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Lipoprotein interconversions during passage through subcutaneous adipose tissue in humans

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Adipose tissue lipoprotein lipase (LPL) is the enzyme responsible for hydrolysis of circulating lipoprotein triacylglycerol (TAG). Successive passages of very-low-density lipoprotein (VLDL) through adipose tissue are thought to produce progressive delipidation of the particles, with the eventual formation of intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL) [1]. The hydrolysis of TAG is accompanied by the movement of other components between lipoprotein fractions. For instance, with the shrinkage of the particles, surface components including cholesterol and, in the case of chylomicrons, apolipoprotein (apo)-A1 may move into high-density lipoprotein (HDL) particles [1].

The evidence for the occurrence of these processes in vivo is still indirect. It is based mostly on the movement of labelled components between fractions, and on studies of lipoprotein lipase action in vitro. We have now studied the changes in lipoproteins during their passage through adipose tissue in vivo in humans, using a recently described technique for measurement of arteriovenous differences across these tissues [2].

Methods

Samples were taken from a vein draining a warmed hand (arterialized) and from a vein draining the subcutaneous tissue of the anterior abdominal wall, as described previously [2]. Three sets of samples were taken after an overnight fast; the subject then ingested a meal of fat (41% total calories), carbohydrate (47%) and protein (12%) (3.1 MJ total) [3]. Further samples were taken at hourly intervals for 6 h. Plasma lipoprotein fractions (chylomicrons, VLDL, IDL, LDL and HDL) were separated by sequential flotation. In these fractions we have measured apo-A1, apo-A2 and apo-B by immunoturbidimetric methods, cholesterol and TAG by enzymic methods. Since the recovery of lipoprotein fractions was not necessarily quantitative, we have analysed the results in terms of ratios of components.

So far we have carried out detailed studies on one obese non-insulin-dependent and one lean insulin-dependent diabetic subject.

Abbreviations used: apo, apolipoprotein; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LPL, lipoprotein lipase; TAG, triacylglycerol; VLDL, very-low-density lipoprotein.

Fig. 1. Changes in the composition of VLDL after ingestion of a meal in a subject with insulin-dependent diabetes

In the insulin-dependent subject (Fig. 1), the arterialized plasma TAG/apo-B ratio in VLDL increased moderately after the baseline period, indicating either the entry of large TAG-rich particles into this density band or the removal of smaller TAG-depleted VLDL particles. In the venous plasma the ratio was lower than that in the arterialized plasma until 180 min, presumably reflecting hydrolysis of TAG by LPL. Later the ratio was greater in venous plasma than in arterialized which must reflect the removal of smaller VLDL particles, perhaps as chylomicrons enter the plasma and compete with larger VLDL particles for hydrolysis.

In contrast, the non-insulin-dependent subject the arterIALIZED TAG/apo-B ratio in VLDL increased steadily after the baseline for 3 h and then decreased. In the venous plasma the ratio was higher than in the arterialized, suggesting disproportionate removal of smaller VLDL particles from this fraction. Kinetic studies show impaired clearance of the large TAG-rich VLDL particles characteristic of non-insulin-dependent diabetes [4].

It would be expected that the hydrolysis of VLDL-TAG would be accompanied by movement of cholesterol into HDL. In the non-insulin-dependent subject, the cholesterol/apo-A1 ratio in HDL was higher in the venous plasma than in the arterialized which could represent such a movement. In the insulin-dependent subject, however, the cholesterol/apo-
AI ratio in HDL was higher in the arterialized plasma. This could represent transfer of apo-AI into HDL exceeding that of cholesterol.

In conclusion, marked changes in the composition of lipoprotein particles occur during their passage through human adipose tissue in vivo. Further studies should help to elucidate the effects of adipose tissue LPL on lipoprotein metabolism.


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Production of insulin-like growth factor-1 by rat hepatocytes is sensitive to nutrients but not growth hormone

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Insulin-like growth factor-1 (IGF-1) is produced mainly by the liver (1) and is thought, at least in part, to mediate the protein anabolic effects of growth hormone (GH). Circulating concentrations of IGF-1, which are closely related to the nutritional status of animals and their rates of growth, are under a number of controls including the proportional complexing of the growth factor to its different binding proteins. It is not clear whether nutrient availability per se exerts a direct control on the hepatic production of IGF-1 or whether relationships in vivo between IGF-1 concentration and nutrition are mediated through indirect endocrine regulation. This paper reports preliminary data on the influence of nutrient availability on the rate of production of IGF-1 by rat hepatocytes.

Hepatocytes were isolated from adult Rowett rats (50 to 55 days old; 240–270 g weight) by perfusion with collagenase [2]. Cells (6.3 x 10⁶) were cultured in Eagle's minimum essential medium (MEM) containing both essential and non-essential amino acids (Sigma: 50 x 20 ml/l and 100 x 10 ml/l solutions, respectively), glutamine (2 mm), and double concentration of glucose (11 mm) plus penicillin (1 x 10⁴ units/l), streptomycin (100 mg/l), amphotericin B (0.25 mg/l), bovine pancreatic trypsin inhibitor (2.5 mg/l), bovine serum albumin (42 mg/l), insulin (97 μg/l), deoxymethasone (39 μg/l), triiodothyronine (102 pg/l), recombinant bovine growth hormone (rbGH) (5 μg/l) and Hepes (20 mm). No serum was used during the preparation and culture of the cells. Cultures were carried out in 9 cm polyethylene dishes at 37°C in a water-saturated atmosphere; the presence of dexamethasone ensured hepatocyte attachment. At the termination of each incubation, supernatant from the culture medium (10 ml) was centrifuged, freeze-dried and taken up in water (0.7 ml), and was later acidified with formic acid (final concentration 0.2 M). This solution was chromatographed on Sephadex G-75 with a formic acid (0.2 M) : NaCl (1.5 mm) mixture as eluant. Fractions corresponding to pure IGF-1 (determined separately using 131I-IGF-1) were collected and prepared for radioimmunoassay.

Fig. 1(a) indicates the time-related production of IGF-1 by hepatocytes over a 50 h incubation. All subsequent studies

Abbreviations used: IGF-1, insulin-like growth factor 1; GH, growth hormone; MEM, minimum essential medium; rbGH, recombinant bovine growth hormone.

Vol. 18