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RESEARCH ARTICLE | *Obesity, Diabetes and Energy Homeostasis*

Physiological and metabolic differences between visceral and subcutaneous adipose tissues in Nile tilapia *(Oreochromis niloticus)*

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Wang YW, Zhang JL, Jiao JG, Du XX, Limbu SM, Qiao F, Zhang ML, Li DL, Du ZY. Physiological and metabolic differences between visceral and subcutaneous adipose tissues in Nile tilapia *(Oreochromis niloticus). Am J Physiol Regul Integr Comp Physiol* 313: R608–R619, 2017. First published August 16, 2017; doi:10.1152/ajpregu.00071.2017.—Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT) have different structures and metabolic functions and play different roles in the regulation of the mammal endocrine system. However, little is known about morphology and physiological and metabolic functions between VAT and SCAT in fish. We compared the morphological, physiological, and biochemical characteristics of VAT and SCAT in Nile tilapia and measured their functions in energy intake flux, lipolytic ability, and gene expression patterns. SCAT contained more large adipocytes and nonadipocytes than VAT in Nile tilapia. VAT had higher lipid content and was the primary site for lipid deposition. Conversely, SCAT had higher hormone-induced lipolytic activity. Furthermore, SCAT had a higher percentage of monounsaturated and lower polyunsaturated fatty acids than VAT. SCAT had higher mitochondrial DNA, gene expression for fatty acid β-oxidation, adipogenesis, and brown adipose tissue characteristics, but it also had a lower gene expression for inflammation and adipocyte differentiation than VAT. SCAT and VAT have different morphological structures, as well as physiological and metabolic functions in fish. VAT is the preferable lipid deposition tissue, whereas SCAT exhibits higher lipid catabolic activity than VAT. The physiological functions of SCAT in fish are commonly overlooked. The present study indicates that SCAT has specific metabolic characteristics that differ from VAT. The differences between VAT and SCAT should be considered in future metabolism studies using fish as models, either in biomedical or aquaculture studies.

Nile tilapia; visceral adipose tissue; subcutaneous adipose tissue; metabolism; gene expression pattern

EXCESS ENERGY is primarily deposited in adipose tissue (AT) in the majority of vertebrates, including fish and mammals. A decrease in blood glucose levels during fasting stimulates lipolysis and release of free fatty acids (FFA) from AT for use by various tissues. This process is vital for the maintenance of cellular function (2). In addition to storage and release of fatty acids, AT also secretes adipokines, such as adiponectin, leptin, and resistin, as part of the classic hormone/receptor systems controlled by the central nervous system (12). Thus, AT is not only a passive reservoir for energy storage, but also a complex and highly active metabolic and endocrine organ (2, 21, 50).

ATs are commonly classified into intra-abdominal adipose tissue (visceral adipose tissue, VAT) and subcutaneous adipose tissue (SCAT) based on their anatomical distribution in humans and mammals. Although the mechanisms for the individual ATs' contributions to metabolic syndromes are not fully understood, the different metabolic characteristics of the two ATs have been the focus for considerable research in mammals. VAT and SCAT have significant variations in anatomical, cellular, molecular, and physiological characteristics (7, 17, 25, 27). Moreover, VAT and SCAT play different roles in the development of metabolic syndromes (45). For example, central obesity caused by an increased amount of VAT is commonly associated with insulin resistance, a high risk of Type 2 diabetes, dyslipidemia, and high mortality (16, 17). In contrast, increased amounts of SCAT, particularly in gluteofemoral regions, is associated with improved insulin sensitivity and lower risk of development of Type 2 diabetes relative to central obesity (12, 27, 45). Ibrahim (27) reviewed the structural and functional differences between VAT and SCAT in mammals: VAT is more cellular, vascular, and innervated; contains a larger number of inflammatory cells; has lower preadipocyte-differentiating capacity; and contains a higher percentage of large adipocytes than SCAT. Moreover, VAT adipocytes are more metabolically active, more sensitive to lipolysis, and more insulin-resistant than SCAT adipocytes. Furthermore, VAT generates FFAs, takes up glucose, and is more sensitive to adrenergic stimulation than SCAT, whereas SCAT plays a greater role in absorption of circulating free fatty acids and triglyceride (27). The known differences between VAT and SCAT from mammals have become an important base in nutritional and clinical health care (33).

Fish that are reared in aquaculture systems commonly have higher levels of fat deposits than wild ones. As in mammals, excess fat accumulation in fish represents an abnormal metabolic situation and impairs fish health, decreases stress resistance, and lowers product quality (8, 14). Consequently, the mechanisms underlying lipid accumulation in fish have attracted significant attention in recent decades (13, 22). Fish

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accumulate fat nonspecifically in organs, such as liver and muscle, as well as in specifically dedicated adipose tissues. A few studies have demonstrated the positive correlation between the masses of both SCAT and VAT and dietary lipid content in zebrafish (31), and Weil et al. (48) observed the different expression of hormone receptors between SCAT and VAT in rainbow trout. In addition, the different AT distribution patterns were found in skeletal muscle among fish species (30). However, it should be noted that the majority of fish lipid studies have only sampled and analyzed VAT (49), whereas other ATs, especially SCAT, have been largely overlooked. Indeed, SCAT has not been scientifically described and studied in the majority of farmed fish. Recently, we used an MRI technique to investigate the distribution of ATs in different fish species (51). Although SCAT is present in the majority of fish species, the amount as a proportion of the total AT differs among species. This suggests that SCAT may be an endocrine organ like VAT and play roles in metabolism in fish. However, the morphological, biochemical, and gene transcriptional characteristics of SCAT in fish are poorly understood.

Nile tilapia (*Oreochromis niloticus*) is an important aquaculture species worldwide and a good fish model for metabolic studies because of its rapid growth, high disease, and stress resistance coupled with availability of its entire genome (23). A number of studies have indicated the optimal dietary lipid level in Nile tilapia is 5% to 7.4% (1, 11, 40, 47, 52). Our recent study has demonstrated that the VAT mass in Nile tilapia, but not hepatic lipid deposition, is significantly increased when dietary fat content is increased from 1 to 13% (26). Moreover, we have recently demonstrated that SCAT exists below the skin under the back fin in Nile tilapia (51). As seen in mammals, we hypothesized that SCAT and VAT in fish would have different functions and that both ATs would exhibit differences in their morphological and biochemical characteristics, as well as their gene expression patterns. Therefore, in the present study, we measured in vivo AT volume, adipocyte size, cellular lipolytic capability, and mRNA expression of a number of genes related to lipid metabolism and inflammation in VAT and SCAT in Nile tilapia, which were first fed either a low- or a high-fat diet (LF and HF, respectively) and subsequently fasted. To the best of our knowledge, this is the first study to document differences in the physiological and metabolic characteristics of visceral and subcutaneous adipose tissues in fish.

MATERIALS AND METHODS

Ethics statement. Procedures were approved by the East China Normal University Animal Research Committee and were consistent with all regulations on fish and animal use for research.

Fish, diets, and sampling. Juvenile all-male Nile tilapia (10–13 g) were obtained from Shanghai Ocean University (Shanghai, China). Before the formal feeding trial, all fish were acclimated in 200-liter tanks and were fed with a commercial diet (protein 48%, lipid 7%) (Kyorin Industry (Shenzhen), Shenzhen, China) at 3% biomass per day for 2 wk before the formal experiment.

To study the effects of different energy intakes between VAT and SCAT in Nile tilapia, two semipurified isonitrogenous experimental diets containing high (150 g/kg diet) or relatively low (40 g/kg diet) lipid contents (HF and LF diet) were formulated and fed to the fish. The LF diet had relatively similar lipid content to the optimal dietary lipid content (50 to 74 g/kg diet) for Nile tilapia (1, 11, 40, 47, 52) to avoid the possible severe impairments caused by too low dietary lipid content. The commercial acclimating diet was not used during the experimental phase because there were no high-fat commercial diets for sale in the market. The protein content of both diets was set at 380 g/kg, because our previous studies have indicated that the diets containing higher than 300 g/kg protein promote normal growth of Nile tilapia under laboratory conditions (35, 36). With the exception of casein and gelatin, which were used as the main protein sources, fish meal (200 g/kg diet) was also supplemented to ensure fast growth of fish. Fish oil and soybean oil were used as lipid sources. The dietary composition was formulated to satisfy the nutrition requirements of Nile tilapia (Table 1). During the diet preparation, all ingredients were thoroughly mixed with oil mixtures, and water was added to produce a stiff dough. The dough was then processed into 3-mm diameter pellets and air-dried at room temperature until the moisture content was less than 10%. After drying was completed, the diets were broken up and sieved into proper pellet sizes followed by subsequent storage at −30°C until required for use.

After the acclimation period, 60 fish with relatively similar masses (12.5 ± 1.1 g) were selected and divided into two groups, each with duplicates (2 tanks per group, 15 fish/tank) and fed with LF and HF diets for 6 wk. To precisely track the growth of individual fish, each fish was embedded with a tracking tag by injecting the chip in the muscle under the back fin using a special syringe. Each tag has a unique radio frequency identification (RFID) code, which could be recognized by a RFID machine (Boise, ID), as described previously (44). Fish in each tank were fed three times per day at a feeding rate of 4% body mass (g). The fish were weighed every 2 wk, and the amount of feed given was adjusted accordingly. After 6 wk, 15 fish in each group were collected randomly from the two tanks. Among the 15 collected fish, 10 fish were first anesthetized and scanned by MRI machine, and then all of the 15 fish were sampled. To investigate the physiological change of both ATs during energy exhaustion, the fish remaining after removal of the 15 fish were fasted for 1 wk until the last sampling day. The experimental design is shown in Fig. 1*B*. During the trial, fish were cultured in freshwater, and the water temperature was maintained at 28 ± 1°C with a 12:12-h light-dark cycle. The pH and total ammonia nitrogen ranged from 7.8 to 8.2 and 0.05 to 0.08 mg/l, respectively.

Table 1. *Composition of the low-fat and high-fat diets*

Ingredients	LF Diet, g/kg	HF Diet, g/kg
Casein	200	200
Gelatin	50	50
Fishmeal	200	200
Fish oil	10	65
Soybean oil	10	65
Corn starch	300	300
Mixed vitamin	18	18
Mixed minerals	28	28
CMC	50	50
Cellulose	118.75	8.75
Choline chloride	5	5
BHT	0.25	0.25
Squid extract	10	10
Total protein	380	380
Total glycogen	300	300
Total lipid	40	150

Vitamin premix (mg or IU/kg): 500,000 IU (international units) vitamin A, 50,000 IU vitamin D_3, 2,500 mg vitamin E, 1,000 mg Vitamin K_3, 5,000 mg Vitamin B_1, 5,000 mg vitamin B_2, 5,000 mg vitamin B_6, 5,000 μg vitamin B_{12}, 25,000 mg inositol, 10,000 mg pantothenic acid, 100,000 mg choline, 25,000 mg niacin, 1,000 mg folic acid, 250 mg biotin, 10,000 mg vitamin C. Mineral premix (g/kg): 314.0 g $CaCO_3$; 469.3 KH_2PO_4; 147.4 g $MgSO_4 \cdot 7H_2O$; 49.8 g NaCl; 10.9 g Fe(II) gluconate; 3.12 g $MnSO_4 \cdot H_2O$; 4.67 g $ZnSO_4 \cdot 7H_2O$; 0.62 g $CuSO_4 \cdot 5H_2O$; 0.16 g KJ; 0.08 g $CoCl \cdot 6H_2O$; 0.06 g NH_4 molybdate; 0.02 g $NaSeO_3$. LF, low fat; HF, high fat. BHT, butylated hydroxytoluene; CMC, carboxymethylcellulose.

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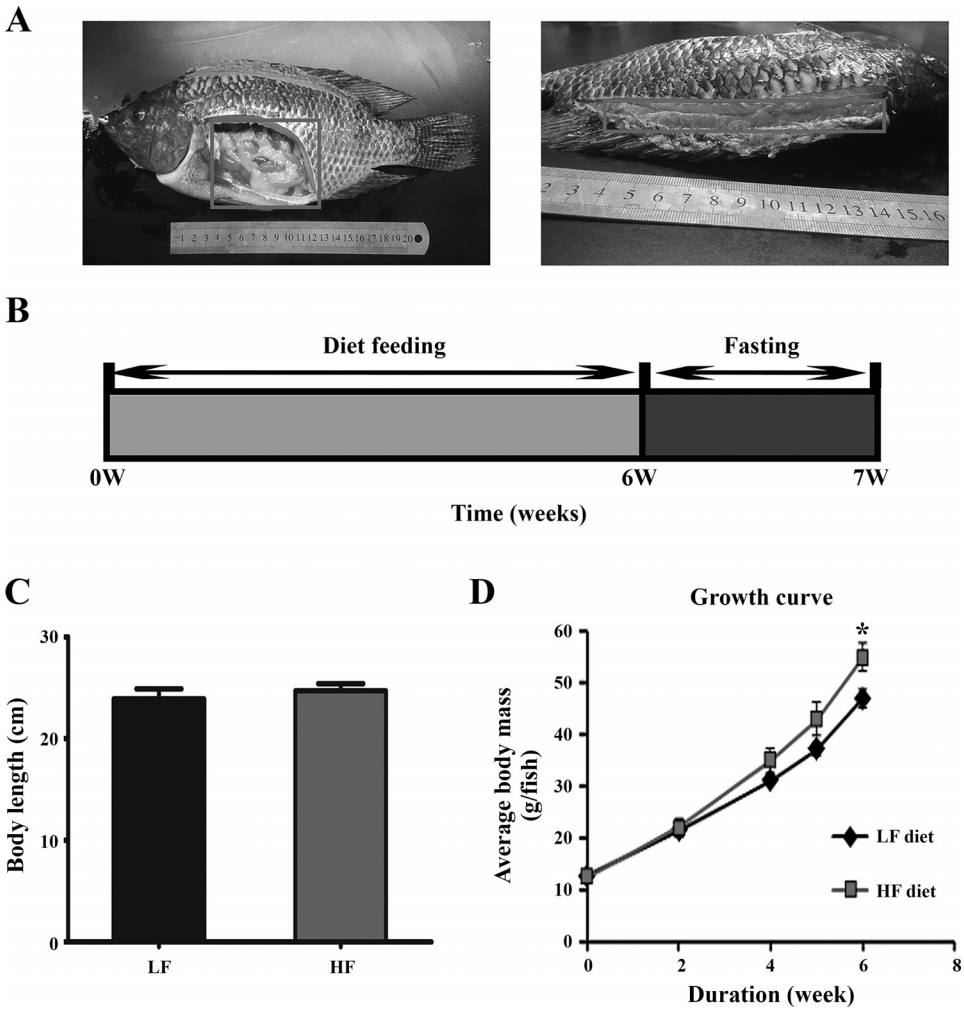


Fig. 1. Position of visceral and subcutaneous adipose tissues, the schedule of the feeding experiment and the growth performance after a 6-wk feeding trial. *A*: position of VAT and SCAT. *B*: schedule of the feeding and fasting experiment. *C*: body length of the fish after a 6-wk feeding trial. *D*: growth curve of the fish fed with high-fat (HF) or low-fat (LF) diet plotting with the average body mass (g/fish) at 0, 2, 4, and 6 wk during the feeding trial All data are presented as means ± SE ($n = 15$). The significant differences between groups were detected using the independent *t*-test. *Statistical significance at $P < 0.05$.

The fish were fasted 24 h before sampling and were anesthetized using MS-222 (7 g/l). The positions of dissection of VAT and SCAT are shown in Fig. 1*A*. Some freshly isolated ATs were collected for histological study, and some were frozen in liquid nitrogen (N_2) and stored at −80°C until analyzed.

In vivo measurement of ATs by MRI. An MRI technique was used to measure the distribution of VAT and SCAT in fish and their response to energy intake flux, 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 protocol and scanning parameters for Nile tilapia AT measurement have been reported in our recent publication (51). Briefly, 10 fish per group (5 fish per tank) were anesthetized with MS-222, and the body surface was dried before MR scanning. The parameters used were field of view (FOV) = 140 × 40 mm^2, matrix = 320 ×320, averages = 1, echo time (TE) = 9.8 ms, and repetition time (TR) = 350 ms. Using the above optimized MRI conditions, we scanned sagittal and coronal slices of 10 fish. The thickness of each scanning slice was 0.2 mm. All images were obtained using T_1-weighted SE imaging. During the assay, the minimum TE value was used in each protocol, and the duration of each scan was less than 10 min. The volume of ATs in each slice with 0.2-mm thickness was calculated according to the method described previously (42), and the total volume of VAT and SCAT in the whole body was calculated by summing the individual AT volumes of the slices.

Histological study and cellular size measurement. At the end of the trial, the freshly isolated ATs were immediately placed in freshly made ice-cold 4% paraformaldehyde for 24 h. The samples were dehydrated in graded series of ethanol, followed by xylene (two times), and embedded in paraffin. Serial section (7 μm in thickness) were prepared, deparaffinized, dehydrated, and stained with hematoxylin and eosin (H&E) by following the standard procedure (12). To compare the sizes of adipocytes between the two ATs, we measured long-axis diameters of at least 200 adipocytes for VAT and SCAT in the H&E slices using the Image-Pro Plus 6.0. The histogram of adipocyte long-axis diameter was made by the Origin 9.0.

Lipid extraction and fatty acid composition analysis. The lipid content and fatty acid composition in tissues could reflect metabolic capability; in particular, the latter is used to indicate fatty acid catabolic preference. Thus, we extracted the total lipid and measured the fatty acid composition of both ATs. The lipid extraction and fatty acid analysis of ATs and isolated adipocytes (APCs) were performed as described previously (18, 19). Briefly, the total lipid of ATs and APCs was extracted using chloroform-methanol 2:1 (vol/vol), and 100 mg/l butylated hydroxytoluene was added as an antioxidant. Each sample was flushed with oxygen-free nitrogen for ~1 min and stored at 4°C for 24-h extraction. Fatty acid methyl esters (FAMEs) were prepared from the lipid fraction mixed with 4 ml containing 15% acetyl chloride–methanol solution in a sealed vial at 70°C for 3 h. After extraction in hexane, 30 μl (50 g/l) C19:0 as a surrogate standard was added. FAMEs were separated by GC using Agilent 7890A GC and 5975C GC/MS, equipped with a fused silica capillary column (Agilent CP-Sil 52 CB). The temperature-time programming was 170°C for 5 min; 240°C for 20 min with all intervening temper-

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ature ramps being at 10°C/min. The FAME levels were quantified by internal standard calibration. The triglyceride content in the extracted lipid from APCs was measured by using thin-layer chromatography, as described previously (15).

Primary adipocyte culture and the measurement of thyroid hormone-induced lipolysis. Lipolysis is one of the basic physiological functions of APCs; therefore, we isolated primary APCs from both ATs and measured the glycerol release from APCs stimulated by thyroid. Both ATs were separated from the fish fed with the commercial diets, which could be regarded as under normal state, because it was difficult to obtain enough APCs from the small mass of SCAT from the LF diet-fed fish and also to avoid potential disturbance of long-term intake of HF diet on normal functions of APCs. Four Nile tilapia were anesthetized with MS-222, and the mature VAT and SCAT APCs were isolated, as described previously (39, 53). Briefly, the AT was cut into 1-mm^3 pieces and incubated for 60 min (28°C) in polypropylene tubes with DMEM-F12 containing collagenase type II (130 U/ml) and 1% BSA. The resulting cell suspension (10^5 cell/ml) was incubated for 1 h (28°C, 5% CO_2) in the absence or presence of thyroid hormone (T3, 3,5,3′-triiodothyronine) (41). At the end of the incubation period, cell-free aliquots of the media were collected for measurement of glycerol release by using the glycerol kit (Jiancheng, Nanjing, China). Considering that the cellular content of triglycerides (TG), which is the source of lipolysis, might be different between two types of APCs, we also measured the TG contents in the isolated mature APCs from VAT and SCAT. This value was used to standardize the APC glycerol release using the formula: (glycerol release/million APC)/(TG content/million APC). For each sample, at least four replicates were analyzed. This experiment was repeated three times. At each time before experimental culture, the cellular viability of APCs was tested by measuring the lactate dehydrogenase leakage, as described previously (12). High cellular viability was obtained by using the mature APC isolation protocol, as described above.

RNA extraction and gene expression. We measured the mRNA expressions of the genes related to fatty acid β-oxidation, lipolysis, adipogenesis, APC differentiation, inflammation, and brown adipocyte markers to compare the molecular characteristics between the two AT forms. Total RNA was extracted from the frozen ATs (50–100 mg) by TRIzol reagent, according to the manufacturer's protocol. The quality of the isolated RNA was assessed by the 260/280 nm absorbance ratio by using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The cDNAs of the total RNA were synthesized using a PrimeScript RT reagent kit with gDNA Eraser (Perfect Real-Time) (TaKaRa, Dalian, China). β-Actin was used as the reference gene. All of the primers used for quantitative RT-PCR (qPCR) were designed to overlap an intron (Table 2). qRT-PCR was conducted by using SYBR Green Premix Ex Taq (TaKaRa) in a CFX96 real-time PCR system (Bio-Rad, Hercules, CA). Quantitative RT-PCR efficiency was between 98% and 102%, and the correlation coefficient was more than 0.97 for each gene. Each PCR was performed in triplicate, and negative controls (no cDNA) were included. The relative cDNA abundance was estimated using the $2^{-\Delta\Delta Ct}$ method: $\Delta\Delta C_t = (Ct_{target} - Ct_{\beta\text{-actin}}) - (Ct_{control} - Ct_{\beta\text{-actin}})$.

Statistical analysis. Results are expressed as means ± SE. All data first passed the Gaussian distribution test, and then the significant differences ($P < 0.05$) of each variable were measured between VAT and SCAT, and between HF and LF, treatments were detected using the independent *t*-test. To explore the potentially existing interactions among different treatments and tissues, two-way or three-way ANOVA analyses were performed in some indexes. All analyses were conducted using the IBM SPSS Statistics 21 (IBM, New York, NY).

Table 2. *Primers used for analysis of gene expression*

Genes	5′-Sense Primer-3′ 5′-Antisense Primer-3′
Fatty acid β-oxidation	
CPT-1b	AAGGGACGTTACTTCAAGGTG
	TCCGACTTGTCTGCCAAGAT
CPT-1a	TTTCCAGGCCTCCTTACCCA
	TTGTACTGCTCATTGTCCAGCAGA
PPARα	CTGATAAAGCTTCGGGCTTCCA
	CGCTCACACTTATCATACTCCAGCT
ACO	AGTCCCACTGTGAGCTCCATCAA
	CAGACCATGGCAGTTTCCAAGA
Mitochondrial gene	
CYB	ACATCGGACGAGGCTTATATTAC
	TAGTTAGGAGGAGGAGAATG
Lipolysis	
ATGL	AAAACGTCCTGGTGACCCAGT
	TAGGAGGAATGATGCCACAGTACA
HSL	AACCTGGATGTCCATTTCTGGAAG
	TCGGTTTACCTTGACTTGAGTGGA
MGL	ACATCGTCAACGCAGACGGATT
	CACAATGTTCCCCAGCTCCAT
Adipogenesis	
FAS	TCATCCAGCAGTTCACTGGCATT
	TGATTAGGTCCACGGCCACA
SREBP1c	TGCAGCAGAGAGACTGTATCCGA
	ACTGCCCTGAATGTGTTCAGACA
APC differentiation	
PPARγ	TGGACTACACAAACATGCACAGC
	CACGGGACTATCTGAGTACTGTGGA
C/EBPα	CTTGGCGGTGAGATCGGAGACA
	GGTGGAACAGGTCAGCCAGGAA
Inflammatory factor related	
TGF-β_1	AAGAGGAGGAGGAATACTTTGCCA
	GAAGCTCATTGAGATGACTTTGGG
IL-10	CAGCAGCAGGAGCATCAGCATT
	CACAGGAGGACGGTCTGAGAAGT
TNFα	CAGAAGCACTAAAGGCGAAGAACA
	TTCTAGATGGATGGCTGCCTTG
IL-1β	GAGCACAGAATTCCAGGATGAAAG
	TGAACTGAGGTGGTCCAGCTGT
MIF	AGCAGAAGCAGGAAGGCGAAGA
	CGGTACATCACCTCTGGCAACATT
Brown adipose tissue marker	
Tbx15	CAAAGCGGGCAGGAGGATGTTT
	GTTGTCCACAGGCACTATGTCCAT
Mylpf	ACGGTCAGAGGATGGCAATAGGAG
	GTGGCGATCTGGATGATTGGATAGC
Hoxc8	CACTGGAGCCAACTTACTACGACTG
	ACATGATGGGATGGGTGCTGAAAG
Reference genes	
β-Actin	CAGGATGCAGAAGGAGATCACA
	CGATCCAGACGGAGTATTTACG

CPT-1b, carnitine palmitoyltransferase 1b; CPT-1a, carnitine palmitoyltransferase 1a; PPARα, peroxisome proliferator-activated receptor α; ACO, acyl-CoA oxidase; CYB, cytochrome *b*; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; MGL, monoacylglycerol lipase; FAS, fatty acid synthase; SREBP1c, sterol regulatory element binding protein-1c; PPARγ, peroxisome proliferator-activated receptor γ; C/EBPα, CCAAT enhancer-binding proteins; TGF-β_1, transforming growth factor-β_1; IL-10, interleukin-10; TNFα, tumor necrosis factor α; IL-1β, interleukin-1β; MIF, macrophage migration inhibitory factor; Tbx15, T-box 15; Mylpf, myosin regulatory light chain; Hoxc8, Homeobox C8.

RESULTS

Effect of dietary lipid content and fasting on body growth and AT mass. After 6 wk, the mean feed intake (g/fish, 48.3 vs. 46.7, HF vs. LF) and fish body length (24.7 cm vs. 23.9 cm, HF vs. LF; Fig. 1*C*) between HF and LF groups was comparable, but the HF group had a higher final body mass than the LF group (average fish mass, 54.8 g vs. 46.9 g, HF vs. LF; Fig. 1*D*). The MRI measurement indicated that the VAT was significantly larger than the SCAT in all treatment groups (Fig. 2, *A–D*). Additionally, the volume of VAT and SCAT was

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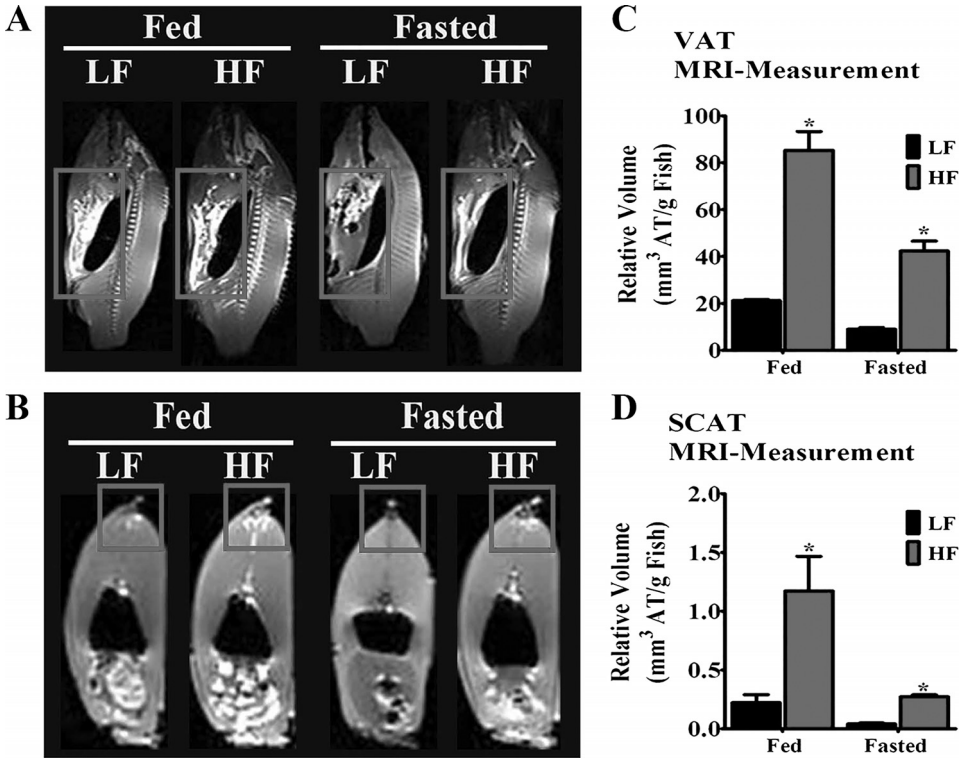


Fig. 2. Quantitative analysis of adipose tissues using MRI. *A* and *B*: position and size of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT; solid line). *C* and *D*: The AT volume-to-body mass ratio. All data are presented as means ± SE ($n = 10$). The significant differences between groups were detected using the independent *t*-test. *Statistical significance at $P < 0.05$.

significantly higher in fish that were fed than those that were fasted and also higher in fish fed with HF diet than those fed with LF diet.

Morphological characteristics of VAT and SCAT. Visually, the abundance of adipocytes in HF-fed fish was higher than in LF-fed fish or fasted fish for both VAT and SCAT. SCAT tended to contain more microvessels and collagen fibers than VAT (Fig. 3*A*). The sizes of VAT adipocytes (APCs) were generally more consistent and more circinal than SCAT APCs; however, SCAT APCs were longer than VAT APCs. The long-axis diameter of VAT APCs in the LF-fed, HF-fed, LF-fasted, and HF-fasted groups was 20–100 μm, 40–120 μm, 20–100 μm, and 40–160 μm, respectively (Fig. 3*B*). The long-axis diameter of SCAT APCs in the LF-fed, HF-fed, LF-fasted, and HF-fasted groups was 20–160 μm, 40–220 μm, 40–160 μm, and 40–220 μm, respectively. The average values of the long-axis diameters of APCs in each group are indicated in Fig. 3*B*. Although no significant differences were found among groups, a trend of long-axis diameters of the SCAT APCs in fed or fasted state being longer than the VAT APCs was observed. This was primarily caused by the presence of a greater number of large APCs in SCAT than in VAT.

Lipid content and hormone-stimulated lipolysis in VAT and SCAT. The lipid content in both ATs and the TG content in VAT increased significantly with increasing dietary lipid content, but the TG contents in SCAT were comparable among groups (Fig. 4, *A* and *B*). The lipid and TG content in VAT were significantly higher than in SCAT. Three-way ANOVA analyses (statistical data not shown) indicated that the total lipid content was significantly affected by diet and AT location, respectively. TG content was significantly affected by nutritional state, diet, and AT location, respectively. However, no interaction was found in total lipid and TG between all of these factors. We further evaluated the lipolytic capability of the APCs from the two ATs in response to thyroxine (T3) stimulation. Two-way ANOVA test indicated that T3 and AT location had significant effects on the results, respectively (statistical data not shown). In fact, T3 stimulation had no effect on glycerol release in the VAT APCs, but stimulated lipolysis in a dose-dependent manner in SCAT APCs (Fig. 4*C*). The glycerol release (per 10^5 cells) from the SCAT APCs was significantly lower than those from the VAT APCs in the control or T3 groups. However, because the TG content in VAT APCs was significantly higher than in SCAT APCs (Fig. 4*D*), after standardization, APC glycerol release from the two types of ATs was comparable in the absence of T3 in the culture medium (Fig. 4*E*). In contrast, in the presence of T3, the SCAT APCs had significantly higher glycerol release than VAT APCs (Fig. 4*E*). Two-way ANOVA test indicated that in Fig. 4*E*, glycerol release was significantly affected by T3 and AT location, and an interaction between the both factors was found (statistical data not shown).

Fatty acid composition of VAT and SCAT. The fatty acid composition differed between the VAT and SCAT, either in the HF diet or LF diet-fed fish and fasted fish (Fig. 5). The most significant difference was the presence of much higher levels of monounsaturated fatty acids (MUFA) and much lower levels of polyunsaturated fatty acids (PUFA) in SCAT relative to VAT, either in HF or LF group. The only exception was in the LF diet/fasted state that the MUFA percentage was lower than that in the HF diet/fasted state (49.7 vs. 62.4%), and the MUFA percentage was comparable between both ATs in the LF diet/fasted state. Except this, the relative differences of FA composition between VAT and SCAT were similar in HF or

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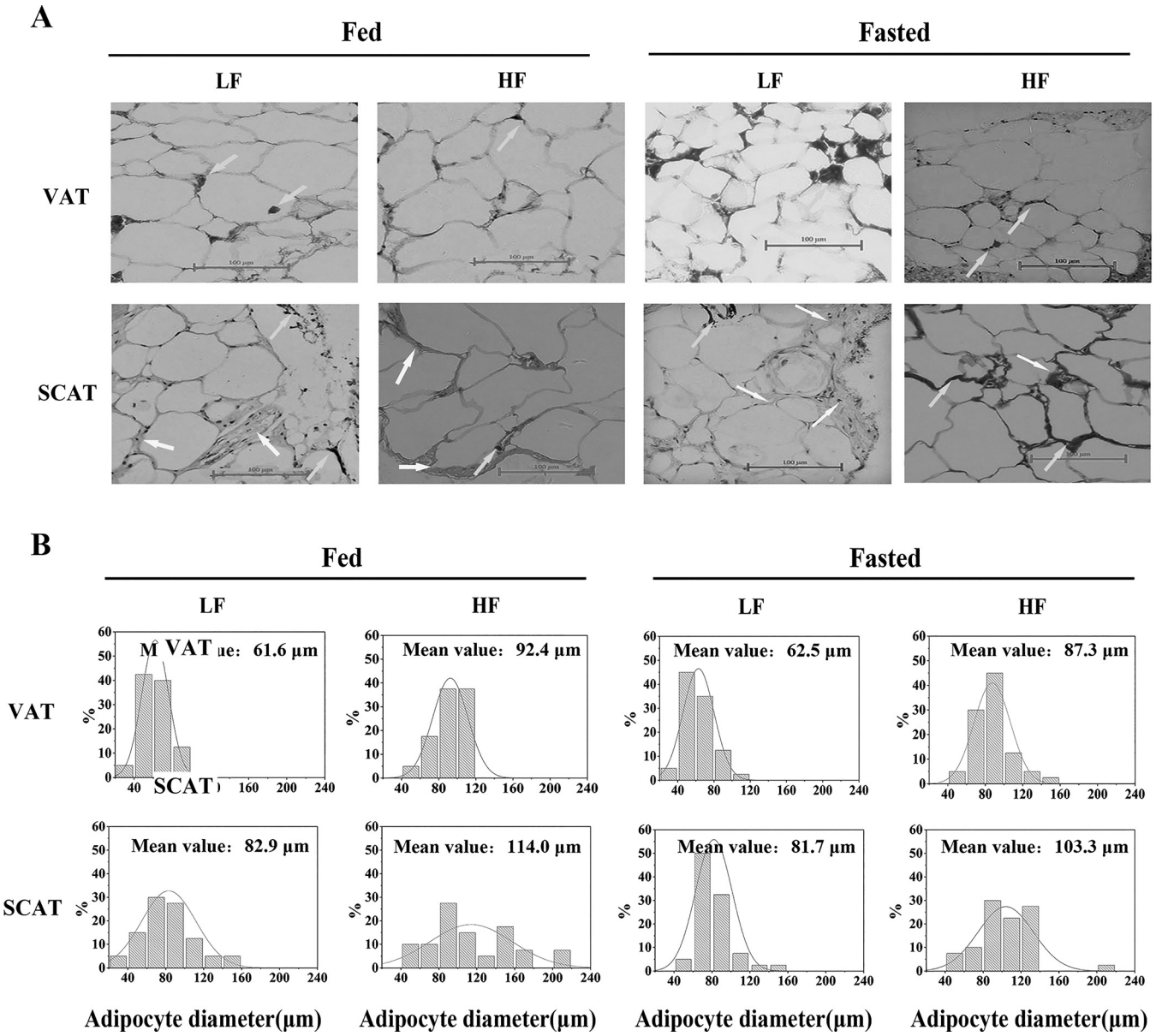


Fig. 3. Histological characteristics of visceral and subcutaneous adipose tissues. *A*: histology characteristics of adipose tissues with hematoxylin-and-eosin staining. The microvessels and collagen fibers are marked by green and yellow arrows, respectively. *B*: histogram of the long-axis diameter of the adipocytes from VAT and SCAT. The mean value of the long-axis diameters of the adipocytes in each group is presented in *B*.

LF feeding states, indicating dietary lipid intake would not largely change the catabolic preference between VAT and SCAT.

Gene expression levels between the two ATs. Fasting significantly increased the expression of the fatty acid β-oxidation-related genes carnitine palmitoyltransferase 1a and 1b (CPT-1a and CPT-1b) but had no effect on peroxisome proliferator-activated receptor α (PPARα) and acyl-CoA oxidase (ACO) (Fig. 6*A*). SCAT had significantly higher expression of CPT-1a in the fasted state and higher PPARα expression and mitochondrial DNA content in fed and fasted states than VAT. However, the expression of ACO was lower in SCAT than in VAT in the fasted state. Among the lipolysis-related genes, adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) were upregulated in VAT during the fasting period, whereas only HSL expression was increased by fasting in SCAT (Fig. 6*B*). SCAT had lower ATGL expression than VAT in the fasted state but had higher levels of monoacylglycerol lipase (MGL) expression than SCAT in both the fed and fasted states. Among the adipogenesis-related genes, fasting significantly decreased the expression of fatty acid synthase (FAS) in both SCAT and VAT but decreased sterol regulatory element binding protein-1c (SREBP-1c) expression only in SCAT (Fig. 6*B*). SCAT had higher expression of SREBP-1c than VAT in the fed state. For the two genes related to APC differentiation, peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT enhancer-binding proteins (C/EBPα), SCAT had significantly lower expression of both genes than VAT in the fed and fasted states, showing VAT has higher potential to be involved in APC differentiation than SCAT.

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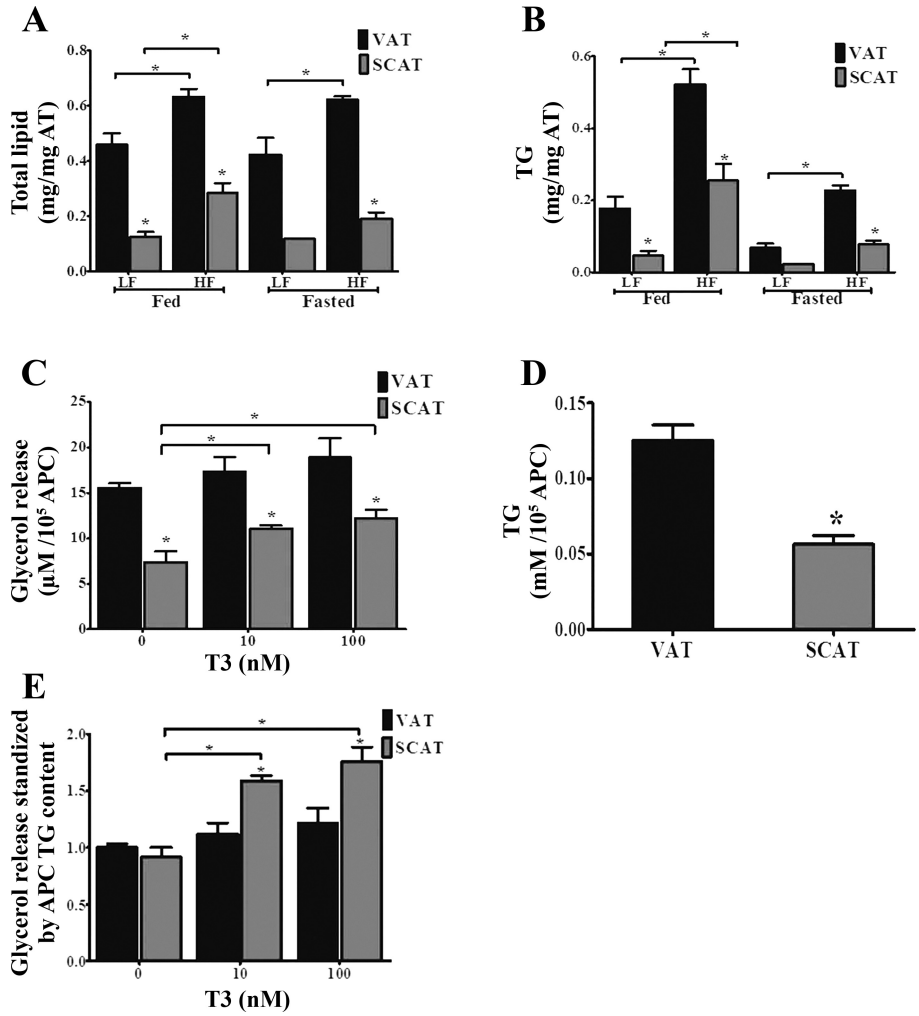


Fig. 4. Lipid concentration of adipose tissues/adipocytes and the hormone-stimulated lipolysis of adipocytes. *A*: the lipid concentrations of VAT and SCAT. *B*: triglyceride. (TG) concentrations of VAT and SCAT. *C*: thyroid hormone (T3, 3,5,3′-triiodothyronine)-stimulated glycerol release of the adipocytes, which were isolated from the Nile tilapia fed with common commercial diet. *D*: TG concentrations of the APCs from VAT and SCAT, which were separated from commercial diet-fed fish. *E*: glycerol release of the adipocytes stimulated by thyroid hormone standardized by the TG content of APCs from two ATs. All data are presented as means ± SE (n = 4–12). The significant differences between groups were detected using the independent t-test. *Statistical significance at $P < 0.05$.

Five inflammation-related genes (transforming growth factor-β1, TGF-β_1; interleukin-10, IL-10; tumor necrosis factor-α, TNF-α; interleukin-1β, IL-1β; and macrophage migration inhibitory factor, MIF) were measured (Fig. 6*D*). Fasting had no significant effect on the expression of these genes. However, TGF-β_1 and IL-10, two anti-inflammation genes, were significantly higher in SCAT than in VAT, but the expression of TNF-α, a well-known inflammatory factor, was significantly higher in VAT than SCAT. The expression of another inflammatory gene, IL-1β, and a microphage marker gene, MIF, had statistically similar expressions between the two AT types. To investigate the cellular characteristics of both APCs, we also measured three marker genes related to white AT (WAT), "brite" (brown in white) AT, and brown AT (BAT) (Fig. 6*E*). The mRNA expression of T-box 15 (Tbx15), a BAT marker, was higher in SCAT than in VAT. A similar trend was also found in myosin regulatory light chain (Mylpf), another gene that normally has higher expression in BAT than in WAT. Noteworthy, the expression of homeobox C8 (Hoxc8), a marker gene for WAT and brite AT, was also higher in SCAT than in VAT.

All gene expression data were also analyzed by two-way ANOVA test (statistical data not shown). Among them, the expression of CPT-1a, CPT-1b, Mt-DNA, ATGL, SREBP-1c, and Tbx15 was significantly affected by nutritional states and AT locations. The expression of PPARα, ACO, MGL, PPARγ, C/EBPα, TGF-β_1, TNF-α, and Hocx8 was only significantly affected by AT location. The expression of HSL and FAS was only significantly affected by nutritional states. The significant interactions of nutritional state and AT location were found in the expressions of CPT-1a, ATGL, and SREBP-1c. These indicate that AT location affected the majority of these genes, suggesting again that SCAT and VAT play different roles in the global metabolic regulation.

DISCUSSION

The different types of ATs are distributed in various positions in animals. However, in fish, the comparison of SCAT and VAT has only been documented in a few species in terms of their anatomic locations and masses. The present results show that VAT is the primary form of AT in Nile tilapia, and SCAT occurs primarily under the dorsal fin and has smaller mass than VAT. Moreover, VAT APCs also contain higher lipid content than SCAT APCs. Therefore, VAT is the primary location for fat deposition in Nile tilapia. Similarly, in other fish species, such as rainbow trout, grass carp, pompano, and large yellow croaker, VAT is also located in the abdominal cavity around the digestive tracts and is the primary type of AT, whereas SCAT is located primarily in the dorsal and

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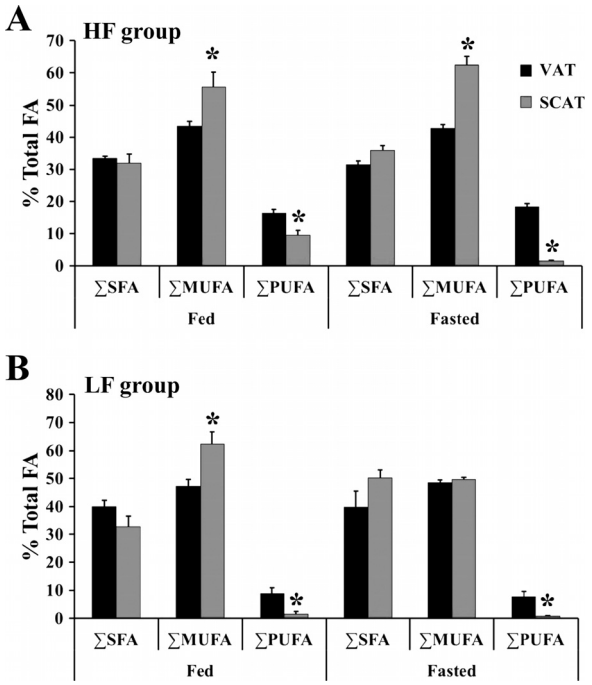


Fig. 5. Fatty acid composition of visceral and subcutaneous adipose tissues in fed or fasted states. *A*: fatty acid composition in HF-diet group. *B*: fatty acid composition in LF-diet group. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. All data are presented as means ± SE ($n = 4$). The significant differences between groups were detected using the independent *t*-test. *Statistical significance at $P < 0.05$.

ventral zones (49, 51). However, in turbot, SCAT located under the skin is the most dominant form of AT (51). This indicates that the distribution patterns of ATs vary among fish species. Therefore, except for Nile tilapia, of which the functional differences between VAT and SCAT have been described in the present study, further investigations should be performed in other species to elucidate the functions of different AT patterns.

VAT and SCAT have various morphological characteristics that likely explain their differences in biological functions. In mammals, VAT contains greater numbers of large APCs than SCAT, which generally contains small APCs (27). Moreover, large mammalian APCs are insulin-resistant and hyperlipolytic, whereas small APCs are more insulin-sensitive and have higher levels of FFA and TG uptake. However, in our study, the sizes of VAT APCs in Nile tilapia were generally more consistent and more circinal than SCAT APCs. Furthermore, SCAT contained a greater number of large and long APCs than VAT. In addition, SCAT APCs, but not VAT APCs, had higher lipolytic activity. These results are contrary to the findings in mammals, highlighting differences in APC structures between SCAT and VAT among various animals. In fact, even among fish species, a species-dependent diversity in APCs exists from VAT and SCAT. For instance, the mean size of collagenase-isolated VAT APCs from rainbow trout and carp was greater than the SCAT APCs (in both the dorsal and ventral regions) (53). However, in Atlantic salmon, there was no difference in the mean size of isolated APCs between SCAT (in the ventral position) and VAT (5). Very recently, a study also reported that SCAT APCs had larger size than VAT APCs in the HF diet-fed zebrafish (31), which is in accordance with the results of our present study. Considering the correlation between APC size and its functions has not been studied in other fish species, the present study calls for further studies to investigate the relationship among morphological characteristics, locations, and functions of different ATs to better understand their physiological roles in animals.

In the present study, we also found that SCAT tends to contain more nonadipocyte components, including more blood cell/vessels residue and collagen fibers than VAT, consistent with results in rats (12). In mammals, these nonadipocyte components also comprise some stromal-vascular cells, such as macrophages, neutrophils, and fibroblasts, which are commonly believed to secrete higher levels of proinflammatory cytokines and eicosanoids than APCs. A number of studies have indicated that the inflammatory characteristics in different ATs are correlated with the infiltration of macrophages (21). However, in the present study, the expression levels of several macrophage-related genes, such as TGF-β_1, TNF-α, and MIF, were lower in SCAT than those in VAT, showing that the composition of nonadipocyte components in Nile tilapia are different from mammals. Since the infiltration of stromal-vascular cells and the related gene markers in fish adipose tissue have also not been intensively studied, the potential filtration of stromal-vascular cells in different ATs in fish requires further investigation.

A number of mammalian and human studies indicate that the deposition and utilization of VAT and SCAT differ depending on nutritional or physiological states (32, 33). In fish, the effects of quantitative and qualitative modification of nutrition on VAT deposition have been well documented (49). Diets containing excess nutrients, including high-fat or high-carbohydrate diets, often induce a significant increase in VAT mass and result in a number of adverse effects (5, 6, 20). Conversely, short- or long-term fasting also decreases VAT mass in some fish species (8). However, until now, there has been no comparison on the changes of VAT and SCAT under different nutritional states and dietary energy content. Our results suggest that both VAT and SCAT masses increase significantly with an increase in dietary lipid content from 40 to 150 g/kg, and also significantly decrease during 1 wk of fasting. Correspondingly, the assay of genes in HF diet-fed fish also revealed an increase in the expression of lipid catabolism-related genes, such as CPT-1a, CPT-1b, ATGL, and HSL, and a decrease in the expression of the adipogenesis-related key gene FAS in both AT types during the fasting period, suggesting again that, VAT and SCAT have different functions in fish, as in mammals. It should be noted that, although both VAT and SCAT masses decreased after 1 wk of fasting, the sizes of the APCs for both ATs were almost comparable before and after fasting. As discussed above, the deposit location and the shape of ATs varies largely among different fish species. In Nile tilapia, the VAT (mesenteric fat) and SCAT both are shaped like a belt revolving around the intestine or existing under the skin, respectively. We observed that 1 wk of fasting could only exhaust some parts of the AT belt, but could not make the whole AT belt disappear. In fact, the sizes of the APCs and the lipid concentrations of the remaining ATs did not significantly differ after 1 wk of fasting (Figs. 3 and 4). The precise mechanisms underlying this phenomenon require further investigation.

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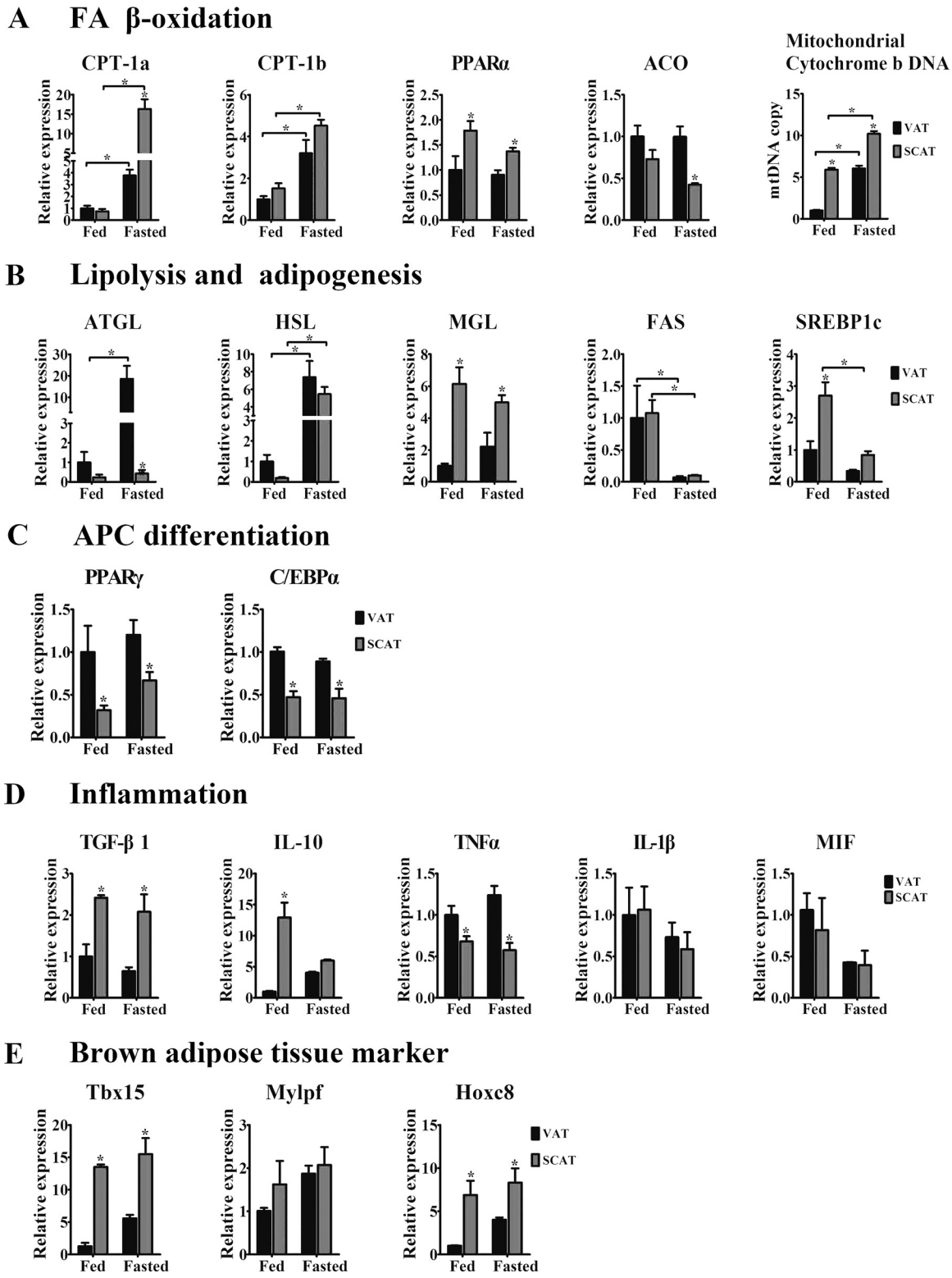


Fig. 6. Gene expression of visceral and subcutaneous adipose tissues in fed or fasted states. *A*: fatty acid β-oxidation related genes and mitochondrial DNA. *B*: lipolysis and adipogenesis-related genes. *C*: adipocyte differentiation-related genes. *D*: inflammation related genes. *E*: brown/brite adipose tissue marker genes. β-actin was used as the reference gene. All data are presented as means ± SE ($n = 6$). The significant differences between groups were detected using the independent *t*-test. *Statistical significance at $P < 0.05$.

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In mammals, the variation in the ability of the different ATs to release FFAs is thought to be the primary reason for the distinction in the physiological functions of the ATs. Elevated FFA concentrations in circulation not only induce insulin resistance (27), but also lead to the accumulation of lipids in nonadipose tissues, resulting in cellular dysregulation and functional impairment in liver (10) and muscle (38). VAT has higher lipolytic ability than SCAT (27), whereas SCAT has a higher contribution to the absorption of FFAs from circulation. However, some studies have also showed that the contribution of VAT lipolysis to systemic FFA availability only accounts for less than 5% of the total FFA burden (34). Thus, it has been argued that SCAT, especially in the upper body, is the major contributor to the supply of FFAs to extra-hepatic tissues in insulin-resistant states. Therefore, the ability to secrete FFAs is an important index for evaluating the contributions of different ATs to systemic energy homeostasis. In the present study, we measured the T3-stimulated lipolytic ability of mature APCs isolated from VAT and SCAT (Fig. 4, *B* and *C*) and found that SCAT APCs had significantly higher lipolytic efficiency than VAT APCs when the results were standardized by APC TG content. This is opposite to the general results in mammalian APCs (27). Because the locations and functions of adipose tissues are likely to be different, even among fish species (4, 47), the variations in APCs between VAT and SCAT are species-dependent. Remarkably, the APCs from both ATs were collected from fish fed a normal commercial diet. Thus, the differences in the lipolytic abilities of the two AT types may reflect a lipolytic difference between VAT and SCAT under a normal state. However, in the HF diet-fed fish, the mRNA expression of ATGL, a key enzyme in AT lipolysis, was higher in VAT than SCAT, although SCAT also had higher mRNA expression of MGL than VAT. Given that VAT is the primary site for lipid deposition in Nile tilapia, the high expression level of ATGL in VAT may provide a protective effect against excess lipid accumulation in VAT. Indeed, we recently found that Nile tilapia can adapt to high energy intake by increasing APC proliferation in VAT, a phenomenon that is partly mediated by increased expression of PPARγ, which is upregulated by the increased lipolysis-sourced free fatty acids in APCs (33). Similarly, PPARγ mRNA expression was higher in VAT than in SCAT in the present study. Although the T3-induced lipolysis test revealed high lipolytic ability of SCAT under a normal state, the total mass and net lipid content of SCAT in Nile tilapia were much less than those of VAT. Thus, VAT is likely to be the dominant contributor of total FFAs circulating in Nile Tilapia. In addition, some researchers have reported that, intramuscular adipose tissue exists in some fish species and plays roles in endocrinological regulation by secreting endocrine hormones such as adiponectin (24, 29, 37). Therefore, adipose tissues in different anatomic locations in fish are important endocrinological organs, and this aspect deserves more study in the future.

In addition to lipolysis, other metabolic processes, including adipogenesis and fatty acid β-oxidation, also play roles in systemic energy homeostasis in APCs (3, 9). In the present study, we measured a number of genes related to adipogenesis and fatty acid β-oxidation. Increased expression of CPT-1a and CPT-1b, which are the key enzymes in fatty acid β-oxidation in SCAT, suggests that SCAT has higher mitochondrial β-oxidation efficiency, which is also evidenced by the higher mitochondrial DNA content in SCAT than VAT. In support of this, the fatty acid composition data indicated that SCAT contained more MUFA and less PUFA than VAT, except that in the LF diet/fasted state, the MUFA percentage was comparable between both ATs. Taking into account that in most animals, MUFA can be efficiently β-oxidized to produce ATP, whereas PUFA is primarily esterified into phospholipids (9), the higher levels of MUFA and lower PUFA content in SCAT, either in HF or LF diet feeding state, also suggest that this tissue plays an important role in lipid catabolism. Of note, as compared with the MUFA percentage (62.4%) in the SCAT in the HF diet/fasted state, the decreased MUFA percentage (49.7%) in the SCAT in the LF diet/fasted state could be explained by the high utilizing efficiency of MUFA when the energy intake was exhausted in the LF diet/fasted situation. This supports the point again that higher MUFA accumulation in SCAT is helpful for energy production. Interestingly, we also found higher expression of BAT/brite AT marker genes in SCAT than in VAT in the present study, which is in accord with results in mammals, in which BAT remodeling predominantly occurs in the subcutaneous depot rather than in the visceral depot (4). It should be noted that these BAT/brite AT marker genes had only been tested in mammals (46), and previous studies have indicated that some BAT-specific proteins, such as uncoupling protein-1 (UCP-1), have different functions and locations between mammals and teleost fish (28). However, although there is no current evidence showing BAT/brite AT existence in teleost fish (28), the possibility that teleost or some species has BAT/brite AT or BAT/brite AT-like ATs cannot be excluded. At least, our present study indicated that SCAT contained more mitochondria and higher β-oxidation efficiency than VAT in Nile tilapia. Furthermore, the higher level of SREBP-1c expression, a key nuclear receptor in lipid synthesis, in SCAT than VAT also suggests that SCAT may have higher lipid synthesis efficiency when excess energy is provided. Some studies have indicated that the metabolism and the expression of related genes are largely changed in different APC differentiation status (32). In the present study, the higher expression of PPARγ and C/EBPα, which are two essential factors in APC differentiation (43), was observed in VAT than in SCAT, indicating that VAT has a higher potential to play a role in APC differentiation than SCAT. However, because the different statuses of APC differentiation simultaneously exist in the whole ATs, the comprehensive effects of these statuses on some given genes in a whole AT are actually difficult to evaluate.

In mammals, excess VAT deposition is associated with elevation of circular inflammatory factors and increased risk of developing metabolic syndromes (30). In the present study, through measuring the mRNA expression of the inflammatory marker genes in VAT and SCAT of Nile tilapia, we found the mRNA expression of $TGF\beta_1$ and IL-10, the two anti-inflammatory genes, were significantly lower in VAT than in SCAT, and, conversely, the mRNA expression of inflammatory factor TNF-α in VAT was significantly higher than in SCAT, suggesting that VAT in Nile tilapia is more likely to be involved in the systemic inflammatory response than SCAT. Considering that, VAT is the primary AT in fish, and excess energy intake mainly induces an increase in VAT mass in fish (26), the potential of VAT-induced systemic inflammation should also be noted.

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Perspectives and Significance

For a long time, the SCAT in fish has not been extensively explored. The present study indicates that SCAT has more large-sized APCs, greater lipolytic efficiency, higher mitochondrial DNA, and higher gene expressions for fatty acid β-oxidation, adipogenesis, and brown adipose tissue, but has lower gene expression for inflammation and adipocyte differentiation than VAT in Nile tilapia. In short, our results mainly suggest that SCAT has higher lipid catabolic activity than VAT in Nile tilapia. The observed structural, biochemical, and physiological differences between VAT and SCAT should be considered in future metabolism research using fish as models, either in biomedical or aquaculture studies.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Z.-Y.D. conceived and designed research; Y.-W.W., J.-L.Z., and J.-G.J. performed experiments; Y.-W.W., J.-L.Z., J.-G.J., X.-X.D., F.Q., M.-L.Z., D.-L.L., and Z.-Y.D. analyzed data; Y.-W.W., X.-X.D., S.M.L., M.-L.Z., D.-L.L., and Z.-Y.D. interpreted results of experiments; Y.-W.W., F.Q., and Z.-Y.D. prepared figures; Y.-W.W., S.M.L., and Z.-Y.D. drafted manuscript; S.M.L. and Z.-Y.D. edited and revised manuscript; Z.-Y.D. approved final version of manuscript.

REFERENCES

1. **Abdel TM, Ahmad M, Khattab Y, Shalaby A.** Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus (L.)*. *Aquaculture* 298: 267–274, 2010. doi:10.1016/j.aquaculture.2009.10.027.
2. **Ahima RS, Flier JS.** Adipose tissue as an endocrine organ. *Trends Endocrinol Metab* 11: 327–332, 2000. doi:10.1016/S1043-2760(00)00301-5.
3. **Böttcher H, Fürst P.** Decreased white fat cell thermogenesis in obese individuals. *Int J Obes Relat Metab Disord* 21: 439–444, 1997. doi:10.1038/sj.ijo.0800425.
4. **Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, Giacobino JP, De Matteis R, Cinti S.** The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *Am J Physiol Endocrinol Metab* 298: E1244–E1253, 2010. doi:10.1152/ajpendo.00600.2009.
5. **Bjerkeng B, Refstie S, Fjalestad KT, Storebakken T, Rødbotten M, Roem AJ.** Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. *Aquaculture* 157: 297–309, 1997. doi:10.1016/S0044-8486(97)00162-2.
6. **Bou M, Todorčević M, Fontanillas R, Capilla E, Gutiérrez J, Navarro I.** Adipose tissue and liver metabolic responses to different levels of dietary carbohydrates in gilthead sea bream (*Sparus aurata*). *Comp Biochem Physiol A Mol Integr Physiol* 175: 72–81, 2014. doi:10.1016/j.cbpa.2014.05.014.
7. **Caserta F, Tchkonia T, Civelek VN, Prentki M, Brown NF, McGarry JD, Forse RA, Corkey BE, Hamilton JA, Kirkland JL.** Fat depot origin affects fatty acid handling in cultured rat and human preadipocytes. *Am J Physiol Endocrinol Metab* 280: E238–E247, 2001.
8. **Chatzifotis S, Panagiotidou M, Papaioannou N, Pavlidis M, Nengas I, Mylonas CC.** Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. *Aquaculture* 307: 65–70, 2010. doi:10.1016/j.aquaculture.2010.07.002.
9. **Chegary M, Brinke H, Ruiter JPN, Wijburg FA, Stoll MSK, Minkler PE, van Weeghel M, Schulz H, Hoppel CL, Wanders RJA, Houten SM.** Mitochondrial long chain fatty acid β-oxidation in man and mouse. *Biochim BiophysActa* 1791: 806–815, 2009. doi:10.1016/j.bbalip.2009.05.006.
10. **Degrace P, Demizieux L, Du ZY, Gresti J, Caverot L, Djaouti L, Jourdan T, Moindrot B, Guilland JC, Hocquette JF, Clouet P.** Regulation of lipid flux between liver and adipose tissue during transient hepatic steatosis in carnitine-depleted rats. *J Biol Chem* 282: 20816–20826, 2007. doi:10.1074/jbc.M611391200.
11. **Deng SX, Tian LX, Liu FJ, Jin SJ, Liang GY, Yang HJ, Du ZY, Liu YJ.** Toxic effects and residue of aflatoxin B1 in tilapia (*Oreochromis niloticus* × *O. aureus*) during long-term dietary exposure. *Aquaculture* 307: 233–240, 2010. doi:10.1016/j.aquaculture.2010.07.029.
12. **Du ZY, Ma T, Lock EJ, Hao Q, Kristiansen K, Frøyland L, Madsen L.** Depot-dependent effects of adipose tissue explants on co-cultured hepatocytes. *PLoS One* 6: e20917, 2011. doi:10.1371/journal.pone.0020917.
13. **Du ZY, Clouet P, Degrace P, Zheng WH, Frøyland L, Tian LX, Liu YJ.** Hypolipidaemic effects of fenofibrate and fasting in the herbivorous grass carp (*Ctenopharyngodon idella*) fed a high-fat diet. *Br J Nutr* 100: 1200–1212, 2008. doi:10.1017/S0007114508986840.
14. **Du ZY, Clouet P, Zheng W-H, Degrace P, Tian LX, Liu YJ.** Biochemical hepatic alterations and body lipid composition in the herbivorous grass carp (*Ctenopharyngodon idella*) fed high-fat diets. *Br J Nutr* 95: 905–915, 2006. doi:10.1079/BJN20061733.
15. **Du ZY, Ma T, Winterthun S, Kristiansen K, Frøyland L, Madsen L.** β-Oxidation modulates metabolic competition between eicosapentaenoic acid and arachidonic acid regulating prostaglandin E(2) synthesis in rat hepatocytes-Kupffer cells. *Biochim Biophys Acta* 1801: 526–536, 2010. doi:10.1016/j.bbalip.2010.01.002.
16. **Einstein FH, Atzmon G, Yang XM, Ma XH, Rincon M, Rudin E, Muzumdar R, Barzilai N.** Differential responses of visceral and subcutaneous fat depots to nutrients. *Diabetes* 54: 672–678, 2005. doi:10.2337/diabetes.54.3.672.
17. **Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW.** Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* 145: 2273–2282, 2004. doi:10.1210/en.2003-1336.
18. **Fauconneau B, Alami DH, Laroche M, Marcel J, Vallot D.** Growth and meat quality relations in carp. *Aquaculture* 129: 265–297, 1995. doi:10.1016/0044-8486(94)00309-C.
19. **Folch J, Lees M, Sloane Stanley GH.** A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226: 497–509, 1957.
20. **Gélineau A, Corraze G, Boujard T, Larroquet L, Kaushik S.** Relation between dietary lipid level and voluntary feed intake, growth, nutrient gain, lipid deposition and hepatic lipogenesis in rainbow trout. *Reprod Nutr Dev* 41: 487–503, 2001. doi:10.1051/rnd:2001103.
21. **Galic S, Oakhill JS, Steinberg GR.** Adipose tissue as an endocrine organ. *Mol Cell Endocrinol* 316: 129–139, 2010. doi:10.1016/j.mce.2009.08.018.
22. **Greene DH, Selivonchick DP.** Lipid metabolism in fish. *Prog Lipid Res* 26: 53–85, 1987. doi:10.1016/0163-7827(87)90008-7.
23. **Guyon R, Rakotomanga M, Azzouzi N, Coutanceau JP, Bonillo C, D'Cotta H, Pepey E, Soler L, Rodier-Goud M, D'Hont A, Conte MA, van Bers NE, Penman DJ, Hitte C, Crooijmans RP, Kocher TD, Ozouf-Costaz C, Baroiller JF, Galibert F.** A high-resolution map of the Nile tilapia genome: a resource for studying cichlids and other percomorphs. *BMC Genomics* 13: 222, 2012. doi:10.1186/1471-2164-13-222.
24. **Han Y, Kaneko G, Nagasaka R, Kondo H, Hirono I, Takahashi SI, Watabe S, Ushio H.** Distribution of adipocyte-related cells in skeletal muscle of rainbow trout *Oncorhynchus mykiss*. *Fish Sci* 79: 143–148, 2013. doi:10.1007/s12562-012-0579-0.
25. **Van Harmelen V, Reynisdottir S, Eriksson P, Thörne A, Hoffstedt J, Lönnqvist F, Arner P.** Leptin secretion from subcutaneous and visceral adipose tissue in women. *Diabetes* 47: 913–917, 1998. doi:10.2337/diabetes.47.6.913.
26. **He AY, Ning LJ, Chen LQ, Chen YL, Xing Q, Li JM, Qiao F, Li DL, Zhang ML, Du ZY.** Systemic adaptation of lipid metabolism in response to low- and high-fat diet in Nile tilapia (*Oreochromis niloticus*). *Physiol Rep* 3: e12485, 2015. doi:10.14814/phy2.12485.
27. **Ibrahim MM.** Subcutaneous and visceral adipose tissue: structural and functional differences. *Obes Rev* 11: 11–18, 2010. doi:10.1111/j.1467-789X.2009.00623.x.

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28. **Jastroch M, Wuertz S, Kloas W, Klingenspor M.** Uncoupling protein 1 in fish uncovers an ancient evolutionary history of mammalian nonshivering thermogenesis. *Physiol Genomics* 22: 150–156, 2005. doi:10.1152/physiolgenomics.00070.2005.
29. **Kaneko G, Shirakami H, Hirano Y, Oba M, Yoshinaga H, Khieokhajonkhet A, Nagasaka R, Kondo H, Hirono I, Ushio H.** Diversity of lipid distribution in fish skeletal muscle. *Zool Sci* 33: 170–178, 2016. doi:10.2108/zs150096.
30. **Kelley DE, Thaete FL, Troost F, Huwe T, Goodpaster BH.** Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. *Am J Physiol Endocrinol Metab* 278: E941–E948, 2000.
31. **Landgraf K, Schuster S, Meusel A, Garten A, Riemer T, Schleinitz D, Kiess W, Körner A.** Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealthy obesity. *BMC Physiol* 17: 4, 2017. doi:10.1186/s12899-017-0031-x.
32. **Liu P, Ji H, Li C, Chen LQ, Du ZY.** Morphology, mitochondrial development and adipogenic-related genes expression during adipocytes differentiation in grass carp (*Ctenopharyngodon idellus*). *Sci Bull* 60: 1241–1251, 2015. doi:10.1007/s11434-015-0833-9.
33. **Muhlhausler BS, Duffield JA, McMillen IC.** Increased maternal nutrition stimulates peroxisome proliferator activated receptor-γ, adiponectin, and leptin messenger ribonucleic acid expression in adipose tissue before birth. *Endocrinology* 148: 878–885, 2007. doi:10.1210/en.2006-1115.
34. **Nielsen S, Guo Z, Johnson CM, Hensrud DD, Jensen MD.** Splanchnic lipolysis in human obesity. *J Clin Invest* 113: 1582–1588, 2004. doi:10.1172/JCI21047.
35. **Ning LJ, He AY, Li JM, Lu DL, Jiao JG, Li LY, Li DL, Zhang ML, Chen LQ, Du ZY.** Mechanisms and metabolic regulation of PPARα activation in Nile tilapia (*Oreochromis niloticus*). *Biochim Biophys Acta* 1861: 1036–1048, 2016. doi:10.1016/j.bbalip.2016.06.005.
36. **Ning LJ, He AY, Lu DL, Li JM, Qiao F, Li DL, Zhang ML, Chen LQ, Du ZY.** Nutritional background changes the hypolipidemic effects of fenofibrate in Nile tilapia (*Oreochromis niloticus*). *Sci Rep* 7: 41706, 2017. doi:10.1038/srep41706.
37. **Nishio S, Gibert Y, Bernard L, Brunet F, Triqueneaux G, Laudet V.** Adiponectin and adiponectin receptor genes are coexpressed during zebrafish embryogenesis and regulated by food deprivation. *Dev Dyn* 237: 1682–1690, 2008. doi:10.1002/dvdy.21559.
38. **Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH.** Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46: 983–988, 1997. doi:10.2337/diab.46.6.983.
39. **Saera-Vila A, Calduch-Giner JA, Navarro I, Pérez-Sánchez J.** Tumour necrosis factor (TNF)α as a regulator of fat tissue mass in the Mediterranean gilthead sea bream (*Sparus aurata L.*). *Comp Biochem Physiol B Biochem Mol Biol* 146: 338–345, 2007. doi:10.1016/j.cbpb.2006.11.003.
40. **Santiago CB, Reyes OS.** Effects of dietary lipid source on reproductive performance and tissue lipid levels of Nile tilapia *Oreochromis niloticus* (*Linnaeus*) broodstock. *J Appl Ichthyology* 9: 33–40, 1993. doi:10.1111/j.1439-0426.1993.tb00385.x.
41. **Schmid AC, Lutz I, Kloas W, Reinecke M.** Thyroid hormone stimulates hepatic IGF-I mRNA expression in a bony fish, the tilapia *Oreochromis mossambicus*, in vitro and in vivo. *Gen Comp Endocrinol* 130: 129–134, 2003. doi:10.1016/S0016-6480(02)00577-4.
42. **Thomas EL, Saeed N, Hajnal JV, Brynes A, Goldstone AP, Frost G, Bell JD.** Magnetic resonance imaging of total body fat. *J Appl Physiol (1985)* 85: 1778–1785, 1998.
43. **Vegusdal A, Sundvold H, Gjøen T, Ruyter B.** An in vitro method for studying the proliferation and differentiation of Atlantic salmon preadipocytes. *Lipids* 38: 289–296, 2003. doi:10.1007/s11745-003-1063-3.
44. **Voulodimos AS, Patrikakis CZ, Sideridis AB, Ntafis VA, Xylouri EM.** A complete farm management system based on animal identification using RFID technology. *Comput Electron Agric* 70: 380–388, 2010. doi:10.1016/j.compag.2009.07.009.
45. **Wajchenberg BL.** Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 21: 697–738, 2000. doi:10.1210/edrv.21.6.0415.
46. **Waldén TB, Hansen IR, Timmons JA, Cannon B, Nedergaard J.** Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. *Am J Physiol Endocrinol Metab* 302: E19–E31, 2012. doi:10.1152/ajpendo.00249.2011.
47. **Wang Y, Liu YJ, Tian LX, Du ZY, Wang JT, Wang S, Xiao WP.** Effects of dietary carbohydrate level on growth and body composition of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquacult Res* 36: 1408–1413, 2005. doi:10.1111/j.1365-2109.2005.01361.x.
48. **Weil C, Lebret V, Gabillard JC.** The IGF/IGFBP system in rainbow trout (*Oncorhynchus mykiss*) adipose tissue: expression related to regional localization and cell type. *Fish Physiol Biochem* 37: 843–852, 2011. doi:10.1007/s10695-011-9482-3.
49. **Weil C, Lefèvre F, Bugeon J.** Characteristics and metabolism of different adipose tissues in fish. *Rev Fish Biol Fish* 23: 157–173, 2013. doi:10.1007/s11160-012-9288-0.
50. **Wozniak SE, Gee LL, Wachtel MS, Frezza EE.** Adipose tissue: the new endocrine organ? A review article. *Dig Dis Sci* 54: 1847–1856, 2009. doi:10.1007/s10620-008-0585-3.
51. **Wu JL, Zhang JL, Du XX, Shen YJ, Lao X, Zhang ML, Chen LQ, Du ZY.** Evaluation of the distribution of adipose tissues in fish using magnetic resonance imaging (MRI). *Aquaculture* 448: 112–122, 2015. doi:10.1016/j.aquaculture.2015.06.002.
52. **Xiong Y, Huang J, Li X, Zhou L, Dong F, Ye H, Gan L.** Deep sequencing of the tilapia (*Oreochromis niloticus*) liver transcriptome response to dietary protein to starch ratio. *Aquaculture* 433: 299–306, 2014. doi:10.1016/j.aquaculture.2014.06.009.
53. **Zhou S, Ackman RG, Morrison C.** Adipocytes and lipid distribution in the muscle tissue of Atlantic salmon (*Salmo salar*). *Can J Fish Aquat Sci* 53: 326–332, 1996. doi:10.1139/f95-197.

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