

# The regulation of glucose metabolism: implications and considerations for the assessment of glucose homeostasis in rodents

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**Kowalski GM, Bruce CR.** The regulation of glucose metabolism: implications and considerations for the assessment of glucose homeostasis in rodents. *Am J Physiol Endocrinol Metab* 307: E859–E871, 2014. First published September 9, 2014; doi:10.1152/ajpendo.00165.2014.—The incidence of insulin resistance and type 2 diabetes (T2D) is increasing at alarming rates. In the quest to understand the underlying causes of and to identify novel therapeutic targets to treat T2D, scientists have become increasingly reliant on the use of rodent models. Here, we provide a discussion on the regulation of rodent glucose metabolism, highlighting key differences and similarities that exist between rodents and humans. In addition, some of the issues and considerations associated with assessing glucose homeostasis and insulin action are outlined. We also discuss the role of the liver vs. skeletal muscle in regulating whole body glucose metabolism in rodents, emphasizing the importance of defective hepatic glucose metabolism in the development of impaired glucose tolerance, insulin resistance, and T2D.

type 2 diabetes; glucose tolerance; gluconeogenesis; insulin resistance; endogenous glucose production

THE INCIDENCE OF TYPE 2 DIABETES (T2D) is reaching epidemic proportions (159). Therefore, it is not surprising that there is an enormous effort among the scientific and medical community to better understand the pathogenesis of this condition. The emergence of modern-day genetic engineering techniques has placed rodents, particularly mice, at the forefront of research focusing on the development and progression of human diseases, including T2D. However, to generate meaningful and clinically relevant data from rodent studies, it is necessary to develop a detailed understanding of how rodent glucose homeostasis is regulated and importantly, to appreciate differences that exist between species, particularly between mice and humans. With increasing reliance on rodents, a number of procedures, such as the glucose tolerance test and euglycemic hyperinsulinemic clamp, have been modified from the clinic to determine glucose homeostasis and metabolic phenotype (4, 7). However, it has become apparent that there is a lack of appreciation and understanding about what these tests actually tell us about glucose metabolism and what causes impaired glucose tolerance and fasting hyperglycemia in rodents. The coordinated regulation of postprandial glucose homeostasis reflects the balance between the ability to stimulate glucose disposal while simultaneously suppressing endogenous glucose production (EGP), which is determined ultimately by three critical components: 1) insulin sensitivity, 2) glucose effectiveness, and 3) insulin secretion. Therefore, the purpose of this review is to discuss the issues and considerations

associated with performing and interpreting tests to assess glucose metabolism in rodents. Although we aim to address the basic concepts in the control of glucose homeostasis in health and metabolic disease, we encourage readers to seek excellent reviews, including those by DeFronzo (51, 53), Rizza (146), Vranic (175), Cherrington (40), and Zierler (182), for further clarification of the intricacies involved in the regulation of glucose homeostasis. Additionally, for more detail about standard practices in performing metabolic experimentation in rodents, please see the comprehensive reviews by Ayala et al. (8) and Hughey et al. (88). Furthermore, we acknowledge the critical role of the  $\beta$ -cell in the pathogenesis of human type 2 diabetes. However, with the exception of genetic mutations or chemically induced destruction of  $\beta$ -cells, rodents have a remarkable ability to maintain the capacity to secrete high levels of insulin and thus do not develop overt T2D, even during the long-term maintenance of the obese and severely insulin-resistant state (3, 38, 179). Therefore, this review will focus on the components of insulin sensitivity and glucose effectiveness.

## *Contribution of Individual Tissues to Glucose Uptake and Energy Metabolism*

To understand the regulation of glucose homeostasis, it is important to appreciate the contribution of individual tissues to both whole body energy expenditure and glucose metabolism. Under postabsorptive conditions in humans, the brain accounts for ~50% of whole body glucose utilization (11, 53). The splanchnic bed contributes an additional 25%, whereas the insulin-dependent tissues, primarily skeletal muscle, account for the remaining 25% of glucose use (11, 53). Although the brain is the major glucose-consuming tissue under basal con-

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ditions in humans, it appears that key species differences do exist. Despite the fact that the rate of brain glucose uptake is relatively high in mice under basal conditions, when accounting for total brain mass ( $\sim 0.4$  g), it makes only a minor contribution to whole body glucose uptake ( $<10\%$  of basal glucose turnover) (32, 96). In further support of these species differences, the contribution of brain to whole body oxygen consumption is markedly lower in rats than in humans, with the human brain exhibiting approximately sixfold greater relative contribution to whole body oxygen use (149). The role of brown adipose tissue (BAT) in regulating energy metabolism may also be an important consideration when comparing species. BAT, which is very well defined in adult rodents, has been shown to play a significant role in contributing to glucose metabolism (32, 46, 96), with studies estimating that as much as 8–12% of insulin-stimulated whole body glucose disposal occurs in rodent BAT (66). Less is known about the role of BAT in humans, but there is currently intense investigation to better elucidate the contribution of BAT to energy metabolism in humans (62, 125).

The widely held view is that skeletal muscle, by virtue of its mass and high metabolic rate, is the predominant site of postprandial glucose disposal. Although this is certainly true under euglycemic hyperinsulinemic clamp conditions where muscle is responsible for 70–80% of glucose disposal, this is not the case under postprandial conditions (53). In fact, following a meal, skeletal muscle and the splanchnic bed (liver and gut) make roughly equal contributions ( $\sim 30\%$  each) in disposing of meal-derived glucose, with the brain accounting for an additional 20% (90, 98, 166, 167). Therefore, when compared with euglycemic hyperinsulinemic clamp conditions, muscle plays a less dominant role in contributing to whole body glucose disposal in the postprandial state (Fig. 1). This is an important yet often overlooked fact. In addition, the notion that the metabolic rate of resting skeletal muscle is high is somewhat misleading. In fact, the resting metabolic rate of skeletal muscle ( $14.5 \text{ kcal}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) is relatively low when compared with that of the heart and kidney ( $440 \text{ kcal}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  each), the brain ( $240 \text{ kcal}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ), and the liver ( $200 \text{ kcal}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) (74, 92, 178). Together the brain, heart, kidneys, and liver account for only  $\sim 6\%$  of total body mass yet contribute to 60–70% of resting energy expenditure, whereas skeletal muscle comprises  $\sim 40\%$  of body mass yet accounts for 20–30% of resting energy expenditure (74, 92, 178). Although these facts clearly highlight that muscle does have an important function in regulating metabolic homeostasis, they also demonstrate that the dominant role it is often proposed to play in contributing to resting energy metabolism and glucose homeostasis needs to be reconsidered. This is clearly highlighted by studies in children with Duchenne muscular dystrophy. Despite having a large (65–75%) reduction in muscle mass and exhibiting hyperinsulinemia, basal glucose turnover has been shown to be normal and glucose tolerance only mildly impaired (73, 85), whereas resting energy expenditure was reduced by only 10% (81). This highlights that the dramatic reduction in muscle tissue, which is insulin responsive and considered to be a quantitatively important site of glucose disposal, does not necessarily compromise postprandial glucose homeostasis. Similarly, transgenic mice with severe muscle wasting have been shown to exhibit normal insulin action and glucose homeostasis (36). However, there is no question that skeletal muscle possesses a unique capacity to increase its energy expenditure during exercise, which has beneficial acute and chronic effects on whole body glucose and energy metabolism (27, 28).

*Metabolic Differences Between Rodents and Humans*

In comparing metabolic studies between different species, an important concept to grasp is that of Kleiber's Law of allometric scaling. Briefly, the observations made by Kleiber (102) in the 1930s concluded that for the majority of animals the basal metabolic rate scales approximately to the power of 0.75 of the animals' mass in kilograms. This can best be explained by using Kleiber's Law to compare a 25-g (0.025 kg) mouse to a 75-kg human. The human is 3,000 times heavier than the mouse, yet the absolute basal metabolic rate of the human is only  $\sim 400$  times greater. So in relative terms, the mouse has a basal metabolic rate that is  $\sim 7.5$ -fold greater than that of the human. It is clear that a mouse is under much greater metabolic constraints than a human. Therefore, it is not surprising that the mouse has blood glucose levels that are markedly higher than that seen in humans (Table 1). This is best illustrated in studies using continuous glucose monitoring that demonstrate that the average blood glucose concentration in free living mice over a 24-h period is  $\sim 8.5$  mM (80), whereas in humans this is maintained at 4.5–5 mM (144). Insulin and free fatty acid (FFA) concentrations are also somewhat higher in mice (Table 1). In addition, a mouse has a resting heart rate of  $\sim 600$  beats/min and a relative basal glucose turnover rate of 20–30  $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , which is 10–15 times greater than that seen in humans (Table 1 and Fig. 2) (7, 87, 132, 171). Moreover, mice deplete hepatic glycogen stores rapidly, such that after a 16- to 24-h fast there is essentially no hepatic glycogen remaining, whereas muscle glycogen stores remain unchanged or are modestly reduced (7, 89). On the other hand, humans and other large mammals such as dogs maintain some liver glycogen stores ( $\sim 10$ – $20\%$  of fed levels), with significant rates of hepatic glycogenolysis observed even

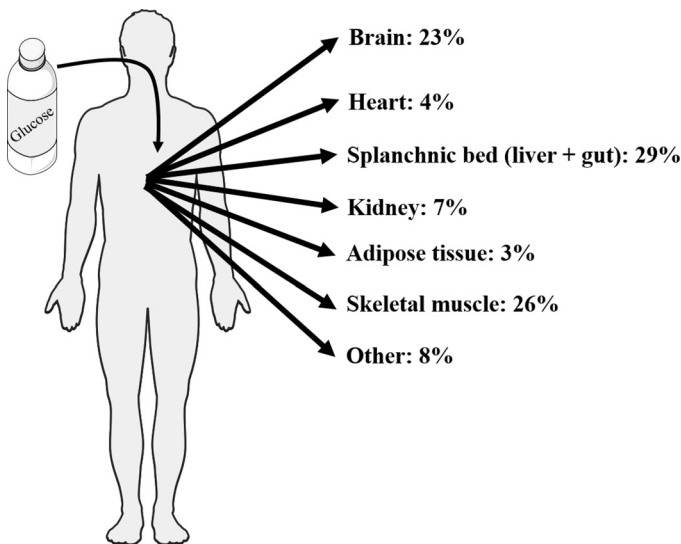


Fig. 1. The contribution of individual tissues and organs to the disposal of an oral glucose load in humans. Data are based on the findings of Jackson et al. (90) and Kelley et al. (98).

Table 1. Comparison of metabolic parameters between humans, rats, and mice

	Human	Rat	Mouse (C57Bl/6)
Body mass, kg	50–100	0.20–0.30	0.02–0.03
Blood glucose, mmol/l	4–6	4–7	7–9
Plasma FFA, mmol/l	0.2–0.6	0.4–0.7	0.5–1.5
Plasma triglyceride, mmol/l	1.0–1.5	1.0–1.5	1.0–1.5
Plasma insulin, pmol/l	40–70	200–300	100–200
Plasma glucagon, ng/l	60–150	45–100	40–150
Liver glycogen, $\mu\text{mol/g}$ wet wt	250–300	150–250	100–200
Muscle glycogen, $\mu\text{mol/g}$ wet wt	55–85	25–40	10–20
Basal EGP, $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$	1.5–2.5	8–15	20–30
Gluconeogenesis, %contribution to EGP	40–50	50	80
Glycogenolysis, %contribution to EGP	50–60	50	20

FFA, free fatty acid; EGP, endogenous glucose production. Typical hormone and metabolite ranges are from venous plasma (nonportal circulation) identified in the literature. Human data (10, 13, 22, 27, 28, 67, 76, 79, 98, 104, 129, 132, 156) are based on overnight (10- to 12-h) fast. Data for rats (33, 38, 41, 56, 93, 105, 126, 128, 137, 151, 152, 154) and mice (4, 5, 7, 10, 16, 35, 80, 116, 171) are based on a 4- to 7-h fast.

after a 42-h fast (40, 86, 130, 132). Accordingly, small mammals such as mice are overwhelmingly reliant on the energetically expensive process of gluconeogenesis to supply their high-glucose demands, with isotopic tracer studies showing that, even after a brief 4-h fast, gluconeogenesis supplies >80% of endogenously produced glucose (Table 1) (35). In contrast, gluconeogenesis contributes ~40–50% of endogenous glucose production in humans following an overnight fast (Table 1) (132). To meet these demands, laboratory mice must eat and drink frequently, with the average interval between food and drinking bouts being ~34 and ~42 min, respectively (total of 36 food and 32 water bouts in 24 h) (75). Thus, mice eat ~4 g and drink ~5 ml/day, which approximates to them eating and drinking their entire body weight in 1 wk (7, 9). The high metabolic rates and extreme reliance on gluconeogenesis in mice (and rats) have implications for understanding and assessing glucose metabolism and insulin sensitivity, which will be subsequently discussed.

#### Assessing Glucose Metabolism in Rodents: Implications and Considerations

A critical factor in metabolic research is the choice of methodology used to examine glucose homeostasis. Although there are a number of techniques available, many of which have been adapted from clinical use in humans, possibly the most important consideration is to recognize the key differences between “physiological” and “pseudophysiological” (i.e., euglycemic hyperinsulinemic clamp, intravenous or intraperitoneal glucose tolerance test, and intravenous insulin tolerance test) techniques to assess glucose metabolism (57). Under physiological conditions, such as during a meal or an oral glucose tolerance test (OGTT), ingested glucose is drained from the small intestine via the mesenteric vein into the portal vein and liver sinusoids. Under these conditions, portal vein glucose and insulin levels are higher (2- to 3-fold) than any other part of the systemic circulation, which cannot be replicated when either glucose, insulin, or both are injected or infused into any other blood vessel or body space (i.e., intraperitoneal injection) apart from the mesenteric or portal vein

(40). The resulting portal vein hyperglycemia and hyperinsulinemia are a strong stimulus for hepatic glucose uptake, metabolism, and storage as glycogen (40, 53). In contrast, an equivalent glucose dose administered intravenously has only small effects on splanchnic glucose uptake (53). Furthermore, oral glucose ingestion results in a higher (~2-fold) insulin secretory response than when administered by intravenous or intraperitoneal injection, which is due to the phenomenon known as the incretin effect (53). The combined postprandial hyperinsulinemia and hyperglycemia act synergistically to stimulate splanchnic glucose uptake, suppress EGP, and stimulate peripheral (muscle, heart, and adipose tissue) glucose uptake (53). It is also important to highlight that following a meal or during an OGTT, EGP is suppressed by ~50% in healthy humans, which contrasts the almost complete suppression that can be achieved under clamp conditions (67, 90, 98, 119, 123, 161). Thus, the route of glucose entry into the body is an important consideration for the assessment and interpretation of glucose homeostasis.

Additionally, hyperglycemia per se (i.e., glucose effectiveness) has potent metabolic effects in the liver and periphery, including muscle (1, 11, 15, 20, 64, 152, 155, 160, 170), and therefore, it should be an important consideration to keep in mind when assessing glucose homeostasis. Glucose effectiveness is defined as the ability of glucose to promote its own metabolism independent of insulin (1), such that hyperglyce-

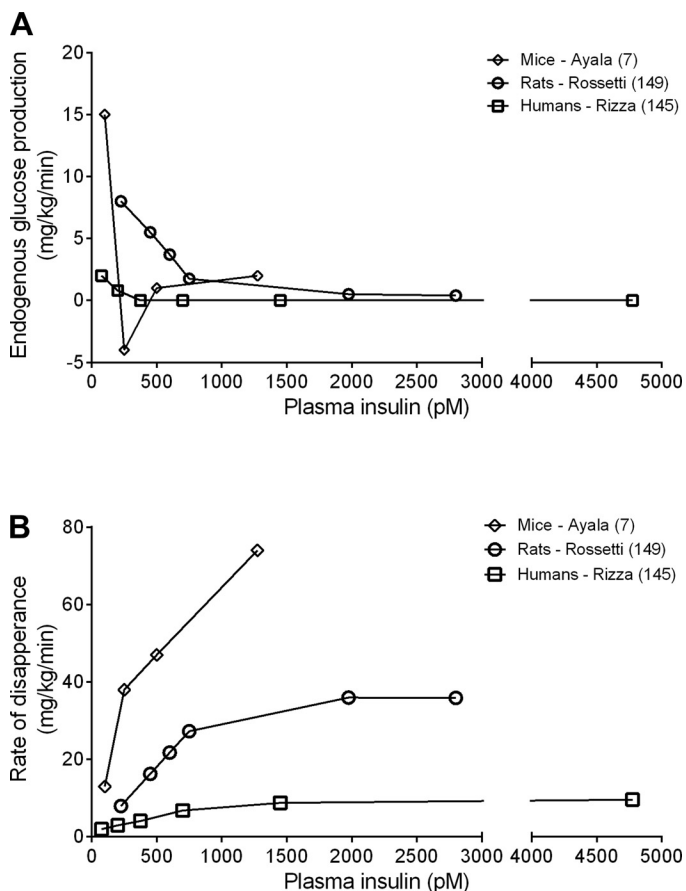


Fig. 2. Effect of insulin dose on endogenous glucose production (A) and rate of glucose disappearance (B) in mice, rats, and humans during the euglycemic hyperinsulinemic clamp.

mia can suppress EGP while stimulating glucose uptake via its mass action. Although the glucose concentration itself is an important factor in stimulating muscle glucose uptake, the question of whether skeletal muscle is truly a “glucose-effective” tissue, at least in humans, is controversial (182, 183). Systemic hyperglycemia has been shown to stimulate muscle glucose uptake independent of insulin secretion (11, 20, 182). However, when hyperglycemia was induced locally in the human forearm in the absence of changes to insulin concentrations, net muscle glucose uptake was not observed, prompting the hypothesis that a “systemic factor” is required to induce muscle glucose uptake during systemic hyperglycemia (182, 183). Regardless, muscle is still heavily reliant on insulin-stimulated glucose transporter (GLUT)4 translocation for increasing glucose uptake above basal levels. In contrast, the liver does not require insulin to promote glucose uptake. Rather, because of the unique GLUT2 transporter and presence of glucokinase, hepatic glucose uptake is directly proportional to portal vein glycemia (40). Thus, in the postprandial state, when portal vein glucose is high, there is net uptake of glucose and suppression of EGP (40). However, the liver is highly insulin sensitive, and although insulin is not required for stimulating glucose uptake, its presence does promote efficient hepatic glucose metabolism by promoting glycogen synthesis and glycolysis (40). Thus, although there are many ways in which to assess glucose metabolism in rodents *in vivo*, it is important to recognize the limitations associated with each of these methods, particularly those related to the use of pseudo-physiological techniques (see below). To overcome many of these limitations, efforts are required to develop innovative and informative approaches to study the regulation of glucose metabolism under dynamic conditions that closely mimic the postprandial state (13, 173).

### *Euglycemic Hyperinsulinemic Clamp*

Although the euglycemic hyperinsulinemic clamp represents a pseudophysiological test, there is no doubt this technique has been fundamental in furthering our understanding of *in vivo* glucose metabolism and insulin action (79, 106, 147). In fact, the strength of the clamp technique is that when combined with the use of glucose tracers, measurements of whole body and tissue-specific insulin sensitivity can be obtained. Unlike other methods such as the glucose or meal tolerance test that assess the coordinated response between insulin secretion, insulin sensitivity, and glucose effectiveness, the clamp remains the “gold standard” technique for assessing insulin sensitivity. The primary purpose of the euglycemic hyperinsulinemic clamp is to match blood glucose and insulin concentrations between study groups to determine insulin sensitivity. In addition to the euglycemic hyperinsulinemic clamp, other clamp techniques are also routinely used in the clinic and in animal research. These include the hyperglycemic clamp and the somatostatin “pancreatic clamp” (40, 54). Although beyond the scope of this review, the aim of the hyperglycemic clamp is to maintain a steady-state level of hyperglycemia by infusing glucose, allowing the assessment of the capacity of the pancreatic  $\beta$ -cells to secrete insulin over an extended period (54). On the other hand, in the pancreatic clamp, somatostatin is infused throughout to block the ability of the pancreas to secrete glucagon and insulin, thus allowing precise control over glycemia indepen-

dent of glucoregulatory hormone action (40). This allows researchers to infuse glucagon and insulin at a desired concentration, often at the basal level, while altering steady-state blood glucose concentrations. The pancreatic clamp technique in particular has been pivotal to our understanding of glucose effectiveness. The pancreatic clamp is often used in human (146) and dog studies (40), and although it has also been performed in rats (133, 153) and mice (116, 150), it is generally not used as frequently. Of note, there is evidence in mice that somatostatin is rather ineffective at inhibiting insulin secretion (32, 134), which may limit its application to the mouse. Furthermore, rat and mouse studies using the somatostatin pancreatic clamp have often failed to infuse/replace glucagon, and they have not reported plasma C-peptide levels to confirm the efficacy of somatostatin action (116, 133, 150, 153), making the data somewhat difficult to interpret. In addition to being technically demanding, there are a number of important issues to take into account when undertaking clamp studies in rodents that will be briefly outlined below.

*Insulin sensitivity vs. insulin responsiveness.* It is important to understand that the physiological and biochemical actions of insulin differ, depending on the degree of hyperinsulinemia that is achieved during the clamp. This phenomenon can be best demonstrated by insulin dose response studies. In humans (12, 24, 79, 147, 168), rats (151, 153), and mice (7), euglycemic hyperinsulinemic clamp studies using progressively higher insulin doses (Fig. 2) have revealed that the liver is markedly more insulin sensitive than muscle, as low-dose insulin can rapidly and maximally suppress EGP even in subjects or animals with diabetes, whereas higher insulin levels are required to stimulate glucose disposal (Fig. 2). However, muscle has a greater capacity to take up glucose at progressively higher insulin doses, such that muscle glucose uptake can continue to increase at insulin concentrations where EGP is already maximally suppressed (7, 12, 24, 79, 147, 151, 153). Furthermore, the prevailing insulin concentration achieved during the clamp can have profound effects on the site of glucose distribution of the glucose disposal process, with small physiological increments in insulin that suppress EGP, predominantly increasing glucose transport (151), inhibiting muscle glycogenolysis (153), and stimulating glucose oxidation (168). However, these actions become saturated, and high physiological to pharmacological increments in insulin shift the site of distribution of glucose disposal toward the stimulation of glycogen synthesis and glucose storage (151, 153, 168). Thus, it is important to appreciate that the measurement of insulin sensitivity during the clamp involves the use of insulin doses that elicit a physiological increase in plasma insulin (95). When infusions result in plasma insulin levels that are in the supraphysiological range, measurements are likely to reflect insulin responsiveness, *i.e.*, the capacity to respond to insulin, rather than insulin sensitivity (95). Likewise, when high physiological insulin concentrations are used, insulin resistance can in fact be masked. This is often the case in obese individuals and patients with T2D, where the suppression of EGP only differs from lean healthy individuals at low insulin concentrations (12, 24, 146). Interestingly, in obese and/or T2D patients, reductions in glucose disposal during the clamp are evident within a broad physiological dose response range (12, 24, 146), yet at supraphysiological insulin concentrations these defects are no longer detectable (24). In rats (91, 106, 151, 153) and

mice (7), who for a given insulin concentration exhibit glucose disappearance rates that are four to 20 times greater than in a human (Fig. 2), whole body glucose disposal and muscle-specific glucose uptake become saturated at insulin infusion rates of  $\sim 14\text{--}20 \text{ mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ . Thus, if one is wishing to detect the presence of insulin resistance, such high insulin infusion rates may mask the insulin resistance due to the compensation achieved by such a high degree of hyperinsulinemia (136). Therefore, to accurately encompass measurements of insulin sensitivity in rodents, it is important to avoid using excessively high ( $<10 \text{ mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) insulin infusions or, alternatively, performing insulin dose response or two-step euglycemic hyperinsulinemic clamp studies.

*Limitations of the euglycemic hyperinsulinemic clamp.* Although the euglycemic hyperinsulinemic clamp remains the gold standard to assess insulin sensitivity, it does have limitations. Possibly the most important is the fact that it does not replicate the dynamic state that occurs under normal postprandial conditions. In addition, the euglycemic hyperinsulinemic clamp may in fact have a bias toward a muscle phenotype, as EGP can be completely suppressed for almost the entire clamp duration, which contrasts what occurs following a meal or during an OGTT (7, 53, 171). Moreover, the relative lack of portal vein hyperinsulinemia and complete absence of both portal and systemic hyperglycemia prevents net hepatic glucose uptake and glycogen synthesis, which normally occur following a meal (40, 42). A similar phenomenon may occur during other tests that employ peripheral insulin injection, such as an intravenous or intraperitoneal insulin tolerance test, techniques that are commonly performed in rodent research.

A further limitation with the clamp technique is deciding upon the glucose concentration at which the experiments should be performed. This is particularly problematic when studying patients or animals that are overtly hyperglycemic. When dealing with groups that differ in glycemia, one must decide whether to clamp all groups at their prevailing blood glucose concentration or to first reduce the glucose levels in the hyperglycemic group to that of the controls with prior insulin infusion. Either way, this will impact on the results, as hyperglycemia has potent metabolic effects in its own right. Indeed, when subjects with T2D were studied under clamped conditions at their prevailing hyperglycemic blood glucose levels (i.e., isoglycemic), whole body glucose disposal and muscle glucose uptake were the same or actually higher than when compared with nondiabetic controls (37, 55, 99, 145, 172). However, when the T2D patients were rendered euglycemic by prior insulin infusion, defects in muscle glucose disposal became evident (55, 99, 172). This demonstrates that hyperglycemia in insulin-resistant subjects with T2D is able to compensate for muscle insulin resistance, ensuring normal or higher absolute rates of muscle glucose uptake in the basal and postprandial states (67, 120, 123, 146), findings that have also been replicated in glucose-intolerant dogs (44, 45) and rats (148). Overall, despite the fact that many studies have shown that defects in muscle insulin action are compensated for by hyperglycemia and hyperinsulinemia, thus resulting in nearly normal muscle glucose uptake, the fact is that this response is inappropriate for the prevailing glucose and insulin levels (52). Furthermore, it should not be interpreted that muscle insulin resistance and the compensatory hyperglycemia and hyperinsulinemia are good for patients in their daily life. In fact, this

is a compensatory response to an underlying pathology and is likely to place a heavy workload on the pancreatic  $\beta$ -cells, ultimately contributing to  $\beta$ -cell failure and glucotoxicity, which leads to diabetic complications such as vascular disease, neuropathy, and kidney failure. However, in contrast to the effects on muscle, hyperglycemia is only able to partially compensate for hepatic insulin resistance, such that hyperglycemia augments but does not restore insulin-mediated suppression of EGP (65, 67, 77, 145). Therefore, loss of hepatic glucose effectiveness has been implicated in the pathogenesis of T2D (19, 83, 84, 115, 121).

The issue of hyperinsulinemia is also an important consideration and is especially relevant when studying the responses to a high-fat diet (HFD). Indeed, investigators often fail to realize or acknowledge that rodents maintained on a HFD exhibit higher plasma insulin levels during the clamp when compared with animals maintained on a low-fat control diet (5, 6, 25, 38, 171), which appears to be attributed to a defect in insulin clearance (38). Because the clamp relies on plasma insulin levels being matched between groups, failure to either adjust the insulin infusion rate to achieve this or simply not report the plasma insulin levels during the clamp can make the interpretation of clamp data difficult.

#### *Use of High-Fat-Fed Rodents to Model Human Obesity and Glucose Intolerance*

To increase our understanding of the mechanisms responsible for defects in glucose metabolism associated with overnutrition and obesity, scientists have turned to rodent models for answers (30, 31, 107, 165). The chronically HFD-fed rat and mouse represent the most commonly used models for metabolic research and often form the basis of genetic and preclinical drug target studies. An important consideration in these studies is the composition of the HFD used (31). Although there is no standardized HFD to induce metabolic disease, it appears that long-chain fatty acids derived from animal fats and  $\omega$ -6/ $\omega$ -9 containing plant oils are effective in causing obesity, hepatic steatosis, and defective glucose homeostasis, whereas diets enriched in marine triglycerides and medium-chain fatty acids are not (31). There are also strain-dependent effects that alter the susceptibility to the detrimental consequences of chronic high-fat feeding (124). In addition, rodent housing temperature can affect energy balance and rates of weight gain in response to a HFD, which can ultimately influence glucose homeostasis (113, 164). Thus, there are a number of facets that need to be taken into account when undertaking dietary intervention studies in rodents to examine the effects on glucose homeostasis.

*Defects underlying the development of glucose intolerance in high-fat fed rodents: the role of liver vs. skeletal muscle.* Despite the extensive use of the HFD model, there is a surprising lack of detail about the underlying factors responsible for the development of defective glucose metabolism. To address this, we recently performed detailed time course studies to document the temporal development of whole body and tissue-specific insulin resistance caused by a HFD in C57Bl/6 mice (171). Glucose intolerance and whole body insulin resistance developed within 7 days of HFD feeding, which was attributed to hepatic but not skeletal muscle insulin resistance, as determined by euglycemic hyperinsulinemic clamp. Skeletal

muscle insulin resistance was first detected after 3 wk of HFD, which was associated with a further reduction in whole body insulin sensitivity, but this failed to exacerbate the glucose intolerance. Although the induction of muscle insulin resistance did not worsen the glucose intolerance, it was associated with the development of hyperinsulinemia under both basal and clamp conditions (171). Interestingly, extending the HFD intervention for  $\leq 16$  wk did not cause any further deterioration in glucose intolerance or whole body and tissue-specific insulin resistance (171).

The findings of diet-induced insulin resistance originating in the liver and not muscle have also been reported in rats (48, 93, 105, 157, 177) and dogs (44, 101). Importantly, it has been shown that short-term overfeeding studies in humans also cause a rapid deterioration in whole body insulin sensitivity that appears to be due to defects in the control of hepatic glucose metabolism rather than effects on muscle insulin action (26, 43, 47). Furthermore, the normalization of glycemic control in T2D individuals after short-term calorie restriction was associated with an improvement in hepatic glucose metabolism, whereas muscle remained insulin resistant (111). Together, these findings highlight that changes in liver glucose metabolism occur rapidly in response to changes in energy balance. However, it should be noted that there is evidence in both rats and mice that muscle may in fact develop insulin resistance after a relatively short exposure to a HFD. Indeed, Lee et al. (110) showed that as little as 3 days of HFD not only impaired the suppression of EGP but also caused defects in insulin-stimulated glucose disposal under euglycemic hyperinsulinemic clamp conditions. However, these data are somewhat difficult to interpret since the plasma insulin during the clamp was not reported, and blood glucose levels were matched during the clamp despite differences in basal glucose levels between the chow- and HFD-fed mice (110). In addition, there was no measurement of tissue-specific glucose uptake from muscle or any other tissue, and rather than the absolute rates of glucose disposal being reported, the insulin-stimulated component of glucose disposal was shown (110). Wang et al. (177) also detected muscle insulin resistance after a relatively short exposure to a HFD (i.e., 7 days) in rats, a time point in which we (171) and others (48, 105, 157) did not observe muscle insulin resistance. Yet they also demonstrated that hepatic insulin resistance developed after 3 days of HFD, which occurred prior to the onset of muscle insulin resistance, which is consistent with our findings in mice (171) and that of others in rats (48, 105, 157). This suggests that the exact appearance of organ-specific insulin resistance in response to a HFD (i.e., 3 vs. 7 days, etc.) may differ slightly in different laboratories, but the order in which these organs develop insulin resistance appears to be consistent. Similar to our findings in mice (171), diet-induced glucose intolerance in dogs was shown to be driven by an inability of the liver to switch from net glucose production to net glucose uptake in the transition to the postprandial state (44, 45). In contrast to the liver, muscle glucose uptake was actually higher in the glucose-intolerant dogs, an effect attributed to the compensatory actions of hyperinsulinemia and hyperglycemia (44, 45). Therefore, at least in mice and possibly other species, it appears that glucose tolerance is more closely related to hepatic rather than muscle insulin action.

*Defects underlying the development of glucose intolerance in high-fat-fed rodents: the role of adipose tissue.* Adipose is considered to be an important gluco-regulatory tissue since it is not only a site of glucose disposal but is also central in regulating plasma FFA levels, which can influence insulin action (18, 21, 23, 71, 108). Indeed, in humans and rodents, an acute elevation in plasma FFAs causes insulin resistance by impairing peripheral glucose disposal and suppression of EGP (23, 108). Conversely, pharmacological inhibition of lipolysis can improve insulin action and glucose metabolism in T2D patients and healthy controls alike (23, 50). Insulin is a potent inhibitor of lipolysis; however, the ability of insulin to lower FFAs has been reported to be impaired in obese and nonobese insulin-resistant and T2D humans and dogs (18, 23, 39, 72, 78). The inappropriate suppression of FFAs has been linked to the inability of insulin to reduce EGP (18, 23, 78). However, the interaction between the suppression of lipolysis and EGP in rodents is equivocal. A number of studies have shown that high-fat-fed rodents appear to retain the ability to suppress lipolysis in response to insulin despite the presence of hepatic insulin resistance (5, 171, 181). In fact, we have shown recently that short- and long-term HFD feeding in mice caused hepatic insulin resistance without the ability of insulin to suppress plasma FFA levels being altered (171). Although insulin's ability to suppress lipolysis may be intact in HFD-fed rodents, severe defects in insulin-stimulated glucose uptake are evident in adipose tissue (105, 171, 181). The reduction in adipose tissue glucose uptake occurs early in response to fat feeding; however, this is not sufficient to cause a reduction in whole body glucose disposal (105, 171), which is consistent with the quantitatively minor contribution of adipose tissue to whole body glucose disposal (53, 70). Taken together, these findings suggest that in high-fat-fed rodents, adipose tissue insulin resistance develops with respect to glucose metabolism, but not necessarily to the control of lipolysis.

#### *Regulation of Glucose Homeostasis: Lessons From Genetically Modified Rodent Models*

The use of genetically modified rodents has not only propelled our understanding of the role that individual genes and proteins play in the maintenance of glucose homeostasis but has also shed light on the role of individual tissues in contributing to whole body glucose metabolism. In fact, a number of surprising observations have been reported, especially surrounding the role of muscle in regulating whole body glucose metabolism. For example, using whole body GLUT4-null mice, Katz et al. (97) made the remarkable discovery that these mice had essentially normal fed and fasted blood glucose levels and exhibited normal glucose tolerance, but they did display postprandial hyperinsulinemia. In contrast, insulin tolerance tests revealed that the GLUT4-null mice were less responsive to insulin, suggesting that GLUT4 is required for insulin responsiveness but not for maintenance of normal glycemia (97). When muscle-specific GLUT4-null mice were subsequently studied, basal blood glucose and plasma insulin levels were actually found to be lower in these mice, whereas glucose tolerance and muscle glucose uptake were normal (63). When placed on a HFD, the muscle-specific GLUT4-knockout (KO) mice developed the same degree of glucose intolerance as wild-type mice but were in fact hyperinsulinemic during the

OGTT, suggesting an adaptive  $\beta$ -cell compensatory response due to the presence of muscle insulin resistance (63).

However, perhaps the most compelling line of evidence questioning the role of muscle insulin action in the control of whole body glucose homeostasis in mice comes from studies conducted on the muscle-specific insulin receptor KO (MIRKO) mouse (29, 100, 117, 180). Despite a lack of muscle insulin receptor signaling, MIRKO mice exhibited normal blood glucose, serum insulin, and glucose tolerance (29, 117). However, euglycemic hyperinsulinemic clamp experiments revealed that MIRKO mice displayed profound whole body insulin resistance, which was attributed to a marked reduction in muscle glucose uptake, as hepatic insulin action remained normal (100). It is important to note that this profound whole body and muscle insulin resistance observed during the clamp occurred despite a marked ( $\sim 2.5$ -fold) compensatory increase in adipose tissue glucose uptake (100), again highlighting the quantitatively minor role adipose tissue plays in contributing to whole body glucose disposal (70, 105, 171). Based on these findings in MIRKO mice, these authors concluded that insulin action in tissues other than muscle appears to be more important in regulating glucose metabolism (29). In a similar regard, mice with muscle-specific deletion of glycogen synthase, the rate-limiting enzyme in glycogen synthesis, displayed reduced muscle glucose uptake during the euglycemic hyperinsulinemic clamp (135). However, glucose tolerance was not compromised but was in fact enhanced compared with control animals despite a complete absence of muscle glycogen (135). However, these mice did show a more sustained increase in serum insulin during the glucose tolerance test, signifying compensation for muscle insulin resistance (135). Collectively, these studies highlight that the complete absence of key proteins involved in skeletal muscle insulin action, glucose uptake, and storage results in various metabolic abnormalities, yet fasting glycemia and glucose tolerance remain largely intact.

Interestingly, when some of these proteins discussed above were deleted specifically from the liver, marked effects on glycemic control were observed. In contrast to MIRKO mice (29, 100, 117, 180), liver-specific insulin receptor KO (LIRKO) mice showed severe glucose intolerance, insulin resistance, hyperinsulinemia ( $\sim 10$ - to 20-fold), and elevated glucose levels in the fed state (68, 122). Unlike MIRKO mice, which only have selective insulin resistance in muscle (100), in addition to having a primary defect in hepatic insulin action, the LIRKO mice also develop peripheral insulin resistance (68). These authors suggested that the primary liver insulin resistance in LIRKO mice can drive secondary insulin resistance in the muscle due to the compensatory hyperinsulinemia required to maintain basal EGP within the normal range (68). Similarly to the comparison of MIRKO and LIRKO mice, liver-specific glycogen synthase KO mice display glucose intolerance (89), whereas muscle-specific glycogen synthase KO mice do not (135). Thus, it appears that the deficiency of key proteins involved in insulin action and glucose metabolism in the mouse liver, but not muscle, has more profound changes to glucose metabolism.

Studies in the genetically obese Zucker fatty rat also point toward defective hepatic metabolism being responsible for the glucose intolerance observed in this model (148). Tracer methodology employed during an OGTT revealed that the rate of glucose disappearance was actually increased in the fatty

Zucker rat when compared with the lean controls due the combined compensatory actions of hyperinsulinemia and hyperglycemia. In contrast, the Zucker fatty rat failed to effectively suppress EGP (148). The ability to improve fasting glycemia and glucose tolerance in the diabetic Zucker fatty rat has also been shown to be determined predominantly by improvements to hepatic and not muscle glucose metabolism (42). Thus, defects in glucose tolerance in this model appear to be the result of defects in the ability to suppress EGP, as opposed to an impaired ability to stimulate peripheral glucose disposal (148). However, it should be noted that much of the investigation of postprandial glucose metabolism following a meal or OGTT in humans and rats was conducted using the constant infusion dual-tracer method. This methodology has been shown to suffer from non-steady-state error, leading to issues in calculating rates of total glucose appearance and disappearance while also giving the impression of a slow reduction in EGP (13, 161, 169). These issues can be overcome only by using the more complex variable infusion triple-tracer approach that is essentially model independent (13, 169).

#### *Understanding the Regulation of EGP*

The findings discussed above highlight the importance of the liver in contributing to the development of defective glucose homeostasis in mice. Therefore, we will briefly outline the mechanisms by which EGP is regulated (Fig. 3). The liver produces glucose via two main pathways, glycogenolysis and gluconeogenesis, with the principal hormone responsible for lowering EGP being insulin (112). The suppressive effects of insulin on EGP are complex and involve both direct and indirect actions (for more details, see Refs. 17, 40, 141, and 175). As discussed previously, in addition to insulin, glucose itself can rapidly and efficiently suppress EGP (152), thus working synergistically with hyperinsulinemia. Hyperglycemia is believed to suppress EGP predominantly via the suppression of glycogenolysis and stimulation of glycolysis (116, 140, 152), whereas insulin's actions are more wide-ranging. Briefly, the direct actions of insulin on EGP represent those that are mediated via insulin binding directly to the hepatocyte insulin receptor. The indirect effects of insulin on EGP involve insulin acting on insulin receptors in other tissues such as the pancreatic  $\alpha$ -cells and adipocytes to suppress glucagon secretion and lipolysis, respectively (40). An additional and somewhat more controversial indirect action of insulin on EGP is via insulin signaling in the central nervous system (112, 141). This central action of insulin has been shown to alter hepatic neural input and suppress EGP in rodents; however, these central actions have not been substantiated in larger mammals such as dogs and humans (139, 141, 158). This lack of reproducibility between species may be an artefact of the methodology employed in the rodent studies or due to species differences in hepatic glucose regulation (141). The direct actions of insulin predominantly regulate hepatic glucose metabolism via the stimulation of glycogen synthesis and glycolysis (59, 142). Indirectly, insulin acts via suppressing and counteracting glucagon action. The processes of glycogen synthesis and glycolysis are also augmented during glucagon suppression, since glucagon is a powerful and rapid activator of glycogenolysis and gluconeogenesis. Thus, insulin counteracts glucagon to reverse net hepatic carbon flow, reducing EGP while stimulat-

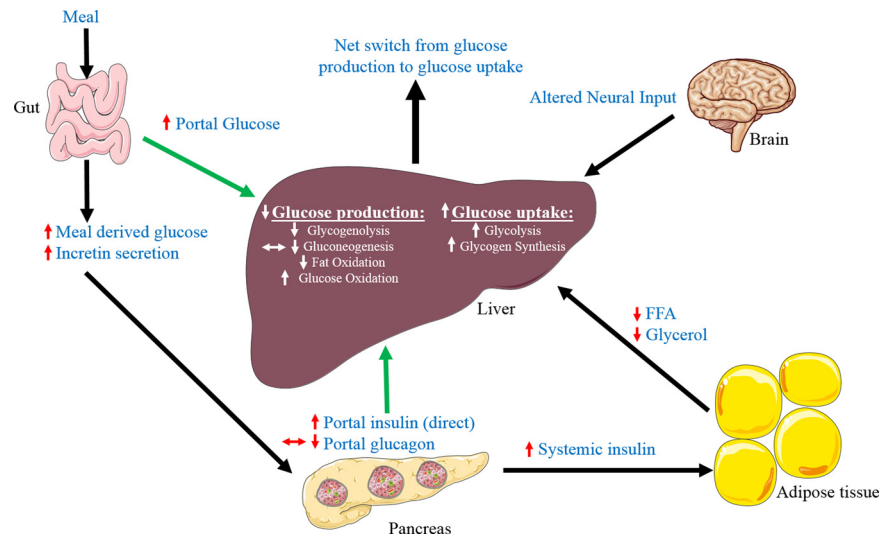


Fig. 3. Regulation of endogenous glucose production. Green arrows represent direct effects on the liver via the hepatic portal circulation. Red arrows represent the following:  $\uparrow$ , an increase;  $\downarrow$ , a decrease;  $\leftrightarrow$ , no change or a modest decrease. FFA, free fatty acid.

ing glucose uptake, metabolism, and storage (40). Furthermore, insulin suppresses adipose tissue lipolysis, thus reducing the availability of plasma FFAs and glycerol, reducing hepatic fatty acid oxidation and the supply of glycerol for gluconeogenesis (40). These changes in substrate supply simultaneously promote glycolysis and glycogen synthesis, which reduces net glycogenolysis.

Chronically, insulin can also inhibit the transcription of gluconeogenic genes such as cytosolic phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in the hepatocyte, with these effects being mediated by insulin signal transduction (112). However, changes in gluconeogenic gene/protein expression often do not correlate with alterations in gluconeogenesis or EGP, particularly in vivo (14, 34, 41, 58, 60, 69, 103, 114, 138, 141, 142, 156, 176). With the emergence of the molecular biology revolution, analysis of insulin signaling and gluconeogenic gene expression is often mistakenly used as a proxy measure of EGP, thus bypassing more difficult isotopic tracer studies to directly measure EGP and hepatic glucose fluxes. It is important to note that physiological hyperinsulinemia begins to suppress EGP within minutes and well before transcriptional changes can manifest into changes at the protein (enzyme) level (13, 49, 60, 61, 90, 142, 169). Furthermore, in humans, postprandial insulin secretion is transient, peaking at  $\sim 1$  h and returning to baseline 3–4 h after a meal (143). Therefore, it is unlikely that transcriptional regulation of gluconeogenesis is responsible for changes in EGP in the postprandial state. In fact, Ramnanan et al. (142) concluded that in dogs, which have similar metabolic characteristics to humans, physiological hyperinsulinemia suppresses EGP predominantly by modulating glycogen metabolism. On the other hand, net gluconeogenesis is only transiently reduced by insulin through an increase in glycolysis and a reduction in fat oxidation independent of transcriptional changes (142). Moreover, the concept of insulin suppressing EGP through a reduction of gluconeogenic gene transcription is incompatible with studies showing that gluconeogenesis is actively used in the postprandial state for hepatic glycogen synthesis via the indirect pathway (109, 118, 127).

Contrary to the commonly held view that insulin suppresses gluconeogenesis, studies examining in vivo metabolic flux in

mice, rats, dogs, and humans have shown overwhelmingly that physiological levels of insulin suppress EGP almost exclusively via the inhibition of glycogenolysis, with gluconeogenesis being largely resistant to suppression by insulin (2, 22, 33, 58, 60, 76, 82, 137, 142, 176). As such, to get persistent reductions in gluconeogenic flux, supraphysiological insulin concentrations are required (2, 22, 58, 60, 76, 82, 137, 142, 176). Indeed, during a euglycemic hyperinsulinemic clamp where hyperinsulinemia is maintained at a constant level over 2–3 h, EGP can be completely suppressed in healthy rodents and humans (7, 38, 147, 171). In line with the fact that small mammals are heavily reliant on gluconeogenesis, it is not surprising that rodent studies have shown only a minor (20–40%) or complete lack of suppression of EGP during physiological hyperglycemia and hyperinsulinemia observed during an OGTT (56, 94, 127, 128, 131, 148, 163). In fact, EGP has been shown to be completely suppressed only in short-term fasted rats following administration of an extremely high (4.8 g/kg) oral glucose load (162). Interestingly, the same glucose load was not able to even remotely suppress EGP following a 30-h fast, indicating that the more fasted or starved a rodent is, the more resistant the liver is to the actions of insulin and hyperglycemia (162). However, as discussed earlier, the dual-tracer constant infusion method, which was employed in this study, is prone to non-steady-state error, which may have affected the estimation of EGP. Interestingly, euglycemic hyperinsulinemic clamp studies have also suggested that 3-day-starved rats exhibit insulin unresponsiveness toward suppression of glucose production (137). Taken together, observations from the literature suggest that the more reliant an animal is on gluconeogenesis, as in rodents, the more resistant the liver is to insulin and hyperglycemia-induced suppression of EGP. Thus, in vivo it seems that gluconeogenesis in mammals remains remarkably constant across a wide variety of metabolic settings, a phenomenon most likely explained via gluconeogenic substrates substituting for one another, depending on their availability (126, 132).

Accordingly, the regulation of EGP in rodents, particularly mice, may differ from that in humans due to the inherent reliance on gluconeogenesis, high glucose turnover rates, and rapid depletion of liver glycogen stores. This is likely to have

important implications for the development and preclinical testing of potential novel antihyperglycemic agents designed to lower EGP by inhibiting gluconeogenesis (174). Indeed, because rodents have a very high reliance on gluconeogenesis, it is possible that inhibitors of this pathway may have pronounced glucose-lowering effects. However, because humans and other large mammals are less reliant on gluconeogenesis (40, 132), any such compound may have more subtle effects on glucose homeostasis.

### Conclusion

The regulation of glucose homeostasis is complex and challenging to study. There is no doubt that our understanding of the mechanisms that control glucose metabolism has benefited from the rapid increase in the use of rodent models in metabolic research. However, it is timely to highlight that although rodents may be a good model to study the basis of human disease, clear differences do exist between species with regard to metabolic regulation. For this reason, it is important for scientists in the field of diabetes research to develop a greater appreciation and understanding of the regulation of glucose homeostasis in rodents. This knowledge is essential to assist with the selection and interpretation of tests used to assess glucose homeostasis and, importantly, to inform on the processes and organs responsible for mediating phenotypic changes in glycemic control. Here, we provide evidence that in chronically high-fat-fed rodents, defects in hepatic glucose metabolism precede those in skeletal muscle, and the development of impaired glucose tolerance is more closely related to dysfunction in hepatic glucohomeostasis as opposed to that in skeletal muscle. However, the field could no doubt benefit from studies to carefully delineate and quantify the role of liver, skeletal muscle, and other organs in contributing to whole body glucose homeostasis in rodents. Finally, there is a need to develop more informative approaches to study glucose metabolism in rodents that closely reflect the dynamic conditions observed in the postprandial state such that the coordinated integration of the glucose disposal and production components, together with insulin secretion, is taken into account. This would be a transformative step not only in advancing our understanding of the regulation of glucose metabolism but also in defining the mechanisms underlying defects in glucose homeostasis in T2D.

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### AUTHOR CONTRIBUTIONS

G.M.K. and C.R.B. prepared figures; G.M.K. and C.R.B. drafted manuscript; G.M.K. and C.R.B. edited and revised manuscript; G.M.K. and C.R.B. approved final version of manuscript.

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