

Hepatic glucose metabolism in humans—its role in health and disease

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The liver is mainly responsible for maintaining normal concentrations of blood glucose by its ability to store glucose as glycogen and to produce glucose from glycogen breakdown or gluconeogenic precursors. During the last decade, new techniques have made it possible to gain further insight into the turnover of hepatic glucose and glycogen in humans. Hepatic glycogen varies from ~200 to ~450 mM between overnight fasted and postprandial conditions. Patients with type-1 diabetes (T1DM), type 2 diabetes (T2DM) or partial agenesis of the pancreas exhibit increased endogenous glucose production and synthesize only 25–45% of hepatic glycogen compared with non-diabetic humans. This defect can be partly restored in T1DM by combined long- and short-term optimized treatment with insulin. In T2DM, increased gluconeogenesis was identified as the main cause of elevated glucose production and fasting hyperglycaemia. These patients also exhibit augmented intracellular lipid accumulation which could hint at a link between deranged glucose and lipid metabolism in insulin-resistant states.

Key words: glycogen synthesis; glycogenolysis; insulin; glucagon; triglycerides; diabetes mellitus; MODY; NASH; liver cirrhosis; glycogen storage disease.

Glucose homeostasis aims to keep concentrations of blood glucose constant within a narrow range, from 3.9 to 5.6 mM. This is maintained by a dynamic equilibrium between endogenous glucose production (EGP) and glucose utilization, which involves a series of cellular metabolic events. These processes are regulated by hormones, mainly by insulin and glucagon, and metabolites such as glucose, free fatty acids (FFA) and carbon-3 compounds, such as lactate, alanine and glycerol.

In vivo, the liver—and, only to a minor extent, the kidney—contribute to both EGP and glucose utilization in humans.¹ The liver is involved in directing glucose fluxes both during transition from the fed to fasted state and under pathological conditions such as diabetes mellitus.²

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Current development of techniques, such as isotopic tracer dilution and nuclear magnetic resonance spectroscopy (NMRS), has made possible the assessment of glucose and glycogen metabolism, thereby providing greater insight into metabolic regulation in humans.^{3,4}

PHYSIOLOGICAL CONDITIONS

Whole-body energy balance remains stable during the episodic changes from nutrient intake to fasting. The principal mechanisms by which nutrients and hormones regulate glucose metabolism are well known. However, their respective contributions—and particularly the intrahepatic fate of glucose and glycogen under various conditions—have only recently been elucidated in humans.

Principal mechanisms of regulation

Intrahepatic glucose fluxes

Glucose uptake

Hepatocytes take up glucose independently of insulin by the low-affinity high-capacity glucose transporter, GLUT-2, which facilitates the entry of glucose in the presence of high concentrations of sinusoidal glucose. There, glucose is rapidly phosphorylated by the hepatic hexokinase isoform, glucokinase, to glucose-6-phosphate. From glucose-6-phosphate, the glucose flux is directed into glycogen via uridine diphosphate (UDP)-glucose (direct pathway of glycogen synthesis), the pentose phosphate shunt—or into glycolysis, yielding carbon-3 compounds such as pyruvate and lactate (Figure 1).

Gluconeogenic and glycolytic flux

Carbon-3 compounds can undergo further oxidation in the tricarboxylic acid cycle or serve as substrates for de novo synthesis of glucose, i.e. glucose-6-phosphoneogenesis, and glycogen, i.e. the indirect or gluconeogenic pathway of glycogen synthesis.⁵ Several

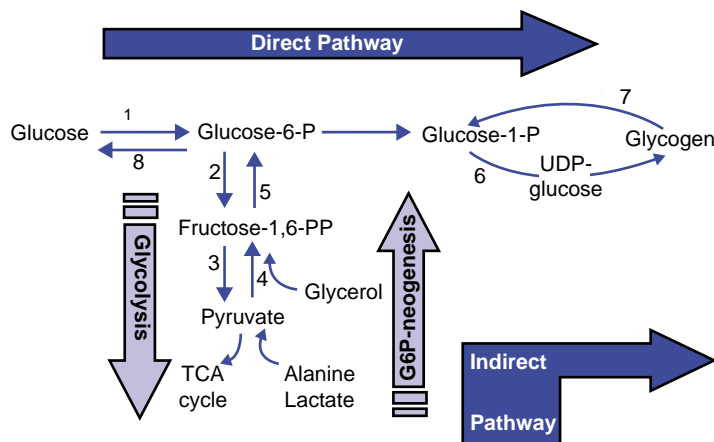


Figure 1. The fate of glucose in the hepatocyte. ¹glucokinase, ²phosphofructo-1-kinase, ³pyruvate kinase, ⁴phosphoenolpyruvate carboxykinase (PEPCK), ⁵fructose-1,6-biphosphatase, ⁶glycogen synthase, ⁷glycogen phosphorylase, ⁸glucose-6-phosphatase.

enzymes regulate substrate cycles between gluconeogenesis exerted by phosphoenolpyruvate carboxykinase (PEPCK) and fructose-1,6-bisphosphatase, and glycolysis exerted by phosphofructo-1-kinase and pyruvate kinase (Figure 1). These substrate cycles provide a system in which rates and direction of flux can be finely tuned by small changes in the concentration of specific effector enzymes and/or by covalent modification of involved enzymes.^{6,7} In addition, the control of metabolic flux can be influenced by changes in the gene expression of these enzymes.⁸

Glycogen synthesis and breakdown

The activities of glycogen synthase and branching enzymes are responsible for the sphaeric shape of glycogen particles which aggregate to larger complexes, the α rosettes.⁹ Degradation of glycogen, i.e. glycogenolysis, requires the concerted action of glycogen phosphorylase and the bifunctional debranching enzyme to release glucose-1-phosphate, which is in equilibrium with glucose-6-phosphate (Figure 1). Of note, glycogen synthase and phosphorylase are both regulated by allosteric effectors, kinases and phosphatases dependent on the hormonal and nutrient environment.^{9–11} Both glycogen synthesis and glycogenolysis can be simultaneously active—resulting in glycogen cycling which is considered to be negligible only in fasting, non-diabetic humans.⁵ In vivo ¹³C nuclear magnetic resonance spectroscopy (NMRS) has enabled the measurement of liver glycogen concentrations and the calculation of rates of glycogen synthesis and glycogenolysis from changes in hepatic glycogen over time in humans (Figure 2).

Glucose release

Glucose-6-phosphatase catalyses the dephosphorylation of glucose-6-phosphate and represents the ultimate step prior to the release of free glucose into hepatic veins, be it derived from glycogenolysis or gluconeogenesis (Figure 1). As only the liver and the kidneys express glucose-6-phosphatase, glucose-6-phosphatase in these tissues can result in the release of glucose by these tissues but only in the release of lactate from skeletal muscle, from where it can shuttle back to the liver (the Cori cycle).

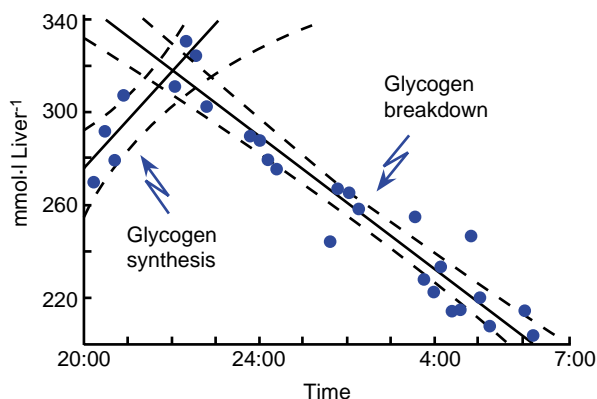


Figure 2. Time course of hepatic glycogen concentrations in one healthy volunteer after ingestion of a 800 kcal liquid meal at 17:00. Rates of net glycogen synthesis and breakdown are presented by the slopes of lines determined from linear regression of the glycogen concentration time curves from 19:30 to 22:30 and from 22:30 to 8:00, respectively.⁸⁷

The portal vein insulin:glucagon ratio and the effect of hyperglycaemia

It is well known that glucose production by the liver is controlled primarily by insulin, glucagon and hyperglycaemia. The drainage of blood from the pancreas into the portal vein ensures direct delivery of islet cell hormones to the liver sinusoids, allowing rapid control by nutritional stimuli.² Even before any nutrient-induced increase in plasma glucose, the cephalic phase of insulin secretion primes the liver and accelerates the endocrine responses to eating.² Plasma glucagon concentrations also increase after ingestion of mixed meals^{2,12,13} but remain unchanged—or even decrease—after ingestion of a pure glucose load.^{14,15} Nevertheless, the portal vein insulin:glucagon ratio rises under both conditions owing to the much more pronounced rise in plasma insulin.

Insulin

Under post-absorptive conditions, insulin concentrations are approximately three-fold higher in the portal vein (~180 pM) than in peripheral plasma.² Similar concentrations of portal vein insulin—130–170 pM—are required for half-maximal stimulation of hepatic glycogen synthesis during hyperglycaemia (~10 mM).¹⁷ Interestingly, similar concentrations of insulin half-maximally suppress EGP,^{18–20} considerably less than the insulin concentration which half-maximally stimulates glucose uptake by peripheral tissues.²

Although the overall flux through glycogen synthase might plateau at high levels of insulin, substantial differences occur in net glycogen synthesis and glycogen cycling. The [¹³C]glucose pulse-[¹²C]glucose chase technique made it possible to assess flux rates through glycogen synthase and phosphorylase, and to calculate net glycogen synthesis from the difference of these fluxes in healthy humans in the presence of various concentrations of glucose, insulin and glucagon.^{21–23} In the presence of euglycaemia

Table 1. Hepatic glycogen metabolism (calculated from the [¹³C]glucose pulse-[¹²C]glucose chase technique) in non-diabetic humans during various conditions of plasma glucose, portal vein insulin and glucagon concentrations either (i) as created by intravenous hormone infusion during somatostatin inhibition of pancreatic secretion (indicated by superscript 'a') or (ii) as calculated assuming a portal vein:peripheral vein concentration gradient of ~3 for insulin¹³² (indicated by superscript 'b'). Superscript 'c' indicates a brief fast of 5–10 hours compared to with overnight fasting for ~12 hours before all other studies.

	Portal vein		Glycogen fluxes ($\mu\text{mol}\cdot\text{l}^{-1}\cdot\text{min}^{-1}$)			Reference
	Insulin (pM)	Glucagon (pg/ml)	Flux through synthase	Flux through phosphorylase	Net glycogen synthesis	
Euglycaemia (~5 mM)	40 ^a	30 ^a	0	270	-270	[22]
	400 ^a	30 ^a	480	290	190	[22]
Hyperglycaemia (~10 mM)	40 ^a	30 ^a	50	50	0	[22]
	400 ^a	30 ^a	390	30	360	[22]
	190 ^a	30 ^a	400	70	330	[21]
	190 ^a	60 ^a	190	120	60	[21]
	~800 ^b	–	430	150	280	[90]
~1300 ^c	–	530	300	240	[90]	

(~ 5 mM), high concentrations of insulin in the portal vein were required for stimulating net glycogen synthesis²² (Table 1). Hyperglycaemia per se inhibited net hepatic glycogenolysis primarily through inhibition of glycogen phosphorylase flux, while hyperinsulinaemia per se inhibited net hepatic glycogenolysis primarily through stimulation of the glycogen synthase flux. Thus, inhibition of glycogen phosphorylase and activation of glycogen synthesis are not necessarily coupled in a reciprocal fashion.

Glucagon

Concentrations of plasma glucagon increase during hypoglycaemia and induce an immediate rise in EGP.²⁵ Likewise, glucagon infusion leads to a rapid but evanescent increase in EGP.²³ Although ~90% of the initial elevation of EGP is accounted for by hepatic glycogenolysis, the subsequent decline in EGP cannot be explained by depletion of glycogen stores in humans^{23,26,27} but rather by gluconeogenesis, as has been shown in dogs.²⁸ Glucagon can augment gluconeogenesis by inhibiting pyruvate kinase²⁹ and phosphofructo-1-kinase³⁰ as well as by stimulating pyruvate carboxylase^{31,32} and PEPCK.³³ In contrast, insulin decreases the activity of pyruvate carboxylase⁷ and the expression of PEPCK³³ and glucose-6-phosphatase.³⁴

The role of glucagon in hepatic glycogen synthesis and glycogenolysis was also studied with the [¹³C]glucose pulse-[¹²C]glucose chase technique.²¹ Suppression of glucagon secretion by somatostatin doubled the glycogen synthase flux and decreased glycogen cycling so that net glycogen synthesis was markedly higher than it was under conditions of fasting glucagonaemia (Table 1). In the presence of fasting peripheral insulinaemia and hypoglucagonaemia, there was negligible hepatic glycogen synthesis and cycling.^{21,22} Thus, small changes in the concentrations of insulin and glucagon in the portal vein independently affect hepatic glycogen synthesis and glycogenolysis.

Other metabolic factors

In addition to splanchnic glucose delivery and hyperglycaemia per se, other nutrients derived from protein or fat intake also participate in the regulation of hepatic glucose metabolism—either by serving as substrates for glucose fluxes (direct or substrate-mediated effect) or by modulating the secretion of pancreatic hormones (indirect or hormone-mediated effect).

Amino acids

It has been suspected that a high intake of protein induces glucose intolerance in humans, mainly by affecting the utilization of gluconeogenic amino acids.³⁵ Alanine—and particularly branched-chain amino acids—can be elevated in insulin-resistant states such as obesity.³⁶ However, when short-term intravenous infusion of an amino acid mixture was used to create postprandial plasma amino acid concentrations, insulin-dependent suppression of EGP did not change¹³³—but amino acid elevation reduced insulin-stimulated glucose disposal by inhibition of skeletal muscle glucose transport or phosphorylation.³⁷

Nevertheless, a high intake of protein could cause hyperglycaemia by increasing EGP in the fasting state.³⁵ This may result from an altered secretion pattern of glucoregulatory hormones^{27,38} and/or stimulation of gluconeogenesis from amino acids.³⁶ To address this issue, the relative contribution of the direct and indirect effects of amino acids to hepatic glucose metabolism was examined in healthy men with or without somatostatin inhibition of pancreatic hormone secretion.²⁷ Combined measurement of hepatic glycogen concentrations and EGP showed that postprandial

amino acid concentrations stimulate the secretion of insulin and glucagon without affecting glycaemia—despite nearly doubled gluconeogenesis. When the secretion of pancreatic hormones was inhibited, amino acid elevation increased EGP by ~30–40% and plasma glucose by ~50%.²⁷ The rise in EGP was associated with ~50–65% increased gluconeogenesis. Thus, only impaired insulin secretion unmasks the direct gluconeogenic—and thereby hyperglycaemic—effect of postprandial amino acid concentrations.

Free fatty acids

Plasma concentrations of free fatty acids (FFAs) are increased in the fasting state, when lipid oxidation determines energy expenditure, and in insulin-resistant populations, in whom FFA concentrations correlate with the magnitude of EGP and hyperglycaemia.³⁹

It is currently under discussion whether FFAs could be responsible for the indirect effects of insulin in suppressing EGP in the dog⁴⁰ and in man.⁴¹ According to the 'single gateway hypothesis', inhibition by insulin of lipolysis in adipocytes would reduce the flux of FFAs and glycerol to the liver⁴², where FFAs stimulate gluconeogenesis via enzyme activation and glycerol by serving as a gluconeogenic precursor.

In order to test direct effects of FFAs on hepatic glucose metabolism, lipid/heparin infusions were performed to achieve postprandial plasma FFA concentrations.⁴³ Under these conditions, FFAs stimulated insulin secretion and induced hyperglycaemia when insulin secretion was prevented by somatostatin. Despite slightly increased gluconeogenesis, lipid/heparin infusion did not increase EGP so that peripheral effects probably account for the observed hyperglycaemia.⁴⁴ When insulin secretion was not inhibited, elevation of plasma FFAs increased gluconeogenesis, but decreased net glycogenolysis by ~85%, suggesting that FFAs may evoke reciprocal changes in gluconeogenesis and glycogenolysis⁴³ which prevent any increase in EGP, as reported for the inhibition of lipolysis by nicotinic acid.⁴⁵

In the presence of fixed concentrations of fasting insulin and glucose, elevation of plasma FFAs increased EGP, which could not be suppressed by subsequent hyperglycaemia.³⁷ Under hyperinsulinaemic–euglycaemic conditions, elevation of plasma FFAs also impaired the insulin-dependent suppression of EGP^{46–48}, which has been primarily attributed to reduced inhibition of hepatic glycogenolysis.⁴⁹

Other endocrine factors

Stress hormones

Stress hormones, such as adrenaline (epinephrine), cortisol and growth hormone, also affect hepatic glucose metabolism, particularly during hypoglycaemia counter-regulation.²⁵ The respective roles of these hormones have been studied extensively and involve both increased gluconeogenesis and glycogenolysis in dogs (Chapter 3, this issue) and humans.^{50–52} Simulating the physiological secretion pattern by variable delivery results in higher metabolic efficacy of these hormones.^{51,53} Considering the relevance of glucocorticoids in clinical therapy, it is of note that high-dose hydrocortisone increases plasma glucose concentrations by augmenting EGP; in humans, this is due entirely to stimulation of gluconeogenesis.⁵⁴

Incretins

In response to nutrient ingestion, the intestine secretes peptides such as glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) which contribute to postprandial insulin secretion and have been shown to cause the incretin effect.⁵⁵ These peptides

further delay gastric emptying and suppress glucagon secretion and appetite.^{56,57} It is therefore not surprising that exogenous administration of GLP-1 ameliorates hyperglycaemia in diabetic patients⁵⁸, and GLP-1 agonists, such as exendin, are currently being tested as antidiabetic agents.⁵⁹

The post-absorptive state

The morning following an overnight fast represents the physiological post-absorptive period which occurs daily in humans. During this period, from 6 to 12 hours after ingestion of a meal, absorption of nutrients is completed, plasma insulin and glucagon concentrations have returned to their baseline levels and fuel consumption is matched by the release of endogenous fuels from storage depots.² Although FFA oxidation becomes the major source of energy supply under these low-insulin conditions, the body continues to consume glucose at rates of $\sim 7\text{--}10$ g/hours with the brain taking up ~ 4 g/hours.^{2,60} Given the small pool of circulating free glucose, the liver needs to switch from glucose storage to glucose production and contributes up to 90% of EGP, the remainder being accounted for by the kidneys. The kidney's contribution to EGP varies over a broad range from 5 to 25% in the early post-absorptive period⁶¹, most likely due to the use of different methods for qualification.⁶² This becomes relevant only after prolonged fasting of 60 hours.¹ Thus, the liver remains the central organ of glucose homeostasis by ensuring rates of EGP of $\sim 1.8\text{--}2.0$ mg/kg/min ($\sim 7\text{--}10$ g/hour).⁶³ This is brought about by releasing glucose resulting from glycogenolysis and glucose-6-phosphonogenesis (Figure 1).

Glycogenolysis

A combination of ^{13}C NMRS and the infusion of $[3\text{-}^3\text{H}]\text{glucose}$ for assessing EGP can be employed to monitor the time course of hepatic glycogen concentrations and EGP (Figure 2). Liver glycogen decreased almost linearly from ~ 400 mM at 4 hours after a 650 kcal meal to ~ 250 mM at 15 hours⁶⁴, which was similar to concentrations obtained by needle biopsy.⁶⁵ Surprisingly, net rates of glycogenolysis contributed to EGP by only $\sim 36\%$ during the first 22 h, by $\sim 18\%$ from 22 to 46 h and by $\sim 4\%$ at 64 h, when the hepatic glycogen concentration had decreased to ~ 40 mM. In order to estimate the maximum contribution of glycogenolysis to EGP, net glycogenolysis was followed for 5–12 h after a 1000 kcal meal, and EGP was measured with $[6,6\text{-}^2\text{H}]\text{glucose}$ ²⁴, which yields a minimum estimate of EGP.⁶⁶ Even under these conditions, net hepatic glycogenolysis contributed only $\sim 45\%$ to EGP, indicating that gluconeogenesis is responsible for at least $\sim 50\%$ glucose production in the early post-absorptive state.²⁴

Gluconeogenesis

Determination of gluconeogenesis as the difference between the ^{13}C NMRS measured rates of glycogen breakdown and EGP yielded a high contribution of gluconeogenesis to EGP, which was in contrast to earlier studies reporting estimates of less than 40% after overnight fasting for 12–14 h.^{67–70} However, these studies were limited by several methodological problems. Hepatic venous catheterization with measurement of uptake of gluconeogenic precursors⁶⁷ neither accounts for splanchnic and intrahepatic precursors nor for renal gluconeogenesis. Tracer-dilution techniques using $[^{14}\text{C}]\text{lactate}$ ⁶⁸ or $[2\text{-}^{14}\text{C}]\text{acetate}$ ⁷¹ suffer from insufficient labelling of

the intrahepatocellular pool or the failure of the tracer to be metabolized in extrahepatic tissues.² On the other hand, mass isotopomer analysis (MIDA) upon administration of uniformly labelled glucose or glycerol^{69,70} relies on untenable assumptions.⁷²

The ²H₂O method circumvented these limitations by calculating the fractional gluconeogenesis in humans from the ratio of ²H enrichments at carbon 5 over that at carbon 2 of plasma glucose upon ingestion of safe doses of ²H₂O.⁷³ This approach is based on the observation that every glucose molecule receives one hydrogen atom from body water at carbon 5 during gluconeogenesis and another hydrogen at carbon 2 during both glycogenolysis and gluconeogenesis. This method yielded a contribution of gluconeogenesis to EGP ranging from 47 to 56% at 14–16 h of fasting in healthy subjects^{74–76} and was in good agreement with the ¹³C NMRS method when these methods were directly compared in healthy humans.^{24,75}

The postprandial state

After ingestion of a meal, i.e. in the postprandial state, the absorbed nutrients stimulate the secretion of insulin and, to a less extent, of glucagon, resulting in a rise in the portal vein insulin:glucagon ratio (Table 1), which is further amplified by incretin and signals from the central nervous system.² Under these conditions, the liver rapidly suppresses EGP and starts to take up and temporarily store glucose. Together with the insulin-stimulated increase in glucose uptake by peripheral tissues such as skeletal muscle, these actions minimize the postprandial rise in plasma glucose concentrations following a meal. Finally, changes in the uptake and release of metabolites such as lactate and alanine could affect postprandial glucose fluxes.⁷⁷

Suppression of EGP

In many studies, oral or intravenous glucose loads were given and EGP was assessed from either splanchnic glucose uptake, which does not allow separation of splanchnic from intrahepatic fluxes, or by tracer techniques. Application of the latter techniques gave a broad range of estimates for residual EGP ranging from ~20 to 55% of fasting EGP for 3–5 h after an oral load of 50–100 g glucose^{15,78–80} (Table 2). Mean residual EGP also varied between ~46 and 79% after ingestion of mixed meals containing 53–83 g carbohydrates.^{14,81,82} This variability is partly explained by the use of constant tracer infusions which result in changes of the specific activity of endogenous glucose under such non-steady-state conditions.²

Variable tracer infusion protocols overcame these limitations by maintaining constant the specific activity of glucose released from the liver (stable tracer:tracee ratio). This approach demonstrated that ingestion of a mixed meal containing 93 or 139 g glucose rapidly suppresses EGP, which thereafter remains at 35% (from 60 to 240 min)⁸³ or 21% (from 60 to 270 min)¹² of fasting EGP (Table 2). Taken together, these findings suggest that previous studies probably underestimated the degree of EGP suppression following ingestion of a meal.

Glycogen synthesis and turnover

Studies on the regulation of hepatic glucose removal were limited to measuring splanchnic glucose uptake by isotopic methods or hepatic vein catheterization^{84,85}, and were therefore unable to determine the fate of glucose within the liver.

Table 2. Endogenous glucose production (EGP; calculated from double-tracer techniques) before and after an oral glucose load or ingestion of a mixed meal in healthy (CON) and in hyperglycaemic (~10–12 mM) type 2 diabetic humans (T2DM).

	Fasting state		Postprandial state			Carbohydrate dose and study duration	Reference
	Insulin (pM)	EGP ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	Insulin (pM)	Suppression of EGP (%)	Appearance of ingested glucose (%)		
CON	72 ± 6*	12.3	~140*	~67	76	Glucose 1 g/kg	[114]
T2DM	138 ± 24	13.9	~290	~54	75	3.5 h	
CON	66 ± 30*	9.1*	~330*	~45	92	Glucose 50 g	[80]
T2DM	84 ± 12	14.9	~150	~39	100	7 h	
CON	89 ± 8*	10.7*	~210*	~46*	72	Glucose 1 g/kg	[114]
T2DM	57 ± 4	12.7	~360	~20	77	5 h	
CON	42 ± 12	7.2*	~330*	~30	71*	Glucose 50 g/meal	[14]
T2DM	64 ± 12	14.4	~60	~30	45	5.5 h	
CON	97 ± 17	~8.9*	~500*	~39	78*	Glucose 50 g/meal	[115]
T2DM	61 ± 7	~11.1	~220	~21	64	5 h	
CON	61 ± 7	10.0	~260	~48	43	Glucose 67 g/meal	[82]
T2DM	81 ± 9	17.1	~180	~30	50	5 h	
CON	50 ± 10	14.4	~270	~45*	–	Glucose 93 g/meal	[83]
T2DM	67 ± 12	16.1	~190	~35	–	8 h	

* P < 0.05 versus T2DM.

When liver glycogen concentrations were monitored closely by ^{13}C NMRS, a rise in liver glycogen from ~210 to 320 mM was observed within the first ~5 h following a meal containing 139 g of glucose.¹² Thus, ~31 g of glucose were deposited as glycogen in the liver, which represents ~19% of the meal carbohydrate.¹² This is consistent with estimates of ~23–26% in recent NMRS studies.^{86–88} By using acetaminophen (paracetamol) to sample the hepatic UDP-glucose pool⁸⁹, the direct pathway was calculated to account for ~50–60% of glucose directly incorporated from plasma glucose into hepatic glycogen.⁸⁶

It is of note that the rate of net glycogen synthesis in these studies does not take into account glycogen cycling under these hyperglycaemic and hyperinsulinaemic conditions. In the presence of comparable hyperglycaemia, the ratio of flux through glycogen breakdown to flux through glycogen synthase yields minimum estimates of glycogen turnover which were markedly higher in the fed state than after 12–14 h of fasting (~60 versus ~30%).⁹⁰ Considering the linear correlation between hepatic glycogen concentration and glycogenolysis, it is conceivable that the higher liver glycogen content in the fed state may itself stimulate glycogen breakdown according to the concept of hepatic autoregulation of glycogen stores.⁹¹

Dynamic variations in hepatic glycogen metabolism

In order to examine changes in hepatic glycogen content during normal eating behaviour, non-diabetic volunteers ingested three mixed meals at 5-h intervals over the course of a day.⁹² Liver glycogen concentrations were measured after each meal and increased from ~275 mM, reaching a peak just before the next meal, up to ~420 mM at 4 h after dinner. This indicates negligible hepatic glycogenolysis during the day time,

so that glucose absorption from meals, and gluconeogenesis, account for the major part of whole-body glucose appearing during the day time, while net hepatic glycogenolysis contributes to whole-body glucose production only during the night.

PATHOLOGICAL CONDITIONS

Type I diabetes mellitus (T1DM)

The elevated rates of fasting EGP in patients with T1DM correlate with the degree of fasting hyperglycaemia^{93,94} and could result from increased glycogenolysis and/or increased gluconeogenesis. Older studies employing the liver biopsy technique reported either decreased⁹⁵, or even increased liver glycogen stores^{96,97} in T1DM. On the other hand, studies using hepatic vein catheterization showed that patients with T1DM have increased splanchnic uptake of substrates such as lactate and glycerol, suggesting augmented gluconeogenesis.^{36,98} Using the isotope-dilution techniques⁷², it was found that the excessive entry of glucose into the peripheral circulation after ingestion of a mixed meal results from insufficient suppression of EGP rather than from defects in the uptake of splanchnic glucose.⁸¹ Of note, intensive insulin therapy in these patients with T1DM restored postprandial suppression of EGP to the rates found in non-diabetic humans.

More recently, application of ¹³C NMRS revealed that concentrations of liver glycogen during fasting are within the normal range in patients with T1DM.^{86,87,92} However, at the end of a day, during which the patients had ingested three mixed meals, T1DM patients on conventional insulin therapy had synthesized only 25–30% liver glycogen compared with that synthesized by non-diabetic humans^{87,92} (Figure 3). These patients with poor metabolic control, as indicated by a mean glycosylated haemoglobin A1c (HbA1c) of ~8.8%, were studied again during a variable insulin infusion resulting in near-normoglycaemia for 24 h. Under these conditions of short-term optimized

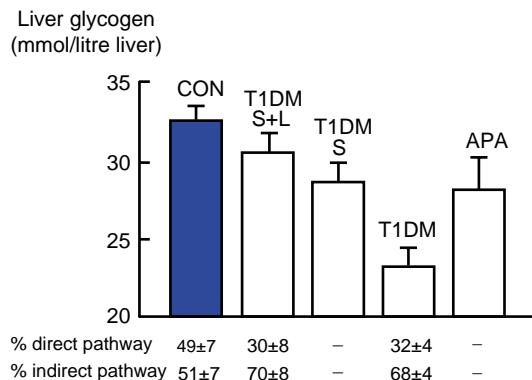


Figure 3. Maximum liver glycogen concentration after ingestion of a mixed meal in healthy volunteers (CON), type I diabetic patients with poor metabolic control before (T1DM) and after short-term improvement of glycaemia by insulin (T1DM S), type I diabetic patients with good short- and long-term metabolic control (T1DM S + L) and patients with agenesis of the dorsal pancreas (APA). Contributions of the direct and indirect (gluconeogenic) pathways of hepatic glycogen synthesis are shown at the bottom.^{13,86,87}

metabolic control, rates of hepatic glycogen synthesis nearly doubled, but were still ~50% lower than in non-diabetic humans⁸⁷ (Figure 3). Likewise, glycogenolysis during the subsequent overnight fast was ~50% in T1DM patients and increased, but remained ~25% lower than in the non-diabetic group. These results help to explain the normal fasting liver glycogen concentrations despite marked defects in glycogen synthesis and glycogenolysis in T1DM.

Intensified insulin therapy in T1DM patients, resulting in long-term optimized metabolic control (mean HbA1c <7% for more than 1 year), results in normal EGP, glycogen synthesis and glycogenolysis under comparable conditions^{86,87} (Figure 3). Although these findings suggest that optimized long- and short-term metabolic control reverses the defects in hepatic glycogen metabolism, these T1DM patients achieved normal rates of postprandial glycogen synthesis only in the presence of doubled concentrations of portal vein insulin and a higher contribution of the gluconeogenic pathway to glycogen synthesis than in non-diabetic volunteers (~70 versus ~50%). Similarly, even conditions of hyperglycaemia, and portal vein insulin:glucagon ratios leading to normalized glycogen synthesis, were not able to abolish the increased gluconeogenic flux to glycogen in T1DM patients (~65 versus ~40%)—which was further associated with decreased rates of hepatic pyruvate oxidation.⁹⁹ The persistence of these defects in well-controlled T1DM is not yet clear, but animal models hint at the involvement of impaired glucokinase activity^{13,100} or increased PEPCK activity.^{101,102} In addition, T1DM may present with peripheral insulin resistance^{94,103} which could augment the availability of gluconeogenic precursors, thus increasing gluconeogenesis and stimulating the flux through the gluconeogenic pathway of glycogen synthesis.

Nevertheless, with regard to clinical practice, these studies showed that excellent long-term metabolic control ensures sufficient postprandial net glycogen accumulation despite the unphysiological, but usual, peripheral route of exogenous insulin delivery. Restoring postprandial hepatic glycogen synthesis in T1DM will ameliorate both blood glucose peaks and prolonged hyperglycaemia after ingestion of mixed meals. Furthermore, this could help to prevent severe hypoglycaemic episodes because glucagon-mediated glycogenolysis, which is the first-line defence against hypoglycaemia²³, depends on the availability of sufficient liver glycogen stores.

Partial agenesis of the pancreas (PAP)

While complete agenesis of the pancreas is not compatible with life¹⁰⁴, partial agenesis of the pancreas can be detected in some patients with diabetes mellitus. A rare form of familial agenesis of the dorsal pancreas in diabetic woman and in both of her non-diabetic sons^{105,106} offered the opportunity to examine the effects of different degrees of endogenous insulin secretion on glucose fluxes after ingestion of a mixed meal (Figure 3). Regardless of differences in glycaemia and insulinaemia among the patients, they all featured ~55 and ~40% reduction in glycogen synthesis and glycogenolysis, respectively.⁸⁸ Gluconeogenesis was ~60% higher in PAP and contributed more to the slightly elevated fasting EGP than in non-diabetic humans (~70 versus ~50%).

Maturity-onset diabetes of the young (MODY)

Another human model of altered insulin secretion is created by mutations in the glucokinase gene on chromosome 7 in autosomal-dominant-inherited diabetes

(MODY-2).¹⁰⁷ ¹³C NMRS studies showed that the moderate postprandial hyperglycaemia of MODY-2 patients is associated with impaired hepatic glycogen synthesis so that, at the end of the day, the glucokinase-deficient subjects had only ~70% of the increment in glycogen observed in healthy subjects.¹³ In addition, the contribution of the gluconeogenic pathway to glycogen synthesis was higher in MODY-2 patients at 4 h after breakfast (~50 versus ~35%). As relative hypoinsulinaemia was present in the MODY-2 patients only during the first 2 hours following breakfast, the lower portal vein insulin:glucagon ratio cannot entirely explain the reduction in liver glycogen synthesis throughout the day. Thus, decreased flux through hepatic glucokinase is probably responsible for the impaired glycogen storage.

Type 2 diabetes mellitus (T2DM)

T2DM is characterized by fasting and/or postprandial hyperglycaemia. Fasting hyperglycaemia was found to be correlated with EGP—which was therefore held responsible for the rise in plasma glucose.^{39,63,79} However, measurement of EGP depends on the experimental conditions and is influenced by, among other factors, the prevailing degree of glycaemia.⁴ The use of refined isotope techniques revised the picture by demonstrating only modestly elevated (~10–25%)^{108,109} or unchanged EGP¹¹⁰ in overt T2DM (Table 2). This small increase in EGP returns to the normal range after insulin-mediated near-normoglycaemia, suggesting a major role for glucose toxicity in the regulation of fasting EGP.^{109,111} Finally, the finding of normal or slightly elevated EGP in the presence of hyperinsulinaemia and hyperglycaemia, which would normally act to suppress EGP, suggests abnormal regulation of EGP in T2DM.⁶³

The mechanism of defective glucose production in poorly controlled T2DM was elucidated by ¹³C NMRS.¹¹² These patients had decreased (89 versus 70%) net hepatic glycogenolysis, so that increased gluconeogenesis almost completely accounted for EGP. Compared with the ²H₂O method, ¹³C NMRS yielded higher estimates for gluconeogenesis in T2DM (84 versus 59%) but similar values in non-diabetic humans (60 versus 56%).⁷⁵ Others calculated slightly higher gluconeogenesis (+10%), also from the ²H₂O method^{49,76}, suggesting substantial glycogen cycling in T2DM.

Ingestion of glucose or mixed meals leads to excessive and prolonged hyperglycaemia in T2DM which has been attributed to decreased glucose-induced insulin secretion, insufficient suppression of glucagon and FFA, and hyperglycaemia per se. Splanchnic glucose output is increased in some patients³⁶ but not all patients.¹¹³ Likewise, splanchnic glucose uptake from enteral glucose is decreased during hyperglycaemia and hyperinsulinaemia in T2DM.¹⁶ Nevertheless, postprandial hyperglycaemia is due to both decreased suppression of EGP and reduced skeletal muscle glucose disposal.^{80,114}

When double-isotope tracer techniques were used, T2DM exhibited an overall increased EGP and initial splanchnic glucose uptake after ingestion of a mixed meal^{14,115} (Table 2). With isotopic dilution technique aiming constant tracer:tracee ratio postprandial, EGP initially decreases to ~50% of fasting values within 30 min in non-diabetic humans and in T2DM patients, who had a ~45% higher EGP during the 2 h following ingestion of a meal.⁸³ Most recently, application of this technique combined with ¹³C NMRS has provided evidence that this EGP suppression is associated with a ~45% reduction in hepatic glycogen synthesis in T2DM patients, despite their excessive hyperglycaemia.¹¹⁶

Non-alcoholic fatty liver disease (NAFLD) and liver cirrhosis

NAFLDs include a broad spectrum of liver diseases ranging from steatosis, fibrosis and non-alcoholic steatohepatitis (NASH) to cirrhosis defined by histological features of alcoholic liver disease in patients with negligible alcohol intake.¹¹⁷ Interestingly, recent studies found that NAFLD is associated with visceral obesity, low high-density lipoprotein cholesterol and elevated plasma triglycerides, suggesting a link with the insulin-resistance syndrome.¹¹⁸ In support of this hypothesis, patients with NAFLD showed a reduction in insulin-stimulated glucose utilization comparable with that in overt T2DM. Despite a normal fasting EGP, the insulin-dependent suppression was similarly decreased in NAFLD and T2DM (−63 and −65% versus non-diabetic subjects: −82% of fasting EGP). Such hepatic insulin resistance was paralleled by a comparable degree of impaired insulin-dependent suppression of plasma FFA.¹¹⁸

Although direct measurement of hepatic fat using liver biopsies is considered the clinical gold standard, hepatocellular lipid concentrations (HCL) can be assessed by ¹H NMRS. HCL correlate with whole-body and hepatic insulin resistance and insulin-dependent suppression of plasma FFA in non-diabetic and T2DM subjects.¹⁰⁹ Moreover, HCL increased during prolonged infusion of insulin¹⁰⁹ and with insulin dosage in T2DM.¹¹⁹ These findings suggest that either augmented portal flux of FFA from visceral fat tissue or increased portal insulin favouring hepatic lipid accumulation could be responsible for the early stages of NAFLD. In addition, serum concentrations of leptin, an adipocyte-derived peptide participating in neuroendocrine appetite regulation, independently predict hepatic steatosis but not fibrosis.¹²⁰ This suggests that cytokines such as leptin could play a role in the early events leading to NAFLD, probably by stimulating hepatic insulin signal transduction¹²¹ and triglyceride synthesis.¹²² On the other hand, a study in three patients with severe lipodystrophy, who were leptin-deficient due to selective loss of fat tissue, featured hyperglycaemia with nearly doubled EGP compared with weight-matched non-diabetic subjects.¹²³ These findings were associated with whole-body and hepatic insulin resistance and augmented HCL, all of which could be almost normalized by chronic leptin treatment. Taken together, aside from its anorectic effect, leptin seems to regulate the metabolic balance between size of visceral fat mass and intracellular lipid stores.

In liver cirrhosis, EGP was found to be decreased in some studies^{124,125}, but not in all.^{126,127} Fasting plasma glucose levels are most often normal, but are less tightly regulated after eating.¹²⁷ Plasma concentrations of insulin, glucagon, lactate and FFA, as well as uptake of gluconeogenic precursors, are increased. In the face of comparable EGP, patients with biopsy-confirmed liver cirrhosis showed a ~80% reduction in net hepatic glycogenolysis measured with ¹³C NMRS.⁷⁴ With the ²H₂O method, gluconeogenesis was higher (~70 versus ~55%) and total glycogenolysis was reduced by only ~30%, indicating substantial glycogen cycling in cirrhotic patients. Glycogen cycling in cirrhosis may result from the rise in plasma concentrations of insulin and glucagon^{21,22,90} or FFA.^{44,45,88}

Glycogen storage diseases (GSDs)

GSDs result from inborn enzyme defects which can affect the liver; these diseases include types I (glucose-6-phosphatase), III (amylo-1,6-glucosidase debranching enzyme), IV (branching enzyme), VI (liver phosphorylase), IX (phosphorylase b kinase) and glycogen synthase deficiency. GSD-I leads to hepatomegaly and hypoglycaemia, even during short periods of fasting. Of note, EGP rates were lower than in healthy

humans, but were not completely diminished.¹²⁸ [U-¹³C]glucose isotopomer analysis of plasma glucose revealed no recycled glucose, indicating that EGP is from non-labelled sources and significant release of hepatic glucose occurs by alternative pathways such as the debranching enzyme amylo-1,6-glucosidase.¹²⁸ In the absence of this enzyme, as in GSD-III, gluconeogenesis will become responsible for EGP. ¹³C NMRS non-invasively confirmed the enlargement of hepatic glycogen pools in GSD-IA¹²⁹ and GSD-IIIA.¹³⁰ This technique also aroused suspicion of GSD from hepatic glycogen accumulation in a boy under 2 years of age prior to successful diagnosis of GSD-VI.¹³¹

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Practice points

- new techniques, such as nuclear magnetic resonance spectroscopy, double isotopic tracer dilution and the ²H₂O method, circumvent limitations of previous approaches and now permit monitoring of intrahepatic glucose metabolism in the human liver
- the liver is mainly responsible for glucose homeostasis by rapidly switching from glucose uptake in the fed state to glucose production during fasting. Small changes in insulin, glucagon and the degree of hyperglycaemia finely tune both hepatic glycogen turnover and glucose production
- reduction in postprandial glycogen storage is not only explained by absolute insulin deficiency but also results from chronic hyperglycaemia and abnormal insulin delivery in diabetes mellitus due to autoimmune processes (type 1 diabetes mellitus), defective glucokinase (MODY-2) and partial agenesis of the pancreas

Research agenda

- further evaluation of hepatic glucose and glycogen metabolism under normal-life conditions in healthy humans and particularly in type 2 diabetes mellitus
- examination of the impact of differently composed diets, individual nutrients and fat-derived cytokines on hepatic glucose metabolism in humans
- testing the mechanism of action and efficacy of new therapeutic drugs designed to improve glucose metabolism in the liver

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