AMERICAN JOURNAL OF PHYSIOLOGY
ENDOCRINOLOGY AND METABOLISM

Am J Physiol Endocrinol Metab 320: E891–E897, 2021.
First published April 5, 2021; doi:10.1152/ajpendo.00628.2020

PERSPECTIVES

Insulin's First 100 Years - Where Next?

Importance of the route of insulin delivery to its control of glucose metabolism

Dale S. Edgerton, Mary C. Moore, Justin M. Gregory, Guillaume Kraft, and Alan D. Cherrington
Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee

Abstract

Pancreatic insulin secretion produces an insulin gradient at the liver compared with the rest of the body (approximately 3:1). This physiological distribution is lost when insulin is injected subcutaneously, causing impaired regulation of hepatic glucose production and whole body glucose uptake, as well as arterial hyperinsulinemia. Thus, the hepatoportal insulin gradient is essential to the normal control of glucose metabolism during both fasting and feeding. Insulin can regulate hepatic glucose production and uptake through multiple mechanisms, but its direct effects on the liver are dominant under physiological conditions. Given the complications associated with iatrogenic hyperinsulinemia in patients treated with insulin, insulin designed to preferentially target the liver may have therapeutic advantages.

CNS insulin; free fatty acids; glucagon; hepatic glucose production; hepatic glucose uptake

INTRODUCTION

Anatomically, the liver is located directly downstream of the pancreas. Consequently, blood entering the liver via the hepatic portal vein is enriched with pancreatic hormones. Because hepatic insulin extraction is normally between 40% and 60%, and pancreatic blood mixes with a relatively small pool of blood in the portal vein, the concentration of insulin entering the liver is approximately threefold higher than that seen throughout the rest of the body (1–3). This liver-to-arterial plasma insulin gradient is important to insulin physiology and is maintained during both fasting and feeding but is lost when patients with diabetes are treated with insulin via a peripheral route (e.g., subcutaneous injection) (1–3). This review will *1*) address the metabolic significance of the hepatic-to-peripheral insulin gradient and *2*) examine the importance of the direct (hepatic insulin receptor) and indirect [adipose, α cell, central nervous system (CNS)] ways in which insulin regulates glucose metabolism in vivo.

Acting as a glucose reservoir, the liver plays a key role in regulating glucose metabolism. During fasting, the liver produces glucose, whereas in the fed state, it stores it. Not only can the liver take up as much as a third of the glucose that is consumed (thus equaling muscle's rate of glucose disposal) (4, 5), but two-thirds of the typical day is spent with the liver in an uptake mode (6). Accordingly, defects in the suppression of hepatic glucose production (HGP) and/or stimulation of hepatic glucose uptake (HGU) are major contributors to diabetes (6–12). Insulin is the key regulator of liver glucose metabolism, but its effectiveness depends on several factors, including how it is distributed across the body. Of note, arterial hyperinsulinemia is associated with metabolic and cardiovascular disease and hypoglycemic risk and is a contributor to type 2 diabetes (2, 13–18).

SIGNIFICANCE OF THE HEPATIC-TO-PERIPHERAL INSULIN GRADIENT

To investigate the importance of the liver-to-arterial insulin gradient on the control of basal HGP, we performed studies using conscious dogs (19). During a pancreatic clamp, somatostatin was infused to disable the endocrine pancreas and both glucagon and insulin were replaced at basal rates into the hepatoportal circulation. After a control period, the route of insulin infusion was switched to a leg vein. Consequently, arterial insulin levels almost doubled, whereas insulin at the liver decreased by 50%, causing the portal vein-to-arterial insulin ratio to decrease from 3 to 0.8 (Fig. 1*A*). This change in insulin distribution caused a rapid increase in HGP (Fig. 1*B*), which peaked at 60 min, and caused a 35% rise in plasma glucose (Fig. 1*C*). Glucose utilization tended to increase but not until after 90 min (Fig. 1*B*), whereas HGP eventually fell due to feedback inhibition by hyperglycemia. Thus, peripherally delivered insulin was not only less effective at acutely limiting HGP but also caused hyperglycemia and peripheral hyperinsulinemia, significant risk factors for metabolic disease.

Additional studies examined the consequences of peripheral insulin delivery using insulin infusion rates that were elevated over basal. In one study (20), after a baseline period, somatostatin was given to disable the pancreas, basal

Correspondence: D. S. Edgerton (dale.edgerton@vanderbilt.edu).
Submitted 14 December 2020 / Revised 24 March 2021 / Accepted 26 March 2021

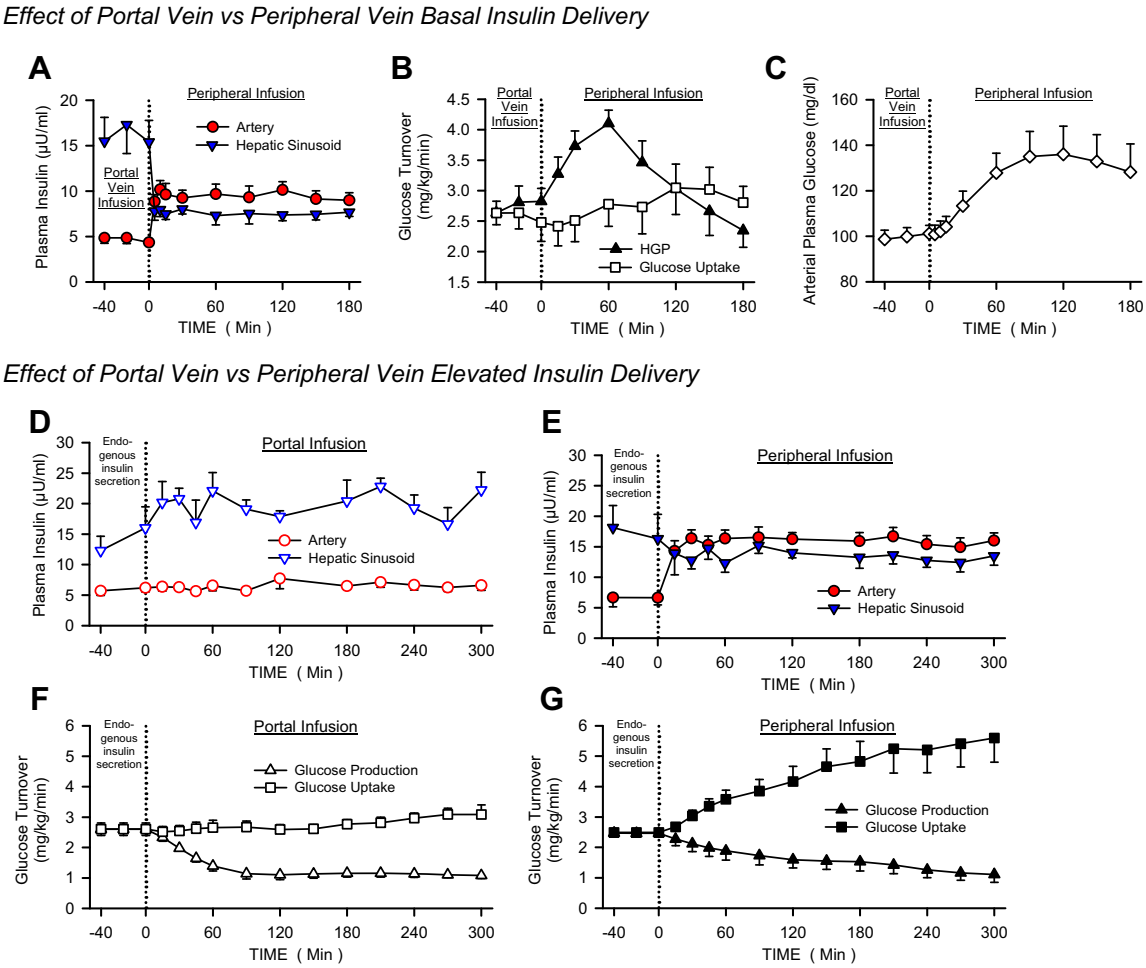


Figure 1. *A–C* adapted from the study by Edgerton et al. (19): arterial and hepatic sinusoidal insulin levels (*A*) during portal (−40 to 0 min) or peripheral vein (0 to 180 min) insulin infusion (basal rate: 200 μU/kg/min from −40 to 180 min). With portal insulin infusion during the basal period (−40 to 0 min), the normal 3 to 1 insulin gradient was present (*A*), and glucose turnover (*B*) was in steady state (i.e., rates of glucose appearance and disappearance were similar). After switching to peripheral insulin delivery (0 to 180 min), arterial insulin levels doubled, whereas hepatic levels fell by 50% (*A*). Consequently, hepatic glucose production (HGP) was rapidly stimulated (*B*), there was a slow and slight rise in whole body glucose uptake over time (*B*), and plasma glucose levels quickly increased (*C*). Published previously in Edgerton et al. (19) Insulin's direct effects on the liver dominate the control of hepatic glucose production. *J Clin Invest* 116: 521–527, 2006. Copyright 2006 by American Society for Clinical Investigation. *D–G* adapted from the study by Edgerton et al. (20): arterial and hepatic sinusoidal insulin levels (*D–E*) during a basal period (−40 to 0 min; endogenous insulin secretion), and during a euglycemic clamp (0 to 300 min) when insulin was infused (300 μU/kg/min) into either the portal vein (*D*) or a peripheral vein (*E*). With portal infusion, HGP was rapidly suppressed, whereas whole body glucose uptake was minimally affected (*F*). With peripheral delivery, HGP was suppressed more slowly and glucose uptake was greatly amplified (*G*). Published previously in Edgerton et al. (20) Changes in glucose and fat metabolism in response to the administration of a hepato-preferential insulin analog. *Diabetes* 63: 3946–3954, 2014. Copyright 2014 by the American Diabetes Association. Data are expressed as means ± SE. $P < 0.05$ vs. baseline for *A*: artery and hepatic sinusoid (5–180 min); *B*: HGP (15–30 min); *C*: glucose (60–180 min); *E*: artery (15–300 min); *F*: HGP (15–300 min) and glucose uptake (240–300 min); *G*: HGP (90–300 min) and glucose uptake (150–300 min).

glucagon was replaced intraportally, insulin was infused at a marginally elevated rate (~30%) into either the portal vein or a peripheral vein, and glucose was infused to maintain euglycemia. With portal infusion (Fig. 1*D*), hepatic insulin exposure increased by 25%, without a detectible change in the exposure of other tissues (i.e., arterial insulin remained flat). In contrast, with peripheral delivery (Fig. 1*E*), arterial insulin increased 2.5-fold, with a slight fall at the liver compared with baseline. Portal insulin infusion caused a rapid, 50% decrease in HGP, with little change in whole body glucose uptake (Fig. 1*F*). With peripheral insulin delivery, the ability of insulin to suppress glucose production was slowed, whereas the stimulation of muscle glucose uptake markedly increased relative to portal insulin delivery (Fig. 1*G*). In addition, there was greater suppression of lipolysis. Thus, small increments in portal vein insulin have major consequences on liver glucose metabolism with little effect on nonhepatic tissues. In contrast, peripherally delivered insulin cannot act on the liver without also having a profound effect on muscle and fat. Given that β cells respond sensitively to small changes in glucose, this means that the fine-tuning of fasting glucose homeostasis primarily reflects insulin's effect on liver rather than muscle or fat.

In another study (21), we evaluated the effect of altering insulin's distribution under postprandial conditions. When insulin was delivered intraportally (4-fold basal) during a hyperglycemic pancreatic clamp, glucose disposal was equally divided between the liver and muscle (46% vs. 44%,

respectively). On the other hand, with the same rate of insulin infused into a peripheral vein, the percentage of glucose taken up by muscle was fourfold greater than that taken up by the liver, and overall HGU was less than half of that which occurred with portal insulin delivery. Thus, portal insulin delivery is vital for the hormone's normal metabolic function in both the fed and fasted state, and altering the hepatic-to-peripheral insulin gradient has deleterious effects on postprandial liver, muscle, and fat metabolism.

DIRECT AND INDIRECT MECHANISMS BY WHICH INSULIN CAN REGULATE HEPATIC GLUCOSE METABOLISM

Insulin can impact the liver by direct interaction with hepatic insulin receptors but also indirectly by acting at adipose tissue to reduce circulating free fatty acids (FFAs), at the pancreatic α cell to lower glucagon, and at the brain to alter neural signaling. In a study designed to assess the liver's response to insulin's direct hepatic versus indirect effects (22), the plasma insulin level was selectively increased either at peripheral tissues (Fig. 2*A*) or at the liver (Fig. 2*B*) during a pancreatic clamp in which plasma glucagon and glucose were kept basal. HGP was suppressed under both conditions (Fig. 2*C*), clearly demonstrating that the liver can respond to either signal.

The role of FFAs in the liver's indirect response to insulin was demonstrated in a study (23) in which arterial insulin was selectively increased in two groups, with no change in insulin in the portal vein (Fig. 2, *D* and *E*). In one group, plasma FFAs were allowed to fall, whereas in the other group, triglycerides were infused to maintain FFAs at the basal level (Fig. 2*F*). Preventing the suppression of FFAs negated much of insulin's inhibitory effect on HGP (Fig. 2*G*). Likewise, in another study (24), the stimulation of HGU was reduced by half when a fall in FFAs was prevented under hyperglycemic, euinsulinemic conditions.

In addition, insulin inhibits the secretion of glucagon, thereby generating potential effects on liver glucose metabolism. In a study (25) in which basal plasma glucagon levels were reduced, whereas insulin remained basal in both the artery and portal vein, HGP was quickly suppressed. In another study (26), a fall in glucagon augmented net hepatic glucose uptake under hyperglycemic hyperinsulinemic conditions. Taken together, these data support a potential α cell role in mediating some of insulin's indirect hepatic effects.

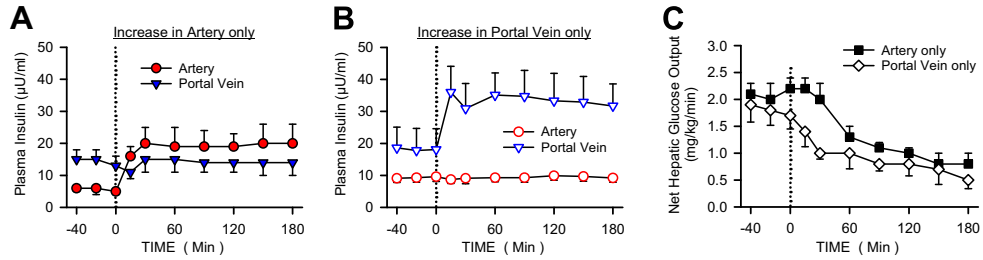
Finally, a brain-liver insulin axis appears to exist across species. In the rodent, insulin's effect on the brain involves activation of hypothalamic insulin signaling, stimulation of vagal hepatic efferent nerves, increased phosphorylation of hepatic signal transducer and activator of transcription 3 (pSTAT3), and suppression of gluconeogenic gene expression, with subsequent reduction of HGP (27–29). In the human, a pharmacological dose of insulin administered intranasally, which preferentially crosses the blood-brain barrier, was able to modestly suppress HGP during a pancreatic clamp (30), in support of a brain effect. In the dog, there is also evidence that brain insulin action can affect hepatic glucose metabolism, at least when insulin in the brain is selectively elevated (31). We infused insulin into the carotid and vertebral arteries at a rate that increased jugular insulin levels 10-fold (Fig. 2*H*), while maintaining basal insulin levels at the liver (Fig. 2*I*). CNS insulin action increased hypothalamic pAkt and hepatic pSTAT3 phosphorylation and altered glucoregulatory gene expression in the liver by 4 h (Fig. 2*J*). These effects were associated with a small reduction in net hepatic glucose balance (Fig. 2*K*), which manifested after 2 h, and appeared to be due to an increase in HGU (31). Blocking hypothalamic insulin signaling with intracerebroventricular infusion of a phosphatidylinositol 3-kinase (PI3K) inhibitor completely eliminated the effects of brain insulin action on the liver (Fig. 2, *J* and *K*).

RELEVANCE OF INSULIN'S INDIRECT EFFECTS IN THE CONTEXT OF PHYSIOLOGICAL HYPERINSULINEMIA

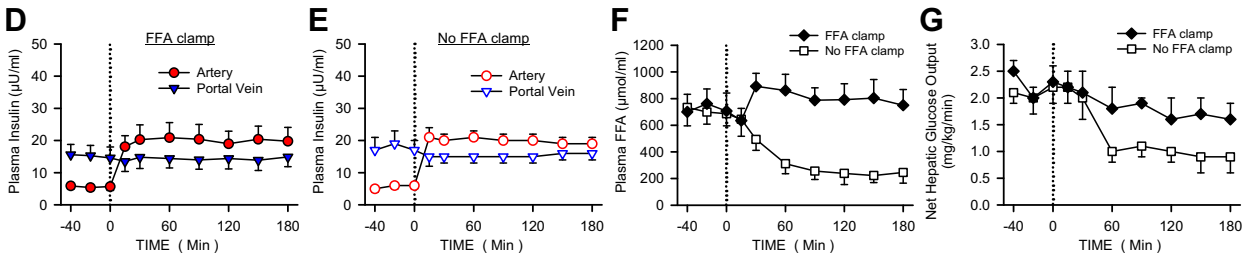
Although the aforementioned studies demonstrate the potential for indirect insulin action to regulate hepatic glucose metabolism, they do not address the significance of these mechanisms under physiological conditions. It should be noted that the normal, 3:1 insulin ratio that exists between the liver and the rest of the body is always eliminated when insulin is administered via a peripheral route (i.e., subcutaneous, peripheral vein, arteries of the brain, intranasally, inhalation, or direct brain injection). This is true even in the absence of somatostatin infusion because exogenous insulin inhibits endogenous insulin secretion (32). Loss of the physiological gradient may explain why some studies have concluded that indirect insulin action is a key controller of HGP. For example, the hepatic effects of a threefold rise in arterial insulin will be entirely indirect if brought about by peripheral insulin infusion, since hepatic insulin levels will remain basal as a result of recirculating insulin that offsets the fall in endogenous insulin secretion (20, 22, 33). It should also be noted that insulin mediates its direct hepatic effects almost entirely by modulating glycogen metabolism (22, 32, 34). Thus, when hepatic glycogen stores are exhausted, insulin's indirect gluconeogenic effects may become more important. Since liver glycogen depletion occurs slowly (over 2 days) and incompletely in the human and dog (35–37), however, this is more likely relevant in rodents that deplete liver glycogen rapidly (hours) and completely (38). With regard to insulin's effects at the brain, it should be noted that since the onset of CNS insulin action is slow (hours), the effect cannot explain the rapid suppression of HGP that occurs in response to insulin (minutes), indicating that brain insulin action does not play a role in the acute suppression of HGP (39, 40). Thus, it is important to consider experimental context when evaluating the physiological relevance of indirect insulin action.

In studies examining the physiological importance of insulin's direct versus indirect effects on the liver, we have shown that direct hepatic insulin action is capable of generating a full response by the liver, even in the absence of insulin's indirect effects (19, 32, 34, 41). For example, during postprandial-like conditions (34), the arterial glucose level was doubled while insulin was infused into the portal vein to increase its level by approximately fourfold throughout the body (Figs. 2*L* and 2*M*). Eliminating insulin's indirect effects

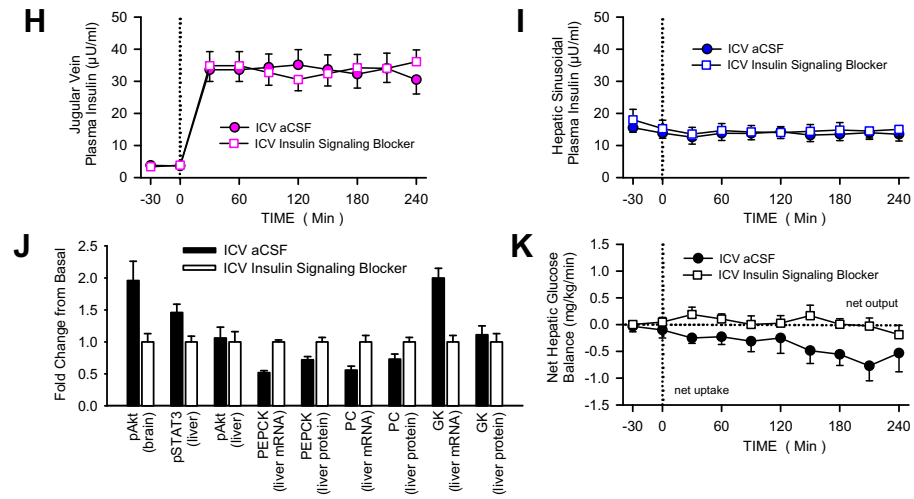
Effect of a Selective Increase in Insulin on Hepatic Glucose Production



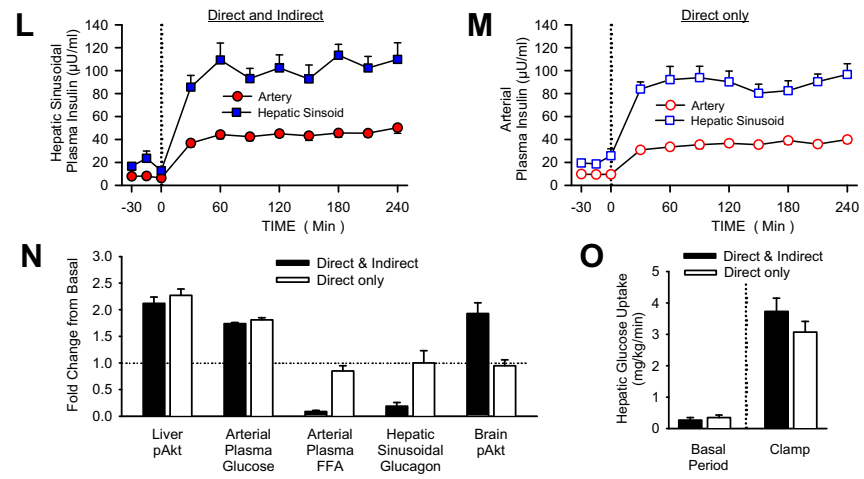
Effect of Free Fatty Acids on Indirect Hepatic Insulin Action



Response to a Selective Increase in Brain Insulin Action



Effect of Eliminating Insulin's Indirect Effects



AJP-Endocrinol Metab • doi:10.1152/ajpendo.00628.2020 • www.ajpendo.org

(Direct only group) by clamping plasma FFA and glucagon levels at basal, while also blocking CNS insulin action (Fig. 2*N*), had no impact on the liver's HGU response (Fig. 2*O*). Likewise, in a hyperinsulinemic euglycemic study (32), blocking insulin's indirect effects, individually or in combination, had no impact on HGP suppression. These data demonstrate that insulin's indirect action is not required for the full acute response of the liver to insulin in vivo.

IMPLICATIONS FOR PEOPLE WITH DIABETES

Insulin therapy is required for patients with type 1 diabetes, who have absolute insulin deficiency, and for many patients with type 2 diabetes, who have relative insulin deficiency. Although large clinical trials have shown that intensive insulin therapy decreases microvascular disease in both forms of diabetes (42, 43), the iatrogenic hyperinsulinemia that results from bypassing first-pass hepatic insulin extraction leads to many unintended metabolic consequences (2, 13–18).

Insulin resistance is an example of one of these complications (2). In previous studies, when healthy patients received a sustained intravenous insulin infusion over multiple days to raise peripheral plasma insulin to levels seen in type 1 and type 2 diabetes, their insulin sensitivity decreased by 17%–53% (44–46). By contrast, restoring portal vein insulin delivery normalizes insulin sensitivity. In one study (47), patients with type 1 diabetes received a pancreas transplant, with the pancreatic vein anastomosed to either the hepatoportal or peripheral circulation. Whereas the group with portal anastomosis had insulin sensitivity nearly matching that of healthy control subjects, insulin sensitivity in the group with peripheral anastomosis was 40% lower. Given that insulin resistance is closely associated with life-limiting cardiovascular risk in type 1 diabetes (48–50) and that iatrogenic hyperinsulinemia is the primary driver of this resistance (2), it is important that future therapies more closely imitate physiological insulin delivery.

Arterial hyperinsulinemia also contributes to glycemic variability, especially in patients with type 1 diabetes. To maintain euglycemia, enough insulin must be delivered to the liver to restrain excessive basal hepatic glucose production and to appropriately stimulate hepatic glucose uptake in the absorptive state (15). To achieve this with subcutaneous insulin, patients must deliver more insulin to peripheral tissues than would be needed with physiological, portal vein insulin delivery. Skeletal muscle represents a large, insulin sensitive mass of tissue that takes up glucose at all glycemic levels. Consequently, errors in peripheral insulin dosing and transient alterations in whole body insulin sensitivity (e.g., from exercise or stress) create greater glycemic variability compared with when insulin enters through the portal circulation. Limiting the overinsulinization of skeletal muscle may be the primary reason a hepatopreferential basal insulin analog reduced glycemic variability compared with a conventional analog in large clinical trials (51).

In the dog, a hepatopreferential insulin analog was found to functionally replicate the effect of endogenous insulin secretion with regard to normal suppression of HGP, despite peripheral delivery (20). In addition, the physiological distribution of glucose across tissues was fully normalized by the analog under postprandial-like conditions (21). These proof-of-principle studies demonstrate that therapeutic strategies that preferentially target the liver may improve diabetic outcomes, including using hepatopreferential or oral insulin

Figure 2. *A–C* adapted from the study by Sindelar et al. (22): following a basal period (−40 to 0 min), plasma insulin levels were selectively elevated, either only at the artery (*A*) or at the hepatic portal vein (*B*). Both indirect (artery only) and direct (portal vein only) insulin action suppressed net hepatic glucose output (*C*), although insulin's direct hepatic effect was more rapid. Published previously in Sindelar et al. (22) A comparison of the effects of selective increases in peripheral or portal insulin on hepatic glucose production in the conscious dog. *Diabetes* 45: 1594–1604, 1996. Copyright 1996 by the American Diabetes Association. *D–G* adapted from the study by Sindelar et al. (23): following a basal period (−40 to 0 min), arterial insulin levels were selectively increased while insulin at the liver was maintained at basal (*D* and *E*; 0 to 180 min). In one group, triglycerides plus heparin were infused to clamp arterial plasma free fatty acids (FFA) at basal, whereas in another the FFA levels were allowed to fall (*F*). Preventing the suppression of FFA eliminated much of insulin's indirect effect on net hepatic glucose output (*G*). Published previously in Sindelar et al. (23) The role of fatty acids in mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog. *Diabetes* 46: 187–196, 1997. Copyright 1997 by the American Diabetes Association. *H–K* adapted from the study by Ramnanan et al. (31): following a basal period (−30 to 0 min), infusion of insulin into the carotid and vertebral arteries increased insulin at the head 10-fold (H; 0 to 240 min), whereas insulin at the liver remained at basal (*I*). Brain insulin action activated the central nervous system (CNS)-liver insulin axis (*J*), increasing the phosphorylation of hypothalamic protein kinase B (p/Akt) and downstream, at the liver, phosphorylation of liver signal transducer and activator of transcription 3 (pSTAT3), without affecting hepatic pAkt. Hepatic gluconeogenic gene expression (phosphoenolpyruvate carboxykinase, PEPCK; and pyruvate carboxylase, PC) was suppressed, whereas glucokinase (GK) expression increased, due to insulin's CNS effect. Changes in liver protein levels were more modest or did not occur. Intraventricular (ICV) infusion of a phosphatidylinositol 3-kinase (PI3K) inhibitor (LY294002) to block the activation of hypothalamic insulin signaling eliminated these effects (*J*). Analysis was performed on tissues collected at 240 min. Increased CNS insulin action was associated with a modest and delayed suppression of net hepatic glucose balance (*K*). Published previously in Ramnanan et al. (31) Brain insulin action augments hepatic glycogen synthesis without suppressing glucose production or gluconeogenesis in dogs. *J Clin Invest* 121: 3713–3723, 2011. Copyright 2011 by American Society for Clinical Investigation. *L–O* adapted from the study by Kraft et al. (34): following a basal period (−30 to 0 min), glucose was infused to double the blood sugar whereas insulin was delivered intraportally (6-fold basal) (*L–M*) to simulate postprandial-like conditions (0 to 240 min). In one group (Direct & Indirect), all of insulin's effects were active, whereas in the other, only its direct effect was present (Direct only). In the latter, triglyceride was infused intravenously, glucagon intraportally, and an insulin receptor antagonist (S961) and K inhibitor (LY294002) were delivered into the third ventricle to prevent insulin induced suppression of plasma FFA and glucagon levels, and activation of brain insulin signaling (*N*). Analysis was performed on tissues collected at 240 min. Elimination of insulin's indirect effects had no impact on the stimulation of HGU (*O*). Published previously in Kraft et al. (34) The importance of the mechanisms by which insulin regulates meal-associated liver glucose uptake in the dog. *Diabetes*. In press. 2021, Copyright 2021 by the American Diabetes Association. Data are expressed as means ± SE. $P < 0.05$ vs. baseline for *A*: artery (15–180 min); *B*: portal vein (15–180 min); *C*: selective increase in arterial insulin (60–180 min) and selective increase in portal vein insulin (15–180 min); *D*: artery (15–180 min); *E*: artery (15–180 min); *F*: no FFA clamp (60–180 min); *G*: no FFA clamp (60–180 min) and FFA clamp (120-180 min); *H*: both groups (30–240 min); and *K*: artificial cerebrospinal fluid (aCSF) (180–240 min). $P < 0.05$ between groups for *J*: hypothalamic pAkt, liver pSTAT3, PEPCK, PC and GK mRNA, and PEPCK and PC protein; *N*: plasma FFA and glucagon and hypothalamic pAkt.

analogs, intraperitoneal insulin delivery, or a glucagon-like peptide-1 (GLP-1) agonist that increases endogenous insulin secretion.

In summary, the hepatoportal insulin gradient created by pancreatic insulin secretion is essential to both the physiological control of fasting HGP and the normal postprandial distribution of glucose across insulin sensitive tissues such as liver and muscle. Although insulin can regulate liver glucose metabolism by multiple redundant mechanisms, direct insulin action is highly sensitive and rapid. This effect is dominant, sufficient, and able to mask insulin's indirect effects. In contrast, indirect insulin action is delayed, reduced in magnitude, often of little physiological consequence, and comes at the considerable therapeutic expense of peripheral hyperinsulinemia. These data suggest that treatments that preferentially target the liver should have full hepatic efficacy and may avoid some of the negative effects associated with iatrogenic hyperinsulinemia caused by peripheral insulin treatment.

GRANTS

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Grants 2RO1-DK18243, 5P60-DK2059, ST32-DK07563, SP60-AM20593, and R01-DK43706.

DISCLOSURES

No conflict of interest for D. S. Edgerton, M. C. Moore, or G. Kraft. A. D. Cherrington has financial interests in Abvance, Biocon, Diakard/Diabetica, Fractyl, Novo Nordisk, and vTv Therapeutics.

AUTHOR CONTRIBUTIONS

D.S.E., M.C.M., G.K., and A.D.C. conceived and designed research; D.S.E., M.C.M., G.K., and A.D.C. performed experiments; D.S.E., M.C.M., G.K., and A.D.C. analyzed data; D.S.E., M.C.M., G.K., and A.D.C. interpreted results of experiments; D.S.E. prepared figures; D.S.E. drafted manuscript; D.S.E., M.C.M., J.M.G., G.K., and A.D.C. edited and revised manuscript; D.S.E., M.C.M., J.M.G., G.K., and A.D.C. approved final version of manuscript.

REFERENCES

1. **Aronoff SL**, **Berkowitz K**, **Shreiner B**, **Want L.** Glucose metabolism and regulation: beyond insulin and glucagon. *Diabetes Spectrum* 17: 183–190, 2004. doi:10.2337/DIASPECT.17.3.183.
2. **Gregory JM**, **Cherrington AD**, **Moore DJ.** The peripheral peril: injected insulin induces insulin insensitivity in type 1 diabetes. *Diabetes* 69: 837–847, 2020. doi:10.2337/dbi19-0026.
3. **Matteucci E**, **Giampietro O**, **Covolan V**, **Giustarini D**, **Fanti P**, **Rossi R.** Insulin administration: present strategies and future directions for a noninvasive (possibly more physiological) delivery. *Drug Des Devel Ther* 9: 3109–3118, 2015. doi:10.2147/DDDT.S79322.
4. **Capaldo B**, **Gastaldelli A**, **Antoniello S**, **Auletta M**, **Pardo F**, **Ciociaro D**, **Guida R**, **Ferrannini E**, **Sacca L.** Splanchnic and leg substrate exchange after ingestion of a natural mixed meal in humans. *Diabetes* 48: 958–966, 1999. doi:10.2337/diabetes.48.5.958.
5. **Ferrannini E**, **Wahren J**, **Felig P**, **DeFronzo RA.** The role of fractional glucose extraction in the regulation of splanchnic glucose metabolism in normal and diabetic man. *Metabolism* 29: 28–35, 1980. doi:10.1016/0026-0495(80)90094-3.
6. **Hwang JH**, **Perseghin G**, **Rothman DL**, **Cline GW**, **Magnusson I**, **Petersen KF**, **Shulman GI.** Impaired net hepatic glycogen synthesis in insulin-dependent diabetic subjects during mixed meal ingestion. A 13C nuclear magnetic resonance spectroscopy study. *J Clin Invest* 95: 783–787, 1995. doi:10.1172/JCI117727.
7. **Basu A**, **Basu R**, **Shah P**, **Vella A**, **Johnson CM**, **Nair KS**, **Jensen MD**, **Schwenk WF**, **Rizza RA.** Effects of type 2 diabetes on the ability of insulin and glucose to regulate splanchnic and muscle glucose metabolism: evidence for a defect in hepatic glucokinase activity. *Diabetes* 49: 272–283, 2000. doi:10.2337/diabetes.49.2.272.
8. **Basu R**, **Basu A**, **Johnson CM**, **Schwenk WF**, **Rizza RA.** Insulin dose-response curves for stimulation of splanchnic glucose uptake and suppression of endogenous glucose production differ in nondiabetic humans and are abnormal in people with type 2 diabetes. *Diabetes* 53: 2042–2050, 2004. doi:10.2337/diabetes.53.8.2042.
9. **Bischof MG**, **Bernroider E**, **Krssak M**, **Krebs M**, **Stingl H**, **Nowotny P**, **Yu C**, **Shulman GI**, **Waldhausl W**, **Roden M.** Hepatic glycogen metabolism in type 1 diabetes after long-term near normoglycemia. *Diabetes* 51: 49–54, 2002. doi:10.2337/diabetes.51.1.49.
10. **Bischof MG**, **Krssak M**, **Krebs M**, **Bernroider E**, **Stingl H**, **Waldhausl W**, **Roden M.** Effects of short-term improvement of insulin treatment and glycemia on hepatic glycogen metabolism in type 1 diabetes. *Diabetes* 50: 392–398, 2001. doi:10.2337/diabetes.50.2.392.
11. **Krssak M**, **Brehm A**, **Bernroider E**, **Anderwald C**, **Nowotny P**, **Man CD**, **Cobelli C**, **Cline GW**, **Shulman GI**, **Waldhausl W**, **Roden M.** Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. *Diabetes* 53: 3048–3056, 2004. doi:10.2337/diabetes.53.12.3048.
12. **Ludvik B**, **Nolan JJ**, **Roberts A**, **Baloga J**, **Joyce M**, **Bell JM**, **Olefsky JM.** Evidence for decreased splanchnic glucose uptake after oral glucose administration in non-insulin-dependent diabetes mellitus. *J Clin Invest* 100: 2354–2361, 1997. doi:10.1172/JCI119775.
13. **Corkey BE.** Banting lecture 2011: hyperinsulinemia: cause or consequence? *Diabetes* 61: 4–13, 2012. doi:10.2337/db11-1483.
14. **Ferrannini E**, **Haffner SM**, **Mitchell BD**, **Stern MP.** Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. *Diabetologia* 34: 416–422, 1991. doi:10.1007/BF00403180.
15. **Gregory JM**, **Kraft G**, **Scott MF**, **Neal DW**, **Farmer B**, **Smith MS**, **Hastings JR**, **Allen EJ**, **Donahue EP**, **Rivera N**, **Winnick JJ**, **Edgerton DS**, **Nishimura E**, **Fledelius C**, **Brand CL**, **Cherrington AD.** Insulin delivery into the peripheral circulation: a key contributor to hypoglycemia in type 1 diabetes. *Diabetes* 64: 3439–3451, 2015. doi:10.2337/db15-0071.
16. **Schwartz SS**, **Jellinger PS**, **Herman ME.** Obviating much of the need for insulin therapy in type 2 diabetes mellitus: a re-assessment of insulin therapy's safety profile. *Postgrad Med* 128: 609–619, 2016. doi:10.1080/00325481.2016.1191955.
17. **Templeman NM**, **Skovso S**, **Page MM**, **Lim GE**, **Johnson JD.** A causal role for hyperinsulinemia in obesity. *J Endocrinol* 232: R173–R183, 2017. doi:10.1530/JOE-16-0449.
18. **Wang X**, **Yu C**, **Zhang B**, **Wang Y.** The injurious effects of hyperinsulinism on blood vessels. *Cell Biochem Biophys* 69: 213–218, 2014. doi:10.1007/s12013-013-9810-6.
19. **Edgerton DS**, **Lautz M**, **Scott M**, **Everett CA**, **Stettler KM**, **Neal DW**, **Chu CA**, **Cherrington AD.** Insulin's direct effects on the liver dominate the control of hepatic glucose production. *J Clin Invest* 116: 521–527, 2006. doi:10.1172/JCI27073.
20. **Edgerton DS**, **Moore MC**, **Winnick JJ**, **Scott M**, **Farmer B**, **Naver H**, **Jeppesen CB**, **Madsen P**, **Kjeldsen TB**, **Nishimura E**, **Brand CL**, **Cherrington AD.** Changes in glucose and fat metabolism in response to the administration of a hepato-preferential insulin analog. *Diabetes* 63: 3946–3954, 2014. doi:10.2337/db14-0266.
21. **Edgerton DS**, **Scott M**, **Farmer B**, **Williams PE**, **Madsen P**, **Kjeldsen T**, **Brand CL**, **Fledelius C**, **Nishimura E**, **Cherrington AD.** Targeting insulin to the liver corrects defects in glucose metabolism caused by peripheral insulin delivery. *JCI Insight* 5: e126974, 2019. doi:10.1172/jci.insight.126974.
22. **Sindelar DK**, **Balcom JH**, **Chu CA**, **Neal DW**, **Cherrington AD.** A comparison of the effects of selective increases in peripheral or portal insulin on hepatic glucose production in the conscious dog. *Diabetes* 45: 1594–1604, 1996. doi:10.2337/diabetes.45.11.1594.
23. **Sindelar DK**, **Chu CA**, **Rohlie M**, **Neal DW**, **Swift LL**, **Cherrington AD.** The role of fatty acids in mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog. *Diabetes* 46: 187–196, 1997. doi:10.2337/diabetes.46.2.187.
24. **Moore MC**, **Satake S**, **Lautz M**, **Soleimanpour SA**, **Neal DW**, **Smith M**, **Cherrington AD.** Nonesterified fatty acids and hepatic glucose metabolism in the conscious dog. *Diabetes* 53: 32–40, 2004. doi:10.2337/diabetes.53.1.32.

25. **Cherrington AD**, **Chiasson JL**, **Liljenquist JE**, **Jennings AS**, **Keller U**, **Lacy WW.** The role of insulin and glucagon in the regulation of basal glucose production in the postabsorptive dog. *J Clin Invest* 58: 1407–1418, 1976. doi:10.1172/JCI108596.
26. **Holste LC**, **Connolly CC**, **Moore MC**, **Neal DW**, **Cherrington AD.** Physiological changes in circulating glucagon alter hepatic glucose disposition during portal glucose delivery. *Am J Physiol Endocrinol Metab* 273: E488–E496, 1997. doi:10.1152/ajpendo.1997.273.3.E488.
27. **Inoue H**, **Ogawa W**, **Asakawa A**, **Okamoto Y**, **Nishizawa A**, **Matsumoto M**, **Teshigawara K**, **Matsuki Y**, **Watanabe E**, **Hiramatsu R**, **Notohara K**, **Katayose K**, **Okamura H**, **Kahn CR**, **Noda T**, **Takeda K**, **Akira S**, **Inui A**, **Kasuga M.** Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. *Cell Metab* 3: 267–275, 2006. doi:10.1016/j.cmet.2006.02.009.
28. **Obici S**, **Zhang BB**, **Karkanias G**, **Rossetti L.** Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Med* 8: 1376–1382, 2002. doi:10.1038/nm1202-798.
29. **Pocai A**, **Lam TK**, **Gutierrez-Juarez R**, **Obici S**, **Schwartz GJ**, **Bryan J**, **Aguilar-Bryan L**, **Rossetti L.** Hypothalamic K(ATP) channels control hepatic glucose production. *Nature* 434: 1026–1031, 2005. doi:10.1038/nature03439.
30. **Dash S**, **Xiao C**, **Morgantini C**, **Koulajian K**, **Lewis GF.** Intranasal insulin suppresses endogenous glucose production in humans compared with placebo in the presence of similar venous insulin concentrations. *Diabetes* 64: 766–774, 2015. doi:10.2337/db14-0685.
31. **Ramnanan CJ**, **Saraswathi V**, **Smith MS**, **Donahue EP**, **Farmer B**, **Farmer TD**, **Neal D**, **Williams PE**, **Lautz M**, **Mari A**, **Cherrington AD**, **Edgerton DS.** Brain insulin action augments hepatic glycogen synthesis without suppressing glucose production or gluconeogenesis in dogs. *J Clin Invest* 121: 3713–3723, 2011. doi:10.1172/JCI45472.
32. **Edgerton DS**, **Kraft G**, **Smith M**, **Farmer B**, **Williams PE**, **Coate KC**, **Printz RL**, **O'Brien RM**, **Cherrington AD.** Insulin's direct hepatic effect explains the inhibition of glucose production caused by insulin secretion. *JCI Insight* 2: e91863, 2017. doi:10.1172/jci.insight.91863.
33. **Farmer TD**, **Jenkins EC**, **O'Brien TP**, **McCoy GA**, **Havlik AE**, **Nass ER**, **Nicholson WE**, **Printz RL**, **Shiota M.** Comparison of the physiological relevance of systemic vs. portal insulin delivery to evaluate whole body glucose flux during an insulin clamp. *Am J Physiol Endocrinol Metab* 308: E206–E222, 2015. doi:10.1152/ajpendo.00406.2014.
34. **Kraft G**, **Coate KC**, **Smith M**, **Farmer B**, **Scott M**, **Cherrington AD**, **Edgerton DS.** The importance of the mechanisms by which insulin regulates meal-associated liver glucose uptake in the dog. *Diabetes*, 2021. doi:10.2337/db20-1271.
35. **Chandramouli V**, **Ekberg K**, **Schumann WC**, **Kalhan SC**, **Wahren J**, **Landau BR.** Quantifying gluconeogenesis during fasting. *Am J Physiol Endocrinol Metab* 273: E1209–E1215, 1997. doi:10.1152/ajpendo.1997.273.6.E1209.
36. **Edgerton DS**, **Ramnanan CJ**, **Grueter CA**, **Johnson KM**, **Lautz M**, **Neal DW**, **Williams PE**, **Cherrington AD.** Effects of insulin on the metabolic control of hepatic gluconeogenesis in vivo. *Diabetes* 58: 2766–2775, 2009. doi:10.2337/db09-0328.
37. **Rothman DL**, **Magnusson I**, **Katz LD**, **Shulman RG**, **Shulman GI.** Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with 13C NMR. *Science* 254: 573–576, 1991. doi:10.1126/science.1948033.
38. **Chen C**, **Williams PF**, **Cooney GJ**, **Caterson ID**, **Turtle JR.** The effects of fasting and refeeding on liver glycogen synthase and phosphorylase in obese and lean mice. *Horm Metab Res* 24: 161–166, 1992. doi:10.1055/s-2007-1003285.
39. **Dash S**, **Xiao C**, **Morgantini C**, **Koulajian K**, **Lewis GF.** Is insulin action in the brain relevant in regulating blood glucose in humans? *J Clin Endocrinol Metab* 100: 2525–2531, 2015. doi:10.1210/jc.2015-1371.
40. **Edgerton DS**, **Cherrington AD.** Is brain insulin action relevant to the control of plasma glucose in humans? *Diabetes* 64: 696–699, 2015. doi:10.2337/db14-1666.
41. **Ramnanan CJ**, **Kraft G**, **Smith MS**, **Farmer B**, **Neal D**, **Williams PE**, **Lautz M**, **Farmer T**, **Donahue EP**, **Cherrington AD**, **Edgerton DS.** Interaction between the central and peripheral effects of insulin in controlling hepatic glucose metabolism in the conscious dog. *Diabetes* 62: 74–84, 2013. doi:10.2337/db12-0148.
42. **Nathan DM**, **Genuth S**, **Lachin J**, **Cleary P**, **Crofford O**, **Davis M**, **Rand L**, **Siebert C**; **Diabetes Control Complications Trial Research Group.** The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329: 977–986, 1993. doi:10.1056/NEJM199309303291401.
43. **UK Prospective Diabetes Study Group**. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352: 837–853, 1998.
44. **Del Prato S**, **Leonetti F**, **Simonson DC**, **Sheehan P**, **Matsuda M**, **DeFronzo RA.** Effect of sustained physiologic hyperinsulinaemia and hyperglycaemia on insulin secretion and insulin sensitivity in man. *Diabetologia* 37: 1025–1035, 1994. doi:10.1007/BF00400466.
45. **Marangou AG**, **Weber KM**, **Boston RC**, **Aitken PM**, **Heggie JC**, **Kirsner RL**, **Best JD**, **Alford FP.** Metabolic consequences of prolonged hyperinsulinemia in humans. Evidence for induction of insulin insensitivity. *Diabetes* 35: 1383–1389, 1986. doi:10.2337/diab.35.12.1383.
46. **Rizza RA**, **Mandarino LJ**, **Genest J**, **Baker BA**, **Gerich JE.** Production of insulin resistance by hyperinsulinaemia in man. *Diabetologia* 28: 70–75, 1985. doi:10.1007/BF00279918.
47. **Carpentier A**, **Patterson BW**, **Uffelman KD**, **Giacca A**, **Vranic M**, **Cattral MS**, **Lewis GF.** The effect of systemic versus portal insulin delivery in pancreas transplantation on insulin action and VLDL metabolism. *Diabetes* 50: 1402–1413, 2001. doi:10.2337/diabetes.50.6.1402.
48. **Nadeau KJ**, **Regensteiner JG**, **Bauer TA**, **Brown MS**, **Dorosz JL**, **Hull A**, **Zeitler P**, **Draznin B**, **Reusch JE.** Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. *J Clin Endocrinol Metab* 95: 513–521, 2010. doi:10.1210/jc.2009-1756.
49. **Rodrigues TC**, **Biavatti K**, **Almeida FK**, **Gross JL.** Coronary artery calcification is associated with insulin resistance index in patients with type 1 diabetes. *Braz J Med Biol Res* 43: 1084–1087, 2010. doi:10.1590/s0100-879x2010007500109.
50. **Schauer IE**, **Snell-Bergeon JK**, **Bergman BC**, **Maahs DM**, **Kretowski A**, **Eckel RH**, **Rewers M.** Insulin resistance, defective insulin-mediated fatty acid suppression, and coronary artery calcification in subjects with and without type 1 diabetes: the CACTI study. *Diabetes* 60: 306–314, 2011. doi:10.2337/db10-0328.
51. **Jacober SJ**, **Prince MJ**, **Beals JM**, **Hartman ML**, **Qu Y**, **Linnebjerg H**, **Garhyan P**, **Haupt A.** Basal insulin peglispro: overview of a novel long-acting insulin with reduced peripheral effect resulting in a hepato-preferential action. *Diabetes Obes Metab* 18: 3–16, 2016. doi:10.1111/dom.12744.