. indicate that the lipoprotein profile present seem to result primarily from the increase in central fat probably due to the development of insulin resistance. The reduction of abdominal fat by medication or lifestyle changes or dietary changes or more physical activity could contribute to improve the lipid profile, and avoid the development of atherosclerosis. Suprijana et al. found a reduction in the levels of TG, total cholesterol, and lipoprotein fractions in rats fed diets enriched with fish oil, which was later observed also by Kim et al.

Similarly, the results observed in this study show that the intake of fatty Amazon fish reduced TG and cholesterol levels. The effects caused by high-fat diets on the metabolism of animals may be influenced by the type of lipids, gender, and treatment period. Patients treated with fish oil rich in omega-6 fatty acids have experienced lower total cholesterol, LDL-c, TG, and VLDL, and higher HDL-c. Similarly, the total cholesterol and TG of the rats fed diets enriched with Amazon fish decreased and the HDL of rats fed diets enriched with mapará and tambaqui increased - these fish contain omega-6 and omega-3 fatty acids. The plasma glucose levels did not differ significantly from those of the control group. Many works have showed that lipid metabolism is regulated by leptin, a protein hormone produced mainly by adipose tissue. According to Nogalska et al., higher expression of the leptin gene partially clarifies the low lysogenic activity of the white adipose tissue of old animals. This finding corroborates our studies which showed low lipogenesis in the adipose tissues and concomitant leptin increase in the rats fed enriched chow.

In conclusion, this study found that diets enriched with fatty Amazon fish changed the lipid metabolism of rats effectively lowering plasma lipids (cholesterol, TG, and LDL) and increasing HDL. These results suggest that regular consumption of fish with these characteristics is beneficial.

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CONTRIBUTORS

FCA SOUZA is the first author; she designed, coordinated and organized all experiments. She carried out an exhaustive review of the literature. In addition, performed statistical analyses and interpretation and was responsible for writing the article. NP GARCIA participated to the experimental design, data analysis in laboratory and participated in the writing of the article. RSA SALES participated to the exhaustive laboratory analysis, particularly for the preparation of the diet and experimental procedures. JPL AGUIAR performed several laboratory analysis, interpretation and participated in the writing of the article. WLP DUNCAN participated in the statistical analysis, writing and reviewing the manuscript. RP CARVALHO is the investigator responsible for the overall study.

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