secretion of VLDL-TG in glucose- and fructose-fed rats. Glyceraldehyde 3-phosphate dehydrogenase; PLFA, phospholipid fatty acid.

The secretion of VLDL-TG in glucose- and fructose-fed rats was studied. In this study, we have tried to evaluate the effects of plasma triacylglycerols [I] which has been attributed to the secretion of VLDL-TG. This agent elicited a rapid, but very small, stimulation of \( ^{40} \text{Ca}^{2+} \) efflux, which was much less than that observed following carbachol addition. Since melittin (1 \( \mu \text{g/ml} \)) on the rate of unidirectional \( ^{40} \text{Ca}^{2+} \) efflux from perfused islets. This agent elicited a rapid, but very small, stimulation of \( ^{40} \text{Ca}^{2+} \) efflux, which was much less than that observed following carbachol addition. Since melittin (1 \( \mu \text{g/ml} \)) a major stimulus for \( ^{40} \text{Ca}^{2+} \) mobilization in islet cells, it may differ from the corresponding protein in islet cells, which may be activated by [AlF\(_4\)] in intact cells (Blackmore et al., 1985).

Fig. 1 also shows the effect of the phospholipase A\(_2\), activator melittin (1 \( \mu \text{g/ml} \)) on the rate of unidirectional \( ^{40} \text{Ca}^{2+} \) efflux from perfused islets of Langerhans.

Isolated rat islets were preloaded with \( ^{40} \text{Ca}^{2+} \) and then infusorion (min). This agent elicited a rapid, but very small, stimulation of \( ^{40} \text{Ca}^{2+} \) efflux, which was much less than that observed following carbachol addition. Since melittin (1 \( \mu \text{g/ml} \) on the rate of unidirectional \( ^{40} \text{Ca}^{2+} \) efflux from perfused islets. This agent elicited a rapid, but very small, stimulation of \( ^{40} \text{Ca}^{2+} \) efflux, which was much less than that observed following carbachol addition. Since melittin (1 \( \mu \text{g/ml} \) on the rate of unidirectional \( ^{40} \text{Ca}^{2+} \) efflux from perfused islets. This agent elicited a rapid, but very small, stimulation of \( ^{40} \text{Ca}^{2+} \) efflux, which was much less than that observed following carbachol addition. Since melittin (1 \( \mu \text{g/ml} \) on the rate of unidirectional \( ^{40} \text{Ca}^{2+} \) efflux from perfused islets. This agent elicited a rapid, but very small, stimulation of \( ^{40} \text{Ca}^{2+} \) efflux, which was much less than that observed following carbachol addition. Since melittin (1 \( \mu \text{g/ml} \) on the rate of unidirectional \( ^{40} \text{Ca}^{2+} \) efflux from perfused islets. This agent elicited a rapid, but very small, stimulation of \( ^{40} \text{Ca}^{2+} \) efflux, which was much less than that observed following carbachol addition. Since melittin (1 \( \mu \text{g/ml} \) on the rate of unidirectional \( ^{40} \text{Ca}^{2+} \) efflux from perfused islets. This agent elicited a rapid, but very small, stimulation of \( ^{40} \text{Ca}^{2+} \) efflux, which was much less than that observed following carbachol addition. Since melittin (1 \( \mu \text{g/ml} \) on the rate of unidirectional \( ^{40} \text{Ca}^{2+} \) efflux from perfused islets. This agent elicited a rapid, but very small, stimulation of \( ^{40} \text{Ca}^{2+} \) ef

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{fig1.png}
\caption{Effect of carbachol, melittin or [AlF\(_4\)] on the rate of \( ^{40} \text{Ca}^{2+} \) efflux from perfused rat islets of Langerhans.}
\end{figure}

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
Agent & Time (min) & \( ^{40} \text{Ca}^{2+} \) efflux (%) \\
\hline
Carbachol & 1 & 200 \\
Melittin & 2 & 250 \\
[AlF\(_4\)] & 3 & 300 \\
\hline
\end{tabular}
\caption{Effect of carbachol, melittin or [AlF\(_4\)] on the rate of \( ^{40} \text{Ca}^{2+} \) efflux from perfused rat islets of Langerhans.}
\end{table}

under these conditions. This, in return, suggests that if a G-protein is involved in receptor-mediated \( ^{40} \text{Ca}^{2+} \) mobilization in islet cells, it may differ from the corresponding protein in hepatocytes which can be activated by [AlF\(_4\)] in intact cells (Blackmore et al., 1985).

Relationships among hepatic lipogenesis, hepatic triacylglycerol secretion and hypertriglyceridaemia in rats fed chronically on fructose- or glucose-rich fat-free diets

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Feeding rats chronically a diet rich in fructose elevates levels of plasma triacylglycerols [1] which has been attributed to increased rates of hepatic lipogenesis [2] and secretion of very-low-density lipoprotein-triaclyglycerols (VLDL-TG) from the liver [3]. In this study we have tried to evaluate the effect of hepatic lipogenesis on VLDL-TG secretion in vivo and the secretion of VLDL-TG in glucose- and fructose-fed rats.

Abbreviations used: VLDL-TG, very-low-density lipoprotein-triaclyglycerol; t.l.c thin layer chromatography; TGFA, triacylglycerol fatty acid; PLFA, phospholipid fatty acid.

Male Sprague-Dawley rats in individual cages (initial body weight 140-150 g) were fed for three weeks on a diet containing 58% by weight of fructose or glucose, 22% casein, 12% non-nutritive fibre with vitamins, minerals and choline chloride as described elsewhere [3]. The rats were maintained in a light-dark cycle, illuminated from 06.00-18.00 h. On the final day, some rats from each dietary group were injected through the tail vein with 0.2 g Triton WR-1339/100 g body weight (20% w/v in saline) between 01.00 and 02.00 h and then 10 min later injected i.p. with 2 mCi \(^{3} \text{H}_{2} \text{O \)} O. Other rats fed glucose or fructose received injections of saline as controls. After 70 min, the rats were killed, samples of liver were removed and plasma lipids were extracted. Only those rats which had plasma concentrations of Triton WR-1339 greater than 5 g/l were accepted for inclusion in the calculations (detection was by u.v. absorption at 279 nm in plasma lipid extracts). Hepatic lipogenesis (newly synthesized fatty acids) was determined from the total incorporation of \(^{3} \text{H}_{2} \text{O \)} into the fatty acids of liver saponifiable lipids [4, 5] and triacyl-

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glycerols secreted in the plasma where 90% of the radioactivity was found in this fraction after separation by t.l.c. (petroleum ether b.p. 40°-60°C + diethyl ether/acetic acid 70: 20 v/v/v). The total rate of triacylglycerol fatty acid (TGFA) secretion from the liver was determined from the difference after 80 min in plasma triacylglycerol concentrations measured colorimetrically [6] between rats either injected with saline or Triton WR-1339.

The plasma triacylglycerol concentration in glucose-fed rats (0.78 ± 0.05 g/l, n = 10) was lower than in those fed fructose (1.33 ± 0.12 g/l, n = 10) without Triton injection. Following Triton, the concentration rose to 4.2 ± 0.7 g/l, n = 4 (glucose) and 7.5 ± 0.11 g/l, n = 3 (fructose). Therefore, the calculated rates of secretion of total TGFA from the liver was greater in the fructose group (Table 1). Greater rates of secretion of newly synthesized TGFA from the liver into plasma were also found in the fructose rats, as were the overall rates of hepatic lipogenesis (Table 1). The normalized rates of hepatic lipogenesis are lower than the total rates of secretion of TGFA. This may reflect different methods of determination of lipogenesis and secretion or the contribution of fatty acids of both hepatic origin (pre-existing stores of TGFA and PLFA) and of non-hepatic origin to the secretion of TGFA.

As indicated, above the plasma triacylglycerol pool was greater in the fructose group, but it was calculated that the fractional turnover rates were similar (0.055 and 0.058 per min - glucose and fructose, respectively) and t (12.6 and 11.9 min - glucose and fructose, respectively).

As may be expected there was a strong positive correlation (r = 0.86, p < 0.01) between the rate of secretion of newly synthesized TGFA into plasma and the rate of hepatic lipogenesis. However, as hepatic lipogenesis increased, so did the total secretion of TGFA until a plateau was reached at higher rates of lipogenesis (0.13 mg fatty acids/min per 100 g body wt).

The rates of TGFA secretion are similar to those found in other studies [7, 8], but here fructose feeding produced a greater increase over the rate in glucose-fed rats. Nevertheless, the rates of secretion were considerably less than those reported for both diets in our earlier work [3] in which [³H]-fatty-acid-labelled VLDL was used to determine rates of secretion. These discrepancies of magnitude cannot be easily reconciled. However, these results clearly establish that enhanced hepatic secretion of VLDL-TG is an important element in fructose-induced hypertriglyceridaemia, and that synthesis of novo of TGFA in the liver makes a significant contribution to the total secretion of VLDL-TG from the liver in rats fed fat-free diets.

Table 1. Hepatic lipogenesis and secretion of triacylglycerols into plasma in rats fed fat-free diets rich in fructose or glucose

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total hepatic lipogenesis* (mg fatty acids min per 100 g body wt.)</th>
<th>Rate of secretion of newly synthesized TGFA in plasma** (mg TGFA)</th>
<th>Increase in total plasma TGFA 80 min after injection of Triton WR-1339† (mg TGFA)</th>
<th>Normalized rate of total TGFA secretion from liver‡ (mg TGFA/min per 100 g body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (n = 4)</td>
<td>0.900 ± 0.009</td>
<td>0.053 ± 0.001</td>
<td>26.4 ± 6.3</td>
<td>0.17 ± 0.048</td>
</tr>
<tr>
<td>Fructose (n = 3)</td>
<td>0.23 ± 0.10</td>
<td>0.087 ± 0.019</td>
<td>45.3 ± 0.58</td>
<td>0.31 ± 0.06</td>
</tr>
</tbody>
</table>

* Incorporates rates of lipogenesis calculated from counts in liver lipids plus the rate of appearance of counts in plasma TGFA**. For a timespan of 60 min was estimated to allow for equilibration of H2O in body water and ** 45 min to allow for a lag in secretion from liver.
† Assuming that plasma volume is 4% of body weight.
‡ Calculated from †. Mean body wt. 207 g (glucose), 200 g (fructose).

Glycation of serum albumin and fibrinogen: identification of modified sites

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Non-enzymic glycosylation (glycation) of blood proteins is a usual occurrence which is accelerated in diabetes. It usually involves the addition of glucose to unprotonated amino groups of proteins with the formation of a Schiff base, which then undergoes an Amadori rearrangement to produce a 1-deoxy, 2 keto-derivative (Harding, 1985). Measurement of the extent of glycation of haemoglobin is now widely used in the management of diabetes as an index of integrated glycaemia of the previous four to six weeks. Albumin has a half-life of 20 days and its glycation reflects the glycaemic status of the preceding 7-14 days, whereas glycated fibrinogen which has a shorter half-life (4 days for fibrinogen) would be expected to respond more quickly to changes in blood glucose levels than either albumin or haemoglobin.

The sites in haemoglobin that are modified have been well studied. The sites in albumin that are modified are less well understood. The identification of modified sites in serum albumin and fibrinogen will contribute to a better understanding of the role of glycation in the development of complications of diabetes.