Chapter 13
Metabolic functions of the porcine liver

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This chapter deals with:

- Anatomy and ultra structure of the porcine liver
- Bile acids and bile secretion
- Metabolic functions of the porcine liver related to glucose, protein, amino acids, lipid and short-chain fatty acid metabolism
- Endocrine and immune functions of the liver
- Hepatic detoxification and scavenging pathways.

1. Introduction

The liver (hepar) is a key organ in the integration of whole body metabolism. All nutrients absorbed to the portal blood are transferred to the liver and subjected to removal from the blood by uptake into liver cells (hepatocytes). Nutrients passing the liver without being taken up and metabolites released from the liver will enter vena cava and be available for metabolism in peripheral tissues. The liver also has a number of other functions: excretion of waste products, secretion of bile, storage of lipids, vitamin A, and glycogen, phagocytosis of particulate matter, detoxification by metabolism, and conjugation of toxins and steroids as well as endocrine functions. The aim of this chapter is to describe the major metabolic functions of the porcine liver.

2. Anatomy of the porcine liver

The liver is the largest gland in the body accounting for approximately 2% of the whole body weight and is situated on the abdominal face of the diaphragm. The porcine liver has four main lobes (Figure 13.1): the right lateral lobe, right medial lobe, left medial lobe, and left lateral lobe. The left lateral lobe is the largest of the four lobes. The right lateral lobe is partly subdivided into

the main lobe and a caudate process. The gallbladder (vesicafellea) is placed in the depression between the quadrate (a minor lobe) and the right medial lobe.

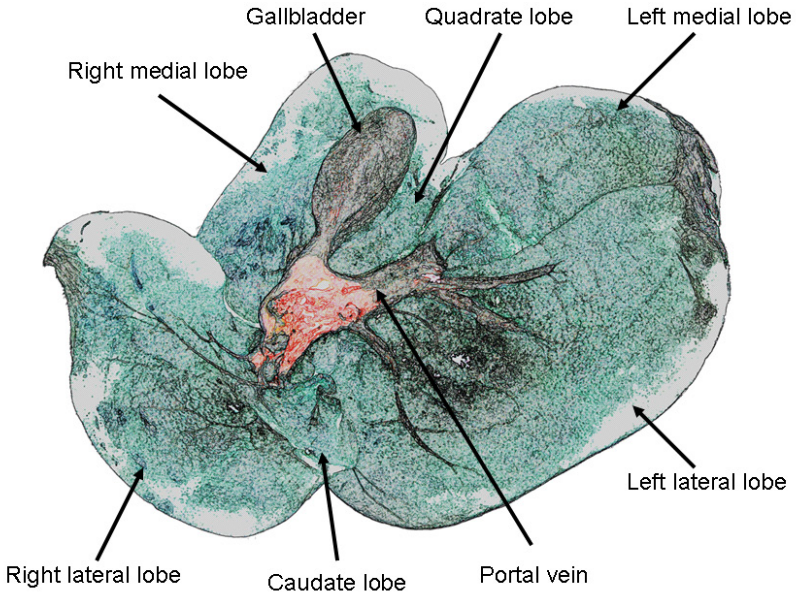

Figure 13.1. Visceral face of the porcine liver. The four major lobes of the liver are the right lateral, right medial, left medial, and left lateral lobes.

The parenchyma (functional tissue of an organ) of the liver is organized into polygonal lobules that are supplied with blood via the portal canal and drained via the central vein forming the smallest branch of the hepatic veins drained into the vena cava (Figure 13.2). The porcine liver lobules have a relatively large amount of interlobular tissue resulting in a firm structure when compared with other animals. Blood passes from the portal vein supplied with a small amount of arterial blood to the central vein along the sinusoids, which are a special porous and discontinuous endothelium permitting blood plasma to enter the space of Disse between the sinusoidal epithelium and the hepatocytes. The special porous endothelium of the liver ensures that hepatocytes are able to extract both small molecules and macromolecules/lipoproteins from the blood; however, red blood cells do not cross the sinusoidal endothelium. All hepatocytes have three different surfaces oriented toward the perisinusoidal space (space of Disse; microvillus surface), the bile canaliculi, and adjacent hepatocytes.

The bile canaliculi originate as expanded extracellular spaces of hepatocytes bordered by tight junctions.

The liver is exposed to antigens originating from food and intestinal microbes, and has important barrier functions mediated by a number of cell types associated with the sinusoid endothelium: Kupffer cells (the largest group of tissue-resident macrophages in the body also has functions in degradation of erythrocytes and lipoprotein metabolism), hepatic stellate cells (store retinol and present antigens for T cells), and dendritic cells (antigen presenting cells). However, in a number of ways the liver has the important function not only to promote an immune response, but also to induce immune tolerance toward a number of substances present in the portal blood maybe in order to limit the attack of the immune system toward neo-antigens produced in the liver.

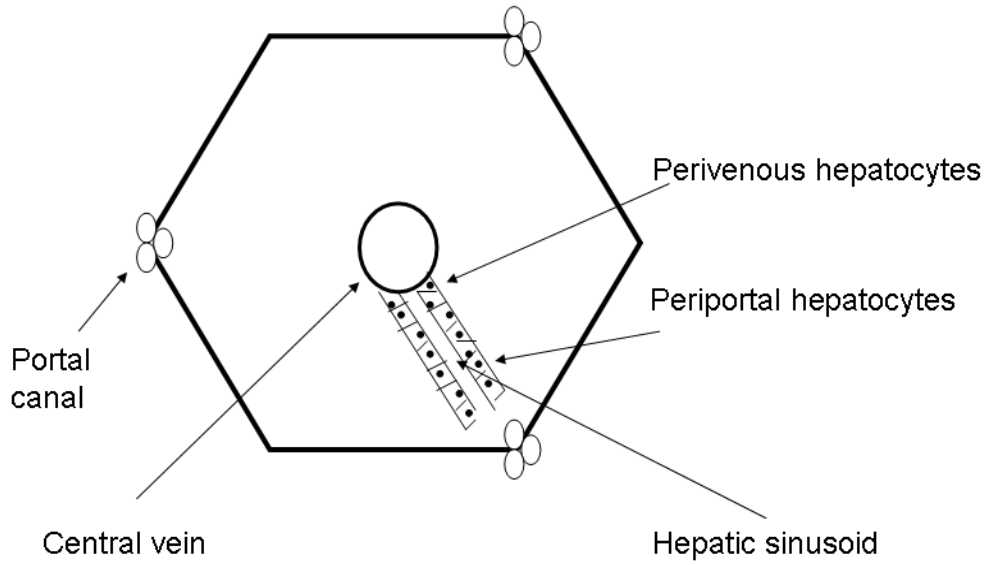

Figure 13.2. Structure of the liver lobule. The portal canal is composed of connective tissue (stroma) carrying small branches of the hepatic portal vein, hepatic artery, bile ducts (originally named the portal triad or Glisson's triad), but in the portal canal also lymph ducts and branches of the vagus nerve are found. Blood passes from the portal canal to the central vein via sinusoids lined with discontinuous endothelium. All hepatocytes are not functionally identical and can be divided into periportal hepatocytes (90-95% of total hepatocytes) and the perivenous hepatocytes (5-10% of total hepatocytes).

The metabolic activity and enzyme pattern of hepatocytes vary along the axis from the portal canal to the central vein. The largest hepatocyte population is named periportal hepatocytes and is located in the area close to the portal canal, and a smaller population of hepatocytes, the perivenous hepatocytes, is located in the sinusoids area close to the central vein.

The small bile ducts of the liver lobules join in the hepatic duct (ductushepaticus) that continues as the bile duct (ductuscholedochus) after bifurcation with the cystic duct (ductuscysticus). Bile flows in both directions in the cystic duct: into the gallbladder from the hepatic duct between meals for storage of bile and from the gallbladder into the bile duct during release of bile from the gallbladder. The bile duct opens into the duodenum relatively shortly after pylorus (distal constriction of the stomach) on the duodenal papillae (papilla duodeni major; in pigs, the pancreatic duct opens on the papillae duodeni minor separated from the papilla duodeni major).

3. Blood and oxygen supply to the liver

Blood flow through the liver is approx. 2.5 L/h per kg whole body weight with the largest contribution from the portal vein. Various estimates exist for the relative contribution from the portal vein and hepatic artery to the total hepatic blood flow; however, data from growing pigs (approx. 60 kg BW) indicate that approx. 85% of the hepatic flow is accounted for by the portal vein flow. With a cardiac output of approx. 6 L/h per kg whole body weight, the liver is receiving approx. 40% of cardiac output. Oxygen is incompletely extracted upon passage of the portal-drained viscera (ap-

prox. 35%) and the extraction of oxygen in the hepatic vein is 50% or more when related to arterial blood, and the liver therefore has access to a much larger amount of oxygen than represented by the relatively small arterial supply. The respiratory quotient (RQ; CO_2 release / O_2 uptake) of the porcine liver is approximately 0.7, which indicates that a substantial amount of oxygen taken up by the liver is used in partial oxidation of metabolites or CO_2 is sequestrated by carboxylation reactions, urea synthesis or excretion in bile. The RQ of the porcine liver is considerably higher than the RQ (~0.50) of the bovine liver reflecting a lower activity of gluconeogensis from propionate and lower activity of ketogenesis in the porcine liver compared with the bovine liver.

4. Bile secretion

Secretion of bile has an important role in digestion and intestinal absorption of fat. A number of xenobiotics (foreign to the body), endogenous compounds (bilirubin, biliverdin), and calcium are also excreted via bile. Bile is continuously secreted by hepatocytes (choleresis), but the rate of formation is low in fasting animals and increases with feeding. Uptake of bile acids in the hepatocytes from plasma stimulates bile secretion by hepatocytes and is a mechanism ensuring an enterohepatic cycling of bile acids. Bile is iso-osmolar with blood plasma and contains bile acids, cholesterol, mucin (from the gallbladder), electrolytes, phosphatidylcholine / lecithin, reduced glutathione (5-10 mM), and calcium (bile is the most important route for calcium excretion in the body).The primary bile acid in porcine bile is hyocholic acid synthesized from cholesterol (as is the case for other bile acids) involving, among others, the cytochrome P450 system (cholesterol and hyocholic acid is shown in Figure 13.3). Within the hepatocytes both newly synthesized and reabsorbed bile acids are conjugated with glycine (pigs are primarily glycine conjugators, but other animals conjugate with taurine or glycine and taurine).

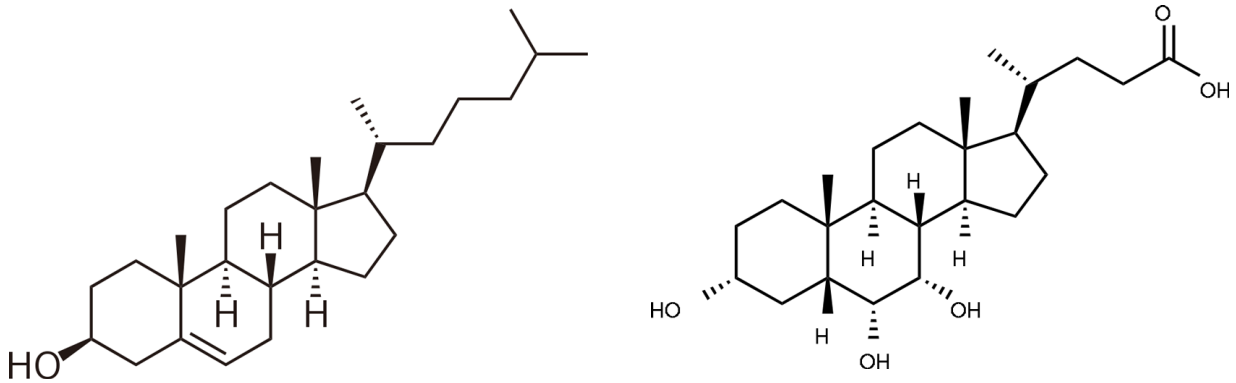

Figure 13.3. Structures of cholesterol (left) and hyocholic acid (right, dominant bile acid in porcine bile). It is apparent that the metabolism of cholesterol into hyocholic acid involves removal of a propionate moiety, reduction of the C-5 double bond, hydroxylation of C-6 and C-7 (involves cytochrome P450). Finally the bile acid will be conjugated with glycine.

Metabolites of haemoglobin, biliverdin (green color) and bilirubin (yellow color) are excreted in bile. The hepatocytes conjugate bilirubin originating from both hepatic and extra hepatic haemoglobin degradation (also spleen and bone marrow remove haemoglobin from old erythrocytes approx. 70 days in pigs) with gluconate or sulfate.

One of the important functions of the liver is to remove chemical substances from the blood and convert them into a more water soluble form; first by cytochrome P450-mediated oxidation (introdu-

ction of hydroxyl groups) and/or then by conjugation with various polar molecules e.g. glucuronate, sulphate and glutathione (form thio ethers with e.g. alkyl halides). A number of membrane transporters in the plasma membranes of hepatocytes are involved in transport from blood plasma to bile. Both sodium-dependent transporters and anion antiport take place on the basolateral membrane (the membrane facing the sinusoids), and the efflux across the apical membrane (facing the bile canaliculi) is probably driven by the ATP-dependent conjugate export pump (EC 3.6.3.44; ABCC2).

Emptying of the gallbladder is controlled by both neural (vagal motor impulses) and by CCK (cholecystokinin) released from the small intestine. The release of CCK to the blood stream is stimulated by the presence of lipids and peptides in the lumen, and decrease the muscular tonus of the sphincter controlling the flow from the bile duct into the duodenum as well as by inducing contraction of gallbladder smooth muscles.

5. Hepatic glucose metabolism

The liver contributes to glucose homeostasis by the ability to store glucose in glycogen (glycogenesis), release glucose stored in glycogen (glycogenolysis), by producing glucose from non-glucose substrates (gluconeogenesis), and by metabolizing glucose. However, as illustrated in Figure 13.4, the net glucose uptake and release in growing pigs is of minor importance relative to glucose disposal in peripheral tissues. In the pig, hepatic lipogenesis (synthesis of long-chain fatty acids) from glucose is insignificant, and hepatic lipogenesis is therefore not involved in postprandial glucose disposal in line with the observed lack of net glucose uptake by the liver after feeding.

Glycogen is stored in the cytosol of hepatocytes as large granules and hydrolyzed to glucose 1-phosphate by the enzyme glycogen phosphorylase (as well as debranching enzyme). Glucose 1-phosphate is converted to glucose 6-phosphate by phosphoglucomutase. The liver is one of two organs (the kidney being the other one) containing physiologically significant activity of glucose 6-phosphatase that enables hydrolysis of glucose 6-phosphate to free glucose and phosphate. After hydrolysis of glucose 6-phosphate to free glucose and phosphate, glucose equilibrates with plasma across thehepatocyte membrane by facilitated transport mediated by a glucose transporter (GLUT2).

Gluconeogenesis is the process of forming glucose from non-glucose carbon and can be regarded as a metabolic strategy where the liver participates in the interorgan exchange of metabolic fuels. The central nervous system, enterocytes and kidney medulla, and in lactating sows also the mammary gland, depend on glucose as substrate, and continuous production of glucose by gluconeogenesis is therefore important during the post-absorptive phase and during fasting. Substrates for gluconeogenesis are amino acids (except leucine and lysine), lactate, glycerol, and propionate (from hindgut fermentation and b-oxidation of odd-chain fatty acids).

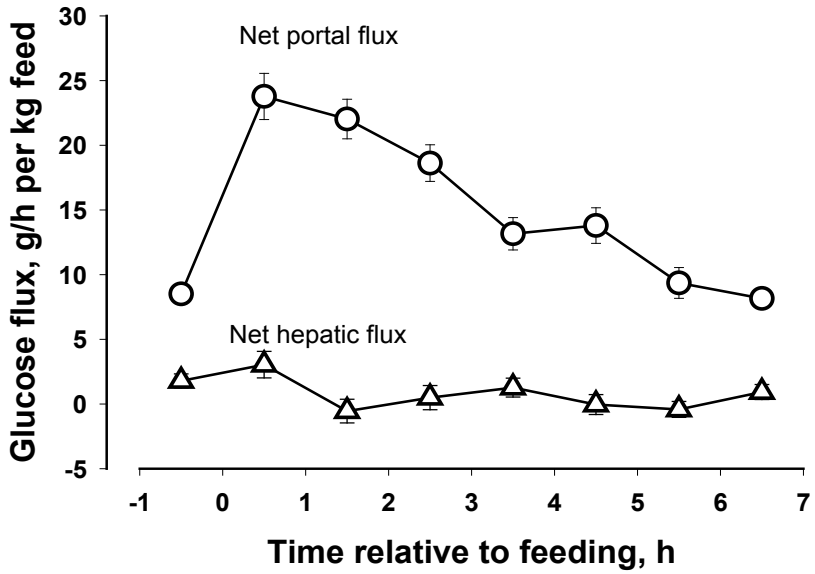

Figure 13.4. Net portal (circles) and net hepatic (triangles) fluxes of glucose in growing pigs (approx. 60 kg BW) fed finishing diets restrictively at 3.6% of BW/day. A positive flux denotes net release and a negative flux net uptake across the tissues. Feed was divided into 3 equally sized meals per day at 8-hour intervals. The figure illustrates that liver metabolism is of minor importance to postprandial glucose disposal and that net storage and mobilization from glycogen storage play a minor role in fed growing pigs. Each data point is the mean of 16 observations ± standard error of the mean.

Gluconeogenesis shares most enzymes with the glycolytic pathway; however, pyruvate carboxylase, phosphoenol-pyruvate carboxylase, fructose 1,6-bisphophatase, and glucose 6-phosphatase are key irreversible enzymes required for gluconeogenesis. In fed growing pigs, hepatic gluconeogenesis has only a minor contribution to total glucose entry as illustrated by the low net hepatic flux of glucose in Figure 13.4. On a net basis, the liver of both growing pigs and sows is net producer of lactate during the prandial as well as the postprandial phases of a normal feeding cycle, and the cori cycle activity in pigs appears to be of limited quantitative importance in total glucose metabolism.

Insulin is generally thought to stimulate hepatic glycogenesis i.e. incorporation of glucose into glycogen and glucagon enhances glycogenolysis through activation of adenylate cyclase. However, it appears that in fed pigs disposal of glucose in the postprandial state occurs primarily in peripheral tissues as skeletal muscles and adipose tissue.

6. Hepatic protein metabolism

In young pigs fed a 20%-protein diet, fractional synthesis of protein (protein synthesis in the liver relative to the total liver protein pool) in the liver is approx. 80% a day. Rates of hepatic protein turnover remain relatively high compared with other tissues, but are lower for older pigs (e.g. approx. 23% a day in finishing pigs). Hepatic protein also undergoes rapid degradation, but due to technical difficulties the rate has not been determined experimentally. In the liver of growing healthy pigs, the rate of protein degradation must be lower than that of protein synthesis. Physiological levels of insulin, growth hormone, IGF-I, glucagons, catecholamines, and glucocorticoids as well as nutrients (e.g. amino acids) are key regulators of hepatic protein synthesis and degradation. The intracellular protein turnover regulates the growth of the liver, the release of functional proteins and

peptides, and the provision of amino acids for metabolic pathways. Indeed, production of proteins (e.g. albumin and acute-phase proteins) for export into the circulation is crucial to the transport of lipids and anti-inflammatory responses.

Plasma contains hundreds of different proteins synthesized in the liver, although peptide hormones and immunoglobolins also originate from tissues other than the liver. Albumin is a general transport protein in plasma (e.g. binds non-esterified fatty acids) responsible for the majority of the colloidal osmotic pressure of plasma (the osmotic pressure of plasma due to the presence of proteins) and is the most abundant plasma protein in healthy animals. Other examples of abundant plasma proteins are transferring (transport Fe) and fibrinogen (clotting factor I). Several proteins in plasma belong to the group called acute-phase reactants, which is a group of proteins that increase in abundance during inflammation. Acute phase reactants have diverse functions i.e. haptoglobin binds free haemoglobin and captures it so that it is not filtrated in the kidney and ends up in urine (which could cause a serious loss of Fe). Capturing of haemoglobin by haptoglobin ensures that it is kept in plasma and can be taken up in the liver upon passage. In inflammation, cytokines affect protein metabolism of both liver and peripheral tissues so that huge amounts of amino acids are directed towards the liver and used for synthesis of acute phase proteins and totally changes the normal physiological utilization of amino acids.

Another important group of proteins synthesized in the liver is apolipoproteins that are important in forming the lipoproteins necessary to transport hydrophobic triacylglycerol in plasma (see below). Both the intestine and the liver produces apolipoproteins, but some, such as apolipoprotein B100, are specific to the liver.

7. Hepatic amino acid metabolism

The liver is the main metabolic crossroad in the body when it comes to integrate carbohydrate and amino acid metabolism. Of particular importance for understanding the role of the liver is the ability of the liver to detoxify ammonia originating both from amino acid catabolism (deamination) and ammonia absorbed from the gastrointestinal tract, especially the hindgut. Figure 13.5 shows the net portal absorption of ammonia in growing pigs and the perfectly mirrored hepatic uptake of ammonia upon passage of the liver. The total amount of ammonia absorbed to the portal blood is removed when the blood passes the liver and a small amount of ammonia released from peripheral tissues is also taken up by the liver. On average, 95% of the urea-N released from the liver could be accounted for by ammonia uptake in fed growing pigs indicating that only a very small amount of amino-N is released in the liver on a net basis when pigs are fed a balanced diet. This is in agreement with a low de novo glucose production in the liver of fed pigs.

The liver does not take up citrulline from the circulation, ie. the intestinally derived citrulline is used by extra-hepatic tissues (primarily kidneys in postweaning pigs) and cells for the synthesis of arginine. Also, uptake of arterial arginine by the liver of mammals (including the pig) is limited due to a low activity of the y^+ transport system. This ensures the availability of circulating arginine to extrahepatic tissues and cells for utilization. With these exceptions, the liver can degrade all amino acids to ammonia and CO_2, and subsequently convert ammonia into urea via the urea cycle. However, under physiological conditions, catabolism of branched-chain amino acids (BCAA) is limited in the liver due to a low activity of BCAA transaminase, and net oxidation of glutamine is negligible in this organ due to the intercellular glutamine-glutamate cycle that involves periportal and perivenous hepatocytes. Degradation of nutritionally essential amino acids by the liver is a potential biochemical basis for the suboptimal efficiency of amino acid utilization for animal growth. At present, little is known about regulation of amino acid catabolism in the liver of animals (including the pig).

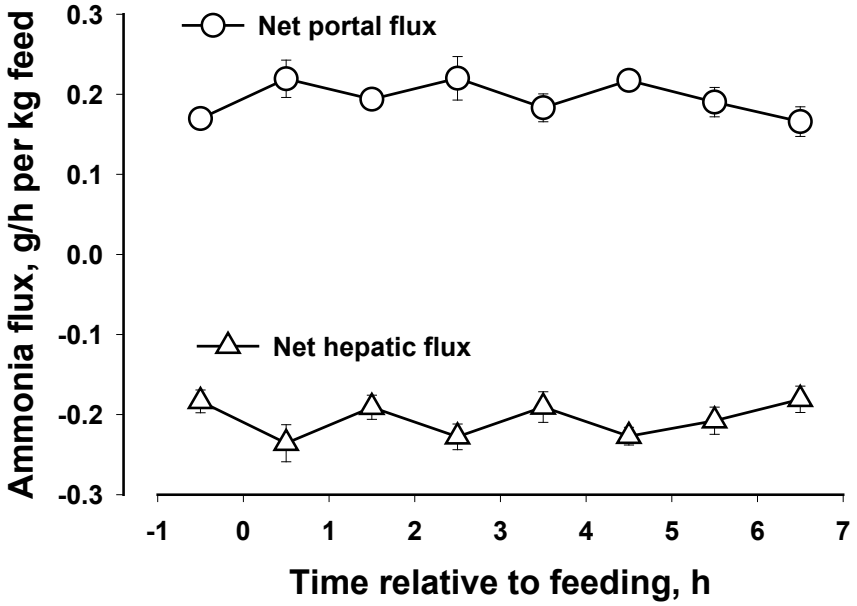

Figure 13.5. Net portal (circles) and net hepatic (triangles) fluxes of ammonia in growing pigs (approx. 60 kg BW) fed finishing diets restrictively at 3.6% of BW/day. A positive flux denotes net release and a negative flux net uptake across the tissues. Feed was divided into 3 equally sized meals per day at 8-hour intervals. The figure illustrates the central function of the liver in metabolizing ammonia released from the gastrointestinal tract. Each data point is the mean of 8 observations ± standard error of the mean.

Although the liver can form citrulline and arginine from ammonia, bicarbonate, and ornithine, there is no net synthesis of arginine by the liver due to an exceedingly high activity of arginase. Arginase (EC 3.5.3.1) catalyzes the degradation of arginine into ornithine and urea, and is the final step in the ornithinecycle (urea cycle). Notably, the porcine liver can synthesize all other so-called ”nutritionally nonessential amino acids”, including alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, ornithine, proline, serine, taurine, and tyrosine. This function of the liver is not shared by other organs. Indeed, synthesis of cysteine and taurine from methionine occurs only in the liver.

The liver can also convert α-ketoacids of all nutritionally essential amino acids (except for histidine, lysine, threonine, and tryptophan that lack transamination) into their respective α-amino acids. Betaine, choline (betaine is the oxidized form of choline and both originate from serine and methyl groups donated by S-adenosylmethionine), creatine (synthesized from arginine and glycine and methylation by S-adenosylmethionine), polyamines, urocanic acid (product of nonoxidative deamination of histidine), and carnitine (synthesized from lysine) are among the important metabolites of amino acids produced by the liver. While the synthesis of amino acids in the liver is likely under the control of hormones (e.g. glucocorticoids, glucagon and catecholamines) and signalling molecules (e.g. nitric oxide), research in this field is very limited.

Figure 13.6 illustrates the large diversity between amino acids in their metabolism in both the portal-drained viscera and the liver. To aid in establishing an overview of the amino acids, we divide the amino acids into 3 groups based on the overall metabolism:

Group 1: For this abundant group of amino acids, the net portal flux can account for a large fraction of the absorbed amount of the respective amino acid, and the liver then takes up a significant part of the amount available to the whole animal (% of net portal flux accounted for by net hepatic uptake): alanine (52%), asparagine (56%), glycine (73%), proline (41%), serine (28%), tyrosine (54%), methionine (17%), phenylalanine (51%), and tryptophan (35%).These glucogenic amino

acids may be converted into glucose or glutamate with the nitrogen also captured in glutamate or captured in urea. Proline is a major source of intra-mitochondrial ornithine via proline oxidase and ornithine aminotransferase to catalyze the turning of the urea cycle for ammonia detoxification. Along with the methionine-derived cysteine, glycine and glutamate are utilized to synthesize glutathione in the liver for both local use and export to the circulation for extrahepatic tissues and cells.

Group 2: Glutamate, glutamine, and aspartate are very abundant in feed protein, but hardly any of these amino acids in the small-intestinal lumen are absorbed to the portal blood. This helps maintain low circulating levels of both aspartate and glutamate, which are very toxic to the brain at elevated concentrations (> 0.1 mM). However, there is a negative net portal flux of glutamine due to its large uptake from arterial blood by the small intestine. Removal of glutamine from arterial blood is also beneficial for neurological function as high concentrations of glutamine (e.g. > 1 mM) for a prolonged period of time inhibit the synthesis of nitric oxide (a major vasodilator) by endothelial cells, resulting in reduced blood flow and consequently reduced supplies of both oxygen and nutrients (including glucose) to the brain. It is also noteworthy that even though the enterocytes metabolized all absorbed glutamate, the net liver release of glutamate (35 g/kg feed) is about twice the net liver release of glucose (19 g/kg feed) in fed growing pigs. Glutamate released from the liver as a result of its endogenous synthesis is therefore a significant carrier of carbon and nitrogen from the liver to the kidneys and other organs. Because the dietary intake of aspartate, glutamate, and glutamine as well as arginine, proline, and glycine by growing pigs fed a typical ration is less than their deposition in body proteins, the liver must synthesize glutamate from i) the catabolism of other non-BCAA amino acids, and ii) ammonia and glucose or glucose precursors.

Group 3: For arginine (7-8% at physiological concentrations, not shown in Figure 13.5), histidine, isoleucine, leucine (9%), lysine (10%), threonine, and valine the liver takes up 10% or less of the net portal flux. For histidine, isoleucine, threonine, and valine a net hepatic uptake could not be detected in growing pigs.Within this third group of amino acids, the branched-chain amino acids (Ile, Leu, and Val) will usually not be taken up in the liver even when supplied in excess because they are primarily deaminated in peripheral tissues. This phenomenon is satisfactorily explained by a low activity of BCAA transaminases at physiological concentrations of BCAA in the liver. As noted above, the near absence of the y^+ transport system (the major transport system for cationic amino acids) accounts for the limited uptake of arginine, histidine, and lysine by the porcine liver, ensuring their availabilities to extra-hepatic tissues and cells for protein synthesis. For all other non-BCAA amino acids in Group 3, a hepatic uptake is expected if the amino acid is supplied in excess.

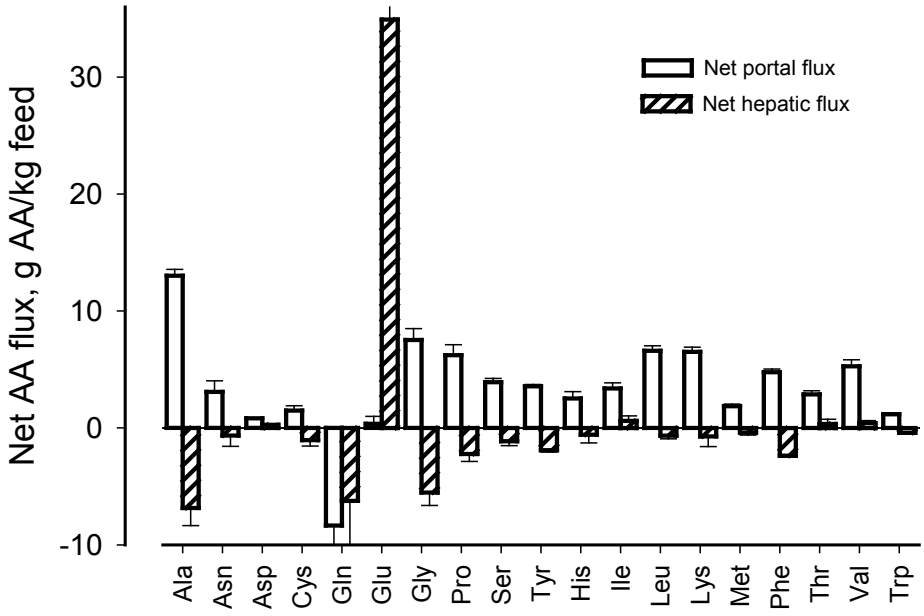

Figure 13.6.Net portal (open bars) and net hepatic (filled bars) fluxes of amino acids in growing pigs (app 60 kg BW) fed finishing diets restrictively at 3.6% of BW/day. Feed was divided into 3 equally sized meals per day at 8-hour intervals. The diets were non-medicated and not supplemented with organic acids. A positive flux denotes net release and a negative flux net uptake across the tissues. The figure illustrates the large variation among different amino acids in their hepatic metabolism. Each data point is the mean of 8 observations ± standard error of the mean.

8. Hepatic lipid metabolism

Among mammals, the pig exhibits two unique aspects of hepatic lipid metabolism. First of all, the porcine liver lacks de novo synthesis of long-chain fatty acids (LCFA) in agreement with low hepatic acetyl-CoA carboxylase activity for converting acetyl-CoA to malonyl-CoA. Rather, white adipose tissue is the major site for LCFA synthesis in pigs. Second, the porcine liver does not synthesize acetoacetate or 3-hydroxybutyrate (ketone bodies) from acetyl-CoA owing to the absence of mitochondrial HMG-CoA synthetase activity (HMG = 3-hydroxy-3-methylglutaryl). Thus, in the pig, the liver does not play a role in catabolising circulating or peripheral fatty acids through partial oxidation of fatty acids to ketone bodies. Concentrations of acetoacetate and 3-hydroxybutyrate in the plasma of post-absorptive, food-deprived, or diabetic pigs are not detectable for 3-hydroxybutyrate (< 0.01 mM) and very low for acetoacetate as compared with concentrations in humans, ruminants, and rodents (up to 10 mM 3-hydroxybutyrate). For this reason, urine concentrations of ketones cannot be used to diagnose the onset of diabetes in pigs.

There has been some interest in the potential relationship between lack of hepatic ketogenesis and high mortality of neonatal pigs. In other milk fed mammalian offspring, ketone bodies appear to have importance as glucose sparing metabolites, but not in piglets. The inability of the liver of piglets to partly oxidize long-chain fatty acids and export the carbon in ketone bodies is also apparent from the total lack of 3-hydroxybutyrate production from both the portal-drained viscera and liver of growing pigs as well as the very low circulating concentrations of ketone bodies even in metabolically challenged lactating sows.

The metabolism of long-chain fatty acids involves transport and activation of fatty acids in the cytosol (formation of CoA thioester via CoA synthetases) and transfer of the fatty acid to the mitochondrion via the carnitineacyltransferase system. Several isoenzymes exist and the dominant activity is expected to be carnitinepalmitoyltransferase with optimum activity for palmitate (C16:0). Oxidation of LCFA to CO_2 and water in the liver provides a significant amount of energy to support its vital functions, particularly under fasting conditions. Very long-chain fatty acids (> C20:0) and some long-chain fatty acids will be shortened in peroxisomes and the shortened fatty acid further oxidized in the mitochondrion. The chain reduction in peroxisomes results in less efficient capture of the energy in the fatty acids because the acyl-CoA oxidases (EC 1.3.3.6) involved in peroxisomalb-oxidation cannot donate electrons to the electron transport chain as in mitochondrial b-oxidation catalyzed by acyl-CoA dehydrogenases. The redox electrons generated from peroxisomalb-oxidation are initially transferred directly to oxygen by the oxidase resulting in production of hydrogen peroxide (H_2O_2). The enzyme catalase (EC 1.11.1.6) is very abundant in peroxisomes and catalyzes the reaction $2\ H_2O_2 = 2\ H_2O + O_2$.

The largest amount of fatty acids is transported between organs in lipoproteins, which are packages of triacylglycerol coated with proteins and phospholipids (Figure 13.7). Instead of oxidizing fatty acids to ketone bodies as in the bovine liver, the pig liver esterifies fatty acids to form triacylglycerol and is assumed to export a major part of the fatty acids extracted and metabolically modified e.g. by chain shortening in very low density lipoproteins (VLDL). The triacylglycerol content of VLDL will be reduced in peripheral tissues catalyzed by lipoprotein lipases and gradually VLDL will be converted to low density proteins (LDL), which in turn are removed by the liver through receptor mediated endocytosis. The LDL pool holds the greatest fraction of circulating cholesterol in the blood of pigs as in humans, and the pig has been used as a model for human atheroschlerosis (thick arterial walls by accumulation of fats and an inflammatory response).

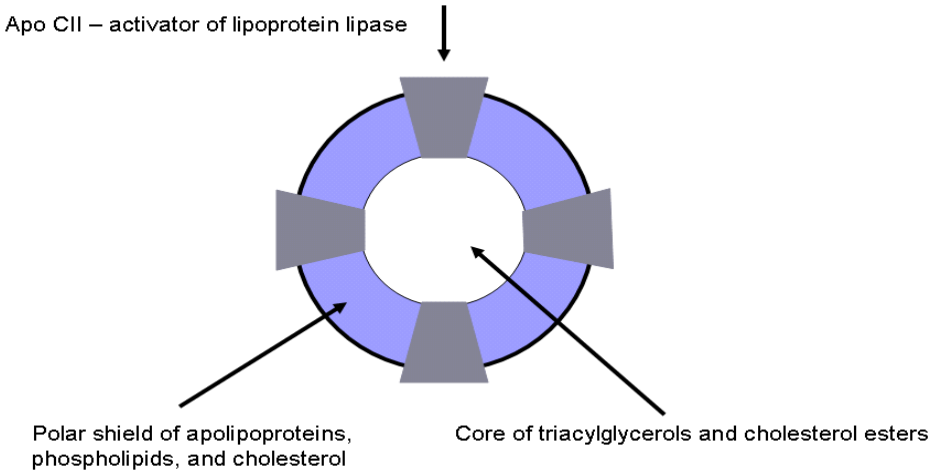

Figure 13.7. Schematic model of very low-density lipoprotein (VLDL) assumed to be released from the porcine liver carrying long-chain fatty acids and cholesterol to peripheral tissues, especially adipose tissue. The ApoCII on the surface of the lipoprotein is assumed to be an important activator of the lipoprotein lipase releasing fatty acids from the lipoprotein.

In other mammals, increasing glucagon would lead to increased fatty acid oxidation and keto-genesis, but not in pigs. This is apparently not due to absence of glucagon receptors, but again indicating low activity of the fatty acid oxidizing pathway. Instead of oxidizing fatty acids to ketones in the liver, fatty acids are re-esterified and probably exported in lipoproteins to peripheral adipose tissues.

9. Hepatic SCFA metabolism

Short-chain fatty acids (SCFA) are produced by microbial fermentation primarily in the hindgut with some contribution also from microbial activity in the stomach. The quantitative most important SCFA are acetate (C2), propionate (C3), butyrate (C4), and valerate (C5). The branched chain SCFA isobutyrate, isovalerate, and 2-methylbutyrate are of less quantitative importance and are primarily products of protein degradation. In growing pigs, the gross energy of SCFA absorbed to the portal blood was 19% of the gross energy of the sum of glucose, and SCFA net absorption and SCFA are therefore significant contributors to energy metabolism in growing pigs and even more so with greater dietary fibre:starch ratios.

All SCFA are subjected to net hepatic extraction in pigs although the liver uptake of acetate (ap-prox. 17% of net portal absorption) is considerably lower compared with the hepatic extraction of propionate (92% of portal absorption), butyrate (59%), and valerate (89%; Figure 13.8).

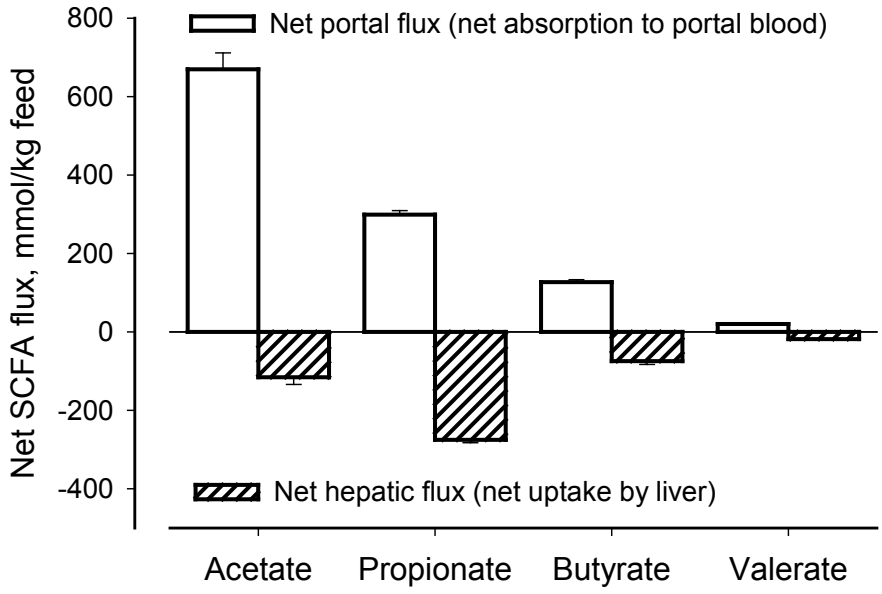

Figure 13.8. Net portal (positive fluxes) and net hepatic (negative) fluxes of acetate, propionate, butyrate, and valerate in growing pigs (approx. 60 kg BW) fed finishing diets restrictively at 3.6% of BW/day. A positive flux denotes net release and a negative flux net uptake across the tissues. Feed was divided into 3 equally sized meals per day at 8-hour intervals. The figure illustrates that the porcine liver removes approx. 17, 92, 59, and 89% of the net portal absorption of acetate, propionate, butyrate, and valerate, and that the liver plays a major role in SCFA metabolism in pigs. Each data point is the mean of 8 observations ± standard error of the mean.

Acetyl-CoA can be oxidized in the TCA cycle (Figure 13.9) located in the mitochondria. In the cytosol of hepatocytes, acetyl-CoA is used for synthesis of cholesterol (not fatty acids, see discussion above), and although this aspect of the porcine liver function is poorly described, the most important function of acetate uptake by the liver might be as substrate for cholesterol synthesis. It is noteworthy that the porcine liver has a net uptake of acetate, whereas ruminant species with a high activity of hepatic ketogenesis have net hepatic release of acetate.

Propionate is as efficiently extracted by the porcine liver as it is in ruminants although this does not seem to be related a dominant role of propionate as a substrate for gluconeogenesis in fed pigs.

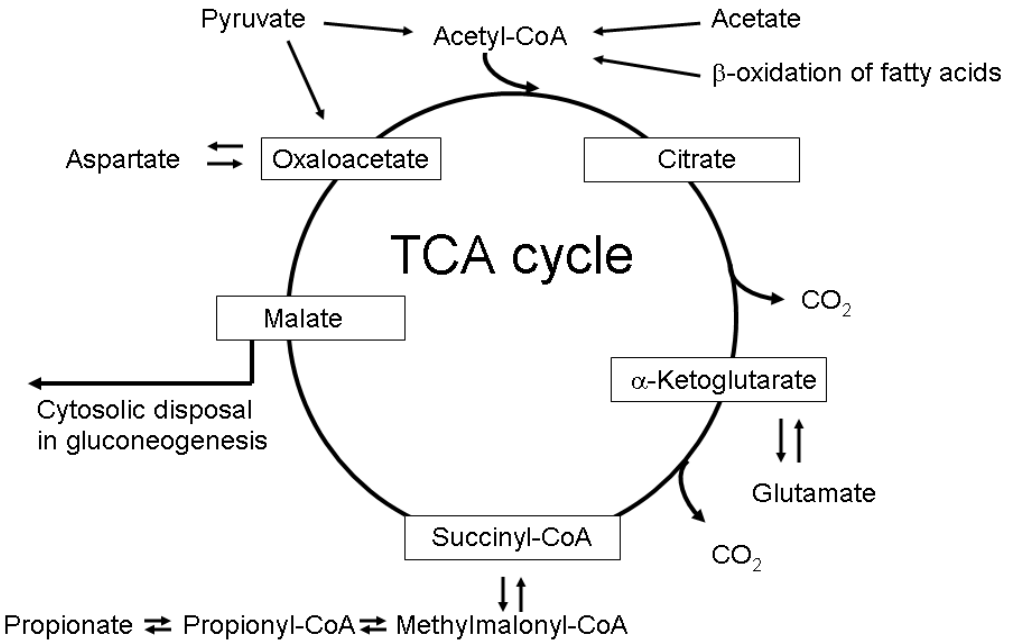

Figure 13.9. Major inputs and outputs of the mitochondrial tricarboxylic acid cycle (citric acid cycle) with special reference to the fundamental different fates of carbon input via acetyl-CoA and propionyl-CoA. Carbon inputs via acetyl-CoA are oxidized in the TCA cycle, whereas carbon input via propionyl-CoA (e.g. propionate) will increase the concentration of TCA intermediates and these have to be disposed either by output in non-essential amino acids (e.g. glutamate) or by export of malate to cytosolic gluconeogenesis.

The calculated 24-h hepatic propionate uptake shown in Figure 13.8 would supply carbon for the production of 58 g glucose/day, which is double the total estimated amount of net release of glucose by the liver of these 60 kg growing pigs. However, as discussed above, the liver in fed growing pigs appears to play a minor role in glucose homeostasis. As shown in Figure 13.9, the principal difference between feeding C2 and C3 carbon units to the TCA cycle is that the C2 (acetyl-CoA) will be completely oxidized in each cycle, whereas the C3-carbons units (and supply of other intermediates) will increase the pool of TCA intermediates and need to be balanced by disposal pathways, eg. export of malate to the cytosol to gluconeogenesis or amination of a-ketoglutarate to form glutamate and export of this amino acids from the liver. It appears as if the function of the liver in propionate metabolism is primary a scavenger ensuring that propionate does not enter peripheral tissues in large amounts where it probably would affect normal cell metabolism.

In ruminants, butyrate would be strongly ketogenic and be metabolized into acetoacetate and 3-hydroxybutyrate by both ruminal epithelium and the liver (in non-ketotic animals 3-hydroxybutyrate is the main hepatic product), but in pigs butyrate is not ketogenic and no release of either 3-hydroxybutyrate or acetoacetate from the liver can be observed. Nor is there any quantitatively important de novo fatty acid synthesis in the porcine liver, which leavesb-oxidation and oxidation of the acetyl-CoA originating from b-oxidation of butyrate as the assumed end point.

10. Endocrine functions of the liver

The liver is anatomically placed at the main metabolic crossroad of the body and the position of the organ enables the liver to obtain a large range of signals related to the metabolic status of the pig. All nutrients transported with the portal blood enter the liver, and it is expected that important nutrient sensors are placed in the liver and portal system although a detailed understanding of the sensors and their signals remains to be obtained. Also, the liver receives neural signals via vagus and endocrine signals from other tissues and cells. Some of the afferent (in-going) signals are actively translated into different efferent signals by the liver making the liver an endocrine gland.

The most prominent example of the endocrine gland function of the liver is the growth hormone (GH; peptide from anterior pituitary gland) induced release and synthesis of insulin-like growth factor I (IGF-I), insulin-like growth factor binding proteins (predominantly IGFBP-3 from liver), and a secondary peptide (acid labile subunit). The GH-IGF-I system is critically important to growth, and although IGF-I is also produced locally in peripheral tissues it appears as if the liver exerts an important regulatory function by integrating metabolic status with the efferent IGF-I signal.

The liver has several functions related to modulation of the activity of other central endocrine signals. The liver is a major organ for converting T4 (thyroxine) to T3 and also secretes thyroxin-binding globulins important for carrying the pool of exchangeable thyroid hormone in the blood. The liver is of primary importance for catabolism of thyroid hormones and is itself a target of T3 that acts as a liver mitogen (stimulates proliferation/cell growth).

The liver extracts a quantitatively important amount of insulin and glucagon released to portal blood whereby the liver modulates the function of these hormones, and important functions of the liver are affected by insulin and glucagon (e.g. glucose release). The liver is apparently a target for glucagon-like peptide I (GLP-I), but otherwise it does not appear that the liver has the same modulating effects on glucagon-like peptides as it has on pancreatic glucagon.

Bile acids synthesized from cholesterol and secreted in bile (see above) have important regulatory functions related to their own synthesis and secretion to bile. However, the bile acids may also function as signalling molecules for the intestine as well as peripheral tissues based on the postprandial increase in blood concentrations of bile acids because the liver extracts less than 100% of the bile acids reabsorbed to the portal blood in each passage of the blood. Bile acids have been suggested to be involved in whole body energy metabolism and insulin sensitivity.

The Greek myth of Prometheus is an excellent symbol of the ability of the liver to regenerate it self. Prometheus was able to regenerate his liver every night after Zeus' eagle had been feasting on it during the day and this myth is a symbol of the ability of the liver to proliferate even if a large fraction of it is removed. Exactly how this re-growth as well as the day to day control of the size of the liver is controlled has not been fully elucidated, but hepatic growth factor (HGF) is one example of regulators involved.

11. Hepatic scavenging pathways

There are two major excretion routes from the body: bile and urine. A number of hydrophobic chemicals, whether their origin are dietary, endogenous pathways, intestinal microbial activity, environmental pollutants or drugs, have to be chemically modified in order to enable to liver or kidneys to excrete them. A common strategy to excrete many hydrophobic compounds are first to introduce new hydroxy groups by monooxygenation (hydroxylation of aliphatic or aromatic carbons, oxidation of nitrogen, hydroxylation of nitrogen or deamination) and then conjugate the products with either glucoronate or sulfate. The key enzyme in the monooxygenation process belongs to the large family of cytochrome P450 monooxygenases. This large group of enzymes is involved in multiple reactions covering the scavenging pathways just mentioned as well as reactions involved in cholesterol metabolism, bile acid synthesis, steroid synthesis and metabolism, vitamin D3 synthesis and metabolism, and retinoic acid hydroxylation. The general reaction scheme is:

$$R\text{-}H + O_2 + NADPH_2 \rightarrow R\text{-}OH + H_2O + NADP$$

where R in R-H often is a carbon, but might as well be a nitrogen or sulphur.

Of particular interest to the pig industry is the hepatic metabolism of skatole (3-methylindole originating from hindgut fermentation of tryptophan). Skatole is hydroxylated by a cytochrome P450 enzyme followed by conjugation with glucoronate or sulphate followed by excretion. The more effectively skatole is extracted and metabolized by the liver, the less will accumulate in back fat and the less likely is it that the meat will suffer from boar taint. Another important component of boar taint is androstenone, which is a pheromone produced by the testis of male pigs. Metabolism of androstenone shows complex interactions with metabolism of skatole. Large variations among male pigs were observed in the hepatic extraction of skatole and thereby susceptibility to development of boar taint, and at present the complex genetic, nutritional and physiological interactions in the hepatic metabolism of skatole and androstenone still await elucidation in order to obtain reliable systems for production of pork from intact males.

Another example of how liver metabolism facilitates excretion of a lipophilic compound is benzoic acid. Benzoic acid is absorbed from the intestine as a product of microbial degradation of phenylalanine and tyrosine, and is also used as a feed additive in the pig industry because of growth promoting properties of benzoic acid through its anti fungal, yeast and bacterial properties. Benzoic acid is also used as an acidifier of urine. Benzoic acid is so lipophilic that its clearance by the kidneys is very low i.e. when the blood passes Bowman's capsule very little benzoic acid will end up in the filtrate because it is associated with lipophilic binding sites on plasma proteins and cells in the blood. This inability of the organism to excrete benzoic acid would lead to accumulation of large amounts of benzoic acid in blood if the liver was not able to extract benzoic acid and conjugate it with glycine to form hippuric acid. Figure 13.10 illustrates the rapid increase in benzoic acid uptake by the liver in pigs fed a diet containing 1% benzoic acid as well as the instant release of an equivalent amount of hippuric acid. Hippuric acid is not only excreted by passively being filtered in the kidney, but is actively being transported into urine along the nephron.

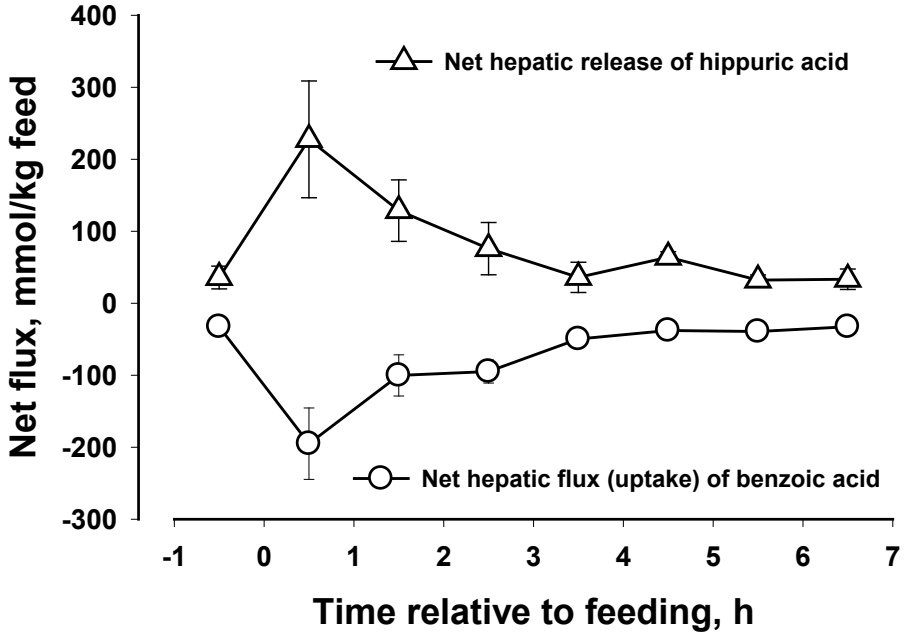

Figure 13.10. Net hepatic flux (uptake; circles) of benzoic acid and net hepatic flux (release; triangles) of hippuric acid in growing pigs (approx. 60 kg BW) fed a finishing diet containing 1% benzoic acid restrictively at 3.6% of BW/day. Feed was divided into 3 equally sized meals per day at 8-hour intervals. The figure illustrates the efficient conversion of benzoic acid to hippuric acid by conjugation with glycine. Each data point is the mean of 4 observations ± standard error of the mean.

12. Summary and perspectives

Multiple functions of the liver relate to its localisation at one of the important metabolic cross-roads of the body, gut – peripheral circulation, and its glandular functions related to bile secretion as well as endocrine and immune functions. Review of the relatively sparse data available for the porcine liver suggests that great care should be taken in extrapolating from general physiology textbooks when considering liver function in pigs. Extent of gluconeogenesis, de novo synthesis of fatty acids, and ketone metabolism are examples of pathways that are expressed very differently between species like pigs, cattle and rats, and these differences must be known in order to make useful metabolic inferences based on metabolomic or nutrition-physiology investigations involving pigs.

13. Suggested reading

1. **Kristensen, N. B., J. V. Nørgaard, S. Wamberg, M. Engbæk, J. A. Fernández, H. D. Zacho, & H. D. Poulsen** (2009) Absorption and metabolism of benzoic acid in growing pigs. J. Anim. Sci. 87:2815-2822.

2. **Wu, G.** (2009) Amino acids: metabolism, functions, and nutrition. Amino Acids 37:1-17.