Organ Specific Lipid Imaging and Quantification with WETSEM™ Technology: A New Tool for Early Evaluation of Drugs for Metabolic Diseases

The Need for Detection of Lipid Accumulation in Tissues

Lipid accumulation in tissues occurs early in the development of systemic metabolic disorders such as insulin resistance and reduced insulin secretion in beta cells. Therefore, the detection and quantification of lipid deposition is an important tool in identifying early onset and development of such disorders. Moreover, the morphology of lipid deposition within cells is suspected to have major effects on organ function; yet current lipid assessment techniques are incapable of providing precise intracellular information.

QuantomiX offers a proprietary technology called WETSEM™ that enables visualization and quantification of lipid bodies in cells or tissue samples. This tool requires only minimal sample preparation, insuring that lipid bodies are preserved intact, and avoiding artifacts caused by standard preparation procedures. High-resolution lipid imaging is thus achieved in a rapid, accurate and convenient manner (1, 2).

Intracellular Lipid Accumulation in Muscle Fibers

At the onset of insulin resistance, skeletal muscle tissue is one of the first to accumulate lipids. The absolute quantity of intramyocellular lipid (IMCL) is not the only determinant of peripheral insulin resistance. Additional parameters such as lipid droplet size, abundance in the proximity of specific organelles (e.g. mitochondria) and their distribution within different muscle fibers, all have an impact on overall metabolism.
Figure 1 shows intracellular lipid body accumulation in the muscle of diet-induced diabetic sand rats (Psammomys Obesus) after three weeks on a high-energy diet (B), vs. control (A).

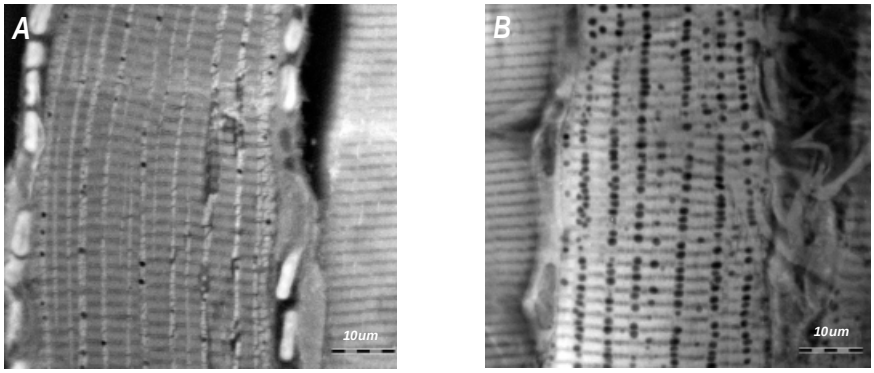

Figure 1: Longitudinal sections of skeletal muscles from the P. Obesus rodent model of diet-induced diabetes showing increased lipid droplet quantity and size in the hyperglycemic animal (B), compared with the control animal (A). Magnification x1600. In collaboration with Prof. N. Kaiser and Prof. I. Raz, Hadassah Medical Center, Israel

Proprietary QuantomiX analysis software was used to quantify the IMCL size distribution, as shown in Figure 2. The experimental groups included four animals per time point (basal, one week, and three weeks on a high-energy diet, as well as after two days on a low energy diet following the three weeks on the high-energy diet).

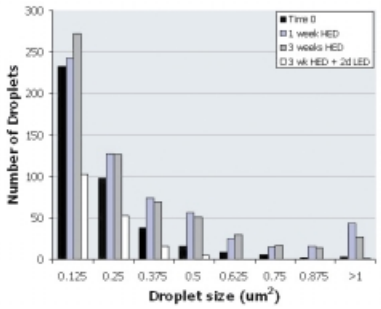

Figure 2: IMCL size distribution. Data was collected for at least 50 muscle fibers per animal; analysis shown for the 10 most lipid dense fibers from each animal.

At baseline (black bar), small lipid droplets are abundant. The development of hyperglycemia (violet and gray bars) is characterized by the appearance of larger lipid droplets. The differentiation between control and hyperglycemic animals is very apparent as the droplet size increases (the x axis). Diet restriction for two days after the animals developed hyperglycemia (white bar) reversed their hyperglycemia, and accordingly, their IMCL state. These results emphasize the tight coupling of lipid accumulation with the appearance of systemic hyperglycemia, specifically in larger lipid droplets within myocytes.

Assessment of Lipid Accumulation in the Liver

Nonalcoholic fatty liver disease (NAFLD), the most common liver disease, is believed, primarily, to be an outcome of insulin resistance. It consists of two stages: 1. fat accumulation within hepatocytes (steatosis), and 2. a local inflammatory response, (NASH or nonalcoholic steatohepatitis). At present, little is known about the natural history or the underlying vulnerability of individual patients in terms of developing progressive liver disease after initial steatosis. Lipid accumulation in the liver was examined in ob/ob mice in comparison to wild-type.

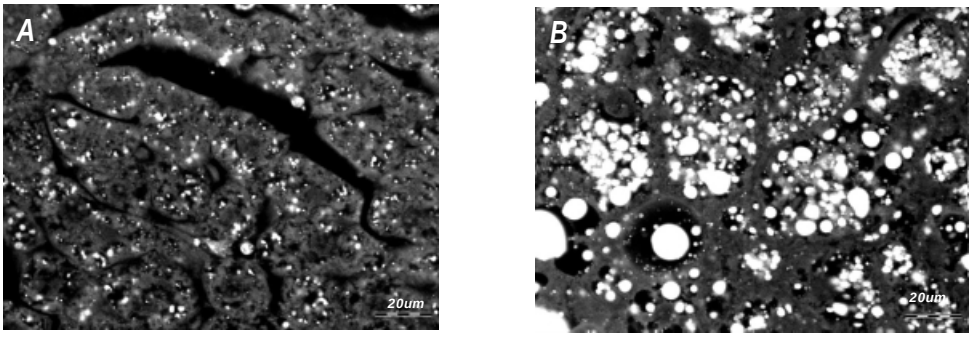

Figure 5: Lipid accumulation in liver tissue from a control wild type (A) and an obese ob/ob mouse (B). Samples were stained with Osmium. Lipid bodies are seen in white. Magnification: x800.

Image analysis software was used to quantify lipid content in liver sections. As shown in Figure 6, lipid accumulation in ob/ob mice was seven times that of age-matched control mice.

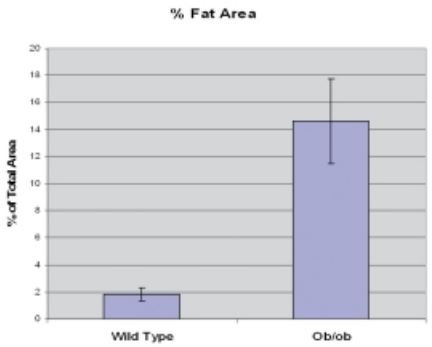

Figure 6: Average lipid accumulation in the livers of three control and three ob/ob mice. The analysis was performed on images taken from two different lobes, three sections per lobe.

White Adipose Tissue: Cell Size Determination

Adipocytes have an enormous capacity to expand to accommodate increasing fat stores. Abnormal enlargement of adipocytes alters their function, which may account for obesity-associated insulin resistance and other metabolic disorders. Increased adipocyte size has been shown to predispose the development of Type 2 diabetes. WETSEM™ technology enables large areas of tissue samples to be scanned for easy, accurate determination of adipocyte size. Further high resolution images can also provide precise morphology of the fat tissue.

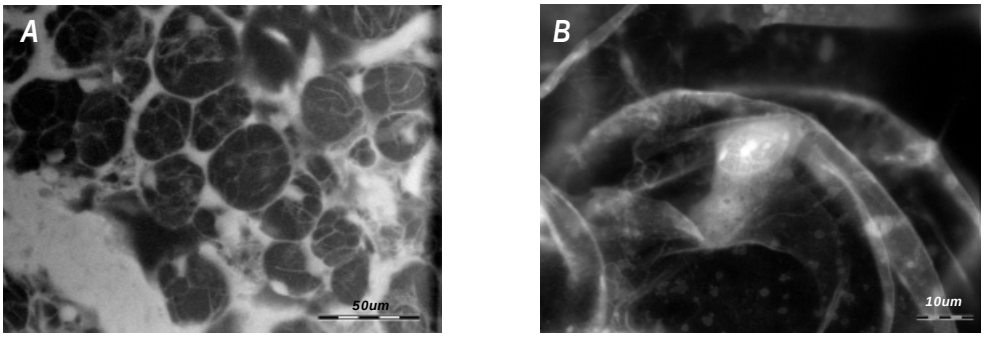

Figure 7: White adipose tissue (unstained) from normal mice, shown in low (A x200) and high (B x1600) magnifications, useful for cell size determination and precise tissue morphology. In collaboration with Prof. S. Cinti, Ancona University, Italy

Human adipocyte size distribution was determined from biopsies sampling different fat regions of the body.

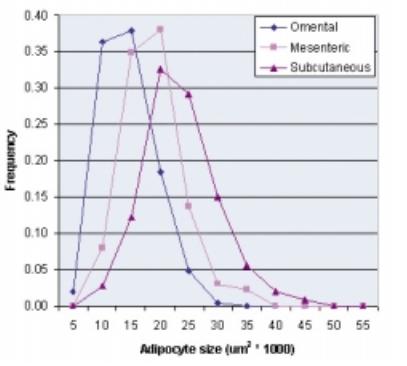

Figure 8: Adipocyte size distribution from three different fat depots (omental, mesenteric and subcutaneous) removed from an obese patient. The samples show that different fat depots are characterized by different cell sizes. Data was collected from at least 200 cells per sample. In collaboration with Drs. B. Corkey and C. Apovian, Boston University Medical Center.

Lipid Body Accumulation in Adipocytes

The differentiation of pre-adipocytes into mature fat cells is a complex process controlled by the interplay of intracellular factors and environmental conditions. Mature adipocytes are characterized by a high lipogenic enzyme content that facilitates the synthesis and cytoplasmic storage of massive amounts of triglycerides. When given the proper hormonal conditions, 3T3-L1 pre-adipocytes undergo differentiation in culture, acquiring the morphological and biochemical characteristics of adipocytes. The monitoring of triglyceride accumulation throughout the differentiation pathway can serve as a phenotypic marker for their state of maturation and, therefore, may be useful in cell-based assays to evaluate the in vitro effects of intervention.

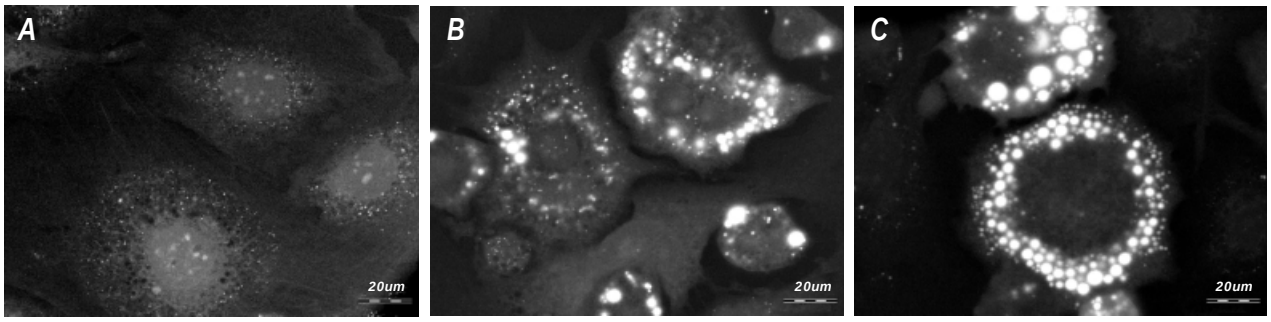

Figure 9: 3T3-L1 cell differentiation (stained with Osmium), showing increased lipid content as cells mature. (A) Basal conditions. (B) After 3 days in culture. (C) after 6 days in culture. All at x 800.

New Opportunities for Drug Development and Beyond

These and other studies have shown the wide-range utility of WETSEM™ technology in lipid research. WETSEM™ is the only comprehensive solution for lipid imaging and quantification for the evaluation of metabolic states. Along with its ability to accurately analyze tissue morphology and molecular immuno-labeling, this capacity opens new opportunities for researchers and drug developers alike.

References

1. *Stephan Thiberge et al. "Scanning electron microscopy of cells and tissues under fully-hydrated conditions." PNAS, 101:3346-3351, 2004.*

2. *Stephan Thiberge et al. "An apparatus for imaging liquids, cells and other wet samples in the scanning electron microscope." Review of Scientific Instruments, 75:2280-2289, 2004.*