Development of Adipose Tissue in the Juvenile Red Sea Bream

Tetsuya Umino, Heisuke Nakagawa and Katsutoshi Arai
Faculty of Applied Biological Science, Hiroshima University, Higashi-hiroshima, Hiroshima, 739 Japan
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Histological observations were performed to clarify the developmental process of the perivisceral adipose tissue in the early life stage of the red sea bream, Pagrus major. The adipocytes were first observed in the pancreas of 37-day-old juveniles. Development of the adipose tissue progressed in the pancreas covering the intestine. Tissue volume (Day 37 to 56) increased acutely with growth of the fish. On the other hand, no significant change was recognized in the average size of adipocytes in this period. This result suggests that hyperplastic growth by active recruitment of precursor cells predominates the early development of the adipose tissue.

Key words: adipose tissue, adipocyte, development, histology, red sea bream

Lipid is known to be the most effective nutrient for fish energy source, because it weighs less and occupies less volume per calorie than either carbohydrate or protein.1 Lipid is also effective as an energy reserve in fish, and is mainly stored in the musculature, liver, and perivisceral adipose tissue. In particular, the adipose tissue is thought to be the most important energy storage compartment. It contains 60 to 85% lipid, and more than 90% of the lipid consists of triglycerides.2-5 The feeding regime of the fish decreases lipid deposition in the perivisceral adipose tissue6-8 and causes alterations of the adipocyte morphology.9,10 The importance of adipose tissue in energy reserve has been recognized in adult fish. However, as far as we know, no studies have been conducted on its development in the early life stages of fish. When and how do fish begin to reserve excessive lipid taken from food? The answer to this question is very important for the improvement of aquaculture, e.g. food management and release size evaluation. This study was conducted to observe the early developmental process of the perivisceral adipose tissue of red sea bream.

Materials and Methods

Fish
Larval and juvenile red sea bream, Pagrus major, were obtained from the Hiroshima Prefecture Fish Farming Center. They were reared at 18.5-20.1°C and fed with Brachionus plicatilis (from 4 to 38 days after hatching), Artemia nauplii (20 to 37 days), and commercial diet (14 to 52 days). Five fish were sampled on each of days 6, 11, 16, 22, 27, 32, 37, 42, 47, 52, and 56 after hatching.

Anatomical Observation
Fish were fixed in 10% buffered formalin and the digestive organs were then removed. For staining of the adipose tissue, the digestive organs were treated with a 2% Sudan III alcohol solution for 30 min, rinsed in 70% alcohol, and differentiated in 95% alcohol.

Microscopy and Volumetric Technique of the Adipose Tissue
Fish were fixed in Bouin’s solution. Whole body embedded in paraffin was cut transversely into serial sections (from the posterior part of the esophagus to the anterior part of the rectum) of 10 μm in thickness and stained with hematoxyline and eosin (H-E). According to the method of Toyoda and Uematsu,11 the volume of the adipose tissue of five fish at each stage was analyzed by a program package consisting of 3D-reconstruction and volume measurement programs (Nikon Cosmozone-2S). The size (diameter) of about 50 adipocytes in at least three non-sequential sections of five individuals at each stage was measured. Changes in the adipose tissue volume and the average size of adipocytes were assessed statistically using a one-way ANOVA.

Transmission Electron Microscopy (TEM)
For electron microscopy, fish were fixed for 1-2 days at 4°C in 2% glutaraldehyde and 2.25% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After rinsing the specimen several times in buffer, they were post-fixed in 1% osmium tetroxide solution for 1.5 h and then dehydrated in a series of graded alcohols. The specimens were placed in propylene oxide for 20 min and embedded in Quetol 812 resin. Semithin sections of 1 μm were cut transversely with a Porter-Blum MT-2B ultramicrotome. Sections were glued to glass slides and stained with 1% toluidine blue solution. Ultrathin sections (silver interference color, corresponding to 60 nm in thickness) for electron microscopy were mounted on copper grids, stained with lead citrate and uranyl acetate prior to examination under an electron microscope at 80 kV (H-600A, Hitachi).

Results and Discussion
According to anatomical observation, the adipose tissue was firstly detected in the right part of the body cavity of 42-day-old juveniles (Fig. 1A). As fish grew older, adipose tissue developed to cover the intestine (52-day-old
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By histological observation in serial sections, the adipocytes were already detectable in the pancreas of 37-day-old fish (Fig. 2A). The inset of Fig. 2A shows a schematic illustration by anatomical observation and 3D-reconstruction. The digestive tract was convoluted already at this stage, although the mid-gut remained straight. Fish also had a well-developed gastric gland in the stomach. Thereafter, the adipose tissue developed in accordance with the morphological changes of digestive tract, typically as follows. In 42-day-old juveniles (Fig. 2B), the number of adipocytes in the pancreas increased. The adipose tissue in the pancreas elongated along the mid-gut toward the rectum. In this stage, the posterior portion of the mid-gut curved slightly. In 47-day-old juveniles (Fig. 2C), the mid-gut elongated and exhibited an S-shape. The adipose tissue also elongated and reached just in front of the rectum. On and after this period, the pancreas was localized on the extreme border of the well-developed adipose tissue. In 52-day-old juvenile (Fig. 2D), the adipose tissue developed further and covered the rectum. These histological results revealed that the adipose tissue appeared and developed in the pancreas.

The histological features of the adipocytes at high magnification of 37-day-old juveniles are shown in Fig. 3. Under light microscopy, the cytoplasm was a thin rim surrounding lipid droplets (Fig. 3A). Osmiophilic lipid droplets were noted in adipocytes (Fig. 3B). When examined under TEM, a single lipid droplet occupied most of the volume of each cell. The nucleus was displaced to one side by the accumulated lipid and the cytoplasm became flattened to a thin rim surrounding the cell (Fig. 3C). On the other hand, some adipocytes which contained small
lipid droplets were noted (Fig. 3D). The cell size of these adipocytes was relatively small, and their nucleus was not flattened against the more abundant cytoplasm. During their early development they may contain multiple lipid droplets, but these ultimately coalesce into a single drop.

In newborn mammals, including man, two distinct types of adipose tissue, white (unilocular) and brown (multilocular) adipose tissue which differ in color and metabolic activity, have been found.\(^{12,13}\) The brown adipose tissue gradually diminishes postnataally by replacement of this brown adipose tissue with white adipose tissue.\(^{12,13}\) Based on the histological features shown in red sea bream (Fig. 3), the adipose tissue in the early developmental stage can be categorized as white adipose tissue.

Changes in the adipose tissue volume and the average size of adipocytes were plotted against total length (Day 37 to 56) in Fig. 4B and 4C, respectively. The adipose tissue volume increased acutely (ANOVA for all combinations, \(P_s=0.11\)) with growth. However, the average cell size showed negligible increase (ANOVA for all combinations, \(P_s=0.52\)). This result suggests that the development of the adipose tissue in this stage is caused by the active recruitment of the adipocytes, instead of hypertrophy of the cells. In rats,\(^{12}\) both hypertrophy and active recruitment of adipocytes (hyperplastic growth) contribute to the growth of the adipose tissue. Bellardi et al.\(^{10}\) working on young rainbow trout Oncorhynchus mykiss, pointed out the coexistence of hypertrophic and hyperplastic manifestations of lipid accumulation in fish. The present results suggest that hyperplastic growth predominates in the early development of the adipose tissue, and hypertrophy is activated as the fish grow older.

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Fig. 4. Growth curve of red sea bream from 37 to 56 days after hatching (A), and changes in the adipose tissue volume (B) and the average size of the adipocytes (C) against the average total length. Data are shown as mean and SE. Sample sizes of the growth, tissue volume, and cell diameter in each stage are 20, 5, and 5, respectively.

References