Changes in individual rates of pancreatic enzyme and isoenzyme biosynthesis in the obese Zucker rat

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Both alterations of enzyme content and a markedly decreased secretory response to selected physiological stimuli have been demonstrated previously in the pancreas of the obese Zucker rat. The purpose of the present investigation was to determine the degree to which alterations of enzyme content could be attributed to changes in enzyme biosynthesis. Amylase content of obese rats was decreased by 50 %, whereas lipase and trypsinogens were significantly increased. However, the decrease in amylase content was less than might have been predicted from the rate of amylase biosynthesis (80 % decrease), and the increases in content of trypsinogen(s) and lipase were greater than would have been predicted from alterations in the absolute rates of biosynthesis. In view of the rapid turnover of pancreatic enzymes under normal conditions, it seems probable that a markedly decreased secretory response to various stimuli leads to an increased content of some enzymes in the pancreas of the obese rat. Ciglitazone treatment, which decreases insulin resistance in obese animals and leads to normalization of glucose metabolism in their pancreatic tissue, restored the enzyme-synthesis rates towards normal, showing that the abnormalities of enzyme synthesis were linked to the insulin resistance rather than to the obese genotype itself. Lipid inclusion bodies were found in acinar cells of obese rats. These bodies have previously been described in acinar cells of starved animals, which, in common with the acinar tissue of the obese Zucker rat, have decreased glucose metabolism.

INTRODUCTION

Several abnormalities of pancreatic acinar function have been identified in the obese Zucker rat. These abnormalities include changes in enzyme content [1,2], down-regulation of specific membrane receptors [3,4] and alterations of enzyme secretion [2,5]. One of the most striking changes in enzyme content is a decrease in amylase [1,2], a change which is seen also in the obese (C57 BL/6J *ob/ob*) mouse [6]. Furthermore, in the obese rat, decreases in mRNA for pancreatic amylase have also been reported [7]. In addition to the decrease in amylase content, variable increases in lipase, trypsinogen and chymotrypsinogen have also been demonstrated [1,2]. However, on account of the decreased response to at least two of the major stimulants of enzyme secretion, or their analogues, namely cholecystokinin and acetylcholine [2–5], it has been difficult to assess the degree to which alterations in enzyme content reflect changes in gene expression and in the rates of enzyme biosynthesis.

The purpose of the present investigation was twofold. The first aim was to assess the rates of enzyme biosynthesis in the pancreas of the obese Zucker rat. Since it has been firmly established that the biosynthetic rates of individual pancreatic enzymes are regulated in very different ways [8–12], it was considered essential that individual rates of enzyme biosynthesis be assessed. The second aim of the project was to determine whether, in association with the biochemical changes, there were any histological changes in the exocrine pancreas of the obese Zucker rat. In order to see whether certain abnormalities were due to insulin resistance, some rats were treated with Ciglitazone {5-[4-(1-methylcyclo-

hexylmethoxybenzyl]thiazolidine-2,4-dione}, a substance which decreases insulin resistance in obese laboratory animals, and which has practically no effect on lean animals [13–15].

EXPERIMENTAL

Animals

Obese (*fa/fa*) and homozygous lean (*Fa/Fa*) female Zucker rats were a gift from Professor B. Jeanrenaud (University of Geneva). Rats were maintained on a standard laboratory chow containing (by wt.) 17 % protein, 3 % lipids, 58.7 % carbohydrate, 4.3 % cellulose, 5 % minerals and 12 % water. A group of obese and a group of lean rats were treated with Ciglitazone (80 mg/kg per day; obtained from Takeda Chemical Industries, Osaka, Japan), which was mixed into the food. Treatment started at 18 weeks of age and lasted for at least 4 weeks. Food intake, plasma glucose [16] and plasma insulin [17] concentrations of treated and control groups were monitored during this period. Animals were maintained in a temperature-controlled room (23 °C) with a 12 h-dark/12 h-light cycle (07:00–19:00 h).

Animals were killed by decapitation in the fed state between 09:30 and 10:30 h. The pancreas was rapidly removed and a portion from the splenic area homogenized before measurements of DNA [18], protein [19], amylase [20], chymotrypsinogen [21], trypsinogen [21] and lipase [measured by a commercially available kit (Monotest 10 lipase) kindly given by Professor F. H. Schmidt, Boehringer G.m.b.H., Mannheim, Germany]. In some animals the remainder of the pancreas was then

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Table 1. Protein and enzyme concentrations in pancreases of adult lean and obese Zucker rats: effect of Ciglitazone treatment

Protein concentration is expressed as mg/g of pancreas, and all enzyme concentrations are as units/mg of protein. The results are expressed as means $\pm$ S.E.M. for the numbers of animals shown in parentheses: *, **, *** indicate significant difference from controls ($P < 0.05$, < 0.02 and < 0.001 respectively).

	Lean		Obese	
	Untreated	Treated	Untreated	Treated
Protein	50.4 $\pm$ 4.3 (13)	47.8 $\pm$ 3.8 (10)	56.6 $\pm$ 2.6 (14)	65.4 $\pm$ 5.3 (10)
Amylase	301 $\pm$ 20 (13)	274 $\pm$ 12 (10)	141 $\pm$ 17 (14)***	267 $\pm$ 22 (10)
Lipase	112 $\pm$ 12 (13)	99 $\pm$ 7 (10)	172 $\pm$ 19 (14)**	149 $\pm$ 17 (10)
Chymotrypsinogens	16.7 $\pm$ 1.4 (13)	17.9 $\pm$ 0.9 (10)	21.3 $\pm$ 2.0 (14)	28.4 $\pm$ 2.2 (10)
Trypsinogens	18.3 $\pm$ 3.0 (7)	21.2 $\pm$ 1.8 (6)	26.1 $\pm$ 2.0 (9)*	26.9 $\pm$ 2.7 (8)

taken for the preparation of dispersed acini and studies on the rates of enzyme biosynthesis.

Acini were prepared by collagenase digestion as previously described in detail [22]. Acini were then preincubated in Krebs–Hepes buffer containing 12.5 mM–Hepes, 135 mM–NaCl, 4.8 mM–KCl, 1.0 mM–CaCl₂, 1.2 mM–KH₂PO₄, 1.2 mM–MgSO₄, 5.0 mM–NaHCO₃, 5 mM–glucose and physiological concentrations of all amino acids [23], pH 7.4, 37 °C for 30 min. The acini were then washed and resuspended in fresh Krebs–Hepes buffer also containing physiological concentrations of amino acids. For the determination of the rates of protein synthesis a mixture of 15 ¹⁴C-labelled amino acids (2.5 μ Ci/ml) was added (algal hydrolysate, Amersham CFB 25; Amersham International, Little Chalfont, Bucks., U.K.) and the acini were incubated at 37 °C for 90 min. During this period protein synthesis is linear with time, and it has been shown previously that discharge of labelled proteins is insignificant (1–2 %) [24]. At the end of the incubation period, the pancreatic acini were homogenized in lysis buffer, and non-reduced proteins were separated by two-dimensional electrophoresis by the method of Scheele [25] as previously described in detail [12]. In brief, in the first dimension proteins were separated according to their apparent pI values (pH range 4.0–9.0), in a gel containing 8 M–urea, and in the second dimension according to their apparent M_r values in 10–20 %–polyacrylamide gradient gels in 0.2 % SDS. The individual protein spots were cut out after staining with Coomassie Blue, homogenized in 2 ml of distilled water, suspended in 1 ml of 5 % SDS and counted for radioactivity in 10 ml of Optifluor scintillant (Packard Instruments G.m.b.H., Frankfurt, Germany). This method of determination of individual rates of enzyme biosynthesis has previously been standardized by using labelled secreted products from pancreatic lobules [10]. Relative rates of synthesis of individual enzymes were calculated from the amount of radioactivity in each spot, expressed as a percentage of the radioactivity incorporated into all the protein spots. The amount of DNA in the sample applied to the gel was determined, and the absolute rate of biosynthesis for individual enzymes or isoenzymes expressed as d.p.m./90 min per μ g of DNA. Two-dimensional gel electrophoresis was carried out at least twice for each sample.

For fine-structural analysis, fragments of the pancreas of lean and obese Zucker rats were fixed by immersion for 2 h in 0.1 M–cacodylate buffer containing 2.5 % (v/v)

formaldehyde and 2.5 % (v/v) glutaraldehyde at pH 7.4. After post-fixation in 1 % OsO₄, standard procedures were used for dehydration and embedding. Light-microscopy was performed on semi-thin sections. From selected areas thin sections were cut, stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 9 S electron microscope.

RESULTS

Effects of Ciglitazone treatment on food intake, plasma glucose and plasma insulin concentrations

We have previously shown that Ciglitazone has no effect on food intake or plasma glucose concentration in Zucker rats [5,7]. In the present experiments this was also the case (results not shown). As expected, Ciglitazone treatment had no effect on plasma insulin of lean rats,

Fig. 1. Separation of exocrine-pancreatic proteins of obese Zucker rats by two-dimensional isoelectric focusing/SDS-gel electrophoresis

Proteins spots were stained with Coomassie Blue and are identified in the Figure by abbreviations described in Table 2.

Table 2. Apparent pI and M_r values of proteins from the exocrine pancreas of the obese Zucker rat

Protein spot*	(Pro)enzyme	pI	M_r
A1/2	Amylase	7.3	55000
L	Lipase	6.8	53000
T1/2	Trypsinogen 1/2	4.5	21000
T3	Trypsinogen 3	7.3	22500
C1	Chymotrypsinogen 1	4.8	25000
C2	Chymotrypsinogen 2	9.0	25000
PE1	Proelastase 1	5.2	26000
PE2	Proelastase 2	8.5	28500
PC	Procarboxypeptidases A, B	5.0–5.4	47000
R	Ribonuclease	9.0	14000
1	GP2	4.7	77500
2		5.8	56000
3	Unidentified proteins	5.8	54000

* See Fig. 1.

but was associated with decreased insulin in obese animals. Thus at the end of the control (untreated animals) or treatment period the plasma insulin concentrations were 0.7 ± 0.1 and 0.7 ± 0.2 ng/ml in untreated and treated lean animals respectively, and 7.2 ± 0.7 and 4.1 ± 0.5 ng/ml for untreated and treated obese rats respectively. In the absence of changes in food intake and plasma glucose concentrations, the decreased plasma insulin concentration with Ciglitazone treatment is taken as evidence of decreased insulin resistance in treated obese rats.

Protein and enzyme concentrations in pancreatic acini

The protein concentration was similar in the pancreases of untreated lean and obese rats (Table 1), and protein content/ μ g of DNA was also similar (results not shown). Amylase concentration was decreased, whereas lipase concentration was increased in the pancreases of obese rats as compared with the lean controls (Table 1). This confirms previous reports from our laboratory [2,7]. In

addition, there was a significant increase in trypsinogen concentration in the pancreases of obese rats. Treatment with Ciglitazone caused no significant change in the pancreatic enzyme content of lean rats. By contrast, it resulted in a significant (90%) increase in amylase concentration in the pancreases of obese rats. There was a smaller increase in chymotrypsinogen and a slight decrease in lipase (Table 1).

Enzyme biosynthesis

(a) **Relative rates.** Smaller groups of animals were chosen at random from the larger groups described above for the studies of enzyme biosynthesis. The Coomassie Blue-staining pattern of pancreatic proteins separated by two-dimensional gel electrophoresis is shown in Fig. 1. This pattern does not differ from that shown previously in the Wistar rat [12]. Table 2 gives the apparent pI and M_r values of individual pancreatic proteins. The relative rates of enzyme biosynthesis are shown in Table 3. It is clear that there were major alterations of these rates in the obese rats. The most striking change was the marked decrease in the relative rate of amylase biosynthesis with respect to other enzymes (Table 3). Thus, whereas in lean animals the relative rate of amylase biosynthesis was 44% of the total, this was decreased to 16% in obese animals, where it was superseded by that for trypsinogens, which had increased from 22% in lean to 33% in obese rats. Lipase accounted for a relatively small percentage of the biosynthetic rate in lean rats, i.e. 1.5%, but this was doubled in the obese rat. Ciglitazone had no effect on the relative rates of enzyme synthesis in lean rats. By contrast, in obese rats, treatment with Ciglitazone was associated with changes in relative rates of biosynthesis, such that the total rates for lipase, procarboxypeptidases, chymotrypsinogens and proelastases were normalized and those for amylase and trypsinogens were partially normalized.

(b) **Absolute rates.** When the total rate of enzyme biosynthesis was expressed as amount of radioactivity incorporated per μ g of DNA, it was revealed that enzyme synthesis was decreased by almost 50% in the obese-rat pancreas (Fig. 2). The concentration of protein

Table 3. Relative rates of enzyme biosynthesis in pancreatic acinar tissue of adult lean and obese Zucker rats: effect of Ciglitazone treatment

Isolated pancreatic acini were incubated in the presence of radioactive amino acids for 90 min. The tissue was homogenized and proteins were separated by two-dimensional (isoelectric focusing/SDS-gel) electrophoresis. Radioactivity was quantified in individual protein spots and expressed as a percentage of radioactivity incorporated into all exocrine proteins. Results are presented as means $\pm$ S.E.M. for the numbers of animals shown in parentheses: *, **, *** indicate significant difference from controls ($P < 0.05$, < 0.005 , < 0.001 respectively).

(Pro)enzyme	Lean		Obese	
	Untreated (4)	Treated (4)	Untreated (6)	Treated (4)
Amylase	44.0 ± 0.8	42.4 ± 0.7	$16.5 \pm 1.8^{***}$	$28.2 \pm 2.2^{***}$
Lipase	1.5 ± 0.2	1.46 ± 0.2	$3.1 \pm 0.2^{***}$	1.7 ± 0.2
Procarboxypeptidases	7.8 ± 0.3	8.4 ± 0.9	$12.2 \pm 0.5^{***}$	9.3 ± 0.7
Chymotrypsinogens	14.4 ± 1.7	14.5 ± 0.7	$19.3 \pm 1.0^*$	16.0 ± 1.3
Proelastases	3.4 ± 0.3	4.0 ± 0.2	$6.1 \pm 0.2^{***}$	4.8 ± 0.3
Trypsinogens	21.9 ± 1.1	20.6 ± 0.9	$33.0 \pm 1.5^{***}$	$26.6 \pm 1.0^{**}$
Ribonuclease	3.5 ± 0.5	2.5 ± 0.3	3.4 ± 0.8	3.4 ± 0.5
Unidentified	1.3 ± 0.5	$4.0 \pm 0.9^*$	3.0 ± 1.0	5.3 ± 1.3

Fig. 2. Absolute rates of incorporation of labelled amino acids into total pancreatic acinar enzymes of adult lean and obese Zucker rats

Ciglitazone treatment was as described in the Experimental section.

per μg of DNA was similar in obese and lean rats (results not shown). In obese animals, Ciglitazone therapy restored the total rate of enzyme synthesis to that found in lean controls. Since Ciglitazone had no effect on either the absolute rate of total enzyme synthesis or on the relative rates of individual enzyme synthesis in lean rats, the two groups of lean rats (treated and untreated) have been considered as one group for determination of the absolute rates of individual enzyme synthesis. As might have been expected from the changes in the relative biosynthetic rates, the greatest alteration in the absolute rate of synthesis was seen for amylase, where there was an 80% decrease, and this alone accounted for almost the whole decrease in total enzyme synthesis (Table 4). This was accompanied by a 20–25% decrease in trypsinogens and chymotrypsinogens and by a 33% increase in lipase. Treatment with Ciglitazone restored these towards normal. For trypsinogens, there was a tendency for overcompensation during Ciglitazone treatment of obese rats (Table 4). When those enzymes which have isoenzymes with widely different pI values were considered in more detail, it was seen that, although the absolute rates of biosynthesis were decreased to a similar degree for cationic and anionic isoenzymes of chymotrypsinogen, this was not the case for trypsinogen (Table 5b). In the latter case, there was an approx. 34% decrease in the absolute rate of synthesis for the anionic isoenzyme, without any change for the cationic isoenzyme(s). Interestingly, Ciglitazone treatment resulted in normalization of the rate for the anionic isoenzyme, while at the same time stimulating the cationic isoenzyme to a biosynthetic rate approx. 33% above that seen in lean rats. Ciglitazone treatment had no effect on the isoenzymes of either chymotrypsinogen or trypsinogen of lean rats (not shown).

Histological findings

No significant difference in the structure of the exocrine pancreas of obese Zucker rats as compared with lean

Table 4. Absolute rates of incorporation of labelled amino acids into individual enzymes of adult lean and obese Zucker rats: effect of Ciglitazone treatment of obese rats

On account of the lack of effect of Ciglitazone on both the absolute rate of total enzyme synthesis (see Fig. 2) and the relative rates of individual enzyme synthesis (Table 3), the two groups of lean rats have been considered as one group for determination of absolute rates of individual enzyme biosynthesis. For calculation of absolute rate of biosynthesis see the Experimental section. Results are presented as means $\pm$ S.E.M. for the numbers of animals shown in parentheses: * indicates significant difference from controls ($P < 0.001$).

(Pro)enzyme	Rate (d.p.m./90 min per μg of DNA)		
	Lean (total) (8)	Obese (untreated) (6)	Obese (treated) (4)
Amylase	2910 $\pm$ 330	621 $\pm$ 171*	1860 $\pm$ 475
Lipase	104 $\pm$ 12	138 $\pm$ 31	101 $\pm$ 13
Procarboxy-peptidases	565 $\pm$ 81	445 $\pm$ 89	612 $\pm$ 147
Chymotrypsinogens	916 $\pm$ 118	696 $\pm$ 139	979 $\pm$ 151
Proelastases	237 $\pm$ 26	225 $\pm$ 38	293 $\pm$ 52
Trypsinogens	1470 $\pm$ 190	1190 $\pm$ 243	1800 $\pm$ 385
Ribonuclease	191 $\pm$ 20	152 $\pm$ 33	208 $\pm$ 31

controls was observed at the light-microscopic level. In low-power electron micrographs in both groups, the acinar cells revealed a similar content of zymogen granules (Fig. 3a). The only difference between the two groups was that, in obese animals, lipid inclusion bodies were regularly observed in the area of the Golgi complex. At higher magnification these bodies were delineated by a membrane and often surrounded by mitochondria (Fig. 3b). Besides circumscribed areas of low electron opacity, electron-dense material with regular spacing, similar to myelin figures, was observed (Fig. 4). After Ciglitazone treatment of obese animals, the size and frequency of the lipid inclusion bodies were markedly decreased (not shown).

DISCUSSION

The results clearly show that, in the Zucker rat, obesity is associated with major changes in the relative rates of biosynthesis of almost all pancreatic enzymes. Furthermore, the absolute rate of biosynthesis for the total of all enzymes was decreased by about 45% in these obese animals. In terms of magnitude, the greatest changes in both the relative and absolute rates were seen with respect to amylase biosynthesis, which was markedly decreased. In fact, the decrease in the absolute rate of total enzyme synthesis was almost entirely due to the decrease in the rate for amylase. This is consistent with our previous finding of decreased mRNA for pancreatic amylase in pancreases of adult obese Zucker rats [7]. As was the case for amylase mRNA, the rate of amylase biosynthesis was partially normalized by treatment with Ciglitazone, showing that this was related to the insulin-resistant state of these animals and was not an expression

Table 5. Relative (a) and absolute (b) rates of incorporation of labelled amino acids into isoenzymes of widely different pI

The relative and absolute rates of synthesis were determined as described in the Experimental section, and are expressed as percentages and as d.p.m./90 min per μg of DNA respectively. The results are expressed as means $\pm$ S.E.M. for the numbers of animals shown in parentheses.

	Cationic pI			Anionic pI		
	Lean total (8)	Obese Untreated (6)	Obese treated (4)	Lean total (8)	Obese untreated (6)	Obese treated (4)
(a) Relative rates						
Chymotrypsinogen	9.7 $\pm$ 0.5	13.5 $\pm$ 1.0	11.5 $\pm$ 1.1	4.2 $\pm$ 0.3	5.8 $\pm$ 0.2	4.5 $\pm$ 0.2
Proelastase	1.9 $\pm$ 0.2	4.0 $\pm$ 0.3	2.8 $\pm$ 0.1	1.8 $\pm$ 0.2	2.1 $\pm$ 0.2	2.0 $\pm$ 0.3
Trypsinogen	12.5 $\pm$ 0.3	22.0 $\pm$ 0.8	17.8 $\pm$ 0.6	11.8 $\pm$ 0.5	11.0 $\pm$ 1.1	8.8 $\pm$ 0.5
(b) Absolute rates						
Chymotrypsinogen	651 $\pm$ 85	482 $\pm$ 92	694 $\pm$ 104	293 $\pm$ 32	215 $\pm$ 49	285 $\pm$ 49
Proelastase	138 $\pm$ 13	145 $\pm$ 25	172 $\pm$ 33	112 $\pm$ 12	81 $\pm$ 15	121 $\pm$ 20
Trypsinogen	858 $\pm$ 107	786 $\pm$ 154	1140 $\pm$ 244	607 $\pm$ 88	402 $\pm$ 93	658 $\pm$ 147

Fig. 3. (a) Fine structure of exocrine pancreas in obese Zucker rat, with normal complement of zymogen granules; (b) the zone of the Golgi complex (G) at higher magnification, showing stacks of Golgi cisternae and condensing vacuoles (CV)

In (a), the arrows indicate inclusion bodies: L = acinar lumen. Magnification $\times 3800$. In (b), the arrows indicate inclusion bodies: note proximity to mitochondria. Magnification $\times 16600$.

of the obese genotype itself. It has been firmly established in the rat that insulinopenic diabetes is associated with decreases in pancreatic amylase protein [26–28] and amylase mRNA [28]. This has been shown also for the mouse, where the various amylase genes are affected to different degrees by insulinopenic diabetes [29]. Furthermore, *in vitro*, insulin treatment of the AR4-2J pancreatic acinar cell line is associated with increased amylase

biosynthesis [30]. All of this underlines the fact that insulin plays a role in the biosynthesis of pancreatic amylase. However, amylase contents are low in the presence of persistent hyperinsulinaemia if obesity and insulin resistance are present [1,2]. Furthermore, if insulin resistance is induced in non-obese rats by feeding them on a high-fat diet, then insulin therapy cannot restore the normal amylase content in diabetic pancreases [31]. A

Fig. 4. Inclusion body at higher magnification, containing electron-opaque droplets surrounded by material with regular stacking (arrowheads)

Magnification $\times 53\,000$.

common feature of both these examples of insulin resistance is decreased glucose metabolism in the exocrine tissue [7,32]. The decreased pancreatic amylase content found in diabetes, obesity and high-fat feeding is probably more closely related to decreased glucose utilization (or increased fatty acid metabolism) than it is to the ambient insulin concentration. Indeed, when AR4-2J cells are incubated in the total absence of glucose, there is a marked decrease in the relative amount of mRNA for amylase [33].

In view of the significant increases in both the relative rates of biosynthesis and the pancreatic content of lipase and trypsinogen, it was interesting to find that changes in the absolute rates of biosynthesis of these enzymes were much more modest. There was a trend for this rate to be increased for lipase and decreased for trypsinogen. These trends were reversed by Ciglitazone treatment. In the pancreases of obese rats, the absolute rate of enzyme biosynthesis was lower than might have been predicted from the pancreatic content of each enzyme in comparison with lean controls. An important factor which contributes to this apparent disequilibrium between synthesis and storage is probably the marked decrease in the secretory response of tissue from obese rats to some physiological stimuli. Thus, it has been shown both *in vivo* [2] and *in vitro* [3–5] that the secretory responses to cholecystokinin and acetylcholine analogues are decreased. Under normal circumstances, turnover of pancreatic enzymes is fairly rapid. Since cholecystokinin and acetylcholine are two of the major physiological stimulants of enzyme secretion, the decreased secretory

responses in the obese rat could account for a higher percentage of enzyme remaining stored in the acinar tissue of these animals. At present we have no explanation for the differential effect of obesity on the various isoenzymes of trypsinogen and proelastase. Alterations in biosynthetic rates of selected isoenzymes have also been noted under experimental conditions where either secretin [12] or caerulein [10] has been infused *in vivo* or where manipulations have been made in the protein and carbohydrate content of the food [8,9]. In contrast with amylase, the changes in the absolute rates of biosynthesis of lipase and trypsinogen are small and, possibly, less important for the alterations of pancreatic content of these enzymes than are the defects which lead to decreased enzyme secretion. Since, in contrast with the situation with amylase biosynthesis, the secretory defect is not closely linked to insulin resistance, it is not ameliorated by Ciglitazone therapy [5].

The lipid inclusion bodies which were found in the pancreases of obese rats are similar to those described by Palade & Schidrowsky [34] in the pancreases of starved guinea pigs. In both cases these inclusions are found in close apposition to mitochondria. It was postulated, for guinea pigs, that the presence of these bodies was probably linked to a further increase in fatty acid metabolism in a tissue where, under normal conditions, endogenous fatty acids are a much more important source of oxidizable substrate than is glucose [35]. We have previously shown that the rate of glucose oxidation by acini from obese Zucker rats is decreased compared with lean controls [7]. This probably leaves the exocrine tissue of obese rats even more dependent on fatty acid metabolism, and in this respect may resemble the situation in starvation. It is interesting that Ciglitazone treatment, which normalizes glucose oxidation rates in the exocrine pancreas of obese rats [7], results in a marked decrease in the size and number of these lipid inclusion bodies.

In summary, therefore, it is clear that changes in enzyme content in the pancreas of the obese Zucker rat cannot, in each case, be attributed entirely to changes in rates of enzyme biosynthesis; the previously described secretory defects must also play a role in altering the rate of turnover of pancreatic enzymes and the percentage which is stored in the pancreas. No major histological defect was seen in the obese-rat pancreases. It is suggested that the lipid inclusion bodies found very close to mitochondria may be the hallmark of acinar tissue in which glucose metabolism is decreased.

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REFERENCES

1. Schneeman, B. O., Inman, M. D. & Stern, J. S. (1983) *J. Nutr.* **113**, 921–925
2. Bruzzone, R., Trimble, E. R., Gjinovci, A. & Renold, A. E. (1984) *Biochem. J.* **219**, 333–336
3. McLaughlin, C. L., Peikin, S. R. & Baile, C. A. (1984) *Physiol. Behav.* **32**, 961–965
4. Praissman, M. & Izzo, R. S. (1986) *Endocrinology* (Baltimore) **119**, 546–553

5. Trimble, E. R. & Bruzzone, R. (1985) *Regul. Pept.* **11**, 227–235
6. Trimble, E. R., Bruzzone, R. & Herberg, L. (1986) *Comp. Biochem. Physiol. A* **83**, 387–390
7. Trimble, E. R., Bruzzone, R. & Belin, D. (1986) *Biochem. J.* **237**, 807–812
8. Wicker, C., Puigserver, A. & Scheele, G. (1984) *Eur. J. Biochem.* **139**, 381–387
9. Schick, J., Verspohl, R., Kern, H. & Scheele, G. (1984) *Am. J. Physiol.* **247**, G611–G616
10. Schick, J., Kern, H. & Scheele, G. (1984) *J. Cell Biol.* **99**, 1569–1574
11. Wicker, C., Puigserver, A., Rausch, U., Scheele, G. & Kern, H. (1985) *Eur. J. Biochem.* **151**, 461–466
12. Rausch, U., Vasiloudes, P., Rüdiger, K. & Kern, H. F. (1985) *Cell Tissue Res.* **242**, 641–644
13. Chang, A. Y., Wyse, B. M. & Gilchrist, B. J. (1983) *Diabetes* **32**, 839–845
14. Fujita, T., Sugiyama, Y., Taketomi, S., Sohda, T., Kawamatsu, Y., Iwatsuka, H. & Suzuoki, Z. (1983) *Diabetes* **32**, 804–810
15. Kobayashi, M., Iwasaki, M., Ohgahu, S., Maegawa, H., Watanabe, N. & Shigeta, Y. (1983) *FEBS Lett.* **163**, 50–53
16. Huggett, A. St. G. & Nixon, D. A. (1957) *Biochem. J.* **66**, 12p
17. Trimble, E. R., Halban, P. A., Wollheim, C. B. & Renold, A. E. (1982) *J. Clin. Invest.* **69**, 405–413
18. Labarca, C. & Paigen, K. (1980) *Anal. Biochem.* **102**, 344–352
19. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
20. Bernfeld, P. (1955) *Methods Enzymol.* **1**, 149–158
21. Hummel, B. C. W. (1959) *Can. J. Biochem. Physiol.* **37**, 1393–1399
22. Bruzzone, R., Halban, P. A., Gjinovci, A. & Trimble, E. R. (1985) *Biochem. J.* **226**, 621–624
23. Eagle, H. (1959) *Science* **130**, 432–438
24. Rausch, U., Vasiloudes, P., Rüdiger, K. & Kern, H. F. (1985) *Cell Tissue Res.* **242**, 633–639
25. Scheele, G. (1975) *J. Biol. Chem.* **250**, 5375–5385
26. Ben Abdeljlil, A., Palla, J. C. & Desnuelle, P. (1965) *Biochem. Biophys. Res. Commun.* **18**, 71–75
27. Söling, H. D. & Unger, K. O. (1972) *Eur. J. Clin. Invest.* **2**, 199–212
28. Korc, M., Owerbach, D., Quinto, C. & Rutter, W. J. (1981) *Science* **213**, 351–353
29. Dranginis, A., Morley, M., Nesbitt, M., Rosenblum, B. B. & Meisler, M. H. (1984) *J. Biol. Chem.* **259**, 12216–12219
30. Mössner, J., Logsdon, C. D., Williams, J. A. & Goldfine, I. D. (1985) *Diabetes* **34**, 891–897
31. Bazin, R. & Lavau, M. (1979) *Digestion* **19**, 386–391
32. Bazin, R. & Lavau, M. (1982) *Am. J. Physiol.* **243**, G448–G454
33. Stratowa, C. & Rutter, W. J. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4292–4296
34. Palade, G. E. & Schidlowsky, G. (1958) *Anat. Rec.* **130**, 352–353
35. Jamieson, J. D. & Palade, G. E. (1968) *J. Cell Biol.* **39**, 589–603

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