Should Clinicians investigate the Serum Bile Acid Metabolome in Patients before and after Treatment with Metformin?

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The bile acid metabolome in serum and urine is altered in type 2 diabetics but may be already changed in insulin resistant subjects or those with impaired glucose tolerance. Interestingly, qualitatively, the alterations in the serum bile acid composition are similar to the changes observed for intrahepatic cholestasis in pregnancy (ICP), which also represents an insulin-resistant condition. ICP, when diagnosed by monitoring serum bile acid composition, is treated with UDCA. UDCA acts as taurine conjugate via a specific receptor site located on integrins. It is hoped in the scientific community that blood-based biomarkers, e.g., a panel of metabolites, including bile acids, can be developed that may be of value for early detection of ICP, but especially for prediction of type 2 diabetes. As discussed elsewhere, present day technology to predict type 2 diabetes from fasting serum samples is expensive and not superior to standard clinical tests such as OGTT or even family history.

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Figure 1: Changes in the Bile Acid Metabolome as a Function of Insulin Resistance

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Metformin is the oldest but still the first line drug for treatment and prevention of type 2 diabetes. The role of bile acids in the antidiabetic activity of the biguanide has attracted attention very recently—although discoveries in this area were made several decades ago. Background information is provided below to stimulate interest of clinicians in these truly fascinating developments.

1.1 Molecular Targets of Metformin

If metformin had to be approved as a new drug under current requirements of the regulatory agencies, a main problem would be to identify the primary molecular target(s) responsible for its multiple actions as an antidiabetic, antiandrogenic, cancer preventing, and even anti-inflammatory drug. At the present time mainly three primary molecular targets have been identified. The first and oldest discovered, still experimentally supported but also questioned, is complex I in mitochondria, which is inhibited by biguanides. Two other targets have been added recently, namely OCT 1 (organic cation transporter 1, SLC22A1) which transports metformin but is also a high-capacity thiamine transporter and mitochondrial glycerol-phosphate dehydrogenase. Whatever the primary biochemical target, most researchers agree that activation of the master regulator of metabolism, AMP-dependent protein kinase (AMPK), via phosphorylation (Phospho-AMPK, pAMPK) is one important biochemical cellular consequence. AMPK may also be even a primary target of metformin. Observations in type 2 diabetes patients, where metformin increased AMPK (and pAMPK) in skeletal muscle and adipose tissue support the role of the master kinase. Without doubt, tissue targets of metformin clearly extend beyond common textbook knowledge that metformin is a “liver specific” antidiabetic.

1.2 Effects of intravenous and acute or chronic oral Dosing of Metformin in Type 2 Diabetes

Acute intravenous infusion of metformin in type 2 diabetics neither changes hepatic glucose production nor peripheral glucose disposition. Oral delivery of a single dose metformin decreased postprandial but not pre-prandial glucose levels, the explanation being that insulin secretion was stimulated by a gut-based mechanism (GSIS, see section 1.8.1). Effects on pre-prandial (fasting) glucose are only observed upon multiple dosing indicating decreased gluconeogenesis in the liver.

1.3 Uptake Mechanisms of Metformin in the Gut

With increasing doses of metformin fractional absorption (the amount of drug reaching the general circulation) is decreasing, suggesting saturable uptake mechanisms in the gut. The mechanisms behind this behaviour have been clarified to a large extent but the consequences for cellular metabolism in enterocytes by “trapped” intracellular metformin of different segments of the intestine were overlooked or even forgotten until recently. Transport of metformin across human small intestinal and colonic tissue from lumen to basolateral direction, measured by using chambers, occurs mainly via a paracellular mechanism and less so by apical to basolateral i.e. transcellular transport. There is considerable confusion about transporters involved. Whereas OCT 3 (SLC23 A3) is an excellent metformin transporter, which is expressed in the human intestine including the colon and localized in the brush border membrane, others find in Caco-2 cell monolayers that PMAT, the plasma membrane monoamine transporter (SLC29A4), serotonin reuptake transporter (SERT; SLC6A4), OCT 1 and a choline transporter—but not OCT-3—are mainly responsible for accumulation in this widely used model system. The inward driving force for these transporters is the extracellular proton concentration, which, in healthy persons, is higher in the colon than in the small intestine. With increasing oral doses a larger fraction of metformin reaches the colon and can be accumulating there. In animal experiments it was observed that enrichment in the intestine also occurs when metformin is given intravenously. Perhaps the serotonin reuptake transporter (SERT), widely distributed in the gut, is in part responsible for metformin uptake from the systemic circulation. High specific uptake of labeled SERT ligands in the intestine of healthy volunteers can be observed. There is one report that exposure of human duodenal biopsies to low concentrations of metformin (1 to 30 micromolar) leads to increased outflow of serotonin. The authors suggest that metformin stimulates the release of serotonin from enterochromaffin cells via another neurotransmitter but did not investigate a possible role of metformin as blocker of reuptake. It seems that this finding has been overlooked as recent animal experiments about the role of duodenal (preatosporptive) activity of metformin and the gut-brain-liver axis concentrated on GLP-1 and AMPK only.

1.4 Upon chronic Dosing a deep Metformin Compartment is building up—especially in the Gut

At steady-state, the distribution volume of metformin in humans can reach up to 4-1/l kg body weight, indicating deep, intracellular compartments. In one of these tissues, especially
the intestine, cellular concentrations must reach even higher values than in the portal vein, estimated to be 40 to 70 micromolar after a single therapeutic dose. Concentrations have been measured in humans, but is not known where the drug is actually distributed in the tissue. If within the cells, mitochondria and other acidic compartments are the most logical locations. In a small clinical study with 8 obese type 2 diabetics, jejunal biopsies after 6-8 weeks chronic dosing (twice 850 mg metformin /day) were taken, after 12 -16 hours of the last dose and 3 hours after the next dose. The pre-dose concentrations in jejunal biopsies were about 250 micromoles/kg and post-dose 4000 micromole/kg. Specific intestinal enrichment may explain the effects of very low oral doses (250 or 500 mg/die) on the prevention of aberrant cryptic foci in the colon.

1.5 A major Tissue Target of Metformin in Type 2 Diabetes is not only the Liver but the Intestine

Indirect evidence is from examinations of metformin- treated patients by 18 F-FDG PET which clearly demonstrate increased diffuse uptake throughout the small but especially in the large intestine. Apparently, as demonstrated in experimental animals, the severe alteration of cellular mitochondrial metabolism by metformin leads to AMPK activation (pAMPK) in small intestine enterocytes and especially in colonocytes. In the colon this is followed by stimulation of glucose uptake via upregulation of GLUT1 from the basolateral membrane as urgently needed for proper functioning of the Krebs cycle and metabolism of short chain fatty acids. In contrast, for more proximal regions of the upper bowel, increased energy demand may be satisfied by upregulation of the sodium-glucose co-transporter 1 (SGLT1) and glucose is mainly imported from the gut lumen.

1.6 Metformin inhibits Bile Salt Reabsorption similar to the ASBT Inhibitors

Once the high enrichment of metformin in the small and large intestine was discovered, functional consequences of this-after chronic dosing- persistent high-concentration drug compartment were sought. In a crossover, placebo -controlled clinical study in patients with type 2 diabetes mellitus, metformin almost doubled faecal bile salt excretion and increased deconjugation about threefold. (For more details, see legend to Figure 2).

In a series experiments performed in rats, metformin blocked the reuptake of bile acids in the ileum by a very significant factor-almost 50%. Taken together, metformin blocks reuptake of bile acids in the ileum and delivers more bile acids to distal regions of the intestine. Of note, inhibitors of the sodium/bile acid co-transporter ASBT (apical sodium-dependent bile acid transporter; SLC10A2 (solute carrier family 10 member 2)) are in development for treatment of type 2 diabetes. The target of metformin in the ileocyte bile acid transporters (apical or basolateral) is yet unknown-but the consequences are apparently similar to the ASBT inhibitors.

1.7 Single Dose Metformin increases GLP-1 Secretion in Healthy Volunteers

In a double-blind study with ten healthy lean young male subjects with no family history of diabetes and after a 10 hour-fast, the volunteers on different days received via a nasogastric tube either placebo or metformin (1.5 g) with concomitant i.v. infusion of placebo (saline) or CCK-8 (0.4 pmol/kg/ min). The results are shown in Figure 3 and prove that oral metformin elicits a rapid (within 10-20 minutes, see figure 2 in reference 1) and sustained (up to 4 hours) release of glucagon-like peptide 1 (GLP-1), which is augmented by bile acids. Without doubt metformin stimulated L-cells and enhanced the effects of bile acids on their receptors. The very early response suggests that the upper intestine-especially the duodenum-is involved. This is supported by animal experiments, which claim a gut-brain-liver axis with a dominant role of duodenal activation of AMPK by metformin and GLP-1 release. The role of afferent nerves for GLP-1 activity in this axis is also emphasized by 1. However, the sustained response, points to involvement of L-cells in more distal segments of the gut, as oral-caecal transit time measured by lactulose breath test is between 60 to 120 min.
1.8 Intestinal L-Cells and the TGR5 Receptor

1.8.1 L-Cells

L-cells (Large cells) are neuroendocrine cells distributed in the entire gut, but especially in the lower intestine. Extending their folded apical membrane deep into the gut lumen they are perfectly adapted to “sense” nutrients and other signals, among them bile acids, passing or produced nearby as in the colon. The mechanisms of the sensing are quite sophisticated. In the upper part of the small intestine (duodenum and jejunum), where glucose from digested carbohydrates is actively taken up by the sodium-glucose co-transporter SGLT1, they depolarize via sodium influx, activate voltage-dependent calcium channels and release, among other compounds, peptide YY and glucagon-like peptide 1 (GLP-1) into the hepato-portal circulation.

Short time (2 h) in vitro experiments with different L-cell lines could not demonstrate any effects of metformin (up to 2 mM) on GLP-1 release. Long term incubations (24 or 48 h) with 0.25 or 0.5 mM metformin increased GLP-1 release without increasing pAMPK whereas higher concentrations (above 1 mM) stimulated AMPK but failed to have effects on GLP-1 release.

Considering the measured tissue concentrations in patients (see section 1.4) any normal oral dose may first stimulate AMPK in the duodenum and jejunum, followed by AMPK-independent insulin and Wnt signaling activation of L-cells, once concentrations reach trough levels. In the upper gut vagaal sensory neurons, communicating with the CNS are activated by GLP-1. Equally important is the presence of GLP-1 receptors on islet beta cells resulting in the gut-potentiation of glucose stimulation of insulin secretion (GSIS) after oral glucose load. In patients with prediabetes with the characteristic loss of the first phase of insulin release and in type 2 with impaired second phase, infusion of GLP-1 can “repair” the defect.

1.8.2 TGR5

The plasma membrane-bound „Takeda“ G-Protein-coupled receptor (TGR5), also known as GP-BAR1 (homo sapiens), was discovered in search for the mechanism by which bile acids suppressed the inflammatory response of macrophages/monocytes. The receptor is widely distributed in human and rodent tissues and activated by secondary bile acids. This suggests to some researchers that the docking site of the receptor co-evolved with the gut microbiome. Surprisingly, although the liver is regarded as the most important organ for feedback bile acid control, hepatocytes do not express TGR5 but sinusoidal and Kupffer cells do. Receptors are in the small and large intestine, including the enteric nervous system, L-cells (which respond by secretion of GLP-1, especially in the colon), uterus, testes, spleen, pituitary and adrenal gland, bone marrow, central and peripheral nervous system as well as brown (rodents), white adipose tissue and skeletal muscle. TGR5 is claimed to be of relevance in metabolic, cardiovascular, hepatic, pancreatic disorders as well as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Last but not least the bile acid-induced pruritus, often the first symptom for ICP, is mediated by this receptor. Numerous compounds are developed that either block or activate TGR5.

1.9 Metformin upon chronic dosing increases faecal Bile Acids, stimulates GLP-1 Secretion, changes the Serum Bile Acid Metabolome and the Microbiome in Type 2 Diabetics

In a truly remarkable crossover study (sponsored by GlaxoSmithKline) with 12 female type 2 diabetics, chronically treated with metformin monotherapy, patients were investigated at 4 time points for duodenal, faecal and serum bile acid profiles, glucose, insulin, gut hormones (including GLP-1) and analysis of the gut microbiome. The first (baseline) set of data (Visit 1) was obtained when patients...
were on their usual, stable dose of metformin. After seven days of stopping the drug was Visit 2. According to other data available in the literature most deep compartments must have lost their metformin at this time point. The authors measured but did not report plasma levels of metformin. When fasting capillary blood glucose had increased by 25% from measurements prior to Visit 1, metformin was re-introduced (Visit 3). After blood glucose levels reached pre-Visit 1 levels, Visit 4 profiling was performed.

In Figure 4 results for bile acids under the conditions „ON” metformin and „OFF” metformin (only Visit 1 and Visit 2) are depicted. Clearly, metformin decreases bile reabsorption during meals, increases faecal bile acids, inhibits fasting bile flow to the duodenum, changes the composition of the circulating serum bile metabolome (decreasing especially cholic acid and its conjugates, which reflects the activity of the 12- alpha- hydroxylase, encoded by CYP8B1) and stimulates GLP-1 secretion: mean active GLP-1 7.36 was 5-fold and total GLP-1 1.8 fold higher under “ON” conditions. Especially intriguing are “favorable” changes in the gut microbiome upon treatment with metformin, which correlated with alterations of the serum bile acid metabolome. The changes confirm earlier cross-sectional data.

Figure 4: Metformin changes the Serum Bile Acid Metabolome and increases Faecal Bile Acid Excretion in Type 2 Diabetics

1.10 Conclusion
Metformin (see Figure 5) acts in the gut by different mechanisms:

First, by releasing GLP-1 from L-cells, which may be independent of AMPK. This occurs rapidly in the duodenum and, perhaps, the jejunum. Animal experimental data point to another effect of metformin in the duodenum, namely by activation of the gut-brain-liver axis via AMPK. Similar findings for AMPK and, additionally, activation of NAD+-dependent deacetylase sirtuin 1 (SIRT1), were reported for resveratrol, which also promotes ASBT degradation via the ubiquitin-proteasome pathway.

Second, via bile acid reabsorption inhibition, enhanced bile acid delivery to distal fractions of the gut leads to activation of TGR5 receptors on L-cells in the colon.

1.11 Recommendation
Since many patients (with prediabetes, type 2 diabetes or polycystic ovary syndrome, PCOS) will receive metformin for the first time (being drug naive), it seems possible to study their serum (or urine?) bile acid metabolome before (baseline) and after 1, 2 or 4 weeks of treatment. An considerable amount of effort has been dedicated to find out which factors, especially genetic variants in the uptake transporters, determine responsiveness. Perhaps, a “positive change” as demonstrated by in the bile acid metabolome, may turn out to be as a surrogate marker for adequate tissue levels and responsiveness.
Legends to the Figures

Figure 1
Changes in the Bile Acid Metabolome as a Function of Insulin Resistance

Data are taken from table 2 of reference1. The study population consisted of 200 healthy, nondiabetic subjects (103 women and 97 men), who were between 30 and 60 years of age. Insulin sensitivity was measured by euglycemic insulin clamp and calculated in units (U) by standard methods. Measurement of the fasting serum bile acid metabolome was with LC/MS. This healthy subject population was divided into quartiles (Q1: most insulin resistant, to Q4: most insulin sensitive), based on clamp-derived insulin sensitivity: Q4 =217 U, Q3 =154 U, Q2 =119 U, Q1 =86 U). Individual serum bile acid concentrations for each quartile were reported as well as classes (12 –alpha – (OH) - BAs, conjugated, non-conjugated) and ratios. The authors also measured the bile acids in a group of 35 type 2 diabetic patients but could not confirm the relationships observed in the healthy cohort. Most likely, as a majority of these patients received metformin, drug treatment “corrected” the insulin- resistant bile metabolome signature (see section 1.9).

Figure 2
Metformin changes the Gut Flora and increases Faecal Bile Acid Excretion in Type 2 Diabetics

In this double-blind, placebo- controlled, cross-over study47 with 23 type 2 diabetic patients oral-cecal transit times were measured first with the lactulose test after 7 days treatment with either placebo or metformin (850 mg twice daily) for 7 days. No significant difference in transit time for the two cohorts was observed, but serum glucose differed by 2.59 mmol/l. Subsequently both cohorts, again after 7 days of placebo or metformin, received 14 C-glycocholate (combined with carrier) orally 1 hour after the usual doses of placebo or metformin. Then a test meal was given and exhaled radioactivity was measured every hour for a period of 6 hours. The graph (data are from figure 3 of 47 ) demonstrates that metformin changed the gut microbiota as deconjugation by bile salt hydrolases was increased significantly. In the insert the mean cumulative (3 days) 14 C-faecal bile salt excretion (numbers are taken from the text), as percentage of administered dose, is depicted. Individual variations in the 11 placebo and 11 metformin treated patients are enormous as shown in figure 4 in reference 47.

Figure 3
Metformin enhances Bile Acid- triggered GLP-1 Secretion in Healthy Volunteers

The cumulative serum GLP-1 increases after i.v. CCK-8, oral metformin (1.5 g) or both is illustrated. The placebo response (277 pM x min) is not shown. Data are taken from the text of 1.

Figure 4
Metformin changes the Serum Bile Acid Metabolome and increases Fecal Bile Acid Excretion in Type 2 Diabetics

Data for the graph are approximate values only which were obtained by enlarging the figures in5 and extrapolating values of the center of data points to the corresponding y-axis. The authors did not answer an e-mailed request for data numbers. For further details and results on the correlation of changes in the bile acid metabolome with gut microbiota, consult reference46.

Figure 5
Metformin and the Gut- an Update

References


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