

### The effect of insulin on the packaging of dietary fats into chylomicrons in intestinal cells

E Allister, S Pal, JCL Mamo

Department of Nutrition, Dietetics & Food Science, Curtin University, Perth, WA, 6102.

Obesity is a common health problem in most Western nations and the prevalence is increasing. Visceral obesity, which is typically seen in overweight men, is frequently a precursor of non-insulin-dependent diabetes mellitus (NIDDM), cardiovascular disease, and premature death. The increased atherosclerotic risk with obesity may relate to several metabolic factors including insulin resistance, hypertension, and dyslipidemia. Recently, ongoing clinical studies in our laboratory have found that obese subjects have postprandial dyslipidemia, specifically a high concentration of chylomicron remnants (CM-r) in plasma, in comparison to lean healthy controls. Chylomicrons (CM) are the vehicle by which dietary fats are transported in blood and CM-r's are considered to be the pro-atherogenic form of this lipoprotein group. A raised plasma concentration of CM-r in obesity may be a consequence of insulin-stimulated overproduction of CM's and hence this study aimed to investigate the influence of insulin on chylomicron production by the intestine *in vitro*. Insulin is known to regulate the production of hepatically derived very low-density lipoprotein (VLDL) and we hypothesise that it may play a similar role in the intestine.

CaCo-2 cells, which are a transformed human intestinal epithelial cell line, were compared to the transformed human hepatic cell line HepG2. In CaCo-2 cells apolipoproteinB<sub>48</sub> (apoB<sub>48</sub>) is synthesised and incorporated into CM's whereas in HepG2 cells apoB<sub>100</sub> is synthesised and incorporated into VLDL. As both CM's and VLDL contain one apoB molecule per particle, measuring this protein provides a quantitative measure of the number of CM's or VLDL that are being produced. Utilising an apoB antibody, the amount of apoB<sub>48</sub> and apoB<sub>100</sub> within the cells and secreted into the media was quantitated by Western Blotting and enhanced chemiluminescence. The amount of insulin binding was compared in the two cell lines by incubating the cells with increasing concentrations of <sup>125</sup>I-insulin. At the same time the level of protein tyrosine kinase (PTK) activity within cells incubated with or without insulin was observed. In HepG2 cells pharmacological levels ( $1 \times 10^{-5}$ M) of insulin significantly inhibited the production of apoB<sub>100</sub> in the cells (62% of control, n = 5) and secreted into the media (63% of control, n = 6). On the other hand, the same dose of insulin did not decrease apoB<sub>48</sub> production in CaCo-2 cells. Both Hep G2 and CaCo-2 cells displayed a similar level of insulin binding (7.9% and 5.1% respectively) and the intracellular cascade (PTK) due to insulin binding to its receptor was induced similarly (4.4 and 3.3-fold increase respectively).

Collectively the data would suggest that insulin regulates the production of apoB<sub>100</sub> in hepatic cells but not apoB<sub>48</sub> in CaCo-2 cells. The observed difference was not due to a lower level of binding of insulin to the two cell lines and indicates that there are different regulatory mechanisms operating on apoB in the different tissues. Therefore, these results perhaps suggest that the raised plasma level of CM-r in obesity may not be a consequence of insulin-stimulated overproduction of CM from the intestine.