# Proteomic and morphological characterization of isolated rainbow trout adipocytes originating from various fat tissues

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In fish, like in other edible species, the repartition of the different fat deposits plays an important role in the determination of carcass quality and flesh organoleptic properties. In mammals, different works report regional differences in fat tissue with respect to cell size, metabolic and secretory functions. So far, in fish, data on adipose cell characteristics, in relation with the origin of fat deposit, are very scarce and concern principally their size distribution. The aim of the present work is to compare, in rainbow trout, the cellularity and proteomic profiles of isolated adipose cells originating from various fat deposits to better understand their functions. The following fat depots were studied : (i) perivisceral fat which constitutes the more important part of non edible residues, subcutaneous (iii) ventral and (iii) dorsal fat deposits which are eliminated when fish fillets are trimmed.

This work has necessitated the setting up of various methods : adipose cell dispersion, extraction and solubilisation of their proteins followed by their separation by bidimensional electrophoresis. Different conditions were tested in order to maximize each previous cited step and the following protocol was chosen. The different adipose tissues (12 g) were collected on fish with a mean body weight of 1.5 kilos and were minced before being submitted to collagenase (2mg/ml) dispersion for 1.5 hr at 12°C. After filtration of the digests through a 200 µm mesh fabric, adipocytes were collected by centrifugation (5 mn, 50g). The collected adipocytes were washed 3 times (5 mn, 50g) in DMEM medium and then, were submitted either to direct microscopy observation to determine cell diameter (with an image analyzer system (Visilog 5.4) or to protein extraction/solubilisation. The latter was performed at 18°C in a glass-glass homogeniser with 500 µl of buffer (40mM TRIS, 8M urea, 2M thiourea, CHAPS 4%, 50 mM DTT, 250 mM EDTA, 1% IPG buffer, protease inhibitors) followed by 2 centrifugations (15 000 g, 30 mn and 100 000 g, 1hr). 100 µg of protein was submitted to bidimensional electrophoresis. IEF was performed by using IPG DryStrips (24 cm, pH, 4-7 L) on IPG-Phor. The 2$^{nd}$ electrophoresis, SDS-PAGE 12.5 % in the format 240 mm x180 mm, was performed on EttanDALT six, followed by silver staining. Scanning of the gels allowed to detect approximately 1500 spots and work is in progress to detect spots differentially expressed in relation with the fat tissue origin of adipocytes. Preliminary results on morphological characteristics of collagenase dispersed adipose cells originating from the different fat tissues indicate that perivisceral adipocytes have a larger mean diameter compared to subcutaneous dorsal and ventral adipocytes.

In conclusion, the whole work would allow, in rainbow trout, to identify specific proteic markers of adipose cells from different fat deposits and would conduct to a better knowledge on the regulation of the regional lipid distribution.

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