INVESTIGATING THE BEHAVIOURAL PROPERTIES OF ADIPOSE TISSUES USING CONFOCAL LASER SCANNING MICROSCOPY

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Introduction

The fatty acid (FA) composition of foodstuffs defines, in part, the healthiness of a food. Many muscle-based foods have long been criticised for having a high level of the undesirable saturated fatty acids in relation to the more desirable polyunsaturated fatty acids. In porcine systems, the ratios of different fatty acid components can be manipulated by varying feeding regimes but this manipulation can lead to adverse changes in back fat texture, taste and oxidation levels (Warnants *et al*., 1996; Wood *et al*, 1999).

The composition of FAs in a tissue is the result of a complex biological process (Large *et al*., 2004). Adipocytes are cells known to be the lipid storage depot for circulating fats in the body. The FAs contained in adipocytes are a mixture of molecules which are either imported into the cells from the blood and lymph or are newly-synthesised within the fat cell.

Using laser-scanning confocal microscopy, we show in this work that we can investigate not only tissue structure, but the behavior of different cells and different levels of maturity, resulting in a better understanding of the properties of adipose tissue.

Materials and Methods

Twenty-one female pigs were slaughtered at an average live weight of 112 kg (± 9 kg). Three days post mortem, samples for immuno-histochemical analysis of subcutaneous fat were taken at the P2 position and immediately frozen in liquid nitrogen. The samples were than stored at -21°C until use.

The actin cytoskeleton was visualized using Rhodamine-Phalloidin (Sigma-Aldrich Inc., St. Louis, USA). A primary monoclonal antibody against Perilipin was obtained from Progen Biotechnik, (Heidelberg; Germany) , and a monoclonal antibody to CD36 was obtained from Cell Science (Canton, MA, USA). Secondary Alexa-conjugated antibodies were purchased from Molecular Probes (Invitrogen, Carlsbad, CA, USA).

Serial sections (60 µm) were cut using a cryostat at -21 °C. Immunolabelling was performed following a standard protocol. Samples were viewed using a confocal laser microscope (SP, Leica Laser Technik GmbH, Heidelberg, Germany) equipped with an argon/krypton laser. During image acquisition, each frame was collected 20 times and averaged. The images shown are a projection of stacks of images in vertical directions at the maximum intensity mode. The resultant grey value of each pixel was the highest value present in the column of pixels in the stack with the same (x, y) position.

Results and Discussion

Existing studies on the activity of adipose tissue use PCR or biochemical methods for analysis. These methods give an inaccurate picture of the tissue as a whole since this adipose tissue is a mixture of cells at different developmental states. Figure 1 shows a typical area of tissue containing both immature brown fat cells and more mature white fat cells.

Figure 1: Co-existence of immature and mature adipocytes in porcine tissue at time of slaughter. Arrows – immature adipocyte.

CD36 is a fatty acid transporter in many cell types and is responsible for the uptake of free fatty acids. The level of CD36 on the adipocyte surface and the clustering of the protein on the cell membrane give information about the activity of the transporter. Figure 2 shows a section of porcine adipose tissue labeled for CD36. Punctate staining for this receptor can be seen surrounding a number of the adipocytes.

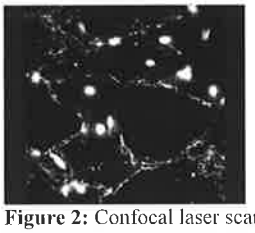

Figure 2: Confocal laser scanning micrograph of the fatty acid transporter CD36 in adipose tissue.

Perilipins are a family of phosphoproteins involved in the transport of lipids out of adipocytes. Perilipins interact with lipases that are responsible for the breakdown and release of triacylglycerides in fat droplets and allow activation of the lipase after hormonal activation. Figure 3 shows the localisation of perilipins on the surface of a white adipocye.

Figure 3: Confocal laser scanning micrograph of perilipins in adipose tissue.

Conclusions

Immunohistochemistry and laser-scanning confocal microscopy are powerful tools to resolve the function of different cells at different maturities in adipose tissue. These techniques can give valuable insights into the properties of adipose tissues with regard to meat eating and processing quality.

References

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