Cellular Aspects of Intestinal Lipoprotein Assembly in *Psammomys Obesus*

A Model of Insulin Resistance and Type 2 Diabetes

Monika Zoltowska,[1] Ehud Ziv,[2] Edgard Delvin,[3] Daniel Sinnett,[4] Ronny Kalman,[2] Carole Garofalo,[1] Ernest Seidman,[4] and Emile Levy[1]

Although postprandial hypertriglyceridemia is a major contributing factor in the development of atherosclerosis, little information is available on the effect of insulin resistance and diabetes on intestinal fat transport. The aim of the present study was to examine intracellular events that govern lipid transport and apolipoprotein (apo) B-48–containing lipoprotein assembly in the small intestine of *Psammomys obesus*, a model of nutritionally induced insulin resistance and type 2 diabetes. Animals with normoglycemia/hyperinsulinemia and hyperglycemia/hyperinsulinemia exhibited high levels of triglycerides (TGs) in the plasma and intestine and postprandial plasma chylomicrons and apo B-48 compared with normoglycemic/normoinsulinemic animals. In vitro studies, using cultured jejunal explants incubated with either [^{14}C]oleic acid or [^{35}S]methionine, revealed their higher efficiency in de novo TG synthesis, apo B-48 biogenesis, and TG-rich lipoprotein assembly. Accordingly, enhanced monoacylglycerol and diacylglycerol acyltransferase activity was also discernible and concomitant with an increased content of L-fatty acid binding protein and in vivo chylomicron production rates. However, both the I-fatty acid binding protein amount and the apo B-48 proteasomal degradative pathway were decreased. Overall, our findings show that the development of an insulin-resistant/diabetic state in *Psammomys obesus* triggers the whole intra-enterocyte machinery, leading to lipoprotein assembly and favoring the intestinal oversecretion of apo B-48-lipoproteins, which may contribute to characteristic hypertriglyceridemia. *Diabetes* 52:2539–2545, 2003

From the [1]Department of Nutrition, Centre de Recherche, Hôpital Sainte-Justine, and Université de Montréal, Montréal, Québec, Canada; the [2]Diabetes Unit, Division of Internal Medicine, Hadassah University Hospital, Jerusalem, Israel; the [3]Department of Biochemistry, Centre de Recherche, Hôpital Sainte-Justine, and Université de Montréal, Montréal, Québec, Canada; and the [4]Department of Pediatrics, Centre de Recherche, Hôpital Sainte-Justine, and Université de Montréal, Montréal, Québec, Canada.

Address correspondence and reprint requests to Dr. Emile Levy, GI-Nutrition Unit, Hôpital Sainte-Justine, 3175 Côte Ste-Catherine Rd., Montreal, Quebec H3T 1C5, Canada. E-mail: levye@justine.umontreal.ca.

Received for publication 1 February 2003 and accepted in revised form 2 July 2003.

apo, apolipoprotein; DGAT, diacylglycerol acyltransferase; FABP, fatty acid binding protein; MGAT, monoacylglycerol acyltransferase; MTP, microsomal triglyceride transfer protein; TG, triglyceride; TLC, thin-layer chromatography.

© 2003 by the American Diabetes Association.

Cardiovascular disease remains the most important complication of type 2 diabetes (1,2). Strong evidence links the increase in diabetic atherosclerosis to dyslipidemia and insulin resistance (3,4). In fact, disturbed lipid metabolism appears to be a central component of both insulin resistance and diabetes (3,4). Impaired plasma triglyceride (TG) clearance and exaggerated liver VLDL production could represent major pathways of the hypertriglyceridemia that characterizes these two conditions. Indeed, a high plasma TG concentration has been reported in insulin resistance and type 2 diabetes, possibly because of the impaired ability of insulin to inhibit lipolysis and reduce hepatic VLDL secretion (5,6). On the other hand, little information is available on the effect of insulin resistance and diabetes on TG-rich lipoprotein production by the small intestine. Few studies have reported a significant relationship between intestinally derived apolipoprotein (apo) B-48 and the progression of atherosclerosis in nondiabetic (7) and more recently diabetic patients (8). Whereas apo B-48–containing intestinally derived lipoproteins seem atherogenic (8), surprisingly, data on the contribution of the intestine to hyperlipidemia in insulin resistance and diabetes are rather limited. Technical difficulties have likely hindered progress in this important area for humans. More recently, fructose-fed hamsters have been used as an animal model of insulin resistance and diabetes to investigate the contribution of hepatic and intestinal lipoproteins to dyslipidemia (9). The development of an insulin-resistant/hyperinsulinemic state in these hamsters was accompanied by the intestinal overproduction of apo B-48–containing particles.

In the present study, we examined whether the development of insulin-resistant and diabetic states led to the intestinal oversecretion of apo B-48 lipoproteins in *Psammomys obesus*. This desert gerbil shows insulin resistance and develops diet-induced obesity-linked diabetes, initially associated with hyperinsulinemia and gradually progressing to severe hyperglycemia.

RESEARCH DESIGN AND METHODS

Animals. *Psammomys obesus* gerbils (2.5–3.5 months old) from the Hebrew University colonies were obtained from Harlan (Jerusalem, Israel). After weaning, *Psammomys obesus* animals were maintained on a low-energy diet containing 2.38 kcal/g (Koffolk, Petach Tikva, Israel) until the beginning of the

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/10/2539/369913/db1003002539.pdf by guest on 10 December 2023

experiments. They were then switched to a high-energy diet (2.93 kcal/g; Weizmann Institute of Science, Rehovot, Israel) for 2 weeks. The *Psammomys obesus* gerbils were housed in individual polypropylene cages in a temperature-controlled room with a 12-h light-dark cycle. Water and food were supplied ad libitum. Animals were classified into three groups (A, B, and C) according to plasma glucose and insulin concentrations. All experimental procedures performed in the study were authorized by the Institutional Animal Care Committee.

Intestinal organ cultures. The jejunum from *Psammomys obesus* was cleared of mesentery, split longitudinally, washed in culture medium, and cut into explants (3 × 7 mm). Five to seven explants were randomly transferred onto lens paper, with the mucosal side facing up in each organ culture dish (Falcon Plastics, Los Angeles, CA). Six dishes were used for each experimental condition. An amount of medium (0.8 ml) sufficient to dampen the lens paper was added. Explants were cultured in serum-free Leibovitz L-15 medium according to the technique described previously (10,11). After a 30-min stabilization period, the medium was replaced with fresh medium containing a final amount of 1.0 μmol/ml unlabeled oleic acid with 0.5 μCi of [^{14}C]-oleic acid (specific activity, 53.9 mCi/mmol; Amersham Pharmacia Biotech) in a micellar mixture (6.6 mmol/l sodium taurocholate, 1 mmol/l oleic acid, 0.5 mmol/l monoolein, 0.1 mmol/l cholesterol, and 0.6 mmol/l phosphatidylcholine) (12). Intestinal explants from *Psammomys obesus* were cultured for 3 h. After this incubation period, tissue integrity was confirmed by morphological (lighted electron microscopy) and biochemical (sucrase activity) studies.

In vivo intestinal fat absorption. To examine whether higher chylomicron secretion contributed to enhanced in vivo lipemia in insulin-resistant and diabetic *Psammomys obesus*, a volume of 2 ml of 2% intralipid was orally administered by gavage in 12-h fasted animals. One hour later, Triton WR-1339 (400 mg/kg body wt) was injected. Arterial blood samples were collected 30, 60, and 90 min after Triton administration. Chylomicrons were isolated by ultracentrifugation as described previously (10,11). TGs were quantified enzymatically, and apo B-48 mass was assessed by analytical SDS-PAGE concomitantly with serial dilutions of internal standard apo B-48. The intensity of staining was quantitated by densitometric scanning. Apo B-48 data were extrapolated from the linear standard curve. TG and apo B-48 secretion rates were performed by multiplying the slope of the concentration increase of TGs (in micromoles per milliliter per minute) and apo B-48 (in micrograms per milliliter per minute) over time by the intravascular distribution volume estimated as 4% of body weight.

Lipid and lipoprotein analyses. Aliquots of explant homogenates and their respective incubation media were lipid-extracted with 2:1 (vol/vol) chloroform-methanol (10–13). Small amounts of lipid standards were added to the samples before the separation of individual lipid classes by one-dimensional thin-layer chromatography (TLC) (silica gel from Eastman Kodak, Rochester, NY) as described previously (10,12). The nonpolar solvent system was 80:20:3 (vol/vol/vol) hexane-diethylether-glacial acetic acid. The radioactivity of the separated fractions was measured in a Beckman liquid scintillation spectrometer. Quenching was corrected using computerized curves generated with external standards. An aliquot of the tissue homogenate was used for protein determinations (10–13). For the determination of secreted lipoproteins, the medium supplemented with anti-proteases was first mixed with a plasma lipid carrier (2:0.6 vol/vol) to efficiently isolate the de novo TG-rich lipoproteins synthesized. The latter were then isolated at a density of 1.006 g/ml by spinning at 100,000*g* for 2.26 h with a tabletop ultracentrifuge (Beckman Instruments, Montreal, PQ, Canada) as described previously (10,11).

Lipid carrier. To provide a carrier for lipoproteins synthesized in vitro, postprandial plasma was obtained from healthy volunteers 3 h after the ingestion of fat (50 g/1.73 m^2) as described previously (10,11).

De novo apolipoprotein synthesis. After incubation with 300 μCi [^{35}S] methionine, jejunal explants were washed (three times) with methionine-free Leibovitz medium and homogenized in PBS containing 1% (wt/vol) Triton X-100, methionine (2 mmol/l), phenylmethylsulfonyl fluoride (1 mmol/l), and benzamidine (1 mmol/l). The homogenates were centrifuged (4°C) at 105,000*g* for 60 min and supernatants subsequently reacted with excess apo B polyclonal antibodies for 18 h at 4°C. Anti–*Psammomys obesus* apo B antiserum was raised in rabbits. Pansorbin was then added, and the mixture was reincubated at 20°C for 60 min. The immunoprecipitates were washed extensively and analyzed by a linear 4–20% acrylamide gradient preceded by a 3% stacking gel as described previously (13). Apo B-48 bands on gels were sectioned and counted after an overnight incubation at 20°C with 1 ml BTS-450 (Beckman) and 10 ml liquid scintillation fluid (Ready Sol. NA, Beckman).

Microsomal TG transfer protein assays. Intestinal microsomes used as the source of microsomal triglyceride transfer protein (MTP) activity were isolated as described previously (14). MTP activity was determined by the transfer of radiolabeled triacylglycerol from donor small unilamellar vesicles (40 nmol egg phosphatidylcholine, 0.08 nmol [^{14}C]triacylglycerol, and 2 nmol cardiolipin) to acceptor small unilamellar vesicles (240 nmol egg phosphatidylcholine and 0.48 nmol triacylglycerol) at 37°C for 1 h. This assay was previously described in detail (14). Lipid transfer activity is expressed as a percentage of TG transfer per unit time under verified linear assay conditions (first-order kinetics). To assess the presence of MTP and evaluate its mass, homogenates of intestinal tissue were prepared for Western blotting as described previously (14). Proteins were separated on a 4–20% gradient SDS-PAGE and electroblotted onto nitrocellulose membranes. Nonspecific binding sites of the membranes were blocked using defatted milk proteins followed by the addition of primary antibodies directed against MTP. The relative amount of primary antibody was detected with horseradish peroxidase–conjugated secondary antibody. Blots were developed, and the mass of MTP was quantitated using an HP Scanjet scanner equipped with a transparency adapter and software.

Enzymatic activities. Activities of monoacylglycerol acyltransferase (MGAT) and diacylglycerol acyltransferase (DGAT) were determined in microsomes as reported by Coleman (15).

Protein mass of I- and L-fatty acid binding protein. Jejunal explants were homogenized and immunoprecipitated with anti–I-fatty acid binding protein (FABP) and anti–L-FABP antibodies raised in rabbits after the injection of recombinant human I- and L-FABP. The immunoprecipitates were run on SDS-PAGE and transferred to nitrocellulose membrane, which was blotted with I-FABP and L-FABP antibodies. Control experiments were performed to confirm the specificity of the antibodies. Quantitation of I- and L-FABP was carried out as described above.

Biochemical analyses. Plasma glucose was determined by an enzymatic glucose analyzer, and insulin levels were assessed by radioimmunoassay using a human primary antibody (Phadesph; Kabi Pharmacia Diagnostics, Uppsala, Sweden). Plasma TG and cholesterol levels were measured colorometrically (Boehringer Mannheim, Mannheim, Germany).

Statistical analysis. Data were statistically analyzed by one-way ANOVA to assess differences in the parameters studied. Differences between mean values were evaluated by Student's two-tailed *t* test.

RESULTS

Body weight and biochemical parameters. As expected, genetically predisposed *Psammomys obesus* on an affluent laboratory rodent diet higher in energy than the desert native food displayed the three characteristic patterns of normoinsulinemia/normoglycemia (group A), hyperinsulinemia/normoglycemia (group B), and hyperinsulinemia/hyperglycemia (group C) (Fig. 1). Furthermore, the latter two animal groups exhibited moderate weight gain (Fig. 1) as well as hypertriglyceridemia and hypercholesterolemia (Fig. 2), despite food intake comparable to controls (~15 g/day).

Intestinal lipid content. The profile of TG and cholesterol in intestinal tissue was determined in *Psammomys obesus* animals. Lipid analysis revealed higher levels of TGs in groups B and C than in group A (Fig. 3). Although cholesterol fractions were also increased in groups B and C, statistical significance was reached only for total cholesterol and free cholesterol in group C compared with group A.

De novo lipid synthesis and status of MGAT and DGAT activities. We measured the activity of MGAT and DGAT to evaluate the effect of insulin resistance and diabetes on the major enzymatic machinery involved in triacylglycerol formation in the intestine. As observed in Fig. 4, MGAT and DGAT activity was elevated in groups B and C compared with group A. Accordingly, the esterification of [^{14}C]oleic acid into complex lipid fractions in jejunal explants of groups B and C exceeded that of group A (Fig. 5*A*). Likewise, the release of labeled TGs, phospholipids, and cholesteryl esters into the medium was higher in group B and C than in group A animals (Fig. 5*B*). The current findings suggest that insulin resistance and diabetes enhanced the intestine's ability to produce lipids in *Psammomys obesus*.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/10/2539/369913/db1003002539.pdf by guest on 10 December 2023

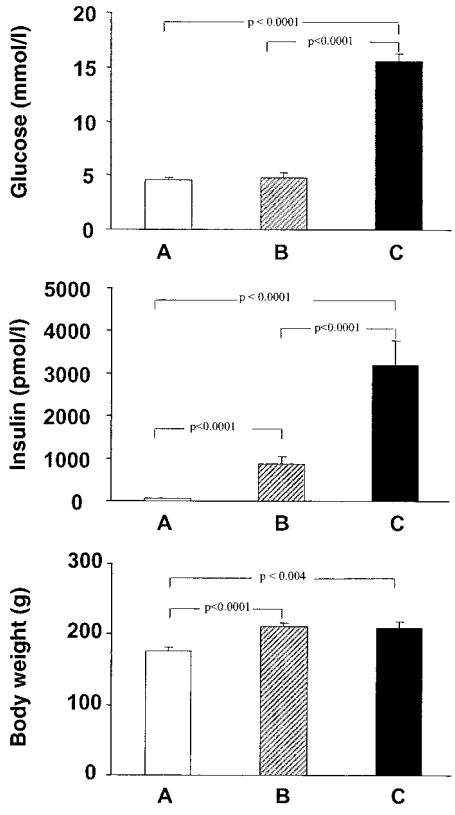

FIG. 1. Body weight, plasma glucose, and insulin concentrations in *Psammomys obesus*. When given free access to laboratory diet, *Psammomys obesus* displays heterogeneous glucose and insulin levels, ranging from animals with normoglycemia and normoinsulinemia to obese diabetic animals with hyperglycemia and hyperinsulinemia. Data are means ± SE for $n = 10$ in group A, $n = 8$ in group B, and $n = 7$ in group C.

In vitro apo B-48 biogenesis and TG-rich lipoprotein assembly. The synthesis of apo B was determined in jejunal explants in culture (Fig. 6). As evidenced by [^{35}S]methionine incorporation, apo B-48 synthesis was significantly increased in the intestine of groups B and C relative to that of group A ($P < 0.02$). With labeled oleic acid used as a substrate, our data revealed significantly ($P < 0.01$) more output of TG-rich lipoproteins in groups B and C than in group A (Fig. 7). However, with the addition of the proteasomal inhibitors (*N*-acetyl-leucyl-norleucinat and lactacystin) to the culture medium, the differences in de novo apo B synthesis in groups A, B, and C disappear. Overall, our observations suggest that the development of insulin resistance and diabetes in *Psammomys obesus* stimulated the intestinal transport of lipids by augmenting apo B-48 synthesis through reduced proteasomal degradation activity, resulting in enhanced TG-rich lipoprotein assembly and secretion.

Microsomal TG transfer protein. Because MTP is necessary for the efficient assembly of TG-rich lipoproteins, we assessed MTP activity and protein expression in the intestine of *Psammomys obesus*. There were no differences in MTP activity and protein expression among groups A, B, and C (Fig. 8). Our results, therefore, suggest that MTP does not play a role in the intestinal overproduction of the TG-rich lipoprotein observed in insulin-resistant and diabetic *Psammomys obesus* animals.

FABP. The next set of experiments was designed to examine the protein expression of the two distinct FABPs, I- and L-FABP, in the intestine in the various *Psammomys obesus* groups. The effect of insulin resistance or diabetes was noted on the abundance of I-FABP, whereas levels of L-FABP were higher in groups B and C than in group A (Fig. 9). This suggests a relationship between the magnitude of L-FABP and the increase in intestinal lipid transport in insulin resistance and diabetes.

In vivo chylomicron-TG and chylomicron-apo B-48 production. To confirm intestinal overproduction in insulin-resistant and diabetic animals, we determined the effect of dietary fat on the increase in chylomicron TG and apo B-48 moieties after the injection of Triton WR-1339. In individual animals, chylomicron-TG and chylomicron–apo B-48 rose linearly with time, irrespective of group, with no tendency to plateau at the time Triton was administered

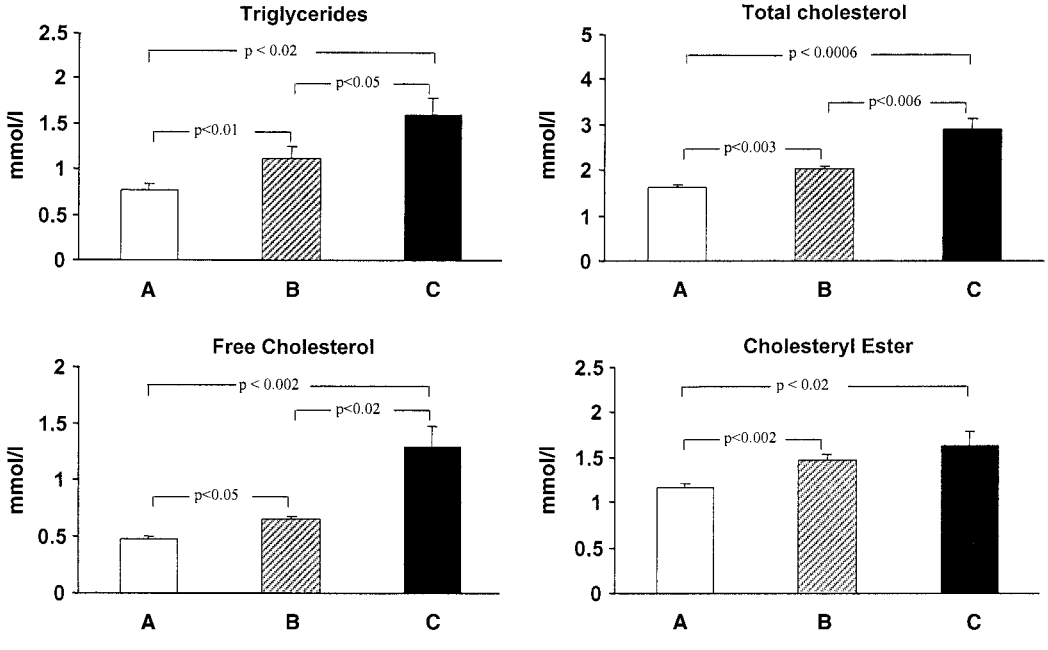

FIG. 2. Plasma lipid concentrations of the three *Psammomys obesus* groups. Data are means ± SE for $n = 6$ in group A, $n = 8$ in group B, and $n = 6$ in group C.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/10/2539/369913/db1003002539.pdf by guest on 10 December 2023

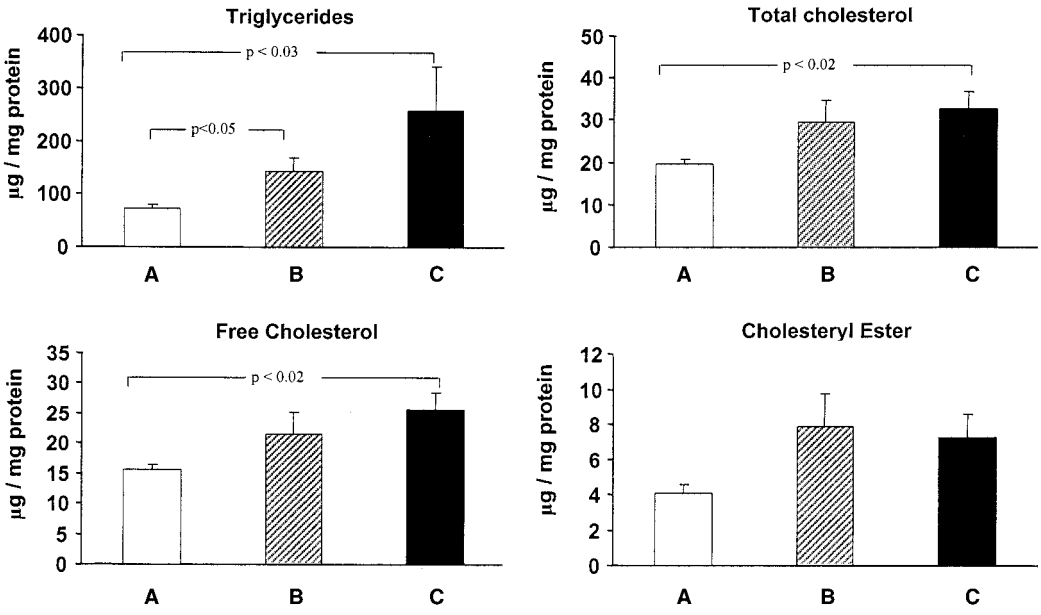

FIG. 3. Lipid content in *Psammomys obesus* intestinal tissue. Intestinal tissue from the three *Psammomys obesus* groups was homogenized and lipids were extracted by chloroform/methanol (2:1, vol/vol). After isolation by TLC, TG and cholesterol fractions were quantitated as described in RESEARCH DESIGN AND METHODS. Data are means ± SE for $n = 6$ in group A, $n = 8$ in group B, and $n = 6$ in group C.

and for the subsequent 90 min (data not shown). Production rates were derived from the slope of the increase over 90 min. After the Triton WR-1339 lipolysis blockade, the levels of chylomicron-TG and chylomicron–apo B-48 were higher in insulin-resistant and diabetic animals compared with normal *Psammomys obesus* (Fig. 10) animals, indicating the efficiency of these abnormal conditions to stimulate in vivo fat transport.

DISCUSSION

Hypertriglyceridemia is very common in diabetes and substantially increases the risk of cardiovascular diseases. Similarly, insulin resistance is associated with elevated plasma TGs and predisposes for cardiovascular complications. Although the regulation of circulating TG-rich lipoproteins is extremely complex in diabetes and insulin resistance, many studies consider increased hepatic VLDL secretion and impaired VLDL clearance mechanisms for dyslipidemia (5,6). It is postulated that the overall effect of insulin resistance and diabetes on VLDL production reflects raised fatty acid flux to the liver (6). Consequently, there is a stimulation of the assembly and output of apo B-100–containing lipoproteins (6,16). The aim of our investigation was to examine whether intestinal TG metabolism is also altered in *Psammomys obesus* animals that are characterized by insulin resistance and diabetes (17,18). We demonstrated that the development of the insulin-resistant/diabetic state is accompanied by *1*) an intestinal increase in lipid content and de novo TG synthesis concomitant with an increased amount of L-FABP and decreased levels of I-FABP; *2*) augmented MGAT and DGAT activity, two enzymes that belong to the primary triacylglycerol synthesis pathway in enterocytes; *3*) elevated apo B-48 biogenesis, probably resulting from reduced proteasomal degradation; *4*) stimulated TG-rich lipoprotein assembly; and *5*) unaltered MTP activity and protein expression.

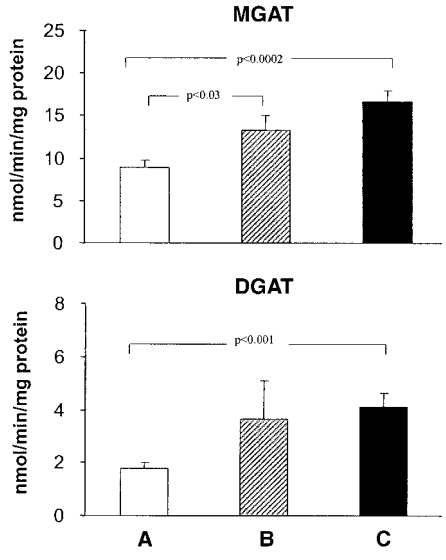

FIG. 4. MGAT and DGAT activity in the small intestine of *Psammomys obesus*. Microsomes were prepared from the intestinal tissue of the three *Psammomys obesus* groups and assayed for the two enzymes. Data are means ± SE for $n = 6$ in group A, $n = 8$ in group B, and $n = 6$ in group C.

Triacylglycerol biosynthesis in the intestine is believed to occur mainly through the monoacylglycerol pathway. Initially, monoacylglycerol and fatty acyl CoA are covalently joined to form diacylglycerol in a reaction catalyzed by MGAT (19,20). Then, diacylglycerol and fatty acyl CoA are used to synthesize triacylglycerol in a reaction catalyzed by DGAT (19,20). In our study, measurement of MGAT and DGAT disclosed a high activity level, which is consistent with the remarkable capacity of the small intestine to produce triacylglycerol in an insulin-resistant and diabetic state. Physiologically, the present findings reflect the intestine's attempt to enhance lipid production under insulin resistance and diabetic conditions. It is generally accepted that the intracellular mechanism of TG-rich lipoprotein assembly requires apo B synthesis and association. The early step in this process is the cotranslational lipidation of apo B that is transiently bound to the endoplasmic reticulum membrane where it is folded. The addition of lipids stabilizes apo B and prevents

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/10/2539/369913/db1003002539.pdf by guest on 10 December 2023

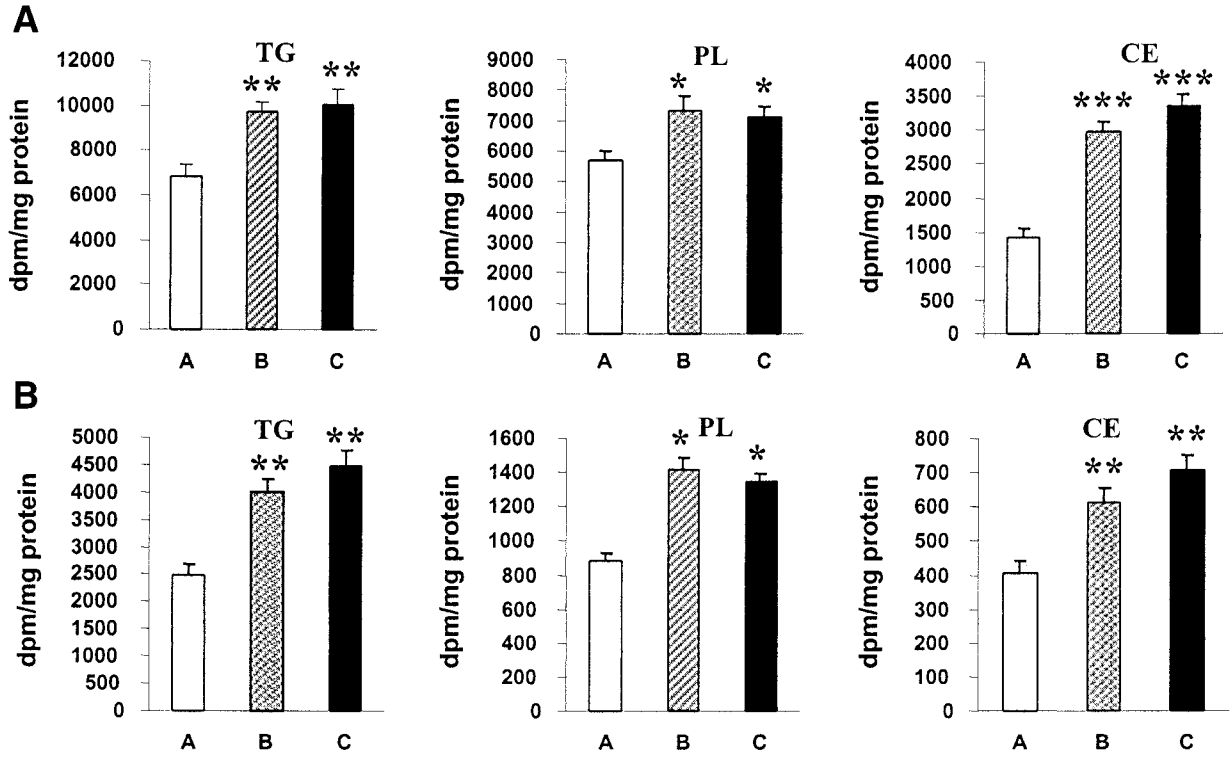

FIG. 5. De novo lipid synthesis and secretion in *Psammomys obesus* intestinal explants. Jejunal explants were incubated with [^{14}C]oleic acid substrate for 3 h. Lipids of tissue homogenates (*A*) and media (*B*) were then extracted with chloroform/methanol (2:1, vol/vol), isolated by TLC, and quantitated as described in RESEARCH DESIGN AND METHODS. Data are means ± SE of three separate experiments. *$P < 0.05$; **$P < 0.02$; ***$P < 0.01$. CE, cholesteryl ester; PL, phospholipid.

its proteolytic degradation via the ubiquitin-dependent proteasomal pathway (21,22). It is, therefore, reasonable to suggest that the augmented TG synthesis in the small intestine of *Psammomys obesus* insulin-resistant and diabetic animals enhanced apo B-48 protection from misfolding and degradation, resulting in the marked secretion of apo B-48–containing TG-rich lipoproteins. Our data argue for an increased efficiency of intestinal TG-rich lipoprotein assembly and secretion and resemble recent observations in fructose-fed hamsters exhibiting chronic hyperinsulinemia and insulin resistance while displaying a significant stability enhancement of newly synthesized apo B with only a minor fraction being sorted to intracellular degradation (10).

MTP is a resident protein in the lumen of the endoplasmic reticulum that facilitates the transfer of lipids from their site of synthesis in the endoplasmic reticulum membrane into the lumen during the assembly of TG-rich lipoproteins (23,24). However, the MTP requirement for

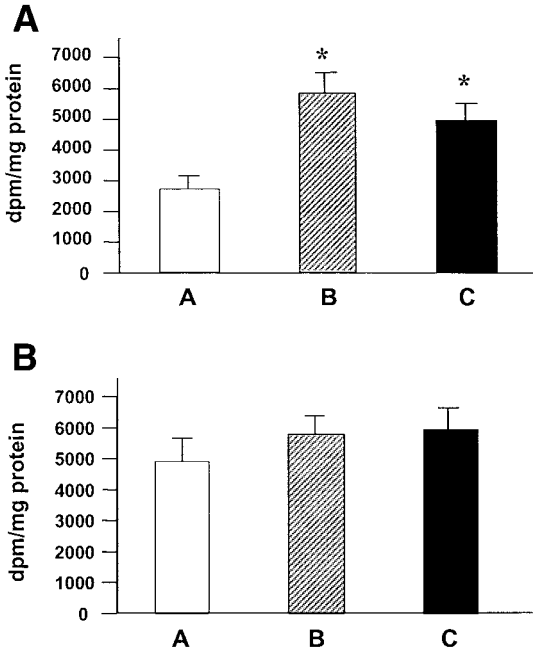

FIG. 6. Apo B-48 synthesis in *Psammomys obesus* intestinal explant. Jejunal explants were incubated for 3 h with methionine-free medium containing [^{35}S]methionine in the presence (*A*) or absence (*B*) of proteasome inhibitors (*N*-acetyl-leucyl-norleucinat [40 μg] and lactacystin [1 μmol/l]). Apo B-48 was then immunoprecipitated and analyzed by gel electrophoresis. Data are means ± SE of three separate experiments. *$P < 0.02$.

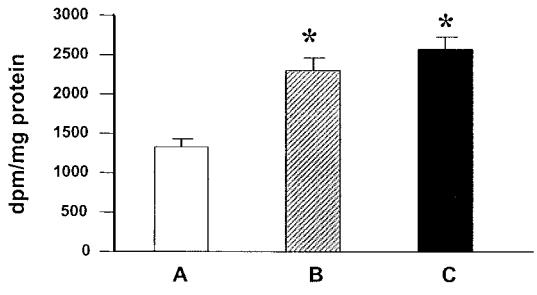

FIG. 7. Production of TG-rich lipoproteins by jejunal explants of *Psammomys obesus*. Jejunal explants were cultured in the presence of [^{14}C]oleic acid. After 3 h of incubation, TG-rich lipoproteins were isolated by ultracentrifugation. Data are means ± SE of three separate experiments. *$P < 0.01$.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/10/2539/369913/db1003002539.pdf by guest on 10 December 2023

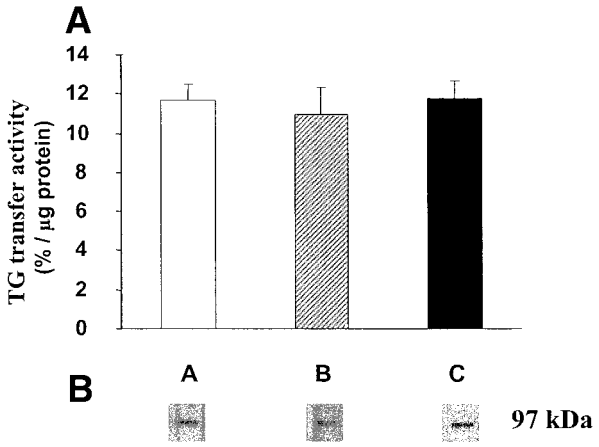

FIG. 8. MTP activity and protein expression in *Psammomys obesus* intestine. Intestinal tissue from the three groups of *Psammomys obesus* was prepared for the determination of MTP activity (*A*) and analysis by Western blot (*B*). Data for TG/transfer are means ± SE for *n* = 6–8/group. Data from a representative experiment of Western blot are illustrated in *B*.

the export of apo B-48 remains controversial. Recent studies have stressed that liver-specific MTP knockout mice still secrete apo B-48, thus excluding the obligatory role of MTP (25,26). Our findings show that *Psammomys obesus* enterocytes are capable of releasing more TG-rich lipoproteins in insulin-resistant and diabetic states without augmenting MTP activity or protein expression. Similar data were obtained in genetically diabetic obese rats (27). Conversely, Haidari et al. (9) suggested that MTP may play an important role in the postprandial dyslipidemia in insulin-resistant hamster and Zucker obese *fa/fa*, respectively (28). Although this discrepancy may be animal species–dependent, additional work is necessary to delineate the role of MTP in apo B-48 delivery, particularly in insulinemic and diabetic states.

FABPs are another set of proteins critical to the transport of fatty acids in the enterocyte (28). Based on a variety of physico-chemical studies, different functions have been proposed for cytosolic FABPs, including fatty acid transport and compartmentalization, the modulation of enzyme activities involved in lipid metabolism, and the protection of cellular integrity from the detrimental effects of hydrophobic fatty acid or other noxious substances (28–30). The physiological significance of the presence of the two FABP forms (L- and I-FABP) in intestinal absorptive cells remains unknown. Present data show a relationship between endogenous L-FABP and TG-rich lipoprotein secretion, both of which are induced in the small intestine of insulin-resistant and diabetic *Psammomys obesus*. On the other hand, I-FABP protein expression appears to be decreased and uncoupled with the stimulated intestinal

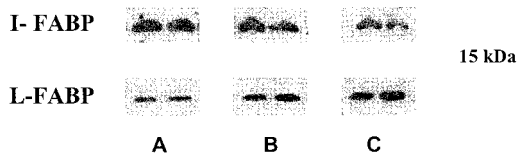

FIG. 9. Western blot analysis of *Psammomys obesus* intestine expressing I-FABP and L-FABP. Protein expression was examined by Western blotting and hybridization. Data are means ± SE of three separate experiments.

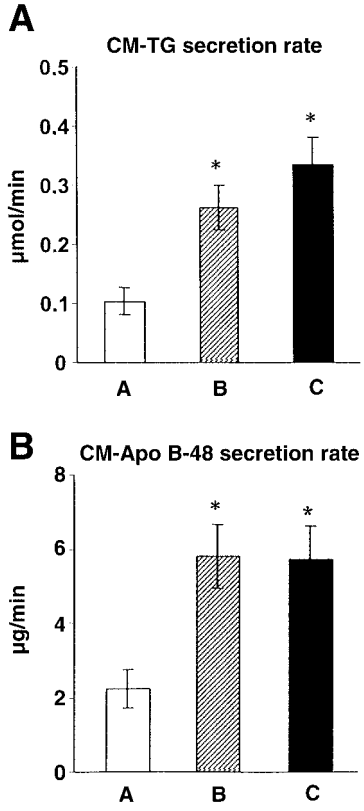

FIG. 10. In vivo production of chylomicron-TGs and chylomicron–apo B-48 in *Psammomys obesus*. The production rate of chylomicron components was assessed after the ultracentrifugation of plasma from postprandial animals treated with Triton WR-1339 as described in RESEARCH DESIGN AND METHODS. Data are means ± SE of three separate experiments in each group. **P* < 0.05 vs. group A.

lipid transport in insulin-resistant and diabetic *Psammomys obesus* animals. Accordingly, recent studies in mice have suggested that I-FABP is not essential for dietary fat absorption (31), and its overexpression may even limit intestinal lipid transport, as noted in Caco-2 cells (32). On the basis of these observations, we suggest that insulin-resistant and diabetic conditions may induce L-FABP that may be involved in the specific targeting of fatty acid to the synthetic pathway, thereby stimulating lipid esterification and TG-rich lipoprotein assembly. It is conceivable that the induction of L-FABP results in more efficient conveyance of fatty acids to the endoplasmic reticulum, which preserves apo B-48 from proteolytic degradation and enhanced chylomicron formation.

Hypertriglyceridemia frequently occurs in individuals with type 2 diabetes and contributes to vascular complications. More and more studies have emphasized the influence of postprandial TG-rich lipoproteins on the acceleration of atherosclerosis and the progression of coronary artery disease (7,33). In the current report, we have shown that the standard diet administered to *Psammomys obesus*, a well-defined model for dietary-induced insulin resistance and type 2 diabetes, provoked increased enterocyte lipid content as well as higher efficiency in intestinal de novo TG synthesis, apo B-48 biogenesis, and chylomicron production. Many differences were noted in these processes between groups B and C and may be due to the degree of insulin resistance and/or hyperglycemia per se. Hyperglycemia is a significant pathophysiological state in

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/10/2539/369913/db1003002539.pdf by guest on 10 December 2023

diabetes that has been suggested to play a key role in the pathogenesis of vascular dysfunction in diabetes via different mechanisms, such as protein glycosylation, sorbitol accumulation, protein kinase increase, oxidative stress, glycotoxicity, and others (34,35). Further studies of this model may provide additional insight into the separate role of insulin resistance and hyperglycemia in the development of these abnormal intestinal fat processes, which may possibly help identify novel approaches to prevention or treatment.

In conclusion, evidence from the *Psammomys obesus* insulin-resistant and diabetic model indicates that intestinal TG-rich lipoprotein overproduction can contribute to the development of hypertriglyceridemia. A number of intracellular factors have been found to be associated with the deregulatory mechanisms, including de novo lipid synthesis, the monoacylglycerol pathway, L-FABP mass, reduced proteasomal degradation of apo B, and lipoprotein assembly. Further understanding of the physiopathology of intestinal lipid transport in insulin resistance and diabetes should facilitate the design of new therapeutic strategies for the prevention and treatment of hyperlipidemia and atherosclerosis.

ACKNOWLEDGMENTS

This study was supported by research grants from the Canadian Institutes of Health Research (MT-10583) and by research scholarship awards from the Fonds de la Recherche en santé du Québec, awarded to E.S. and E.L.

We acknowledge the expert secretarial assistance of Schohraya Spahis.

REFERENCES

1. Stamler J, Vaccaro O, Neaton JD, Wentworth D: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434–444, 1993
2. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339:229–234, 1998
3. Beckman JA, Creager MA, Libby P: Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 287:2570–2581, 2002
4. Reaven GM: Multiple CHD risk factors in type 2 diabetes: beyond hyperglycaemia. *Diabetes Obes Metab* 4 (Suppl. 1):S13–S18, 2002
5. Howard BV: Insulin resistance and lipid metabolism. *Am J Cardiol* 84:28J–32J, 1999
6. Ginsberg HN: Insulin resistance and cardiovascular disease. *J Clin Invest* 106:453–458, 2000
7. Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A: Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* 106:83–97, 1994
8. Mero N, Malmstrom R, Steiner G, Taskinen MR, Syvanne M: Postprandial metabolism of apolipoprotein B-48- and B-100-containing particles in type 2 diabetes mellitus: relations to angiographically verified severity of coronary artery disease. *Atherosclerosis* 150:167–177, 2000
9. Haidari M, Leung N, Mahbub F, Uffelman KD, Kohen-Avramoglu R, Lewis GF, Adeli K: Fasting and postprandial overproduction of intestinally derived lipoproteins in an animal model of insulin resistance: evidence that chronic fructose feeding in the hamster is accompanied by enhanced intestinal *de novo* lipogenesis and apo B-48-containing lipoprotein. *J Biol Chem* 277:31646–31655, 2002
10. Levy E, Thibault L, Menard D: Intestinal lipids and lipoproteins in the human fetus: modulation by epidermal growth factor. *J Lipid Res* 33:1607–1617, 1992
11. Levy E, Menard D, Delvin E, Stan S, Mitchell G, Lambert M, Ziv E, Feoli-Fonseca JC, Seidman E: The polymorphism at codon 54 of the FABP2 gene increases fat absorption in human intestinal explants. *J Biol Chem* 276:39679–39684, 2001
12. Levy E, Marcel Y, Deckelbaum RJ, Milne R, Lepage G, Seidman E, Bendayan M, Roy CC: Intestinal apo B synthesis, lipids, and lipoproteins in chylomicron retention disease. *J Lipid Res* 28:1263–1274, 1987
13. Levy E, Sinnett D, Thibault L, Nguyen TD, Delvin E, Menard D: Insulin modulation of newly synthesized apolipoproteins B-100 and B-48 in human fetal intestine: gene expression and mRNA editing are not involved. *FEBS Lett* 393:253–258, 1996
14. Levy E, Stan S, Delvin E, Menard D, Shoulders C, Garofalo C, Slight I, Seidman E, Mayer G, Bendayan M: Localization of microsomal triglyceride transfer protein in the Golgi: possible role in the assembly of chylomicrons. *J Biol Chem* 277:16470–16477, 2002
15. Coleman RA: Diacylglycerol acyltransferase and monoacylglycerol acyltransferase from liver and intestine. *Methods Enzymol* 209:98–104, 1992
16. Dixon JL, Ginsberg HN: Regulation of hepatic secretion of apolipoprotein B-containing lipoproteins: information obtained from cultured liver cells. *J Lipid Res* 34:167–179, 1993
17. Ikeda Y, Olsen GS, Ziv E, Hansen LL, Busch AK, Hansen BF, Shafrir E, Mosthaf-Seedorf L: Cellular mechanism of nutritionally induced insulin resistance in *Psammomys obesus*: overexpression of protein kinase Cepsilon in skeletal muscle precedes the onset of hyperinsulinemia and hyperglycemia. *Diabetes* 50:584–592, 2001
18. Walder K, McMillan J, Lapsys N, Kriketos A, Trevaskis J, Civitarese A, Southon A, Zimmet P, Collier G: Calpain 3 gene expression in skeletal muscle is associated with body fat content and measures of insulin resistance. *Int J Obes Relat Metab Disord* 26:442–449, 2002
19. Tso P: Intestinal lipid absorption. In *Physiology of the Gastrointestinal Tract.* Johnson LR, Ed. New York, Raven, 1994, p. 1867–1907
20. Levy E, Mehran M, Seidman E: Caco-2 cells as a model for intestinal lipoprotein synthesis and secretion. *FASEB J* 9:626–635, 1995
21. Yeung SJ, Chen SH, Chan L: Ubiquitin-proteasome pathway mediates intracellular degradation of apolipoprotein B. *Biochemistry* 35:13843–13848, 1996
22. Fisher EA, Zhou M, Mitchell DM, Wu X, Omura S, Wang H, Goldberg AL, Ginsberg HN: The degradation of apolipoprotein B100 is mediated by the ubiquitin-proteasome pathway and involves heat shock protein 70. *J Biol Chem* 272:20427–20434, 1997
23. Wetterau JR, Zilversmit DB: A triglyceride and cholesteryl ester transfer protein associated with liver microsomes. *J Biol Chem* 259:10863–10866, 1984
24. Sharp D, Blinderman L, Combs KA, Kienzle B, Ricci B, Wager-Smith K, Gil CM, Turck CW, Bouma ME, Rader DJ: Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinaemia. *Nature* 365:65–69, 1993
25. Raabe M, Flynn LM, Zlot CH, Wong JS, Veniant MM, Hamilton RL, Young SG: Knockout of the abetalipoproteinemia gene in mice: reduced lipoprotein secretion in heterozygotes and embryonic lethality in homozygotes. *Proc Natl Acad Sci U S A* 95:8686–8691, 1998
26. Raabe M, Veniant MM, Sullivan MA, Zlot CH, Bjorkegren J, Nielsen LB, Wong JS, Hamilton RL, Young SG: Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice. *J Clin Invest* 103:1287–1298, 1999
27. Hayashi H, Sato Y, Kanai S, Ichikawa M, Funakoshi A, Miyasaka K: Increased lymphatic lipid transport in genetically diabetic obese rats. *Am J Physiol Gastrointest Liver Physiol* 282:G69–G76, 2002
28. Davidson NO, Magun AM: Intestinal lipid absorption. In *Textbook of Gastroenterology.* Yamada T, Alpers DH, Owyang C, Powell DW, Silverstain FE, Eds. Philadelphia, Lippincott, 1993, p. 428–455
29. Angellon LB, Toth MJ, Thomson AB: Intracellular lipid binding proteins of the small intestine. *Mol Cell Biochem* 239:79–82, 2002
30. Bordewick U, Heese M, Borchers T, Robenek H, Spener F: Compartmentation of hepatic fatty-acid-binding protein in liver cells and its effect on microsomal phosphatidic acid biosynthesis. *Biol Chem Hoppe Seyler* 370:229–238, 1989
31. Vassileva G, Huwyler L, Poirier K, Agellon LB, Toth MJ: The intestinal fatty acid binding protein is not essential for dietary fat absorption in mice. *FASEB J* 14:2040–2046, 2000
32. Gedde-Dahl A, Kulseth MA, Ranheim T, Drevon CA, Rustan AC: Reduced secretion of triacylglycerol in CaCo-2 cells transfected with intestinal fatty acid-binding protein. *Lipids* 37:61–68, 2002
33. McNamara JR, Shah PK, Nakajima K, Cupples LA, Wilson PW, Ordovas JM, Schaefer EJ: Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: results from the Framingham Heart Study. *Atherosclerosis* 54:229–236, 2001
34. Pinget M, Boullu-Sanchis S: Physiological basis of insulin secretion abnormalities. *Diabete Metab* 28:4S21–4S32, 2002
35. Esmatjes E, Vidal J: Heart pathology of extracardiac origin. XI. Cardiac repercussions of diabetes mellitus. *Rev Esp Cardiol* 51:661–670, 1998

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/10/2539/369913/db1003002539.pdf by guest on 10 December 2023