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Increase of active transport of conjugated bile salts in streptozotocin-diabetic rat small intestine[1]

W. F. CASPARY

From the Division of Gastroenterology and Metabolism, Department of Medicine, University of Göttingen, Germany

SUMMARY Active transport of conjugated bile salts, Na-tauro- and Na-glycocholate and D-galactose, was examined in the small intestine of streptozotocin-diabetic rats by an *in-vitro* technique.

Tissue uptake and mucosal to serosal transport of conjugated bile salts and D-galactose was enhanced in diabetic rat ileum. The minimal transport capacity for conjugated bile salts in the jejunum did not differ between diabetic and control intestine. D-galactose transport and transport of 3-0-methyl-glucose were, however, enhanced in diabetic jejunum as well. Kinetic analysis of the initial uptake rates for conjugated bile salts revealed that the maximal transport capacity (Vmax) was enhanced in diabetic ileum.

In accordance with earlier results on the effect of experimental diabetes mellitus on digestive-absorptive functions it is suggested that experimental diabetes mellitus increases the transport capacity of active, Na^+-dependent intestinal transport processes in general.

Numerous studies have been published on the effect of insulin and experimental diabetes mellitus on facilitated diffusional membrane transport systems operating, for example, in red cells, fat cells, and muscle, but only a few reports consider the effect of insulin and experimental diabetes mellitus on active, energy-requiring transport systems operating in the small intestine or the renal tubular epithelial cells (Schultz and Curran, 1970). Increase of active hexose transport has been reported to occur at all levels of the diabetic small intestine (Crane, 1961; Aulsenbrook, 1965; Flores and Schedl, 1968; Olson and Rosenberg, 1970; Leese and Mansford, 1971; Caspary, Rhein, and Creutzfeldt, 1972b). Hexose transport capacity was most markedly increased in the distal parts of diabetic small intestine, where hexose transport is known to proceed more sluggishly than in the jejunum (Leese and Mansford, 1971).

Brush border digestive enzymes have recently been shown to be increased in streptozotocin- and alloxan-diabetic rat small intestine as well (Olson and Rogers, 1971; Caspary, Rhein, and Creutzfeldt, 1972a; Younoszai and Schedl, 1972).

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Since in addition to increased hexose absorption active transport of amino acids is stimulated in diabetic rat small intestine (Olson and Rosenberg, 1970; Caspary *et al*, 1972b), it seemed likely that during the course of experimental diabetes mellitus active transport systems in general are operating at higher transport rates rather than stimulating hexose transport capacity specifically.

As bile salts are very efficiently reabsorbed in the ileum due to the additive effects of several transport mechanisms, namely, ionic and non-ionic diffusion, and active, Na^+-dependent transport, we examined the effect of streptozotocin-diabetes on the active transport capacity for conjugated bile salts, tauro- and glycocholate, in rat ileum by an *in vitro* technique.

Methods

Female Wistar rats (200 ± 20 g) were injected intravenously via the saphenous vein with 70 mg/kg of streptozotocin (Upjohn Company, Kalamazoo, Michigan, USA, lot no. 9681-GGS-118 FI U 9885), dissolved in citrate buffer (pH 4·5). Controls received citrate buffer alone. Rats with a heavy glycosuria, corresponding to blood glucose levels between 350 and 500 mg%, were used for the experiments five to six days after the administration of streptozotocin.

Pair-feeding revealed that up to six days after giving streptozotocin experimental results were not affected by altered food consumption. Diabetic rats consumed for up to five or six days of streptozotocin-diabetes the same amounts of food as did the controls.

After killing by decapitation the intestine was removed, washed with saline and everted over a glass rod. Everted sacs were prepared according to the method of Wilson and Wiseman (1954). Small intestine up to 15 cm proximal to the ileocecal valve was considered to be ileum and was used for the everted sac experiments. Intestinal tissue 40-50 cm beyond the pylorus was regarded as jejunum. The serosal compartment of the everted sac preparations measuring 7 cm in length was filled with 0·5 ml Krebs-Henseleit phosphate buffer and the appropriate substrates. The serosal compartment contained the same substrate concentration as was added to the mucosal compartment (10 ml). Incubations were carried out for 45 minutes under pure oxygen. In experiments measuring tissue uptake of conjugated bile salts at shorter incubation intervals, segments of everted small intestine measuring approximately 1 cm were incubated in Krebs-Henseleit phosphate buffer. Processing of the incubated tissue was according to the method of Crane and Mandelstam (1960) as modified in a more recent publication (Caspary, Stevenson, and Crane, 1969). D-mannitol was used to correct for extracellular space (Caspary *et al*, 1969). Results are expressed in:

$$\text{Percentage filling} = 100 \times \frac{\mu\text{moles / ml tissue water}}{\mu\text{moles / ml medium}}$$

assuming a water content of approximately 80% of the tissue wet weight (Crane and Mandelstam, 1960). In everted sac experiments accumulation of the substrate in the serosal compartment is expressed in S/M-ratio (μmoles of substrate per ml serosal fluid/ μmoles per ml of mucosal medium).

Gas chromatographic examinations showed that Na-taurocholate (Serva, Heidelberg, Germany) contained less than 0·1% contamination. The major contaminants were chenodeoxycholic acid (0·004%) and cholic acid (0·062%).

^{3}H-Na-taurocholate and ^{14}C-glycocholate were obtained from New England Nuclear. More than 95% of the radioactivity in tissue water extracts or serosal medium could be identified as unmetabolized conjugated bile salts (Caspary, 1973). Radioactivity of the mucosal and serosal fluid and the tissue water extracts were assayed by a Packard liquid scintillation system using the channel ratio method for quench correction. Toluene-Triton-X-100 was used in the scintillation liquid.

The significances of the differences between each series of experiments were calculated by the paired and unpaired t test. The P values given in the text represent the lowest values obtained. The number of animals used in the experiments is given in the legends of the graphs (=n).

Results

After incubating the everted gut sacs from diabetic rat ileum with conjugated bile salts a significantly higher accumulation of tauro-(P=0·002) and glycocholate (P=0·002) was achieved in the serosal compartment of diabetic ileum compared with intestine from controls (fig 1). Alongside the increased accumulation of conjugated bile salts D-galactose was

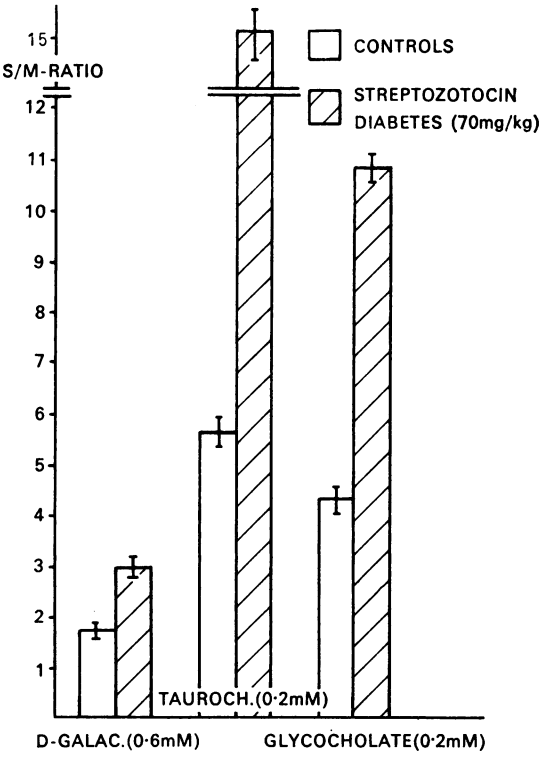

Fig 1 *Effect of streptozotocin-diabetes on the accumulation of conjugated bile salts and D-galactose in the serosal compartment of everted sacs prepared from rat ileum.*

Everted gut sacs from streptozotocin-diabetic rats and controls were incubated for 45 min in Krebs-Henseleit phosphate buffer. Substrates at the concentrations indicated in the graphs were added to the mucosal and serosal compartment. S/M-ratio = μmole of substrate per ml serosal fluid/ μmole of substrate per ml mucosal fluid.

Results are means ± SEM (n=8)

accumulated to a greater extent in the serosal compartment of everted diabetic rat ileum ($P=0.05$). Accumulation of conjugated bile salts in the tissue water space of intestinal mucosa from diabetic rat small intestine was markedly increased in the ileum ($P=0.002$), as was accumulation of 3-0-methyl-D-glucose in the jejunum ($P=0.005$) (fig 2). The increase in tissue uptake and mucosal to serosal transport of conjugated bile salts in diabetic small intestine could only be demonstrated in the ileum, whereas no significant differences in the minimal rates of taurocholate could be observed in the jejunum ($P=0.2$) (fig 2). 3-O-methyl-D-glucose uptake was, however, markedly increased in the jejunum of diabetic rats ($P=0.005$) (fig 2). Measurement of the extracellular space, tissue water space, and of the minimal uptake rates of conjugated bile salts in the jejunum did not give different results for intestine from diabetic rats or controls, indicating that experimental diabetes mellitus does not seem to alter the passive mucosal permeability for conjugated bile salts in the jejunum (fig 2).

In order to show that diabetes of short duration stimulates active, Na^+-dependent transport processes rather than passive diffusional movements we measured tissue uptake of Na-taurocholate in the ileum of diabetic rats and controls under conditions of reduced mucosal Na^+ concentrations and in the presence of a decoupling agent, 2·4-dinitrophenol. If Na^+ was replaced by D-mannitol ($P=0.002$), choline chloride ($P=0.005$), or if 2·4-dinitrophenol was present ($P=0.002$) (fig 3) tissue uptake of taurocholate was markedly depressed. Under the conditions of the depressed uptake of taurocholate, while Na^+ was replaced differences could no longer be observed between taurocholate uptake rates in diabetic and control intestine (fig 3).

Time-dependent uptake of tauro- and glycocholate was also markedly enhanced in diabetic rat ileum at all incubation intervals tested, suggesting that influx of conjugated bile salts is increased at the very early incubation intervals rather than efflux decreased (fig 4).

In order to test whether diabetes mellitus increases transport of conjugated bile salts by increasing the maximal transport capacity or by affecting the carrier binding affinity for bile salts, we incubated intestinal segments of rat ileum with taurocholate over a wide concentration range. Under the conditions used tissue uptake was still proceeding in a linear fashion thus representing initial rates of uptake. Tissue uptake rates were increased in diabetic rat ileum at all concentrations of taurocholate used in the mucosal medium (fig 5). If these results are plotted according to Lineweaver-Burk it can be seen that the maximal transport capacity (Vmax) for taurocholate is increased in diabetic ileum but transport Km (fig 6) is unaffected. These results are in accord with the kinetic analysis of D-galactose transport in diabetic rat jejunum (Olson and Rosenberg, 1970; Caspary *et al*, 1972b) and suggest that more carriers per unit weight of intestine are operating in diabetic ileum to transfer conjugated bile salts across the brush border membrane or that more energy is available for the active transfer process; the nature of the carriers, however,

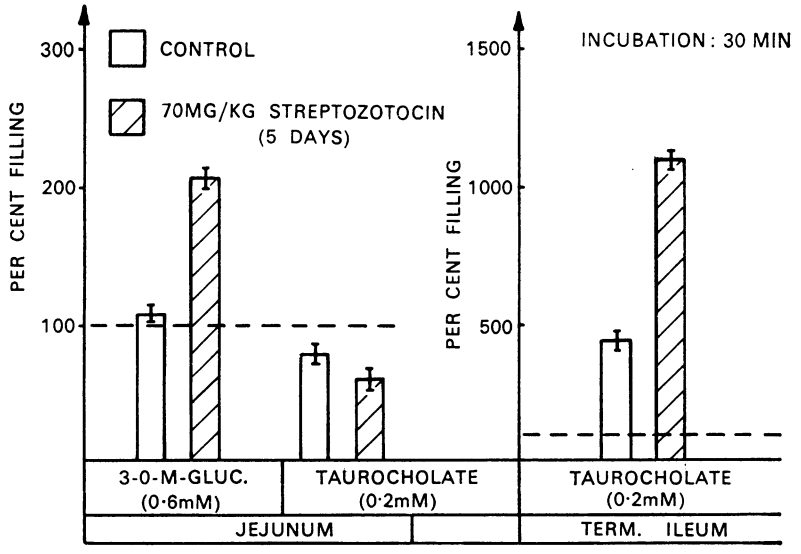

Fig 2 *Effect of streptozotocin-diabetes on tissue uptake of 3-O-methyl-D-glucose in jejunum and of taurocholate in jejunum and ileum.*

Segments of everted rat small intestine from streptozotocin-diabetic rats and controls were incubated for 30 min in Krebs-Henseleit phosphate buffer. Results are means ± SEM ($n=7$) and are expressed as 'per cent filling'

$$\left(100 \times \frac{\mu mole/ml\ tissue\ w}{\mu mole/ml\ muc\ med}\right)$$

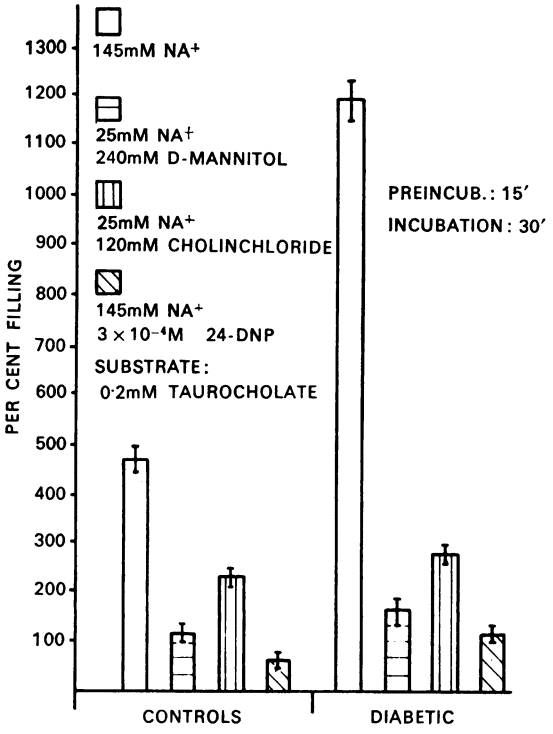

Fig 3 *Influence of Na^+-replacement and 2·4-dinitrophenol on tissue uptake of taurocholate in ileum of streptozotocin-diabetic rats and controls.*

Segments of ileal small intestine were incubated in a modified Krebs-Henseleit phosphate buffer in which 120 mM Na^+ was replaced by D-mannitol or choline chloride. In one set of experiments 2.4.-dinitrophenol ($3\times 10^{-4}M$) was added. Tissue was preincubated under these conditions for 15 min.; then 0·2 mM ^{3}H-taurocholate was added and the incubation was continued for a further 30 minutes.

Results are means ± SEM ($n=8$)

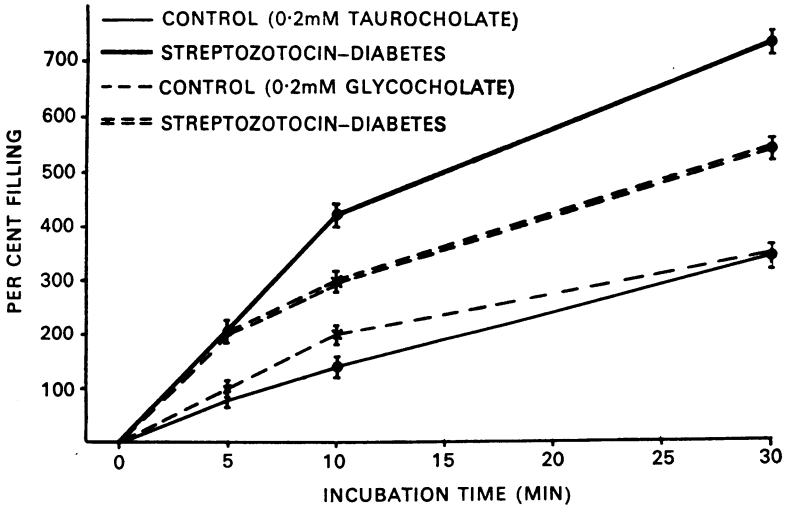

Fig 4 *Time course of tissue uptake of tauro- and glycocholate by ileal segments from streptozotocin-diabetic rats and controls.*

Results are means ± SEM ($n=7$)

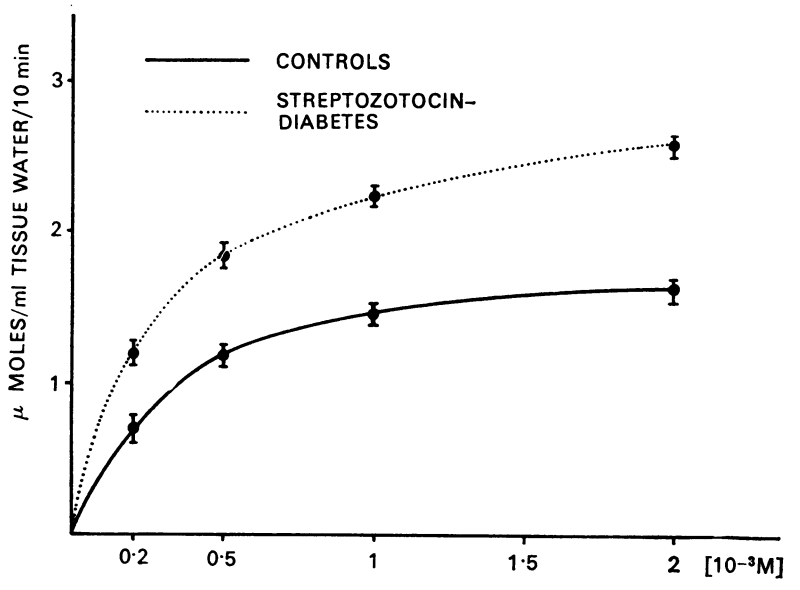

Fig 5 *The effect of increasing concentrations of taurocholate in the incubation medium upon the tissue uptake of taurocholate by ileal segments of small intestine from streptozotocin-diabetic rats and controls.*

Segments of ileal small intestine were incubated for 10 min at different concentrations of taurocholate ($0{\cdot}2 \times 10^{-3}M$) in the incubation medium.

Results are means ± SEM ($n=6$)

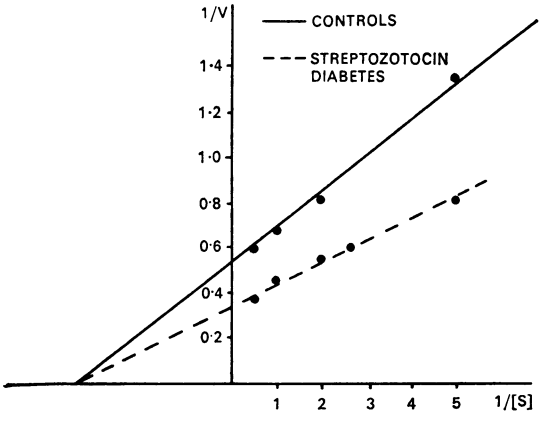

Fig 6 *Concentration-dependent tissue uptake of taurocholate by ileal segments of small intestine from streptozotocin-diabetic rats and controls.*

The data from fig 5, representing initial rates of uptake, are plotted according to Lineweaver-Burk [S] = [taurocholate ($10^{-3}M$)], V = tissue uptake (μmole/ml tissue water / 10 min. The mean values from fig 5 are used.

expressed by their substrate affinity (Km) does not seem to be altered by the conditions of experimental diabetes mellitus.

Discussion

Conjugated bile salts are known to be absorbed in the ileum of rats by an active, Na^+-dependent transport process resulting in an accumulation of the transported substrate against an electrochemical concentration gradient in the mucosal epithelial cells or in the serosal compartment of an everted sac preparation (Lack and Weiner, 1961; Dietschy, Salomon, and Siperstein, 1966; Holt, 1966; Dietschy, 1967)) whereas a mechanism of ionic and nonionic diffusion is operating for transfer of bile salts along the entire small intestine. The amount of bile salts absorbed by nonionic diffusion largely depends on the pK values of the particular bile acids. The high reabsorptive capacity of the ileum for conjugated bile salts is due to the additive effect of the three transport mechanisms mentioned; the active transport system in the ileum is contributing the major portion of the reabsorptive capacity (Lack and Weiner, 1961; Dietschy *et al*, 1966; Dietschy, 1967).

An increase of intestinal hexose transport capacity can be observed at all levels of experimental diabetic rat small intestine (Crane, 1961; Aulsenbrook, 1965; Flores and Schedl, 1968; Olson and Rosenberg, 1970; Leese and Mansford, 1971; Caspary *et al*, 1972b). Glucose absorption was maximally stimulated in diabetic ileum where hexoses normally are absorbed at lower rates than in the jejunum (Leese and Mansford, 1971; Caspary *et al*, 1972b). Our results are in agreement with the results in the literature (Crane, 1961; Aulsenbrook, 1965; Flores and Schedl, 1968; Olson and Rosenberg, 1970) that tissue uptake of actively transported hexoses was enhanced per unit weight of intestine in the jejunum as

well as in the ileum of diabetic small intestine. The increase in active, energy-dependent tissue uptake and mucosal to serosal transport of conjugated bile salts could be observed in the ileum of diabetic rat small intestime. The minimal amount of absorption achieved in the jejunum, however, by the mechanism of passive diffusion was not affected by streptozotocin-diabetes. These findings, in addition to the data presented for ileal taurocholate absorption under conditions of energy deprivation and reduction of mucosal Na^+-concentration, confirm that experimental diabetes mellitus stimulates active, Na^+-dependent transport processes rather than diffusional solute movements in the small intestine of rats.

Kinetic analysis of the uptake mechanism revealed that maximal transport capacity (Vmax) is increased rather than carrier affinity for conjugated bile salts. Similar kinetic data have been obtained for active hexose absorption in alloxan- (Olson and Rosenberg, 1970) and streptozotocin-diabetic rat jejunum (Caspary *et al*, 1972b).

Since experimental diabetes mellitus does not only stimulate intestinal transport functions for hexoses and amino acids and digestive functions (Olson and Rogers, 1971; Caspary *et al*, 1972a; Younoszai and Schedl, 1972) but also active transport of bile salts in the ileum, we have to assume that experimental diabetes mellitus affects energy-requiring transport systems in general.

Hyperphagia developing in the later stages of experimental diabetes mellitus (Jervis and Levin, 1966: Schedl and Wilson, 1971) is very unlikely to be responsible for the increased transport capacity per unit weight of intestinal tissue as food consumption did not differ between diabetic animals and controls five to six days after the induction of streptozotocin-diabetes. Since diabetic animals are losing weight due to unaltered food consumption and glycosuria, these animals might be in a condition of metabolic imbalance, possibly similar to the status of semi-starvation where hexose absorption is said to be increased (Kershaw, Neame, and Wiseman, 1960). But a recent observation casts doubt on the hypothesis that starvation might be responsible for the increased transport capacity of conjugated bile salts. Wall and Baker (1972) found a decreased uptake and a lower mucosal-to-serosal transport rate in addition to a decreased taurocholate induced transmural potential difference (Pd) in the ileum of rats which were starved for 24 hours. Under the conditions of a 24-hour fast hexose absorption would be increased with the same methods applied (Kershaw *et al*, 1960). Brush border digestive enzymes have been found to be decreased in the small intestine of starved rats as well (McNeill and Hamilton, 1971), whereas in experimental diabetes mellitus brush border hydrolase activity is markedly enhanced (Olson and Rogers, 1971; Caspary *et al*, 1972a; Younoszai and Schedl, 1972). In the later stages of experimental diabetes (after five to six days) a marked hypertrophy of the small intestine develops in the hyperphagic diabetic animals (Schedl and Wilson, 1971). During this stage hexose absorption is still enhanced due to the hypertrophy of the intestine. Specific transport capacity (absorption/per unit wet weight of intestine), however, is only increased in diabetic rat small intestine during the first days of experimental diabetes mellitus (up to the fifth or eighth day). During this time there is no significant difference in intestinal weight between diabetic animals and controls (Schedl and Wilson, 1971). The mechanism responsible for the increased transport rates and the increase in digestive enzymatic activity in diabetic rat small intestine still remains obscure.

Experiments *in vivo* will be necessary to show whether the observed increase of ileal bile salt reabsorption in experimental diabetes mellitus will have an effect on bile salt pool size or synthesis rate. The ratio of the pool size to the synthesis rate of bile salts is determined by the efficiency of ileal reabsorption (Hofmann, 1966). An additional factor regulating pool size is now believed to be the frequency of enterohepatic circulation (Low-Beer and Pomare, 1973; Northfield and Hofmann, 1973). Interruption of the enterohepatic circulation results in increased bile acid synthesis by the liver. Under the conditions of an increased reabsorptive capacity for bile salts in diabetic small intestine, bile salt synthesis by the liver might therefore be expected to be decreased. Finally, since no data are available on bile acid synthesis rates in streptozotocin-diabetes, the increased transport capacity for conjugated bile salts could be an adaptive process of the ileum due to an increased bile acid synthesis rate in the liver with a consequent increase in the size of the bile salt pool requiring absorption.

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