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Changes in Glucose and Fat Metabolism in Response to the Administration of a Hepato-Preferential Insulin Analog

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PHARMACOLOGY AND THERAPEUTICS

Endogenous insulin secretion exposes the liver to three times higher insulin concentrations than the rest of the body. Because subcutaneous insulin delivery eliminates this gradient and is associated with metabolic abnormalities, functionally restoring the physiologic gradient may provide therapeutic benefits. The effects of recombinant human insulin (HI) delivered intraportally or peripherally were compared with an acylated insulin model compound (insulin-327) in dogs. During somatostatin and basal portal vein glucagon infusion, insulin was infused portally (PoHI; 1.8 pmol/kg/min; *n* = 7) or peripherally (PeHI; 1.8 pmol/kg/min; *n* = 8) and insulin-327 (Pe327; 7.2 pmol/kg/min; *n* = 5) was infused peripherally. Euglycemia was maintained by glucose infusion. While the effects on liver glucose metabolism were greatest in the PoHI and Pe327 groups, nonhepatic glucose uptake increased most in the PeHI group. Suppression of lipolysis was greater during PeHI than PoHI and was delayed in Pe327 infusion. Thus small increments in portal vein insulin have major consequences on the liver, with little effect on nonhepatic glucose metabolism, whereas insulin delivered peripherally cannot act on the liver without also affecting nonhepatic tissues. Pe327 functionally restored the physiologic portal–arterial gradient and thereby produced hepato-preferential effects.

Secretion of insulin into the hepatic portal vein results in preferential exposure of the liver to insulin. Studies of humans (1–3), dogs (4–7), and rats (8) have shown that insulin concentrations are typically two- to fourfold greater in the portal vein than in the arterial circulation during steady-state conditions. Subcutaneous injection of insulin into diabetic patients cannot recreate the normal portal-to-arterial insulin gradient, leading instead to relative overinsulinization of peripheral tissues (such as muscle, fat, and the vasculature) and relative underinsulinization of the liver (9). Exposure of nonhepatic tissues to excess insulin has metabolic and therapeutic implications. For example, arterial hyperinsulinemia may cause insulin resistance (leading to hyperglycemia) (10–12), coagulation abnormalities (13,14), weight gain (15–17), and alterations in body fat distribution and lipid metabolism, leading to hypertriglyceridemia and low HDL levels (18). Excess insulin is also a risk factor for hypoglycemia, hypertension, atherosclerosis, and long-term micro- and macrovascular complications, including coronary and ischemic heart disease (18–29). At the same time, hepatic hypoinsulinemia contributes to excessive glucose production (30) and can alter the growth hormone–IGF-I axis (16,31,32). In comparison with peripheral insulin administration, portal vein or intraperitoneal insulin delivery improved glucose control (reducing daily glucose fluctuations and the frequency of serious hypoglycemic episodes), normalized hepatic glucose production, limited weight gain, and decreased the requirement for antihypertensive therapy in patients with type 1 or 2 diabetes mellitus (33–41). The hope, therefore, is that a peripherally delivered, hepato-preferential insulin analog could functionally restore the physiologic insulin gradient between the liver and peripheral tissues and thereby help correct the metabolic abnormalities associated with subcutaneous insulin delivery.

Several therapeutic strategies are being used to address the need for a more physiologic distribution of insulin

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action, including implantation of continuous intraperitoneal insulin pumps (38) and the development of oral insulin analogs (42). Another approach is to modify insulin, making it hepato-preferential despite peripheral delivery (43,44). As recently reviewed (9), insulin must first cross the tight endothelial capillary barrier at muscle and fat to reach its receptor, whereas the fenestrated sinusoids of the liver are relatively open to larger plasma constituents. This study tested the effect of an insulin molecule (insulin-327), which was acylated with a 22-carbon-length fatty diacid to promote strong but reversible binding to plasma albumin. The intention was to make it hepato-preferential by altering distribution such that insulin-327 levels would be relatively higher in the interstitial space of the liver compared with muscle and fat.

While previous studies have demonstrated metabolic advantages of portal versus peripheral insulin delivery, in this study it was necessary to quantify the magnitude of the abnormalities in glucose metabolism and lipolysis when regular (unmodified) human insulin (HI) is administered peripherally (as occurs in the subcutaneous treatment of diabetic patients) as opposed to intraportally (as occurs with endogenous secretion) at the same rate. These responses then were used to assess the hepato-preferential effectiveness of peripherally delivered insulin-327. With peripheral delivery of regular insulin there was reduced suppression of glucose production and greater stimulation of glucose uptake relative to the effects of the same amount of insulin infused intraportally. On the other hand, the consequences of peripheral delivery of acylated insulin-327 more closely resembled those of portal insulin infusion.

RESEARCH DESIGN AND METHODS

Animals and Surgical Procedures

Studies of 20 conscious dogs of either sex (20–23 kg) that were fasted for 18 h were carried out. The surgical and animal care facilities met the standards published by the American Association for Assessment and Accreditation of Laboratory Animal Care, and diet and housing were provided as previously described (45). The protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Approximately 16 days before study the animals underwent surgery for placement of a sampling catheter in a femoral artery and portal vein infusion catheters in the splenic and jejunal veins. In addition, in a subset of animals (n = 4 in each group) sampling catheters also were inserted into the hepatic and portal veins, and ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic portal vein and the hepatic artery, as described previously (45). The proximal ends of the catheters and flow probes were tucked into subcutaneous pockets at the end of the surgical procedure. All dogs were determined to be healthy before experimentation, as indicated by *1*) leukocyte count $<$18,000/mm^3; *2*) hematocrit $>$35%; and *3*) good appetite (consuming at least 75% of the daily ration). The morning of the experiment the catheters and flow probe leads were exteriorized from their subcutaneous pockets under local anesthesia. Intravenous (IV) catheters also were inserted into peripheral leg veins for infusion of glucose and hormones as necessary.

Experimental Design

Each experiment consisted of a 100-min tracer equilibration period (−140 to −40 min), a 40-min period for basal sample collection (−40 to 0 min), and a 300-min experimental period (0–300 min). At −140 min, a primed continuous IV infusion of [3-^{3}H]-glucose (42 μCi prime and 0.35 μCi/min continuous rate; PerkinElmer, Shelton, CT) was started to calculate endogenous glucose production and uptake. At 0 min, somatostatin (0.8 μg/kg/min; Bachem, Torrance, CA) was infused to suppress pancreatic insulin and glucagon secretion, and glucagon was replaced intraportally at a basal rate (0.5 ng/kg/min). Also at 0 min, regular HI (1.8 pmol/kg/min) was infused into either the portal vein (PoHI; n = 7) or a peripheral vein (PeHI; n = 8). In an additional group, insulin-327 (Novo Nordisk A/S, Copenhagen, Denmark) was infused into a peripheral vein (Pe327; 7.2 pmol/kg/min; n = 5). Glucose was infused intravenously as needed to maintain euglycemia in each group. Based on pilot studies (data not shown), the insulin-327 infusion rate was selected so as to require a glucose infusion rate that was approximately equal to the rate necessary in the PeHI group.

Hematocrit, plasma glucose, [3-^{3}H]glucose, insulin, glucagon, cortisol, nonesterified fatty acids (NEFAs), and blood glycerol concentrations were determined as described previously (45).

Insulin-327

Insulin-327 (A22Lys[N^{ε}(S)-(22,42-dicarboxy-10,19,24-trioxo-3,6,12,15-tetraoxa-9,18,23-triazadotetracontan-1-oyl)], B29Arg, desB30 HI) is an acylated model compound designed to test the pharmacodynamic effects on hepatic versus nonhepatic glucose metabolism. Fatty acid acylation of the insulin molecule promotes binding to albumin, which leads to a protracted mode of action in a manner similar to insulin detemir (46).

Calculations and Data Analysis

Net hepatic glucose balance was calculated in a subset of animals (n = 4 in each group) as $LOAD_{out} - LOAD_{in}$. The $LOAD_{in} = ([A] \times F_A) + ([P] \times F_P)$ and $LOAD_{out} = ([H] \times F_H)$, where A, P, and H refer to the arterial, portal vein, and hepatic vein glucose concentrations, respectively, and F_A, F_P, and F_H refer to the arterial, portal vein, and hepatic vein (total liver) blood flow. Nonhepatic glucose uptake equaled the glucose infusion rate minus net hepatic glucose balance; the rate was corrected for changes in the size of the glucose pool using a pool fraction of 0.65 mL/kg (47) and assuming that the volume of distribution for glucose equaled the volume of the extracellular fluid, or ~22% of the dog's weight (48). For all glucose balance

calculations, glucose concentrations were converted from plasma to blood values using correction factors (ratio of the blood to the plasma concentration) previously established in our laboratory (49,50). Glucose turnover, used to estimate endogenous glucose production and whole-body glucose uptake, was measured using 3-^{3}H glucose infusion based on the circulatory model described by Mari et al. (51).

The approximate plasma insulin concentration entering the liver sinusoids was calculated in a subset of animals (n = 4 in each group) using the equation [A] × $\%F_A$ + [P] × $\%F_P$, where [A] and [P] are arterial and portal vein hormone concentrations, respectively, and $\%F_A$ and $\%F_P$ are the respective percent contributions of arterial and portal flow to total hepatic blood flow, respectively. Whole-body (arterial) insulin clearance was determined by dividing the insulin infusion rate by its arterial concentration. Net hepatic insulin fractional extraction was calculated by dividing net hepatic insulin uptake by hepatic insulin $LOAD_{in}$.

Hepatic insulin preferentiality was determined by dividing the average increase in nonhepatic glucose uptake by the average decrease in net hepatic glucose balance and by dividing the average increase in tracer-determined glucose uptake by the average decrease in endogenous glucose production during insulin infusion.

Statistical Analysis

Statistical comparisons were carried out with SigmaStat (Systat Software, San Jose, CA) using ANOVA for repeated measures and Student-Newman-Keuls post hoc analysis. Statistical significance was accepted when $P < 0.05$. Data are expressed as mean ± SEM.

RESULTS

During the basal period, portal vein canine insulin concentrations (measured in a subset of animals in each group) were approximately threefold greater than arterial insulin concentrations as a result of β-cell secretion (2.8 ± 0.7-, 2.8 ± 0.5-, and 3.2 ± 0.6-fold in the PoHI, PeHI, and Pe327 groups, respectively). Somatostatin, which completely eliminates endogenous insulin secretion when given at this rate in the dog (52), was infused during the experimental period to ensure that all the insulin in the circulation at that time was of exogenous origin. Infusion of insulin into the portal vein (PoHI) at 1.8 pmol/kg/min did not result in a detectable increase in the arterial insulin concentration, whereas there was a 27% increase in portal vein insulin (107 ± 7 to 147 ± 9 pmol/L; Fig. 1*A*). The concentrations of insulin entering the liver (combination of the arterial and portal vein concentrations) were 95 ± 5 and 119 ± 8 pmol/L during the basal and experimental periods, respectively, whereas the concentrations leaving (hepatic vein) were 50 ± 7 and 54 ± 7 pmol/L. Peripheral infusion of insulin (PeHI) at 1.8 pmol/kg/min increased arterial concentrations about 2.5-fold (39 ± 7 to 95 ± 8 pmol/L; Fig. 1*B*) and led to a small decrease (19%) in insulin in the portal vein. In this case, the concentrations of insulin entering the liver were 84 ± 19 and 81 ± 9 pmol/L and the concentrations leaving the liver were 45 ± 13 and 41 ± 10 pmol/L in the two periods, respectively. The portal-to-arterial insulin gradient remained >3 during the experimental period in the PoHI group, whereas it was <1 in the PeHI group.

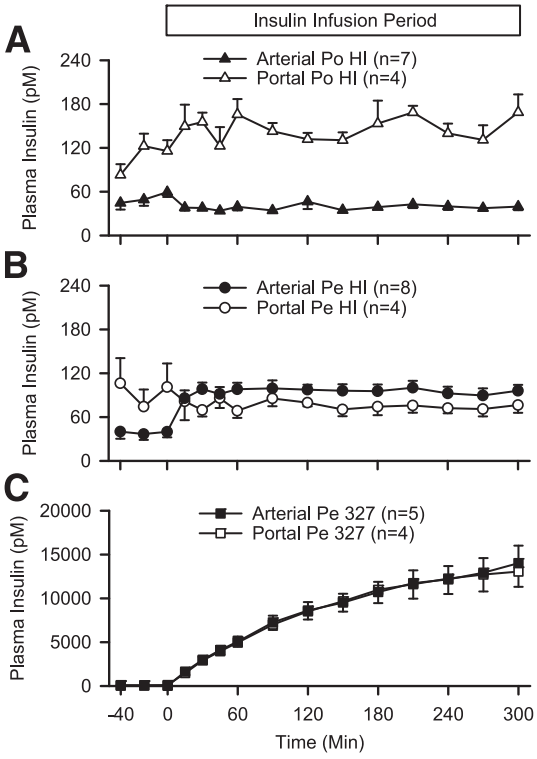

Figure 1—Arterial and portal vein insulin concentrations in conscious dogs fasted overnight during the basal (−40 to 0 min) and experimental periods (0–300 min) in the PoHI (*A*), PeHI (*B*), and Pe327 groups (*C*) (mean ± SEM; n = 7, 8, 5 for arterial insulin in the 3 groups, respectively; portal insulin also was measured in a subset of 4 animals from each group).

In the Pe327 group the arterial, portal, and hepatic vein concentrations of insulin-327 gradually increased to 13,036, 12,674, and 10,850 pmol/L, respectively, by the last hour of the experiment (Fig. 1*C*). It should be noted that these represent the total (bound and free) concentrations of the compound, the majority of which was presumably inactive (unable to bind to the insulin receptor) because of albumin binding. The portal-to-arterial insulin-327 gradient was <1 during the experimental period.

Whole-body insulin clearance was significantly greater in the PoHI compared with the PeHI group (51 ± 5 vs. 20 ± 2 mL/kg/min, respectively) because of differences in liver insulin exposure rather than effects on the hepatic fractional extraction of insulin (53 ± 5 vs. 51 ± 6%, respectively). Steady-state plasma insulin concentrations were rapidly achieved in the HI groups (<30 min), whereas insulin-327 concentrations were still increasing

slightly even during the fifth hour of infusion. The dissimilarity in kinetics between HI and insulin-327 was primarily a function of the markedly lower clearance of Pe327 (1 ± 0 mL/kg/min during the infusion period), which was presumably secondary to binding of insulin-327 to albumin. Plasma glucagon and cortisol concentrations were basal and similar between groups throughout the experiment (Table 1).

Arterial plasma glucose concentrations remained basal (approximately 110 mg/dL) in all groups during the clamp period (Fig. 2*A*). Despite identical molar HI infusion rates, more than twice as much glucose was required to maintain euglycemia in the PeHI group (4.3 ± 0.9 mg/kg/min; last hour of the clamp; Fig. 2*B*) as in the PoHI group (1.9 ± 0.3 mg/kg/min). The molar insulin-327 infusion rate was chosen so that the glucose infusion rate in the Pe327 group (4.1 ± 0.3 mg/kg/min) matched what was required in the PeHI group.

Tracer-determined endogenous glucose production (Fig. 3*A*) was rapidly inhibited by the small increase in the insulin concentration that occurred within the liver sinusoids when insulin was infused intraportally. Because there was little change in the arterial plasma insulin concentrations, whole-body glucose uptake (Fig. 3*B*) remained essentially unaltered in the PoHI group. In contrast, peripheral vein insulin infusion rapidly stimulated glucose uptake, whereas glucose production was suppressed more slowly. Peripheral delivery of insulin-327, on the other hand, inhibited endogenous glucose production as rapidly as portal vein insulin infusion but to a greater extent. In addition, stimulation of glucose uptake was delayed for several hours in the Pe327 group, after which glucose uptake increased to an intermediate rate compared with the PoHI and PeHI groups.

Net hepatic glucose balance and nonhepatic glucose uptake were measured in a subset of animals from each group (Fig. 4), and the data are in close agreement with the glucose turnover results. Portal insulin delivery rapidly and completely inhibited net hepatic glucose output, whereas there was only a minimal effect on nonhepatic glucose uptake. Peripheral delivery of HI, in contrast, had a modest effect on net hepatic glucose balance, which tended to become apparent during the last hour of the study. Nonhepatic glucose uptake, however, was stimulated immediately, and it increased steadily. Pe327 rapidly suppressed net hepatic glucose output such that there was actually a low rate of glucose uptake by the liver at the end of the experiment. The stimulation of nonhepatic glucose uptake by insulin-327 was delayed, eventually ending up intermediate to the rates in the PoHI and PeHI groups.

Insulin can indirectly modulate hepatic and muscle glucose metabolism by regulating lipolysis. There was a slow and subtle suppressive effect of intraportal insulin administration on lipolysis, consistent with the notion that there was actually a small increase in arterial insulin concentrations in that group. In contrast, peripheral insulin infusion rapidly (<60 min) reduced arterial NEFA and glycerol concentrations (Fig. 5). During the first hour of the clamp the NEFA and glycerol concentrations in the Pe327 group were similar to those observed in the PoHI group. During the second hour their concentrations began to decline, and by the end of the third hour they had converged with the concentrations found in the PeHI group.

An index of the hepato-preferential nature of insulin (whether resulting from the portal versus peripheral route of delivery of HI or modification of the hormone) can be derived by comparing insulin's effects on the liver relative to muscle. Linear regression of the increase in whole-body glucose uptake compared with the decrease in endogenous glucose production in different time intervals during the clamp studies illustrates this relationship (Fig. 6). Whereas portal vein insulin inhibited glucose production without much of a consequence on glucose uptake (indicated by a flat regression line), this was not the case for peripheral HI. The steeper slope of the PeHI regression line indicates that when insulin is delivered peripherally it cannot decrease glucose production without also increasing glucose uptake. Pe327 was clearly hepato-preferential compared with PeHI early in the infusion period (Fig. 6*A*) but became less so as the experiment went on (Fig. 6*B*).

DISCUSSION

This study first quantified the effects of the route of insulin delivery (intraportal or peripheral) on hepatic and

Table 1—Plasma glucagon and cortisol concentrations between groups during the basal and experimental periods

		Experimental period (min)				
	Basal period	60	120	180	240	300
Arterial plasma glucagon (pg/mL)						
PoHI	50 ± 4	53 ± 4	52 ± 3	50 ± 2	48 ± 4	49 ± 4
PeHI	48 ± 4	53 ± 6	52 ± 6	48 ± 6	46 ± 4	47 ± 4
Pe327	44 ± 7	43 ± 2	41 ± 2	40 ± 2	41 ± 2	39 ± 1
Arterial plasma cortisol (μg/dL)						
PoHI	3 ± 1	3 ± 1	4 ± 2	2 ± 0	3 ± 1	2 ± 1
PeHI	4 ± 1	4 ± 0	3 ± 1	3 ± 1	4 ± 1	4 ± 1
Pe327	4 ± 1	3 ± 1	3 ± 0	3 ± 0	4 ± 1	3 ± 1

Data are mean ± SEM; n = 7, 8, and 5 in the PoHI, PeHI, and Pe327 groups, respectively.

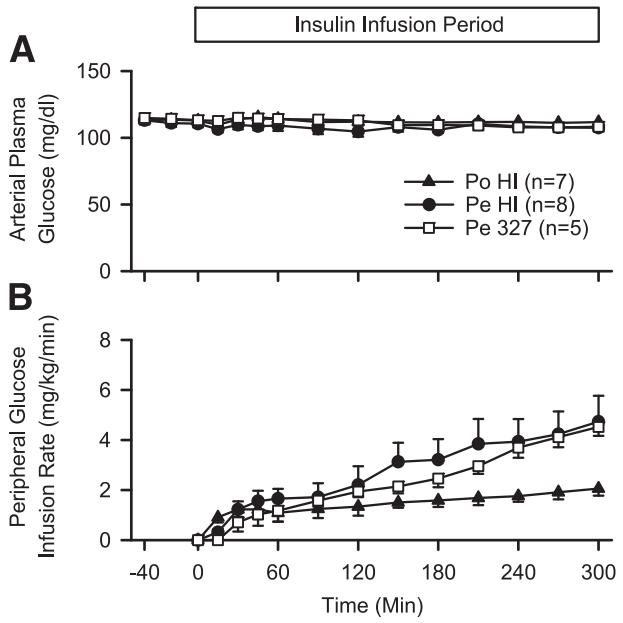

Figure 2—Arterial plasma glucose concentration (*A*) and peripheral vein glucose infusion rate (*B*) in conscious dogs fasted overnight during the basal (−40 to 0 min) and experimental periods (0–300 min) in the PoHI, PeHI, and Pe327 groups (mean ± SEM; $n = 7, 8, 5$ in the 3 groups, respectively).

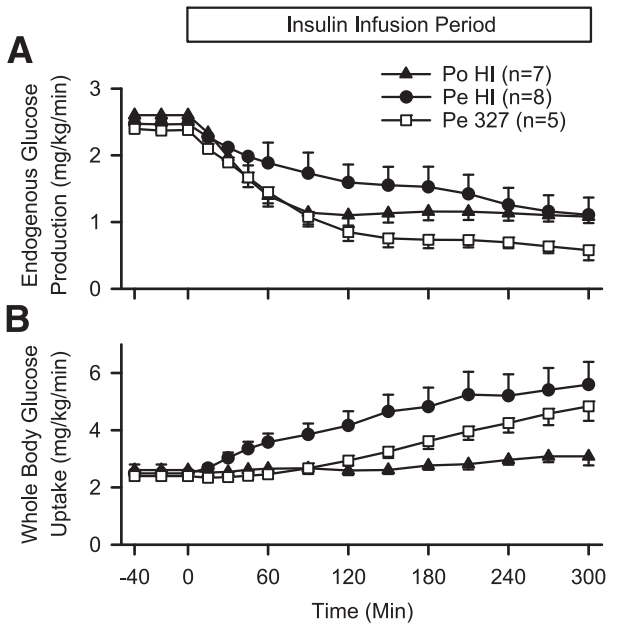

Figure 3—Tracer-determined endogenous glucose production (*A*) and whole-body glucose uptake (*B*) in conscious dogs fasted overnight during the basal (−40 to 0 min) and experimental periods (0–300 min) in the PoHI, PeHI, and Pe327 groups (mean ± SEM; $n = 7, 8, 5$ in the 3 groups, respectively). Panel *A*: $P < 0.05$ for PeHI vs. PoHI at 90 min and for PeHI vs. Pe327 between 120 and 180 min. Panel *B*: $P < 0.05$ for PeHI vs. PoHI between 120 and 300 min, for PeHI vs. Pe327 between 90 and 210 min, and for PoHI vs. Pe327 between 240 and 300 min.

nonhepatic glucose metabolism. It next examined the effects of a prototype insulin analog, which was modified to act hepato-preferentially despite being infused peripherally. As a result of the direct effects of insulin on the liver, portal vein insulin infusion caused a more rapid and pronounced suppression of hepatic glucose production and less of an increase in glucose uptake when compared with the effect of the same amount of insulin delivered peripherally. In fact, even when the suppression of glucose production was nearly complete, portal vein insulin infusion had little, if any, effect on peripheral glucose metabolism. In contrast, peripheral delivery of insulin could not appropriately reduce hepatic glucose production without also increasing glucose uptake by nonhepatic tissues. The metabolic response to infusion of insulin-327 more closely resembled that of portal rather than peripheral vein insulin infusion, especially during the first several hours of administration.

Peripheral hyperinsulinemia resulting from subcutaneous insulin injection is associated with negative clinical consequences, including hypoglycemia, excessive glycemic fluctuations, insulin resistance, weight gain, hypertension, atherosclerosis, and micro- and macrovascular disease—complications that may be reduced when the portal-to-arterial insulin gradient is normalized. For example, because portally delivered insulin has a greater effect on the liver than on peripheral tissues, and muscle has a very large capacity to take up glucose even when the blood glucose is low, hepato-preferential insulin may reduce the risk of hypoglycemia, as shown in studies in which insulin was delivered into the peritoneum (the majority of intraperitoneal insulin is absorbed into the hepatic portal vein) (34,36). Thus hepato-preferential insulin analogs may provide clinical benefits. At the same time, not all studies have shown a superiority of intraportal versus peripheral delivery of insulin. For example, some investigators found that drainage into the portal vein following pancreatic transplant did not offer a major metabolic advantage over a systemic shunt, even though the patients were exposed to peripheral hyperinsulinemia with the latter (20,53–56). It is likely, however, that some of the complications of subcutaneous insulin injection are avoided in these transplant patients because the insulin concentrations would be much more precisely regulated in the closed-loop system involving pancreatic insulin secretion. Nevertheless, it will be important to more clearly establish the efficacy of hepato-preferential insulin analogs in the clinical setting.

Insulin suppresses hepatic glucose production through both its direct (hepatic) and indirect (primarily via the inhibition of lipolysis) effects (4,6), although the direct effects of the hormone have been shown to be dominant (30). This observation was confirmed in the current study. Despite rapid and pronounced suppression of lipolysis and elevated concentrations of insulin in the brain during peripheral insulin delivery, portal vein insulin infusion reduced hepatic glucose production to a greater extent. Likewise, the hepato-preferential effects of Pe327 delivery

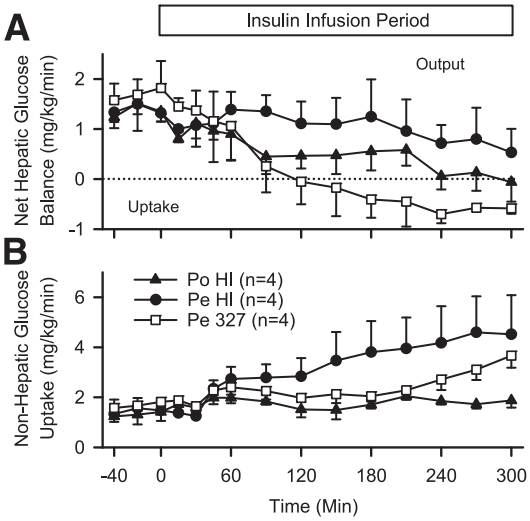

Figure 4—Net hepatic glucose balance (*A*) and nonhepatic glucose uptake (*B*) in conscious dogs fasted overnight during the basal (−40 to 0 min) and experimental periods (0–300 min) in the PoHI, PeHI, and Pe327 groups (mean ± SEM; n = 4, 4, 4 in the 3 groups, respectively). Panel *A*: $P < 0.05$ for PeHI vs. Pe327 at 180 min. Panel *B*: $P < 0.05$ for PeHI vs. PoHI between 180 and 300 min, for PeHI vs. Pe327 between 180 and 210 min, and for PoHI vs. Pe327 at 300 min.

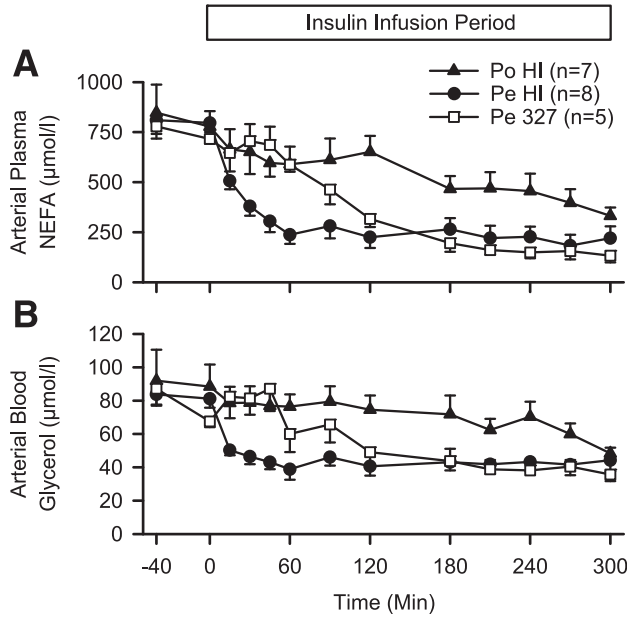

Figure 5—Arterial plasma NEFAs (*A*) and arterial blood glycerol (*B*) in conscious dogs fasted overnight during the basal (−40 to 0 min) and experimental periods (0–300 min) in the PoHI, PeHI, and Pe327 groups (mean ± SEM; n = 7, 8, 5 in the 3 groups, respectively). Panel *A*: $P < 0.05$ for PeHI vs. PoHI between 30 and 270 min, for PeHI vs. Pe327 between 30 and 60 min, and for PoHI vs. Pe327 between 120 and 240 min. Panel *B*: $P < 0.05$ for PeHI vs. PoHI between 15 and 240 min, for PeHI vs. Pe327 between 15 and 45 min, and for PoHI vs. Pe327 between 120 and 240 min.

were not reliant on the suppression of lipolysis; glucose production began to decrease in response to the analog before a decrease in circulating NEFAs. In addition, later in the study, when NEFA concentrations were very similar in the PeHI and Pe327 groups, insulin-327 still had a greater suppressive effect on glucose production. Because NEFAs play an important role in hypoglycemic counterregulation (57), the more physiologic effect of a hepato-preferential insulin analog on lipolysis may also provide a clinical benefit.

Issues such as kinetic properties, duration of action, and stability of the formulation will play a role in determining the clinical suitability of the insulin analogs that will be developed. While acylated insulin (insulin-327), PEGylated insulin (LY2605541), and thyroxyl insulin each demonstrate hepato-preferential effects, differences in the experimental designs used to characterize each of the analogs, such as unprimed (insulin-327) or primed (43) IV infusion and subcutaneous delivery (44), as well as unmatched pharmacodynamics (e.g., glucose infusion rates) between studies make it difficult to directly compare the action of these analogs. The purpose of this study was to determine whether acylation would impart hepato-preferentiality rather than to characterize the analog in a clinical setting. Further modification of the molecule will be necessary to create kinetics suitable for therapeutic intervention.

Duration of effect and hormone concentration are other important considerations when evaluating the effects of an analog. While the hepato-preferential effects of PoHI were fairly steady over time, infusion of insulin-327 led to time-dependent changes in glucose uptake by nonhepatic tissues and in lipolysis (i.e., the effects of insulin-327 became less hepato-preferential as the experiment progressed). The magnitude of insulin's effect on liver, muscle, or fat will depend both on its concentration (with higher concentrations favoring a larger effect on muscle) and on its rate of movement across the interstitial barrier in each tissue. Because insulin-327 levels increased progressively in this study, the relative importance of time versus concentration with regard hepatic insulin preferentiality cannot be distinguished. While the liver is very sensitive to subtle changes in portal vein insulin concentrations (as demonstrated in this study), these effects saturate at a much lower insulin concentration than does the effect on muscle (58). Maximal suppression of endogenous glucose production (from about 3 to 0 mg/kg/min) occurs quite quickly (in minutes) and at relatively low concentrations of insulin (i.e., threefold basal), whereas glucose uptake by muscle and fat continues to increase over a broad range of increments in insulin, as well as over time (eventually exceeding 20 mg/kg/min after several hours at high concentrations in the dog) (59). Thus, as the circulating level and time of exposure increase, insulin becomes less hepato-preferential. Of note, because the dose of insulin-327 was chosen to match the glucose requirement of the PeHI group, the study was intentionally biased toward not observing

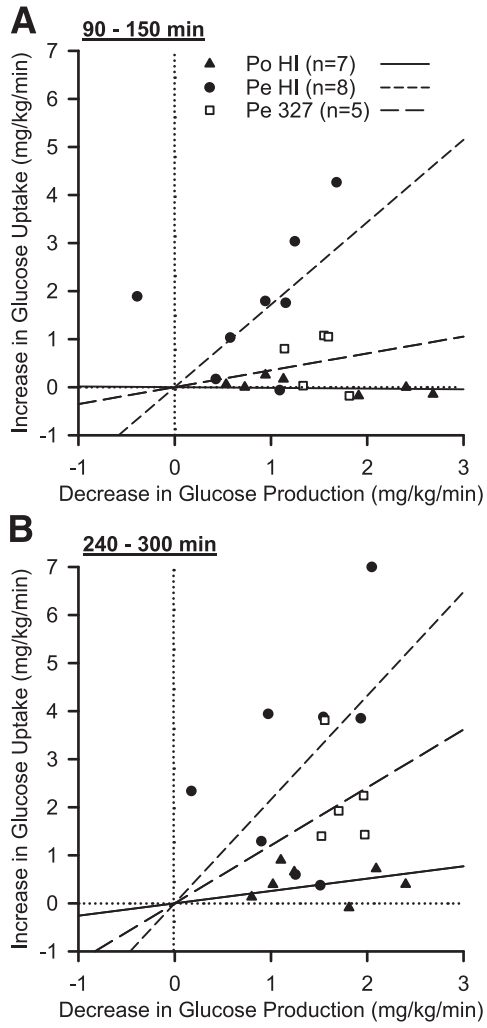

Figure 6—Change from basal tracer-determined glucose uptake relative to change from basal endogenous glucose production in conscious dogs fasted overnight between 90 and 150 min (*A*) and 240 and 300 min (*B*) of the experimental period in the PoHI (solid regression line), PeHI (medium dashed line), and Pe327 groups (large dashed line) (mean ± SEM; n = 7, 8, 5 in the 3 groups, respectively).

hepato-preferential effects. It is interesting to observe that the hepatic effects of Pe327 were apparent almost immediately, as occurred with portal insulin infusion. This is likely because of the fenestrated nature of the liver, which allows large plasma constituents (such as albumin-bound insulin) greater access to hepatic insulin receptors (9). While the concept that albumin-bound insulin can give rise to hepato-preferential effects has been previously examined using insulin detemir (60), because of the longer length of the fatty acid in insulin-327 (C-22 versus C-14 in insulin detemir), there is a stronger interaction with plasma albumin, which is expected to give rise to more pronounced hepatic preferentiality. Later in the study, when the concentrations of insulin-327 had increased substantially, hepato-preferentiality was reduced. Greater hepato-preferentiality may have been maintained if insulin-327 had been rapidly brought to a steady state with primed infusion and then maintained at a lower level.

In addition, compared with inhibition of tracer-determined glucose production, changes in net hepatic glucose balance provide a greater dynamic range for the assessment of hepato-preferentiality; this parameter incorporates both reduction of liver glucose output and stimulation of hepatic glucose uptake. Thus, when comparing the average increase in tracer-determined glucose uptake with the average decrease in production between 90 and 150 min (1.5 to 2.5 h into the insulin infusion period), this ratio was 0.01 in the PoHI group (reflecting very little change in uptake) and 2.06 and 0.37 in the PeHI and Pe327 groups, respectively, suggesting that although Pe327 was not as hepato-preferential as PoHI, it was much more so than PeHI. On the other hand, when the average increase in nonhepatic glucose uptake was compared with the decrease in net hepatic glucose balance during the same time period, the PoHI and Pe327 ratios were both more similar to each other and more different from PeHI (0.33, 7.94, and 0.26 in the PoHI, PeHI, and Pe327 groups, respectively).

This study clearly demonstrates the differential effects of intraportal compared with peripheral insulin delivery on glucose kinetics. Small increments in insulin within the portal vein have major consequences on the liver, with little effect on nonhepatic glucose metabolism. In contrast, when insulin is delivered peripherally it cannot act on the liver without also generating glucoregulatory effects at nonhepatic tissues. In addition, insulin-327 demonstrates that fatty acid conjugation to a modified insulin backbone is an approach that can produce hepato-preferential effects that help correct the metabolic abnormalities associated with peripheral insulin delivery. Further studies will be required to assess the clinical benefits of such analogs.

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Author Contributions. D.S.E. designed and carried out the studies, interpreted data, and drafted the manuscript. M.C.M., J.J.W., M.S., and B.F. participated in the studies and reviewed the manuscript. H.N., C.B.J., P.M., T.B.K., E.N., and C.L.B. contributed to the development of insulin-327, designed the studies, and reviewed the manuscript. A.D.C. designed the studies, interpreted data, and wrote the manuscript. A.D.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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