The Role of Noradrenaline on the Lipid Metabolism of Water- and Air-Breathing Fish Species.

Heeswijk, J.C.F. van

Adrenergic Control of Adipose Lipolysis in 3 Teleost Fish Species of Different Ecological Background.

J.C.F. van Heeswijk[1], G.J. Vianen[1], F. Medale[2], S. Kaushik[2], J. Pérez-Sánchez[3], and G.E.E.J.M. van den Thillart[1].

1: Department of Integrative Zoology, Institute of Biology Leiden, Leiden University, PO Box 9516, 2300 RA, Leiden, the Netherlands.
2: Laboratoire de Nutrition des Poissons, Unitée Mixte INRA-IFREMER, 64310 Saint-Pée-sur-Nivelle, France.
3: Instituto de Acuicultura de Torre de la Sal (CSIC), 12595 Ribera de Cabanes, Castellón, Spain.

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Abstract

Adipocytes were isolated from the mesenteric adipose tissue from three fish species of different ecological backgrounds: the warm freshwater African catfish (*Clarias gariepinus*), the cold freshwater Rainbow trout (*Oncorhynchus mykiss*) and the warm seawater Gilthead sea bream (*Sparus aurata*). There were large differences in the basal FFA (free fatty acids) and glycerol release: catfish > trout >> sea bream. The ratio FFA:glycerol was 5.0 ± 0.8 for trout and 7.1 ± 3.4 for catfish and 3.0 ± 0.4 for sea bream, indicating that triglycerides in sea bream were completely hydrolysed while in the two freshwater species only partial hydrolysis occurred. The adipocytes were incubated with adrenaline and noradrenaline (α- and β-agonists), and isoproterenol (nonselective β-agonist, not with trout). As no adrenergic reduction was found in adipocytes of sea bream, no hormone-sensitive lipase is functionally present in this species. In both freshwater species, all catecholamines mediated a reduction in lipolytic rate, indicating the presence of hormone-sensitive lipase. The ratio FFA:glycerol for adipocytes of African catfish approached 3 upon adrenergic stimulation, due to a stronger inhibition of FFA than of glycerol release. For the first time, it is demonstrated that not only noradrenaline, but also adrenaline reduces the lipolytic rate in fish adipocytes.

Introduction

Lipid metabolism and its hormonal regulation in fish have received relative little attention (Van den Thillart *et al*., 2002). Liver, adipose tissue and muscle are quantitatively the major sites involved in lipid metabolism in fish. The liver functions mainly as a processing site in receiving dietary lipids and excreting lipoproteins, while storage of lipids in the muscles serves to meet local demands (Sheridan, 1994). The adipose tissue is the dominant and most dynamic site for storage and mobilisation of free fatty acids (FFA; Van den Thillart *et al*., 2002).

Isolation of mammalian adipocytes has become a classically used technique in studying adipose tissue lipolysis since Rodbell (1964) published a suitable isolation procedure. Studies performed on isolated fish adipocytes are however still rare, but already in the 60's Farkas (1967a,b, 1969a) studied the effect of catecholamines on the FFA release by slices of adipose tissue. In 1985, Murat *et al*. and Christiansen *et al*. published studies with isolated fish adipocytes but very few data and a short incubation period, respectively, limited the value of these studies. In 2002, Vianen *et al*. published a detailed study in which the mammalian adipocyte isolation was adapted for use in fish. This study was also the first pharmacological work on the adrenergic control of lipolysis in fish adipocytes and it demonstrated that β-adrenergic stimulation reduced the FFA release in adipocytes of tilapia (*Oreochromis mossambicus*). This is in corroboration with the *in vivo* effect of β-adrenergic stimulation as found in the carp (*Cyprinus carpio*), where it mediated a decrease in plasma FFA levels (Van den Thillart *et al*., 2001). Van den Thillart *et al*. (2001) hypothesised that this reducing effect of β-adrenergic stimulation serves as a protective mechanism against fatty acid poisoning in case of hypoxia.

The β-adrenergic inhibition of adipose lipolysis was mediated solely by noradrenaline, which was the dominant catecholamine under hypoxia as opposed to low and stable adrenaline levels (Vianen *et al*., 2002). When adrenaline is injected into fish, it even mediates a complete opposite effect as compared to noradrenaline, i.e. a significant increase in plasma FFA levels (Van Raaij *et al*., 1995). As both catecholamines stimulate β-adrenoceptors, Van den Thillart *et al*. (2001) hypothesised that lipolysis-stimulating β-adrenoceptors are present on the liver, while lipolysis-inhibiting β-adrenoceptors are present on the adipose tissue. These findings on the adrenergic effect on lipolysis in fish are in sharp contrast with the adrenergic effect on mammalian adipocytes, where both catecholamines exert a well-known stimulatory effect on lipolysis (Fain and Garcia-Sainz, 1983).

As lipolysis in adipocytes of only one tropical species (tilapia) has been studied up to now (Vianen *et al*., 2002), our primary objective was to obtain comparative data on the lipolytic rate in adipocytes of three other fish species of different ecological background: the warm freshwater African catfish (*Clarias gariepinus*), the cold freshwater Rainbow trout (*Oncorhynchus mykiss*) and the warm seawater Gilthead sea bream (*Sparus aurata*). Additionally, the effect of both endogenous catecholamines (adrenaline and noradrenaline) as well as of the nonselective β-agonist (isoproterenol) on lipolysis was studied using isolated adipocytes of the three earlier mentioned species.

Materials and methods

Experimental animals

Juvenile Rainbow trout (*Oncorhynchus mykiss*) were kept in a flow-through system in 130-l tanks with a water temperature of 17 ± 1ºC at the Donzacq fish farm of INRA, France. The fish were fed for 40 days an experimental diet, based on a commercial composition containing fishmeal (Vianen *et al*., 2003). After this 40-day period, the fish were transferred to the experimental facilities of INRA, St-Pee, France. The adipocyte isolation and incubations started after a 10-day acclimatisation period. At both facilities, the fish were hand-fed twice a day (at 9 a.m. and 3 p.m.) to visual satiation resulting in a feeding rate of 2.0%. The growth performance of the trout was normal. The final weight of the trout was 100.5 ± 4.7 gr with a mesenteric fat content of 2.03 ± 0.09 gr, being 2.05 ± 0.08 % of the body weight.

Juvenile Gilthead sea bream (*Sparus aurata*) were purchased from a commercial fish farm (Cupimar, Cadiz, Spain). The fish were kept in a flow-through system in 500-l tanks with a water temperature of 24 ± 1ºC at the experimental facilities of the CSIC Institute of Aquaculture, Torre de la Sal, Spain. The fish were fed for 37 days with an experimental diet based on a commercial composition containing fishmeal (Gómez-Requini *et al*., 2004). The fish were hand-fed once a day (at 9 a.m.) to visual satiation resulting in a feeding rate of 1.7%. The growth performance of the sea bream was normal. The final weight of the sea bream was 166.9 ± 2.4 gr with a mesenteric fat content of 2.61 ± 0.08 gr, being 1.57 ± 0.05 % of the body weight.

African catfish (*Clarias gariepinus*) were purchased from a commercial fish farm (Gresnigt, Vleuten, The Netherlands). The fish were kept in a recirculation system in 400-l tanks with a water temperature of 26 ± 0.5ºC at the experimental facilities of Leiden University, Leiden, The

Netherlands. The fish were hand-fed once a day (at 10-11 a.m.) at a feeding rate of 1% with a commercial catfish diet (Biomeerval, Trouvit, Putten, The Netherlands). The final weight of the catfish was 1307 ± 100 gr with a mesenteric fat content of 36.60 ± 7.13 gr, being 2.71 ± 0.40 % of the body weight. Like the trout and sea bream, the catfish were in a non-reproductive phase.

Isolation and incubation procedure

Trout. Adipocytes were isolated and incubated according to the procedure described by Vianen *et al*. (2002). All handling of fat tissue and adipocytes took place at room temperature. Five to six trout were killed by a blow to the head. The mesenteric adipose tissue was dissected immediately and placed in Krebs-Henseleit buffer. This buffer was pregassed with 5% CO_2 in O_2 at 17ºC and contained in mM: 117.5 NaCl, 5.6 KCl, 1.18 $MgSO_4$, 2.52 $CaCl_2$, 1.28 NaH_2PO_4, 25.0 $NaHCO_3$ and 5.5 D-Mannitol; the physiologically inert mannitol was used instead of glucose to reduce reesterification to a minimum. When the adipose tissue of all fish was dissected, the portions were pooled and cut into pieces of 1 mm^3 with a tissue chopper (Fine Mechanical Department, Leiden University, The Netherlands). The minced fat tissue was then incubated in a Teflon dish containing 20 ml of Krebs-Henseleit buffer with BSA (1% w/v) and collagenase (type II, 130 iu/ml) to allow enzymatic digestion. Collagenase incubation took 90 min in a shaking water bath at 17ºC under an atmosphere of 5% CO_2 in O_2. After 90 min, the content of the Teflon dish was filtered over a nylon cloth and washed two times with Krebs-Henseleit buffer with BSA (1% w/v). The remaining cell cake was left to compact for 15 min. Portions of 100 µl of compacted cells were incubated for 6 h with or without hormones in a total volume of 3 ml Krebs-Henseleit buffer with BSA (2% w/v) and 0.050 mg $Na_2S_2O_5$ per ml as antioxidant. All incubations were done in triplicates in the same water bath as in the isolation procedure and under the same conditions. The basal FFA and glycerol release by the adipocytes was measured as the concentration difference in the medium before and after 2, 4 and 6 h. Adrenaline and noradrenaline (10^{-5} and 10^{-6} M) were added with as a vehicle Krebs-Henseleit buffer with 0.050 mg $Na_2S_2O_5$ per ml. The medium of the hormone incubations was sampled at the end of the 6-h incubation period. All samples were immediately frozen on dry ice and subsequently stored at –80ºC until analysis.

Sea bream. The isolation and incubation procedure for sea bream adipocytes was essentially the same as for trout, albeit that the incubation temperature (temperature of the waterbath) was 24ºC. All handling of fat tissue and adipocytes took place at room temperature. As sea bream is a

seawater fish, the osmolarity of the plasma was 375 mOsm, which is 84 mOsm higher than the Krebs-Henseleit buffer of 291 mOsm. Hence, an extra 42 mM NaCl was added to the Krebs-Henseleit buffer. Again, 5-6 fish were killed by a blow to the head and the spinal cord was cut. Hormone incubations were identical to the incubation with trout adipocytes with additional incubations with the nonselective β-agonist isoproterenol (10^{-5} and 10^{-6} M). All medium samples were immediately frozen in liquid nitrogen and subsequently stored at –80ºC until analysis.

African catfish. The isolation and incubation procedure for African catfish adipocytes was essentially the same as for trout, albeit that the incubation temperature was 26ºC. Collagenase type V was used instead of type II, because preliminary experiments showed a higher cell yield when using collagenase type V. All handling of fat tissue and adipocytes took place in a climatised room at 27ºC to prevent coagulation of the lipids and thereby damaging the adipocytes. The same Krebs-Henseleit buffer as in the trout isolation was used. The same type of incubations as with the sea bream adipocytes was done. The FFA concentration was measured at t= 0, ½, 1, 1½, 2½ and 5 h; the glycerol release over time was not measured. Both FFA and glycerol concentrations were measured after 5 h to establish the basal release over 5 h and to evaluate the effect of the different catecholamines. All samples were frozen and stored at –80ºC until analysis.

Chemicals and analysis

Bovine Serum Albumin fraction V (BSA), collagenase type II and V, isoproterenol-hydrochloride, adrenaline-bitartrate and noradrenaline-bitartrate were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands).

The FFA concentration in the incubation medium was measured using a commercial kit from WAKO (NEFA-c method; Instruchemie, Delfzijl, The Netherlands). The NEFA-c method is based on oxidative formation of a coloured adduct which is stoichiometric with the FFA concentration; as catecholamines can also be oxidised, high catecholamine concentrations ($>10^{-6}$ M) can lead to an underestimation of FFA concentrations. With catfish adipocytes, the FFA concentrations were sufficiently high to allow correction for the catecholamine interference. The glycerol concentration was measured using a commercial bioluminescence kit (CLS II kit, Roche, Almere, the Netherlands) according to the method of Hellmer *et al*. (1989).

Data analysis and statistics

All data are presented as the mean ± SEM. The basal lipolytic rate was calculated as the mean release during the complete incubation period. All data on incubations with hormones were normalised relative to this basal release rate. As the experiments with the adipocytes of these poikilothermic animals were performed at different temperatures, the basal release rates by trout and sea bream adipocytes were corrected to 26ºC using the correction factors of Winberg (1971), multiplication by 2.15 and 1.18, respectively. Using Sigmaplot 5.00, a regression analysis on the mean lipolytic data over time was performed according to the equation: $Y= a\cdot(1-e^{-b\cdot X})$. This type of equation implies decreasing release rates over time and thus the maximal release rate was calculated as the slope of the curve at 0 h.

Statistical differences ($p<0.05$) were tested using Sigmastat 2.03. Differences between treatments and the basal release were tested with a Repeated Measures Anova on Ranks according to Dunnett's method. Differences between different concentrations of the same hormones or different catecholamines of the same concentration (trout) were tested with a Wilcoxon Signed Rank test. Differences between different catecholamines of the same concentration (sea bream and African catfish) were tested with a Repeated Measures Anova on Ranks according to Student-Newman-Keuls method.

Results

Basal lipolysis

Large differences were found in the mean basal FFA and glycerol release, being the amounts released during the incubation period (Table 1). The highest basal FFA and glycerol release rates were by catfish adipocytes, while the release rates by trout adipocytes were around 4x lower. When corrected to the same temperature of 26ºC, the differences were much smaller i.e. 1.6 to 2.2x lower. Sea bream adipocytes released very low amounts of FFA and glycerol, respectively 33x and 12x less than catfish adipocytes. As the temperature difference was small, temperature correction had only a small effect.

The increase in both the FFA (Fig. 1) as well as the glycerol release (not shown) over time was best described with an exponential curve. Hence, the slope of a curve at 0 h represented the maximal release rate as opposed to the mean release rate as given in Table 1. The maximal

Table 1. Mean basal release of FFA and glycerol during the incubation period (in nmol/ml cells/h) by adipocytes of Gilthead sea bream (*n*= 8; 24ºC), Rainbow trout (*n*= 8; 17ºC) and African catfish (*n*= 5; 26ºC). The mean basal release rates of trout and sea bream were corrected according to Winberg (1971) to a theoretical value (in italics) at 26ºC.

	FFA release	*Corrected to 26ºC*	Glycerol release	*Corrected to 26ºC*	Ratio FFA:Glycerol
Sea bream	32.3 ± 8.9	*38.0*	10.3 ± 2.0	*12.1*	3.0 ± 0.4
Trout	231.5 ± 39.0	*498.0*	37.7 ± 5.6	*81.1*	5.5 ± 1.1
Catfish	1084.4 ± 522.5	*1084.4*	128.0 ± 50.7	*128.0*	7.1 ± 3.4

lipolytic rate for trout adipocytes at 17ºC was 283.6 nmol FFA and 78.9 nmol glycerol/ml cells/h, for sea bream at 24ºC 56.6 nmol FFA and 24.8 nmol glycerol/ml/h, and for catfish at 26ºC 1122.1 nmol FFA/ml cells/h.

Adipocytes of sea bream released FFA and glycerol in a ratio of 3.0 ± 0.4, which indicates complete hydrolysis. Adipocytes of trout and catfish, on the other, hand released relatively more FFA than glycerol, namely in a ratio of 5.5 ± 1.1 and 7.1 ± 3.4, respectively. These ratios were significantly higher than 3, which indicate partial hydrolysis of the lipids.

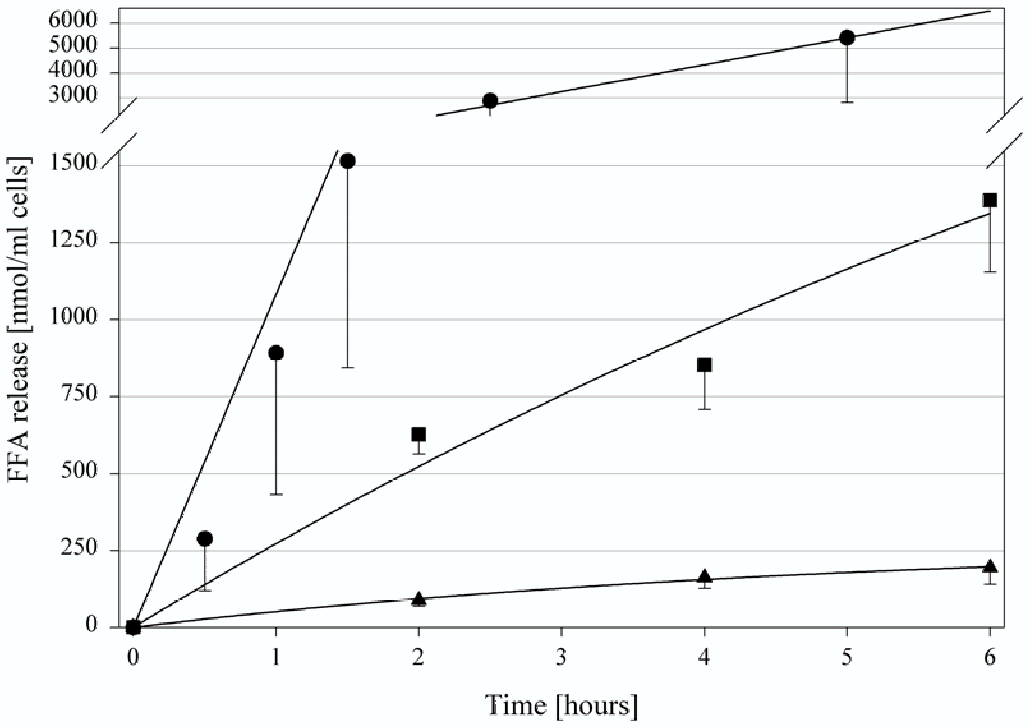

Fig. 1. The amount of FFA released by adipocytes of Gilthead sea bream (▲; *n*= 8), Rainbow trout (■; *n*= 8) and African catfish (●; *n*= 5) over time. Exponential regressions were based on the formula: $Y= a\cdot(1-e^{-b\cdot X})$; R^2 were 0.974, 0.996 and 0.993, respectively.

Table 2. The adrenergic effect on the glycerol release by adipocytes of Rainbow trout (*n*= 7), Gilthead sea bream (*n*= 8) and African catfish (*n*= 5). For catfish adipocytes, also FFA release rates and the ratio FFA:glycerol are given (see Materials and Methods). Data are normalised relative to the mean basal release (100%) as given in Table 1.

	Trout	Sea bream	Catfish		
	Glycerol	Glycerol	Glycerol	FFA	Ratio FFA:Glycerol
ADR 10^{-5} M	72,6±14,4%*	110,7±10,7%	48.2±16.1%*	21.7 ± 6.6%*	3.0 ± 0.7
ADR 10^{-6} M	94,0±13,1%#	107,2±14,5%	38.7±6.7%*	24.0 ± 7.3%*	3.8 ± 0.9
NA 10^{-5} M	72,6±12,7%*	101,4±8,5%	45.3±10.5%*	19.8 ± 5.2%*	3.0 ± 0.9*
NA 10^{-6} M	81,5±16,0%	107,9±16,9%	67.6±11.4%*$	31.3 ± 8.5%*	3.4 ± 0.3*
ISO 10^{-5} M	---	95,7±19,6%	44.2±11.7%*	24.6 ± 5.8%*	3.8 ± 1.3
ISO 10^{-6} M	---	125,5±24,1%	47.7±16.7%*	24.8 ± 6.8%*	3.8 ± 1.2

ADR = Adrenaline; NA = Noradrenaline; ISO = Isoproterenol (nonselective β-agonist). * $p<0.05$ vs. basal release; # $p<0.05$ ADR 10^{-6} M vs. ADR 10^{-5} M; $ $p<0.05$ NA 10^{-6} M vs. ISO 10^{-6} M as well as vs. ADR 10^{-6} M.

Effects of catecholamines

In trout adipocytes, lipolysis was significantly reduced by both catecholamines at 10^{-5} M to 72.6% of the basal glycerol release (Table 2). At 10^{-6} M, no significant adrenergic effect was found. The glycerol release of 94.0 ± 13.1% for adrenaline 10^{-6} M was significantly different from the rate at 10^{-5} M. No significant difference was found between the effects of both concentrations of noradrenaline.

In sea bream adipocytes, all three catecholamines had no significant effects on lipolysis; the mean glycerol release varied between 95.7 ± 19.6% and 125.5 ± 24.1% of the basal release.

In catfish adipocytes, all three catecholamines had a clear and significant reducing effect on the lipolytic rate. At 10^{-5} M, there were no significant differences between the catecholamines; the glycerol release varied from 44.2 to 48.2% and the FFA release varied from 19.8 to 24.6%. At 10^{-6} M, the reduction in glycerol release by noradrenaline (67.6 ± 11.4%) was significantly different from the reductions by adrenaline and isoproterenol (38.7 ± 6.7% and 47.7 ± 16.7%, respectively). As compared to basal conditions, stimulation by noradrenaline significantly reduced the ratio FFA:glycerol from 7.1 to 3.0 and 3.4 at 10^{-5} and 10^{-6} M, respectively. The ratio FFA:glycerol after incubation with adrenaline and isoproterenol were in the same range, but these ratios were not significantly different from the basal release.

Discussion

Ratio FFA:glycerol release

In mammals, two enzymes are involved in triglyceride degradation: hormone-sensitive lipase (HSL) hydrolyses triglycerides to di- and subsequently monoglycerides, while monoglyceride lipase (MGL) hydrolyses the thus formed monoglycerides to glycerol (Vaughan *et al*., 1964). Hence, the ratio of FFA:glycerol is 3 when triglycerides are completely hydrolysed. Altered ratios indicate incomplete hydrolysis (ratio >3) or reesterification of fatty acids (ratio <3) as glycerol is not intracellularly recycled because adipocytes essentially lack glycerokinase (Margolis and Vaughan, 1962). Our data on adipocytes of the freshwater species, trout and African catfish, fit the presence of such a dual enzymatic breakdown of triglycerides in these fish species. As the ratio FFA:glycerol release in trout and catfish adipocytes was over 3, relatively less glycerol than FFA was released and thus partial glycerides accumulated. Hence, MGL was rate-limiting in the trout and catfish adipocytes in our study. In mammals, HSL is normally rate-limiting (Coppack *et al*., 1994) and hardly any di- and monoglycerides are formed in the course of triglyceride hydrolysis (Liebel and Hirsch, 1985). However, also in mammals the deletion of glucose led to a ratio FFA:glycerol higher than 3 due to a constant FFA release and a diminished glycerol release (Naito and Okada, 1975). These results suggest that MGL activity is stimulated by glucose. Upon stimulation by noradrenaline, the ratio FFA:glycerol produced by adipocytes of catfish was significantly reduced from 7.1 to 3. In view of the dual enzymatic breakdown as present in mammalian adipocytes, this means that the HSL activity was lowered and was equal to or below the MGL activity.

In mammals, the ratio FFA:glycerol is normally well below 3 (Vaughan and Steinberg, 1963) as the large majority of released FFA is reesterified (Wolfe *et al*., 1990). Also in trout *in vivo* results indicate that hydrolysed FFA are recycled intracellularly (Bernard *et al*., 1999). The observed high FFA:glycerol ratio suggests that lipolysis resulted for a large part in the formation of di- and/or monoglycerides. Normally, di- and monoglycerides can be used for reesterification (Jungas and Ball, 1963), but based on the high ratio in trout and catfish reesterification was marginal. Since we applied mannitol instead of glucose in the medium, reesterification with glucose can be excluded. Comparable high FFA:glycerol ratios (9.9 ± 3.6) are reported for rat adipocytes when no glucose was present in the medium (Naito and Okada, 1975). Coleman *et al*. (2000) stated that lipolytic products are most likely not freely available within the cell but

channelled into distinctive pathways. A spatial division would simply make di- and monoglycerides unavailable for reesterification as opposed to the free-moving glucose.

In sea bream adipocytes, the ratio between FFA and glycerol was around 3, indicating that the lipid degradation was complete. In sea bream, catecholamines had no effect on lipolysis, so no HSL was functional. Hence, another triglyceride lipase had to be present in sea bream adipocytes or HSL was in a state in which its activity could not be affected by hormones. In HSL-deficient mice, triglycerides hydrolysis was still active implying the presence of a second type of triglyceride lipase (Haemmerle *et al*., 2002). Obviously, the low lipolytic rate shows that mesenteric fat can hardly be mobilised. The major function of this lipid storage may be for later gonadal development.

The data presented in this study give strong indications that in trout and catfish adipocytes, like in mammalian adipocytes, a dual enzymatic breakdown of triglycerides was present.

Basal lipolysis

Large differences were found in the basal lipolytic rate between the three species tested in this study. After temperature correction, the basal FFA release by catfish adipocytes (~1100 nmol/ml/h) was about 2 times higher than the release by trout adipocytes (~500 nmol/ml/h), but a massive 25 times higher than the release by sea bream adipocytes (~40 nmol/ml/h). The basal FFA release of tilapia adipocytes was around 1000-1200 nmol/ml cells/h (after Vianen *et al*., 2002, and corrected to 26ºC), which is in the same order as the lipolytic rate of catfish adipocytes. Although glucose was included in the medium used for tilapia adipocytes (Vianen *et al*., 2002), which could have facilitated reesterification, this probably had little effect on the FFA release as was observed in rat adipocytes (Naito and Okada, 1975).

Possible explanations for the differences in lipolytic rate of these 3 freshwater species can be found in the nutritional state and/or age of the animals. First, the nutritional state can have a large impact on the lipolytic rate as an inverse correlation exists between nutritional state and hepatic lipase activity (Sheridan and Mommsen, 1991); the feeding rates of the catfish and the tilapia were equal (1% BW), but the feeding rate of the trout was twice as high (2% BW). Second, in rats the lipolytic rate increased during growth and ageing due to an increased triglyceride content of the fat cells (Hartman *et al*., 1971). As the catfish (1300 gr) and the tilapia (400-700 gr, Vianen *et al*., 2002) were considerably larger and closer to adulthood than the juvenile trout (100 gr), it is likely that, assuming a comparable total number of adipocytes, the fat

cells of the catfish and tilapia were larger, and thus contained more triglycerides per cell than the trout adipocytes. This was confirmed by microscopical observations that catfish adipocytes (70-100 μm) were on average larger than trout adipocytes (<50 μm).

There are no other papers on the lipolytic rate of isolated fish adipocytes, but some on slices of adipose tissue. However, the release of lipolytic metabolites by slices gives an underestimation of the true lipolytic rate. This is because diffusion is impaired by interstitial barriers (Angel *et al*., 1971). Slices of adipose tissue of trout of 12 months old released around 290 nmol/ml/h to the medium at 14ºC (Eilertson and Sheridan, 1994), equivalent to 830 nmol/ml/h at 26ºC. These fish were however starved 3 to 4 days (Sheridan and Mommsen, 1991), which may have increased the lipolytic rate. The FFA release by adipose tissue of tigerfish (*Hoplias malabaricus*) was maximally 270 nmol/ml/h (Migliorini *et al*., 1992). Considering that the animals in this study were adult fish on a low feeding rate, the difference with the lipolytic rate of tilapia (1000-1200 nmol/ml/h; Vianen *et al*., 2002) and African catfish (1100 nmol/ml/h; this study) is considerable. Remarkably, the low lipolytic rate of adipose tissue of tigerfish was accompanied by a lack of response to adrenaline, which corresponds to the results with sea bream adipocytes in this study.

The extremely low lipolytic rate in the sea bream was surprising as these animals contained a considerable amount of mesenteric fat (1.6% BW). The Antarctic icefish (*Chaenocephalus aceratus*) has about the same amount of adipose tissue (1.50% of the BW) as the sea bream in our study and also the adipose tissue of the icefish lacks any significant lipase activity (Sidell and Hazel, 2002). In many seawater species, the liver functions as the main storage site of triglycerides (Henderson and Tocher, 1987). In sea bream, the liver has an almost 10 times higher HSL activity than the adipose tissue, while the opposite was found in trout (G.J. Vianen, unpublished results). Likewise in several Antarctic marine fish species, the lipase capacity of the liver is considerably larger than of the adipose tissue (Sidell and Hazel, 2002). Altogether, this suggests a dominant role of the liver over the adipose tissue in marine fish species.

Our study clearly demonstrates that the lipolytic rate of marine sea bream was very low while the lipolytic rates in adipocytes of both freshwater species, trout and African catfish, were higher and comparable to each other, irrespective of their ecological background.

Effect of catecholamines

Both adrenaline and noradrenaline reduced lipolysis in adipocytes of trout and catfish. A reduction by noradrenaline has been reported for adipose tissue of several freshwater fish species

(see Farkas, 1967a, 1969a; Vianen *et al*., 2002). Although Farkas (1969a) reported that also adrenaline reduced the FFA production in fish adipose tissue, he did not provide any statistical basis as presented in this study for trout and catfish. Both in tilapia (Vianen *et al*., 2002) and African catfish (this study), lipolysis was reduced by specific activation of β-adrenoceptors. Although adrenaline is like noradrenaline a β-agonist, it is surprising that adrenaline was capable of reducing adipose lipolysis. *In vivo* experiments with carp showed that FFA levels were only reduced by noradrenaline but not by adrenaline (Van Raaij *et al.*, 1995). Most likely, stimulatory β-adrenoceptors present on fish liver mediated an enhanced hepatic FFA release (Sheridan, 1987; Van den Thillart *et al*., 2001). There is a clear difference in affinity between noradrenaline and adrenaline for specific β-receptors (Tetens *et al*., 1988). Hence, at physiological catecholamine concentrations ($<10^{-6}$ M) differential effects of both catecholamines, meaning a noradrenaline mediated inhibition of adipose lipolysis and adrenaline mediated stimulation of hepatic lipolysis as observed by Van Raaij *et al*. (1995), are likely to be present.

The adrenergic reduction of lipolysis functions as a possible protective mechanism during hypoxia (Van den Thillart *et al*., 2002). The sea bream is a demersal marine species living in coastal water, where it may come in contact with hypoxia (Thetmeyer *et al*., 1999). Because the lipolytic rate in the adipocytes of this species was however extremely low, a reduction of adipose lipolysis has most likely no physiological relevant effect on plasma FFA levels. As stated earlier, the liver is a more important site for lipid mobilisation and thereby a more likely site for adrenergic action in the sea bream.

Catecholamines strongly reduced lipolysis in adipocytes of African catfish. For the air-breathing African catfish, water submersion reflects hypoxic conditions (Chapter 3). African catfish live in stagnant warm water, in which the oxygen concentrations drop frequently; especially because African catfish is a bottom dwelling ambush hunter, it is likely that this species frequently has to shift to a hypoxic metabolism and thus displays a strong adrenergic reduction of adipose lipolysis. Compared to African catfish, the adrenergic reduction of adipose lipolysis in trout was smaller and only significant at the highest catecholamine concentration of 10^{-5} M. Maximal plasma concentrations of catecholamines in hypoxic trout are around 10^{-7} M (Butler *et al*., 1986; Vianen *et al*., 2001). Hence, it is questionable if the adrenergic reduction of lipolysis in adipose tissue of trout is physiological relevant. However, noradrenaline concentrations can locally reach the micromolar range due to overflow from adrenergic nerve terminals (Schömig and Richardt, 1990). Mammalian adipose tissue is directly innervated (Bartness and Bamshad, 1998), but for fish adipose tissue this is unknown. Trout lives in running

cold water, which has a constant high level of oxygen and therefore, this species will seldomly come in contact with hypoxia. Van Raaij *et al*. (1996a) observed no consistent depression of plasma FFA levels in hypoxic trout as was seen in hypoxic carp. Vianen (1999) found in hypoxic trout a marker reduction in plasma FFA levels together with increased levels of noradrenaline. Haman *et al*. (1997) showed that although plasma FFA levels stayed constant during hypoxia in trout, the mobilisation of FFA was indeed inhibited. These results support our findings with trout adipocytes of moderate inhibition of lipolysis by catecholamines.

This study demonstrates that in contrast to sea bream, in trout and African catfish adipocytes the lipolytic activity is reduced upon adrenergic stimulation, indicating the presence of HSL.

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