

2.3 Metabolism

2.3.1 Carbohydrates and the liver

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Introduction

After a carbohydrate-containing meal, the liver maintains plasma glucose concentration within a narrow range by taking up one-quarter to one-third of the absorbed glucose, oxidizing some of it and storing the rest as glycogen or converting it into fat. In the postabsorptive state, the liver provides much needed glucose to the central nervous system and other glucose-utilizing tissues by breaking down glycogen (a process called glycogenolysis, GL) and/or by new formation of glucose from non-glucose precursors (a process called gluconeogenesis, GNG). Disturbances of any one of these processes can result in either hyperglycaemia or hypoglycaemia. This chapter focuses on glucose, the physiologically most important carbohydrate, and gives a brief overview of the pivotal role of the liver in glucose metabolism under normal and abnormal conditions.

Hepatic glucose metabolism in the postprandial state

After a meal, the liver plays a pivotal role in maintaining blood glucose homeostasis by regulating, minute by minute, hepatic glucose production and uptake.

Glucose production

After a mixed meal (~ 60% carbohydrate, ~ 20% fat and ~ 15% protein), endogenous glucose production (EGP; over 90% of which comes from the liver, with the remainder derived from the kidneys) falls to very low rates (0–20% of basal) (reviewed in [1]). During this period, blood sugar concentrations are predominantly maintained by absorption of meal-derived glucose, while EGP decreases to 20% or less of basal (postabsorptive

rates). As intestinal absorption of carbohydrate decreases several hours after the meal, EGP rises slowly towards basal levels.

These changes in EGP are controlled primarily by two pancreatic hormones, insulin and glucagon.

Insulin is secreted by the pancreatic β cells directly into the portal circulation. The liver extracts and degrades between 50% and 80% of the insulin entering it on first pass [2,3] and, thus, even basal insulin concentrations are approximately three times higher in the portal than in the peripheral circulation [1]. Insulin secretion is stimulated by changes in blood levels of glucose, fatty acids and amino acids (with glucose being the strongest secretagogue). Insulin secretion can be modified by an as yet unidentified portal signal (presumably mediated by the parasympathetic nervous system) [4], by several gastrointestinal hormones including glucose-dependent intestinal peptide (GIP) and glucagon-like peptide 1 (GLP1) [2,5], and by the central nervous system (cephalic phase).

Hepatic glucose production is exquisitely sensitive to changes in insulin levels. The four- to 10-fold rise in hepatic sinusoidal insulin typically seen after a carbohydrate-rich meal almost completely inhibits hepatic glucose production. There is little information on the effect of insulin on renal glucose production, but insulin presumably suppresses renal glucose production as well. Insulin suppresses EGP through direct and indirect actions. In the direct pathway, insulin lowers glucose production primarily by stimulating glycogen synthesis [6]. In the indirect pathway, by inhibiting proteolysis and lipolysis, insulin decreases the supply of gluconeogenic precursors such as amino acids and free fatty acids (FFAs) to the liver. This results in a decrease in glucose production, presumably by reducing GNG [7,8]. In addition, insulin can lower hepatic GNG and blood glucose levels by direct action on adenosine triphosphate (ATP)-sensitive K channels in the mediobasal hypothalamus [9].

Glucagon is secreted by the pancreatic α cells. Like insulin, glucagon is secreted directly into the portal circulation but, unlike insulin, the liver degrades only ~ 15–25% of the glucagon entering it [10]. Glucagon secretion is stimulated by

hypoglycaemia, amino acids, and sympathetic and parasympathetic nervous stimulation. Glucagon secretion is decreased by hyperglycaemia, and high FFA and somatostatin levels. Glucagon rapidly increases EGP by promoting glycogenolysis via an increase in the phosphorylase reaction [6]. After a mixed meal containing carbohydrates, protein and fat, both insulin and glucagon concentrations rise in the portal circulation, but the rise in insulin exceeds that of glucagon. The net effect is an increase in the insulin/glucagon ratio and a sharp decrease in EGP. After a protein/fat-rich meal containing little or no carbohydrates, glucagon rises much more than insulin. The low insulin/glucagon ratio results in a rise in EGP, which prevents the hypoglycaemia that would occur if the amino acid-stimulated insulin secretion was unopposed by a larger amino acid-stimulated glucagon secretion.

Glucose uptake

The liver takes up one-quarter to one-third of an ingested glucose load during the initial 4–5 h after ingestion of a mixed meal. Most of this glucose is stored as glycogen; the remainder is oxidized or converted into fat.

Postprandial glycogen synthesis is driven by portal venous hyperglycaemia and by high insulin/glucagon ratios. Hyperinsulinaemia stimulates glycogen synthase flux, while hyperglycaemia inhibits glycogen phosphorylase flux [6]. Thus, postprandial hyperglycaemia and hyperinsulinaemia together stimulate glycogen synthesis and inhibit GL, resulting in a rapid and profound decrease in EGP.

Glycogen is synthesized via two different pathways. The direct pathway (glucose to glucose-6-phosphate to glucose-1-phosphate to UDP glucose to glycogen) accounts for ~ 50% of glycogen synthesis during the postabsorptive phase and increases postprandially to 60–70% of glycogen synthesis, while the indirect pathway (three carbon glucose precursors to glucose-6-phosphate to glucose-1-phosphate to UDP glucose to glycogen) accounts for the remainder [1].

Hepatic glucose metabolism in the postabsorptive state

During the postabsorptive state, all macronutrients have ceased to enter the circulation from the digestive tract and all meal-associated changes in hormone secretion have returned to basal. For practical purposes, it is the time after an overnight fast and before breakfast. Under these conditions, the liver of a healthy person weighing ~ 70 kg produces glucose at a rate of ~ 10 g/h, of which ~ 6 g/h is taken up by the central nervous system, with the rest going to all other tissues including skeletal muscle, adipose tissue, red blood cells, renal medulla, etc. [11]. The postabsorptive blood glucose concentration of a person weighing 70 kg is ~ 90 mg/dL, and ~ 19 g of glucose, i.e. less than a 2-h supply, is present in the extracellular space (assumed to be ~ 30% of body weight).

The vital role of the liver in supplying glucose for use mainly in the central nervous system during an overnight fast is regulated by insulin and glucagon. The low postabsorptive insulin levels reduce glycogen synthesis to very low rates and allow glucagon to stimulate GL, resulting in an increase in EGP to ~ 10 g/h. Hence, it has been estimated that, after an overnight fast, basal plasma glucagon levels are responsible for 75% or more of EGP [12].

Gluconeogenesis (GNG) and glycogenolysis (GL)

EGP has two components. GNG, which is the formation of glucose from non-glucose precursors (lactate, pyruvate, glucogenic amino acids and glycerol), and GL, which is glucose derived from the breakdown of glycogen stored in the liver. Despite its physiological importance, accurate and quantitative information from human subjects on rates of GNG and GL is limited, primarily because of methodological problems. Specifically, the labelled precursors used to measure GNG are diluted to an unpredictable degree in the oxalocetic acid pool, which is shared by GNG and the tricarboxylic acid cycle [13]. Recently, however, several methods have become available that allow non-invasive measurement of *in vivo* rates of GNG and GL in humans [14–17]. Of those, the $^2\text{H}_2\text{O}$ method, which was developed and validated by Landau *et al.* [17] to measure GNG, is currently most widely used. This method depends on the incorporation of ^2H from $^2\text{H}_2\text{O}$ into carbon 5 of glucose (Fig. 1). It measures GNG from all sources, including lactate, pyruvate, glucogenic amino acids and glycerol, and avoids the problems related to precursor dilution. On the other hand, the $^2\text{H}_2\text{O}$ method determines only GNG-derived glucose that enters the glucose space, that is the glucose-6-phosphate to glucose flux. It does not detect GNG-derived glucose that is deposited in glycogen or cycles from glucose-6-phosphate to glycogen and back to glucose-6-phosphate and thus does not enter the blood. When GNG is determined by this method, GL can be calculated by subtracting

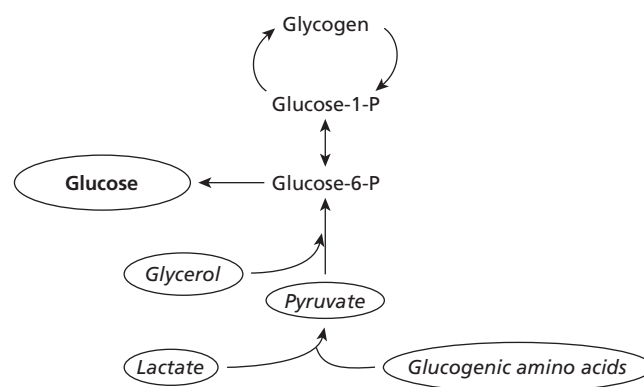
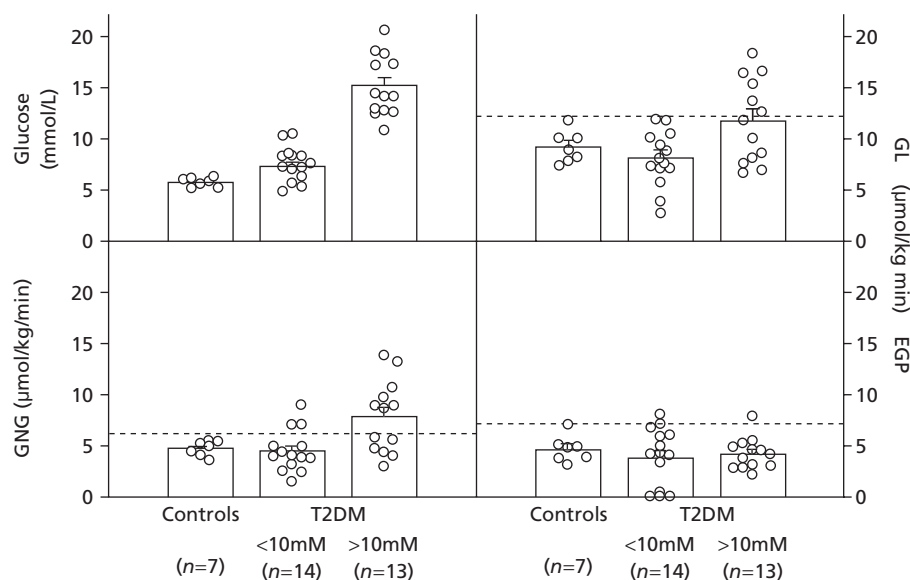


Fig. 1 Schematic representation of GNG as measured with $^2\text{H}_2\text{O}$. This method determines glucose derived from pyruvate and glycerol, which enters the glucose space.

Fig. 2 Plasma glucose, GNG, GL and EGP in 14 patients with type 2 diabetes mellitus (T2DM) who had fasting plasma glucose levels of < 10 mM, in 13 patients with T2DM with fasting plasma glucose levels of > 10 mM and in seven non-diabetic control subjects. Shown are means \pm SE. Broken horizontal lines represent mean \pm 2 SD of control subjects. The data show that higher rates of GNG and EGP are seen mostly in patients with fasting plasma glucose levels > 10 mmol/L who have more than modest degrees of insulin deficiency [26].



GNG from EGP ($GL = EGP - GNG$). GL can also be obtained by directly measuring liver glycogen concentration with ^{13}C -nuclear magnetic resonance spectroscopy in combination with magnetic resonance imaging of the liver volume and isotopic measurement of EGP [14]. Use of these newer methods have shown the following:

1 In healthy people after an overnight fast, GNG and GL contribute about equally to EGP. As fasting progresses, the absolute contribution of GNG (in $\mu\text{mol/h}$) remains essentially unchanged, whereas GL decreases as glycogen stores become depleted. As a result, after more than 40 h of fasting, GNG accounts for over 90% of EGP.

2 Acutely rising serum insulin levels reduce EGP primarily by suppressing GL and, to a much lesser extent, GNG [18,19].

3 Acute elevations of plasma FFA levels raise GNG and lower GL, whereas acute lowering of FFA levels lower GNG and raise GL [20]. Because of the reciprocal changes in GNG and GL, EGP remains essentially unchanged [21]. This so-called hepatic autoregulation of EGP seems to be regulated by insulin in the following way. Rising FFA levels stimulate GNG and insulin secretion. The rise in insulin levels suppresses GL.

4 Abnormally elevated plasma FFA levels cause hepatic insulin resistance, i.e. they inhibit insulin-mediated suppression of EGP, which is due to inhibition by FFA of the normal insulin suppression of GL [19].

5 After a mixed meal, net hepatic glycogen synthesis increases by ~ 50% resulting in about 20% of the ingested glucose being deposited as glycogen in the liver [22].

Diabetes

Patients with type 2 diabetes frequently have higher rates of GNG and EGP than non-diabetic control subjects [23–26]. There is, however, a great deal of overlap in GNG and EGP

between diabetic patients and non-diabetic control subjects, and absolutely higher than normal rates of GNG and EGP are usually seen only in patients with fasting plasma glucose concentrations of > 10 mmol/L [26] (Fig. 2). It needs to be recognized, however, that the ‘normal’ rates of GNG and EGP in patients with type 2 diabetes are abnormal in view of the fact that insulin and glucose levels are commonly elevated in these patients and that both hyperglycaemia and hyperinsulinaemia inhibit EGP in healthy subjects. In addition, patients with type 2 diabetes also seem to have dysfunctional hepatic autoregulation. For instance, when plasma FFA levels were lowered in patients with type 2 diabetes, these patients were unable to compensate for the decrease in GNG with an increase in GL; when FFA levels were raised, GNG rose but GL did not decrease appropriately [27].

In patients with type 1 diabetes mellitus, injection of insulin resulted in an acute decrease in EGP, which was due to a decrease in GL with little change in GNG (glucose concentrations were clamped at 6.5 mmol/L) [28]. In patients with type 1 diabetes during a phase of acute insulin deficiency, which developed 4–8 h after the last insulin injection, EGP rose from 9.5 to 14.3 mmol/L, which was again due to an increase in GL with little change in GNG, and glucose rose from 6.2 to 10.5 mmol/L. Thus, acute regulation of EGP in patients with type 1 diabetes is brought about primarily by changes in GL with little or no participation of GNG [28].

The reason for the increased EGP in patients with type 2 diabetes is incompletely compensated hepatic insulin resistance. Although the causes of the hepatic insulin resistance are not completely understood, there are known defects in postreceptor insulin signalling. The normally functioning direct and indirect insulin pathways (see above), which result in insulin-induced suppression of EGP, involve tyrosine phosphorylation of several intracellular proteins including insulin receptor substrates (IRS-1/2), phosphoinositide-3 kinase (PI3K),

phosphoinositide-dependent kinase (PKC-1), protein kinase B (Akt) and glycogen synthase kinase-3 (GSK-3) [29]. In obesity-associated insulin resistance, the most common type of hepatic insulin resistance, instead of tyrosine phosphorylation, there is serine phosphorylation of IRS-1/2 by several serine kinases including pyruvate kinase C (PKC) and I κ B kinase (IKK). Serine phosphorylation results in degradation of IRS-1/2 and thereby interrupts insulin signalling [30].

Non-alcoholic fatty liver disease (NAFLD) (see also Chapter 13)

NAFLD refers to a series of liver disorders ranging from simple steatosis to steatohepatitis, advanced fibrosis and cirrhosis in patients who do not abuse ethanol. In the United States, steatosis has been estimated to occur in more than two-thirds of the obese population and in more than 90% of morbidly obese subjects. Steatohepatitis affects ~ 20% of obese and ~ 50% of morbidly obese people. All obese diabetic patients have at least some degree of steatosis, while ~ 50% have steatohepatitis and ~ 90% have cirrhosis (reviewed in [31]).

Hepatic insulin resistance and increased generation of inflammatory cytokines are currently believed to be important for the development of steatohepatitis. In that respect, high fat feeding in rodents results in hepatic and systemic insulin resistance, as well as hepatic steatosis associated with subacute hepatic inflammation, activation of the proinflammatory NF- κ B pathway and the production of several cytokines [32].

Cirrhosis

Cirrhosis of the liver is the result of many different causes, including hepatitis induced by toxins (most frequently by ethanol), NAFLD (see above), by viral infections (hepatitis A, B and C) [33] and by autoimmune inflammatory disorders (see Section 6, Cirrhosis). Thus, it is a heterogeneous disorder, which makes discussion of carbohydrate metabolism in cirrhosis difficult. In general, however, many patients with moderate liver disease, who are in a reasonably normal nutritional state, have normal or near-normal EGP rates (1.8–2.2 mg/kg/min after an overnight fast) and blood glucose levels. As most of these patients are insulin resistant [34], their normal rate of EGP and blood glucose levels are the result of compensated hepatic, as well as peripheral, insulin resistance. A significant proportion of cirrhotic patients, however, are glucose intolerant, indicating that they are unable to meet the increased insulin requirements of a carbohydrate load [35].

Several studies have reported that the contribution of GL to EGP is decreased and the contribution of GNG is increased in patients with cirrhosis. For instance, Petersen *et al.* [35] have determined changes in hepatic glycogen content with 13 C-nuclear magnetic resonance spectroscopy [14] before and after an overnight fast and found that net hepatic GL was 3.5 times lower (13% vs. 40% of EGP) in cirrhotic patients than in healthy

control subjects whereas GNG was increased (87% vs. 60% of EGP). Comparable results were obtained in the same patients when GNG was measured with the 2 H $_2$ O method [35]. Earlier, Owen *et al.* [36] had determined EGP and GNG by measuring arterial venous differences of glucose and GNG precursors across the liver. They found that, after an overnight fast, GNG accounted for 67% and GL for 33% of hepatic glucose production in cirrhotic patients. This was an increase in GNG and a decrease in GL compared with healthy subjects studied with the same technique by Wahren *et al.* [37]. The reason for the relative decrease in GL is not clear, but may be related to a decrease in glycogen content in patients with cirrhosis of the liver. Thus, it appears that many patients with cirrhosis of the liver maintain a relatively normal hepatic glucose production by increasing GNG to compensate for a decrease in GL. This may be one reason why these patients have a tendency to become protein depleted.

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2.3.2 Lipoprotein metabolism

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Introduction

Lipoproteins are macromolecular aggregates of lipids and proteins that function to transport otherwise insoluble lipid molecules through the plasma. This chapter will discuss the structure and function of lipoproteins. Emphasis will be placed on the transport of triglycerides and cholesterol, which constitute the principal lipids carried by lipoprotein particles.

Triglycerides, which consist of three fatty acids esterified to a glycerol molecule, are insoluble in water [1]. Triglycerides are either absorbed from the diet following a meal or assembled by the liver. Lipoproteins transport triglycerides to muscles, which utilize the fatty acids as a key source of energy. Triglycerides are also transported to adipose tissue, where the fatty acids are taken up by adipocytes, reassembled and stored for later use by the body.

Cholesterol is a critical regulator of membrane structure and function. Its concentration in membranes preserves bilayer fluidity and governs the formation of microdomains. Microdomains facilitate the association of plasma membrane proteins that participate in critical cell functions, including signal transduction and receptor–ligand binding. In addition to its role in membrane biology, cholesterol is the substrate for bile salt and steroid hormone biosynthesis (see also Chapter 2.3.6) [2]. Oxidized cholesterol molecules (i.e. oxysterols) serve as ligands for nuclear hormone receptors, which regulate cellular lipid metabolism [3]. Although cholesterol is absorbed in substantial amounts by the intestine, there does not appear to be a dietary cholesterol requirement. This is because virtually all cells in the body synthesize cholesterol molecules.

An important determinant of the physical state of cholesterol is whether the hydroxyl group is esterified to a long-chain fatty acid. For cholesterol to reside in membranes, this hydroxyl group must be unesterified. Molecules of esterified cholesterol (i.e. cholesteryl esters) are too insoluble even to be accommodated within membrane bilayers in more than trace quantities.

The liver is the only organ capable of degrading cholesterol and eliminating it from the body. As a result, excess cholesterol