The how and why of glycogen ultrastructure - implications for physiology and disease

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Glycogen is a branched polymer of glucose and exists in all cells, but in mammals the primary reserves are in skeletal muscle and the liver. In skeletal muscle, glycogen provides glucose to meet the energetic requirements associated with movement, and is the major destination of insulin-dependent glucose transport. In liver, glycogen incorporates excess glucose from either dietary or gluconeogenic sources and contributes to hepatic glucose output in response to hypoglycemia. Because of its central role in carbohydrate metabolism, glycogen is highly regulated. There are three potential modes of regulation. The catalytic activities of glycogen metabolic enzymes are controlled by a combination of post-translational modification and allosteric activators (reviewed in Roach *et al.*, 2012). Secondly, glycogen-associated proteins form a dynamic carbohydrate / protein complex (Stapleton *et al.*, 2010) that in some cases translocate between the glycogen particle and other cellular structures and finally, the glycogen particle associates with specific cellular structures, particularly sarcoplasmic reticulum, myofibrils, mitochondria, endoplasmic reticulum membranes and polyribosomes (Nielsen *et al.*, 2011, Rybicka, 1996). The glycogen particle therefore is a sub-cellular compartment that has the required composition to maintain the macromolecule and respond to the metabolic and synthetic requirements of the cell.

Glycogen particle structure consists of two types of particles; individual particles known as β-particles containing up to 55,000 glucosyl units and varying in diameter in a tissue-dependent manner from 10 to 50 nm (Ryu *et al.*, 2009). β-particles that aggregate in two or more particles are known as α-particles and can reach diameters of up to 500 nm in the liver. The molecular basis for why certain β-particles have the ability to aggregate is under investigation in our laboratories. Until recently the only available glycogen structure was that of α-particles found in the liver. However, using a non-denaturing method, we have shown that human muscle glycogen consists entirely of β-particles (Ryu *et al.*, 2009) whilst murine cardiac muscle contains both α and β-particles (Besford *et al.*, 2012). These data suggest tissue-specific regulation not only of glycogen metabolism but also glycogen particle structure formation.

A limiting factor in understanding glycogen metabolism is purifying sufficient glycogen from human muscle biopsies or animal tissue. To this end we are developing new methodology to enhance our glycogen yields for subsequent proteomic, electron microscopy and branching analysis studies using a combination of techniques including mass spectrometry, high performance gel filtration, transmission electron microscopy and nuclear magnetic resonance (NMR). This multidiscipline approach will enable new insights into glycogen metabolism at a molecular level. For example, we will be able to compare skeletal muscle glycogen particle and branching structures together with their associated proteomes between trained and sedentary individuals. In addition, we will investigate whether impaired glycogen metabolism found in diabetic cardiac, renal or hepatic tissue alters glycogen particle ultrastructure or associated proteins.

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