ELSEVIER

CrossMark

Evaluation of the distribution of adipose tissues in fish using magnetic resonance imaging (MRI)

Jun-Lin Wu [a,1], Ji-Lei Zhang [b,1], Xiao-Xia Du [b], Yong-Ju Shen [c], Xun Lao [a], Mei-Ling Zhang [a], Li-Qiao Chen [a], Zhen-Yu Du [a,*]

[a] Laboratory of Aquaculture Nutrition and Environmental Health (LANEH), School of Life Sciences, East China Normal University, 200241 Shanghai, China
[b] Shanghai Key Laboratory of Magnetic Resonance, Department of Physics, East China Normal University, Shanghai 200062, China
[c] Shanghai Tenth People's Hospital, Tongji University, Shanghai 200072, China

ABSTRACT

The accumulation of excess fat in fish would impair fish health and has been a serious problem in cultured fishes. Adipose tissues are specific tissues for fat deposit in many fish species, therefore the knowledge of the anatomic distribution and total mass of adipose tissues is the basic premise to study the mechanism of fat accumulation in economic fishes. However, this issue has not been well documented. To investigate the morphology of adipose tissues in fishes, a T_1-weighted magnetic resonance imaging (MRI) technique was developed to scan grass carp, turbot, tilapia, pompano and large yellow croaker. Three-dimensional (3-D) images that quantitatively integrated the total volumes of adipose tissues in these fish were constructed for the first time. From 2-D and 3-D MRI images, we identified two patterns of adipose tissue distribution in fish: visceral adipose tissue dominant (grass carp, tilapia, pompano and large yellow croaker) and subcutaneous adipose tissue dominant (turbot). The volumes of adipose tissues assessed by MRI were highly consistent with those obtained by traditional dissection. In a fasting experiment on tilapia, the MRI signal of mesenteric adipose tissues successfully distinguished between fish before and after 28-d starvation. Although our MRI technique has limitations for measuring lipid in organs not specifically dedicated to fat storage, e.g., liver, muscle and intestine, the method will help researchers to gain insights into the distribution, size, volume and shape of the adipose tissues in intact and live fish. This could be a powerful tool in future studies of fish lipid metabolism.

Statement of relevance to aquaculture: In aquaculture, the accumulation of excess fat in fish, which is mainly sourced from unbalanced diets or feeding strategy, would impair fish health and has been a serious problem in cultured fishes. Adipose tissues are specific tissues for fat deposit in many fishes, therefore the knowledge of the anatomic distribution and total mass of adipose tissues is the basic premise to study the mechanism of fat accumulation in economic fishes. However, this issue has not been well documented. Our present work investigated the distribution and content of adipose tissues in five economic fishes with different feed habits and farming environments by using the MRI technique. Through this work, we described the shape, distribution and mass of adipose tissues in different economic fishes for the first time. We also developed an ideal MRI scanning method for live fish, which largely expends the application of the MRI technique in nutrient physiological study in aquatic animals. Because the adipose tissue has been widely accepted to be closely related to nutrition status and diets in the most of farmed animals, including economic fishes in aquaculture, our present study has close relevance to aquaculture.

1. Introduction

Currently, most of economic fish are farmed and fed with formulated diets. However, excess fat accumulation in fish, which is mainly caused by excess energy intake and the nutrient-unbalanced diets, has been a serious problem for worldwide aquaculture. As in mammals, excess fat accumulation in fish represents an abnormal metabolic situation and impairs fish health, decreases stress resistance, and lowers product quality (Chatzifotis et al., 2010; Du et al., 2006). Therefore, the mechanisms underlying lipid accumulation in fish have received much

attention in recent decades (Du et al., 2008; Greene and Selivonchick, 1987). In most of the animals, adipose tissue is a specific tissue for fat deposit. In mammals, adipose tissue distribution is a strong predictor of the occurrence of the metabolic syndrome in the context of obesity (Wang et al., 2013a). Fish are known to accumulate fat nonspecifically in organs such as the liver and muscle, as well as in specifically dedicated adipose tissues. However, as compared with the liver and muscle which have relatively fixed anatomic location and shape, adipose tissues in fish are often studied without considering their anatomic distribution, shape, size and location. Therefore, researchers should first obtain accurate information concerning the content and anatomic distribution of adipose tissues in intact fish before delving into deeper mechanism of lipid metabolism.

At present, the technical approaches commonly used to determine fat in fish metabolic studies are: 1) chemical measurement of the quantity of lipid in whole fish or tissues by organic solvent extraction (Folch et al., 1957) and 2) observation or measurement of stained lipid droplets in tissue slices using histological methods (Gu et al., 2014). However, the size, shape, quantities and distribution of adipose tissue vary greatly among fish species and neither of these methods provides overall information of adipose tissues in intact fish, particularly in large fish. In recent years, some indirect invasive and noninvasive techniques have been developed to measure the content and location of fat in fish, particularly in the field of food science, where fish flesh is the primary organ of interest (Collewet et al., 2013). Noninvasive techniques for detection of fat in intact fishes include computer-aided optics, X-ray tomography and magnetic resonance imaging (MRI) (Mathiassen et al., 2011). Of these, X-ray CT has been widely applied in detecting the characteristics of different body tissues, including bones, fat tissues and so on, with fast scanning speed (Chang et al., 2014a; Wang et al., 2013b). However, X-ray CT has radiation which would potentially bring adverse effects on small lower animals, such as fish, especially in their juvenile or brood fish stages. Compared to the X-ray method, the MRI method has the advantage of the lack of ionizing radiation. Therefore, the use of MRI instead of X-ray based methods avoids the exposure of the small living animals to X-rays, thus eliminating any exposure to potentially damaging ionizing radiation (Chatham and Blackband, 2001). Besides, MRI also provides information on the anatomic location of fat deposits in different planes and also offers the opportunity to measure total fat volume (Toussaint et al., 2005).

Clinically, MRI has been routinely used in disease diagnosis. By examining the hydrogen density and relaxation behavior of tissues in strong radiofrequency magnetic fields, MRI can clearly recognize abnormal characteristics of target tissues compared with healthy tissues, without surgical intervention. Because MRI can safely and rapidly provide visible images of internal organs and tissues in an intact body, the technique has also been used in a number of animal researches (Guan et al., 2012; Tinsley et al., 2004). Recently, with the assistance of powerful software, several 3-D MRI digital models of human organs, including brain and bones, have been developed (Annweiler et al., 2014; Chang et al., 2014b). In fish, MRI has been used for quantification and localization of fat in Atlantic mackerel (Brix et al., 2009) and for describing the distribution of fat in the flesh of brown trout (Toussaint et al., 2005) and rainbow trout (Collewet et al., 2013). However, these studies were mainly focused on the fat in fish flesh, including subcutaneous fat between the skin and muscle, but the visceral adipose tissues, which are the main fat deposit place in most of the fishes, have not been thoroughly investigated. Moreover, each of these studies concerned only a single fish species, and the differences of adipose tissues between different fish species are not available yet. In addition, the fish used in these previous MRI studies were dead. Till now, the MRI technique has not been tested in live fish and this largely limits the application of the MRI technique in fish physiological studies, in which live fish are often necessary.

To investigate the anatomic location of adipose tissues in different fishes, this study first standardized an MRI method to represent adipose tissues in five fish species of different sizes, shapes and feeding habits.

The method was then used to build 2-D and 3-D models of fish adipose tissues. The standardized MRI method was further validated in a tilapia fasting experiment. In order to expand the application of this MRI method in the physiological study of aquatic animals, this MRI method was also tested in live fishes.

2. Materials and methods

2.1. Ethics statement

The experiments were conducted following the Guidelines of the Animal Care of the East China Normal University and were approved by the Ethics Committee of the university.

2.2. Sample preparation

Five species of live fish of commercial size were selected from a local fish market in Shanghai. These were grass carp (*Ctenopharyngodon idella*, mean weight: 1302.2 ± 236 g; $N = 6$), turbot (*Scophthalmus maximus*, mean weight: 336 ± 15 g; $N = 6$), large yellow croaker (*Pseudosciaena crocea*, mean weight: 434 ± 16 g; $N = 6$), golden pompano (*Trachinotus ovatus*, mean weight: 400 ± 15 g; $N = 6$), and tilapia (*Oreochromis niloticus*, mean weight: 492 ± 67 g; $N = 6$). On the same day, all fish were anesthetized with MS-222 and the body surface was dried by absorbent paper prior to MR scanning. After MR scanning, at least three fish were dissected and the related chemical analysis of tissues was carried out. For the fish needed for the follow-up study, they were put into water for resuscitation.

2.3. MRI parameter optimization

Three experiments were designed to optimize and standardize the MRI parameters for fish measurements. MRI was carried out using a Siemens 3.0 T Trio Tim MRI system with a 12-channel head coil located at the Shanghai Key Laboratory of Magnetic Resonance, East China Normal University, Shanghai, China. The first experiment compared the use of longitudinal relaxation time (T_1) and transverse relaxation time (T_2) to discriminate fat signals from the signals generated by other tissues in the fish. Fish were scanned with a series of spin echo (SE) T_1-weighted images and one SE multi-slice multi-echo T_2-weighted image. The parameters of the T_1-weighted images were: field of view (FOV) $= 300 \times 300$ mm^2, matrix $= 256 \times 256$, six slices with slice thickness $= 1.5$ mm, averages $= 1$, concatenations $= 1$, echo time (TE) $= 13$ ms, and repetition time (TR) $= 21$ ms, 50 ms, 100 ms, 300 ms, 600 ms, 1000 ms, 1500 ms, 2000 ms, and 3000 ms. The parameters of the T_2 weighted image were: FOV $= 300 \times 300$ mm^2, matrix $= 256 \times 256$, six slices and slice thickness $= 1.5$ mm, averages $= 1$, concatenations $= 1$, TR $= 4000$ ms, and TE $= 15.2$ ms, 30.4 ms, 45.6 ms, 60.8 ms, 76.0 ms, 91.2 ms, 106.4 ms, 121.6 ms, 136.8 ms, and 152 ms. The T_1 and T_2 values of the adipose tissues, muscle and liver were suitable for making images. The T_1 values were 378.3 ± 10.2 ms for the adipose tissues, 1272.6 ± 40.2 ms for the muscle and 964.4 ± 42.9 ms for the liver. The T_2 values were 112.7 ± 5.1 ms for the adipose tissues, 61.1 ± 1.3 ms for the muscle and 58.6 ± 7.2 ms for the liver. That is, the T_1 value of fatty tissues was shorter, and the T_2 value of adipose tissues was longer than for other tissues, respectively. Therefore, the shortest TR and TE corresponding to the T_1 value of fat should be appropriate for detection of the adipose tissue distribution using T_1-weighted images.

The second experiment was designed to obtain the optimum TR value. A grass carp was scanned using the following parameters: FOV $= 420 \times 420$ mm^2, 10 slices and slice thickness $= 2.0$ mm, matrix $= 320 \times 320$, averages $= 1$, concatenations $= 2$, TE $= 9.4$ ms, and four values of TR (250 ms, 350 ms, 500 ms, 800 ms) were used. The TA (acquisition time) of four TRs was 187 s, 259 s, 368 s, 586 s, respectively. We introduced SNR (signal-to-noise ratio) efficiency of