Letters to the Editor

Chylomicron apolipoprotein B catabolism

Dear Sir,

Haffner et al. [1] studied the catabolism of chylomicron apolipoprotein (apo) B in normal subjects and lipaemic diabetic and non-diabetic patients. They found much longer residence times in the plasma for chylomicron apo B in normal subjects, compared with studies performed at the Cardiovascular Research Institute, San Francisco [2]. Haffner et al. reported in normal subjects a biphasic disappearance of chylomicron apo B with initial residence times of 1.2–1.9 h and, in the slower second phase, residence times from 3.6 up to 35.7 h. From these data initial half-lives of 50–80 min can be calculated. In contrast, the initial half-life of the intestinal apo B-48 reported by us was 5–15 min [2]. The residence times found by Haffner et al. are probably incorrect for the following reasons: firstly, autologous chylomicrons were injected, which were isolated from the blood 4–5 h after ingesting whipping cream; this material would be partially metabolized by lipoprotein lipase (LPL) and contains remnant particles with longer half-lives. In our studies, we used 'nascent' chylomicrons from a donor with genetically determined LPL-deficiency. Secondly, the decay curves of chylomicron apo B were expressed by Haffner et al. as the fraction of the value found 10 min after injection, instead of calculating the disappearance as a percentage of the injected dose; because of the rapid initial removal of the chylomicron apo B, neglecting this first period will greatly influence the results. Thirdly, Haffner et al. determined radioactivity in total chylomicron apo B with the tetramethylurea method, without discriminating between intestinal apo B (B-48) and apo B of hepatic origin (B-100). Although the authors claim that the chylomicrons were not contaminated with VLDL, this is hard to accept because of the large overlap in the properties and size of both lipoproteins. Indeed, we found in our preparation of triglyceride-rich lipoproteins, isolated under similar conditions (10^6 g) as described by Haffner et al., appreciable amounts of B-100, even in a subfraction containing particles with the largest diameter [2]. It is therefore more accurate to measure the specific marker of intestinal lipoproteins, namely B-48.

Yours sincerely,

A. F. H. Stalenhoef

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Reply from the authors

Dear Sir,

We welcome the contribution of Stalenhoef et al. [1] since, as mentioned in our paper [2], we were concerned that the tetramethylurea method measures both B-48 and B-100 and only 14 subjects were studied (five normal subjects, four hypertriglycaemic control subjects and five diabetic patients). We are pleased that Stalenhoef et al. have also reported that lipoprotein lipase (LPL)-deficient subjects have much longer residence times than normal individuals (normal subjects, $n=3$, residence time 5–15 min, LPL-deficient subjects, $n=2$; subject 'FL' essentially no removal for several hours and subject 'JC' slow removal) which are results similar to ours. We admit that using the 10 min value rather than the injected dose disappearance rate does give a high estimate of the residence time in normal subjects, but this would underestimate the difference between normal and lipaemic diabetic subjects, which was the major point of our paper. Contamination of chylomicrons with VLDL apolipoprotein B (B-100) would also increase the residence time, but we stand by our observation that no detectable B-100 was found in our chylomicron preparations. Our method can detect contamination as low as 5%. We are uncertain whether the estimates of residence time given by Stalenhoef et al. [1] or our own results are closer to the actual values, because differences in methodology were not addressed by Stalenhoef. The number of subjects studied was very small and estimates of residence times are likely to be imprecise. No data were given to compute confidence intervals for residence times. Our 95% confidence intervals to the mean residence times were 38 min, 93 min (DF=4). If we had as few subjects as Stalenhoef, the 95% confidence interval would be 12.4 min, 109 min (DF=2). We cannot compare residence times because Stalenhoef did not describe the method used to compute the residence times. We used the modification of the Phair model [3] with data analysis carried out by the SAAM computer program [4]. The Stalenhoef design was essentially an isologous-autologous study, where chylomicrons isolated from LPL-deficient subjects were injected into other subjects. Isologous materials can be removed much more rapidly than autologous material because the recipient may not have a similar pool of isologous chylomicrons.

Thus, while we acknowledge that our methodology was not as sophisticated as that of Stalenhoef et al. and residence times in normal subjects were likely to be overestimated, the major conclusion in both papers, that residence times are greater in LPL-deficient individuals (diabetic in our study and non-diabetic in that of Stalenhoef) than in normal individuals by more than an order of magnitude, is similar. We emphasise that subject number 6 in our study had a similar pool size (triglycerides 21.9 nmol/l) as seen in lipaemic diabetic subjects, and had a very short residence time. Thus, the increased residence time was due to LPL deficiency rather than enlarged pool size. Furthermore, we have also shown that apolipoprotein B from our injected chylomicrons did not appear in LDL [5]. We look forward to hearing further results from Stalenhoef et al.

Yours sincerely,

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Hyperinsulinism in iron overload

Dear Sir,

Niederau et al. have demonstrated hyperinsulinism in association with normal glucose tolerance in pre-cirrhotic patients with haemochromatosis [1]. Almost identical observations and conclusions were obtained by us in 15 younger patients with transfusional iron overload, five of whom had cirrhosis and nine had severe hepatic fibrosis [2]. Oral glucose tolerance tests showed plasma glucose concentrations which were within the normal range in all patients except one, who had 'frank' diabetes. There was, however, a unique pattern of a secondary rise of glucose concentrations at 120 min in seven of these patients, all of whom had glucose tolerance curves within the normal range. Thus, the 120 min glucose concentrations (5.9 ± 0.3 mmol/l) were significantly higher ($p < 0.02$) than those at 90 min (4.7 ± 0.2), whilst the latter were lower ($p < 0.01$) than the peak concentrations achieved at 30 min (6.2 ± 0.5 mmol/l). Such a pattern is not recognised

Fig. 1. The median IRI concentrations in patients with iron overload (○·····○) and control subjects (●—●) following a glucose challenge. Ranges of IRI concentrations (mU/l): control subjects: 0 min: 0–10; 30 min: 12–38; 60 min: 13–39; 90 min: 30–72; 120 min: 3–23. Patients: 0 min: 18–44; 30 min: 38–116; 60 min: 35–101; 90 min: 30–72; 120 min: 30–80. The difference between the two curves was statistically significant at all points of measurement ($p < 0.01$)

or associated with any condition. Fasting immunoreactive insulin (IRI) concentration was greater than 20 mU/l (age- and sex-matched control subjects: 10 mU/l) in all patients except two, one of whom had diabetes. The response of IRI to glucose was markedly greater than that seen in control subjects (Fig. 1).

These data are directly comparable to those presented by Niederau et al. [1]. The presence of fasting and post-glucose hyperinsulinism suggests the presence of insulin resistance as the initial abnormality of glucose metabolism following iron overload. This is caused by inadequate insulin extraction by the liver. The development of diabetes probably follows a relative decompensation of the pancreatic β -cell due to continuing iron overload and injury of the pancreas. It is of interest that the only patient in our series, who had diabetes, had a fasting IRI concentration greater than that in control subjects but less than that in non-diabetic patients with iron overload. It is also of relevance that in patients with iron overload, the development of cirrhosis is not necessarily associated with a significant further abnormality of glucose metabolism, since cirrhotic patients showed glucose tolerance and IRI response not significantly different from those seen in patients without cirrhosis. Thus, pancreatic iron overload with β -cell destruction and not cirrhosis is the final crucial step in the pathogenesis of diabetes due to iron overload.

Since there is increasing evidence that there is an abnormality of exocrine pancreatic function in diabetes [3–6], we have also investigated the presence of an exocrine pancreatic deficit caused by iron overload. There has hitherto been no evidence to show exocrine pancreatic deficit due to iron overload in such patients ante mortem. Using recently developed assay techniques, we were able to show that serum immunoreactive trypsin concentrations and pancreatic lipase activity are markedly lower in patients with iron overload and are comparable with those found in diabetes [7, 8] and severe chronic pancreatitis [9]. It is of interest, however, that neither diabetic patients nor patients with iron overload have pancreatic steatorrhoea.

Yours sincerely,

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