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Comparison of insulins detemir and glargine: effects on glucose disposal, hepatic glucose release and the central nervous system

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Abstract

Aims—The effects of insulins detemir (Det) and glargine (Glar) on endogenous glucose production (EGP) and net hepatic glucose output (NHGO) were compared.

Materials and methods—Arteriovenous difference and tracer ([3-³H]glucose) techniques were employed during a 2-step hyperinsulinemic euglycemic clamp in conscious dogs (6 groups, $n=5-6$ /group). After equilibration and basal sampling (0–120 min), somatostatin was infused and basal glucagon was replaced intraportally. Det or Glar were infused via portal vein (Po), peripheral vein (IV), or bilateral carotid and vertebral arteries (H) at 0.1 and 0.3 mU·kg⁻¹·min⁻¹ (Low Insulin; Glar vs Det, respectively, 120–420 min) and 4× the Low Insulin rate (High Insulin; 420–540 min).

Results—NHGO and EGP were suppressed and glucose R_d and infusion rate were stimulated similarly by Det and Glar at both Low and High Insulin with each infusion route. Nonesterified fatty acid concentrations during Low Insulin were 202±37 vs 323±75 μM in DetPo and GlarPo ($P<0.05$), and 125±39 vs 263±48 μM in DetIV and GlarIV, respectively ($P<0.05$). In DetH vs GlarH, pAkt/Akt (1.7±0.2 vs 1.0±0.2) and pSTAT3/STAT3 (1.4±0.2 vs 1.0±0.1) were significantly increased in the liver but not in the hypothalamus.

Conclusions—Insulins detemir and glargine have similar net effects on acute regulation of hepatic glucose metabolism in vivo regardless of delivery route. Portal and IV detemir delivery reduces circulating NEFA to a greater extent than glargine, and head detemir infusion enhances molecular signaling in the liver. These findings indicate a need for further examination of insulin detemir's central and hepatic effects.

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Conflict of Interest/Author Contributions: M.C. Moore directed the design and conduct of the studies, the interpretation of the data, and the preparation of the manuscript; she was the recipient of an investigator-initiated grant from Novo Nordisk. S. Boysen was in charge of detemir analysis. She is an employee of Novo Nordisk A/S. The other contributors were involved in data collection and analysis and have contributed to the preparation of the manuscript. They have no disclosures.

Keywords

insulin analogues; insulin therapy; insulin signaling; liver; hepatic glucose production; STAT3

Introduction

Basal-bolus insulin therapy is a common treatment in type 1 diabetes and is also used in some patients with type 2 diabetes that have progressed to insulin therapy. Insulin glargine and insulin detemir, two newer insulin analogues designed for basal coverage, have recently been compared to the older NPH insulin in a meta-analysis [1]. Both insulin analogues resulted in lower HBA_{1c} concentrations than NPH, and in addition insulin detemir resulted in both a reduction in episodes of severe and of nocturnal hypoglycemia and a smaller weight gain compared with NPH [1].

An important role of an insulin preparation meant to provide basal insulin concentrations and action is to restrain endogenous glucose production (EGP), which is primarily hepatic glucose production [2]. Although insulin detemir has been found to be effective in regulating EGP, insulin detemir has been shown to have a greater effect than NPH insulin on EGP at equivalent hypoglycemia [3], the manner in which it acts at the liver is not established. However, hypothalamic insulin signaling offers a possible unifying explanation for insulin detemir's ability to regulate EGP while averting weight gain. In rats, a decrease in insulin receptor number in the arcuate nucleus resulted in hyperphagia, as well as impaired insulin-mediated suppression of EGP [4]. Thus the finding of rapid, enhanced action of insulin detemir on insulin signaling in the hypothalamus [5] suggests that insulin detemir might act in this manner to regulate EGP. Nevertheless, it is impossible from the data available to rule out other mechanisms, i.e., insulin detemir's effect could be a product of indirect effects on the liver mediated by lipolytic suppression [6] and/or a direct action on the hepatocyte [7].

The question of which effect(s) of insulin detemir – indirect via modulation of circulating NEFA and/or actions in the central nervous system or direct actions on the liver – are most significant in regulation of EGP remains unanswered. Since insulin glargine and insulin detemir are completely different in their approach to creating basal insulin coverage, it is also of interest to compare their effects in a model in which it is possible to measure hepatic glucose balance. The dog model is one that has been used in numerous studies of direct vs indirect actions of insulin on the liver [8-11], because it is one of the few models available that allows simultaneous sampling from the artery, portal vein, and hepatic vein. In addition, measurement of insulin in the venous effluent from the brain is possible in the dog. Moreover, taking samples of liver and hypothalamic tissue at the end of study enable comparison of insulins detemir and glargine with regard to insulin signaling in the two tissues. Therefore these studies were carried out in the chronically catheterized conscious dog, with the goal of comparing the effects of insulin detemir and insulin glargine on hepatic glucose metabolism and insulin signaling. The insulin analogues were infused into the portal vein, the normal route of insulin delivery to the liver; into a peripheral vein, allowing assessment of indirect effects on the liver; and into the carotid and vertebral arteries to allow assessment of the effects of an enrichment of insulin in the central nervous system.

Materials and Methods

Animals and surgical procedures

Studies were carried out on 33 conscious 18-h-fasted adult mongrel male dogs weighing 22±1 kg. Diet and housing were as previously described [12], and the protocol was approved

by the Vanderbilt University Institutional Animal Care and Use Committee. Approximately 16 days before study, each dog underwent a laparotomy for placement of ultrasonic flow probes (Transonic Systems, Ithaca, NY) around the portal vein and the hepatic artery for measurement of total hepatic blood flow, as well as insertion of silicone rubber catheters for sampling in a hepatic vein, the portal vein, and a femoral artery and for infusion into a splenic and a jejunal vein as described in detail elsewhere [12]. A subset of 11 of the dogs (Head or H groups) underwent a second surgery 7-8 days after the first surgery for insertion of infusion catheters bilaterally into the carotid and vertebral arteries and a sampling catheter into the left jugular vein [8]. Criteria for health of the animals prior to study were as previously described [8]. On the morning of the study, catheters and flow probe leads were exteriorized from their subcutaneous pockets under local anesthesia.

Experimental design

Each experiment consisted of a 90-min equilibration period (0-90 min), a 30-min basal period (90 to 120 min), and a 420-min experimental period divided into two sub-periods (Low Insulin, 120-420 min; High Insulin, 420-540 min). At 0 min, a primed ($39 \mu\text{Ci/kg}$), continuous ($0.014 \mu\text{Ci}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) infusion of [$3\text{-}^3\text{H}$]glucose was begun. At 0 min, a constant peripheral infusion of somatostatin ($0.8 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; Bachem, Torrance, CA) was begun to suppress endogenous insulin and glucagon secretion. In 4 pilot studies, two with insulin detemir and two with insulin glargine, the insulins were infused stepwise at a range of rates so as to determine the infusion rate that would reduce the EGP approximately 50% when the infusate was delivered intraportally. The rates determined were 0.3 and $0.1 \mu\text{U}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for insulins glargine (Glar; sanofi-aventis, Paris, France) and detemir (Det; Novo Nordisk, Bagsværd, Denmark), respectively. Insulin was infused at these rates from 0-300 min (Period 1, or P1) in the subsequent studies, and it was delivered into either the portal vein via the splenic and jejunal catheters (GlarPo and DetPo groups; $n = 6/\text{group}$), into a peripheral vein (GlarIV and DetIV groups, $n = 5/\text{group}$, respectively), or bilaterally into the carotid and vertebral arteries (GlarH and DetH; $n = 5$ and 6 , respectively; 60% into the carotid and 40% into the vertebral arteries). In the dog, infusion into the head arteries perfuses the entire brain vasculature [13]. The relative potency of insulin detemir varies by species due to differences in albumin binding, with the potency being relatively greater in the dog than in the human [14, 15]. The relative potency difference between insulins detemir and glargine is similar to that between insulin detemir and regular insulin when administered IV to dogs. In all experiments, glargine infusate was prepared by adding the insulin to normal saline, pH 5.5, containing 3% v:v of the dog's own plasma, and insulin detemir was prepared in a solution containing NaCl, 140 mM; polysorbate 20, 70 ppm; and NaH_2PO_4 , 5mM, pH 7.4. Beginning at 0 min, glucose was infused via a peripheral vein as needed to maintain euglycemia, and glucagon was replaced intraportally in basal amounts ($0.57 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; GlucaGen, Novo Nordisk) beginning at 120 min. From 300-420 min (Period 2, or P2), the infusion rates of the insulin analogues were increased 4-fold, to stimulate whole body glucose disposal.

Femoral artery, portal vein, and hepatic vein blood samples were taken every 15-30 min throughout the study; arterial samples were also taken every 5 min throughout the experimental period to monitor the plasma glucose level and allow appropriate adjustment of the glucose infusion rate [12]. Blood pressure and heart rate were obtained continuously with an arterial transducer (DigiMed, Micro-Med Inc, Louisville, KY). At the end of study, the animal was anesthetized, and biopsies from the liver and hypothalamus were rapidly removed and frozen in liquid nitrogen.

Hematocrit; blood lactate, and glycerol; and plasma glucose, nonesterified fatty acids (NEFA), canine insulin, glucagon, and cortisol were measured as described previously [16]. Insulin detemir was assayed by a modified LOCI immunoassay with an analogue-specific

antibody using the method described by Poulsen and Jensen [17]. Insulin concentrations in the Glar groups were analyzed by RIA as previously described [18]. Analysis of immunoreactive canine C-peptide was determined via double-antibody radioimmunoassay (Millipore, Billerica, MA) and found to be below the limit of detection during the clamp period, confirming that there was no endogenous insulin secretion in any of the groups. Activation of PI3K signal transduction was assessed by measuring phosphoserine Akt (serine residue 473) in hypothalamic and liver extracts by ELISA assay (Biosource, Camarillo, CA). Total and phosphorylated Akt and STAT3 were also assessed by Western blot and densitometry quantification. The pSTAT3 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and all other antibodies were purchased from Cell Signaling (Beverly, MA).

Calculations and data analysis

The hepatic load_{in} was calculated as $(A \times AF) + (P \times PF)$, where A and P are the arterial and portal vein glucose concentrations and AF and PF are the hepatic artery and portal vein blood flows, respectively. Load_{out} was calculated as $H \times HBF$, where H is the hepatic vein glucose concentration and HBF is total hepatic blood flow. Net hepatic balance (NHB) was load_{out} minus load_{in}. Net hepatic fractional extraction was calculated as $NHB \div load_{in}$, and nonhepatic glucose uptake equaled the total glucose infusion rate minus NHB of glucose, adjusted for changes in the glucose pool. Conversion factor for glucose concentrations: mmol/l = mg/dl $\times$ 0.05551; conversion of glucose load and balance data: $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} = \text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1} \times 5.551$. For all glucose balance calculations, glucose concentrations were converted from plasma to blood values by using correction factors (ratio of the blood to the plasma concentration) previously established in our laboratory [12, 19]. Hepatic sinusoidal concentrations of insulin and glucagon were calculated as for load_{in}, with the results divided by the total hepatic flow.

EGP and glucose disappearance (R_d) were calculated with a 2-compartment model using dog parameters [20, 21].

Statistical analysis

Data are presented as means $\pm$ SE. Time course data were analyzed with repeated-measures ANOVA, and Tukey's test was used for *post hoc* comparisons (SigmaStat, Systat Software, San Jose, CA). Statistical significance was accepted at $P < 0.05$. The data presented for P1 or P2 are the means of the last hour of each period.

Results

Hormone concentrations

During the basal period and during portal insulin infusion of insulin glargine, the hepatic sinusoidal plasma insulin concentration was approximately 2-3 times as great as the arterial plasma concentration as a result of the higher portal vs arterial insulin concentrations (Fig. 1; summarized in Table 1). However, during IV or head Glar infusion, the arterial-portal vein gradient was reversed, so that the arterial concentration was greater than the hepatic sinusoidal concentration ($P < 0.05$ for both P1 and P2 with both infusion routes). There were no differences in the arterial or hepatic sinusoidal insulin concentrations in the GlarH and GlarIV groups. The insulin glargine concentrations in the brain of the GlarH dogs, as reflected in the jugular vein concentrations (Table 1), were approximately 2- and 1.5-fold greater (Low and High Insulin infusion periods, respectively) than the arterial concentrations in GlarH and GlarIV. During Det infusion, there were no significant differences between the arterial and hepatic sinusoidal Det concentrations with any route of delivery, and there was

no difference between the jugular vein concentrations and the arterial or hepatic sinusoidal concentrations in DetH.

There were no significant differences among groups in the concentrations of plasma glucagon (Table 1) or cortisol (data not shown), and both glucagon and cortisol remained at basal concentrations throughout the experiments.

Glucose concentrations and metabolism

Arterial plasma glucose concentrations were clamped at basal levels in all groups (Fig. 2). Hepatic blood flow did not differ significantly among groups at any time (data not shown). Basal net hepatic glucose outputs (NHGO) in GlarPo and DetPo were 1.8 ± 0.2 and 1.6 ± 0.1 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively; during Low Insulin they declined to 0.5 ± 0.2 and 0.8 ± 0.2 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively (NS between groups). During High Insulin they fell to rates no different than zero in both groups. In GlarIV and DetIV, NHGO fell to 0.2 ± 0.3 and 0.5 ± 0.5 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during Low Insulin; during High Insulin both groups exhibited net hepatic glucose uptake (0.8 ± 0.3 and 0.3 ± 0.3 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (NS between groups). The responses in GlarH and DetH were not different from those in GlarIV and DetIV. Similarly, the fall in EGP, the increase in glucose disappearance (R_d), and the glucose infusion rates (GIR) did not differ between GlarPo and DetPo, between GlarIV and DetIV, or between GlarH and DetH (Fig. 2). In addition, there were no differences between GlarIV and GlarH or between DetIV and DetH in NHGO, EGP, glucose R_d , or GIR.

Nonesterified fatty acids (NEFA) and glycerol

NEFA concentrations declined from basal in all groups during insulin analogue infusion (Fig. 3). During Low Insulin the concentrations were significantly less in DetPo than in the corresponding Glar groups. The change from basal in DetIV at the low rate of insulin infusion was also significantly greater than that in GlarIV (907 ± 82 vs 613 ± 44 $\mu\text{mol/l}$, $P < 0.05$). During High Insulin, there were no evident differences among groups in NEFA concentrations. Glycerol concentrations fell significantly below basal in all groups during Low Insulin and remained suppressed in all groups during High Insulin. There were no significant differences between Glar and Det with any infusion route. Net hepatic glycerol uptake fell in parallel with the concentrations and did not differ among the groups (Table 2). The rate of net hepatic glycerol uptake during the basal period was high (3.1 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in one animal in the DetH group, and it remained elevated during insulin infusion, increasing the variance in that group. If the data from that animal were excluded, net hepatic glycerol uptake in the DetH group would have averaged 1.7 ± 0.2 , 0.8 ± 0.1 , and 0.8 ± 0.1 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during the Basal, Low Insulin and High Insulin periods, respectively (repeated measures ANOVA: $P = 0.41$ vs GlarH if the outlier animal is excluded, compared with $P = 0.27$ when that animal is included). Th3 dog that was an outlier in terms of glycerol was not dropped from the analysis, however, because all of his other data were similar to those in the other animals in the group.

Arterial blood lactate concentrations did not differ between the analogues with any infusion route (Table 2). All groups exhibited net hepatic lactate uptake or a very low rate of net hepatic lactate output in the basal period. During Low Insulin, all groups exhibited net hepatic lactate output, with the rate tending to wane during High Insulin.

Akt (protein kinase B or PKB) and signal transducer and activator of transcription 3 (STAT3)

ELISA and Western blotting indicated that phosphorylation of Akt in the liver but not the hypothalamus was significantly greater in DetH than in GlarH (liver data in Fig. 4; data for the hypothalamus not shown). There were no differences between Det and Glar when

delivered via the other routes. STAT3 phosphorylation was also significantly enhanced in liver but not hypothalamus of DetH vs GlarH; again, there were no differences between Det and Glar when they were delivered intraportally or IV (Fig. 4). The data passed the tests for normality and equal variances, and therefore a t-test was used for comparing differences between analogues with the different delivery routes. Nevertheless, the differences between groups were modest and the variances tended to be large. Therefore, the 95% confidence intervals for the data are also presented in Table 3. As in Fig. 4, the data in Table 3 are consistent with a small effect of head infusion of Det but not Glar to enhance phosphorylation of Akt and STAT3 in the liver.

Discussion

The first step in carrying out these experiments was establishing biological equivalence of the analogues in the dog model, since insulin detemir is known to have different potencies in different species [15]. To do this, pilot studies were carried out to establish analogue infusion rates that would suppress tracer-determined glucose EGP 50% during portal delivery. These infusion rates were subsequently used during the Low Insulin infusion period in all experiments. During the High Insulin period, the infusion rates were increased 4-fold to stimulate glucose R_d ; the rates were selected to fall on the linear portion of the insulin dose-response [2]. As expected, the suppression of glucose EGP and of NHGO in the GlarPo and DetPo groups were not different in either the Low or High Insulin infusion periods. Neither were glucose R_d or the GIRs different during the two infusion periods.

Infusion of regular insulin into the portal vein under clamp conditions in the dog maintains the normal arterial-portal insulin gradient (ie, insulin concentrations in the portal vein $\approx$ 3-fold higher than those in the artery) [e.g., 8]. The gut extracts $\approx$ 30% of the insulin presented to it (whether the insulin is endogenous or is infused as regular insulin), and therefore the portal vein concentration is lower than that in the artery during peripheral venous regular insulin infusion [e.g., 8]. Because the portal vein contributes $\approx$ 80% of the total hepatic blood in the absence of clamp conditions and $\approx$ 75% of the total during somatostatin infusion, the hepatic sinusoidal insulin concentration follows the pattern of that in the portal vein. The current data demonstrate that these typical responses with endogenous and regular insulin were also observed in GlarPo and GlarIV. Insulin extraction by the brain is extremely low [13, 22, 23], and thus the circulating concentrations of Glar in GlarIV vs GlarH were not different. The jugular vein Glar concentrations were significantly higher than those in the artery in the GlarH group, however, similar to the gradient observed previously in the dog during head infusion of regular insulin [24]. Approximately 98-99% of insulin detemir in the circulation is albumin-bound [25, 26], with the large pool of serum albumin delaying detemir clearance and maintaining relatively high circulating concentrations as it serves as a depot for the analogue [14, 15]. With endogenous insulin secretion or the infusion of regular insulin, the canine liver extracts $\approx$ 50-60% of the insulin presented to it and the human liver may extract somewhat more [27-30]. In the current investigation, the extraction of insulin glargine was similar to that of regular insulin, resulting in portal concentrations that were $\approx$ 3-fold higher than those in the artery during portal insulin infusion. Because of the large albumin-bound fraction, there was no systematic gradient of insulin detemir among any of the blood vessels with any of the routes of infusion. At steady state, the absolute rate of liver and peripheral clearance of insulin detemir has been shown to similar to that of regular insulin, but because the concentration of insulin detemir is much higher, the percentage cleared is much less [31]. It is possible that a different time course of action for the two insulin analogues might impact their ability to stimulate phosphorylation of hepatic Akt and STAT3 when infused into the head arteries. Generally the onset and time course of glargine and detemir action on glucose metabolism has been found to be very similar in various species when the insulin analogues are administered subcutaneously [15, 32, 33]. The

modification of the insulin molecule in insulin glargine serves to delay its absorption from the subcutaneous tissue [34], but once in the bloodstream, its action is remarkably similar to that of regular insulin, and it has a similar rapid rate of activation [35]. Thus, there is no evidence that insulin detemir would act more quickly than insulin glargine. Nevertheless, the uptake of insulin detemir and glargine into the central nervous system has not been compared, and we cannot rule out a faster action of detemir in that tissue. The hypothalamic impact of regular insulin on EGP is apparently slow, requiring hours for its effects to be manifest [4].

There were no significant differences in NHGO, EGP, glucose R_d , or GIRs between GlarIV and DetIV or between GlarH and DetH. In addition, the effects of GlarIV vs GlarH and DetIV vs DetH were not significantly different. Thus, the physiologic effects of the insulin analogues on hepatic and whole body glucose metabolism during acute administration were indistinguishable, regardless of the route of delivery at the infusion rates utilized. With both analogues, EGP was suppressed more ($P<0.05$) at the Low Insulin step by portal insulin infusion than by peripheral IV and head infusion, consistent with previous results using regular insulin [8]. Insulin's direct effects appear to dominate indirect effects over control of hepatic glucose production.

In spite of the lack of differential effect on glucose metabolism, insulin detemir infusion into a peripheral vein resulted in greater suppression of NEFA during the Low Insulin infusion period. Differences in NEFA concentrations with the two forms of insulin might affect EGP in at least two ways. First, NEFAs serve as a metabolic fuel for gluconeogenesis. Since gluconeogenesis is less readily repressed by insulin than glycogenolysis, higher NEFA concentrations would tend to increase EGP unless a compensatory fall in glycogenolysis occurred [36, 37]. Alternatively, long-chain fatty acids apparently serve as a signal of nutrient availability from the peripheral tissues to the CNS, thus indirectly affecting the regulation or dysregulation of EGP [38-40]. The enhanced suppression of NEFA in DetPo and DetIV vs the corresponding Glar groups was not accompanied by increased suppression of arterial glycerol concentrations. This indicates that insulin detemir was not superior to insulin glargine in suppression of lipolysis but instead might have increased the clearance of NEFA. Lipolysis is very sensitive to even small increases in insulin [41], which may explain why there is no differential effect of the two analogues on the suppression of glycerol concentrations. It is likely that the levels of both of the analogues were sufficient to suppress lipolysis substantially. In contrast to the Po and IV routes of insulin delivery, DetH did not suppress plasma NEFA concentrations more than GlarH during Low Insulin infusion. It is not clear why this would occur, because the circulating concentrations of insulins detemir and glargine during head infusion were not different from their concentrations during IV infusion. Recent evidence indicates that brain insulin controls lipolysis and lipogenesis in adipose tissue [42], but NEFA concentrations are not sensitive indicators of peripheral lipid flux since they, unlike glycerol, can undergo reesterification in peripheral adipose tissue.

Total and phosphorylated Akt in liver and hypothalamus was examined because of its critical role in the insulin signaling pathway that controls most of insulin's metabolic actions [43]. STAT3 was assessed because central actions of insulin are reported to include the stimulation of STAT3 phosphorylation in the liver [44]. Activation (phosphorylation) of STAT3 in the liver suppresses the expression of the transcriptional co-activator of gluconeogenesis PGC-1 α and gluconeogenic genes, including phospho-*enol*-pyruvatecarboxylase and glucose 6-phosphatase [45, 46]. Infusion of insulin detemir into the head, but not into the portal or peripheral circulation, significantly increased both pAkt/Akt and pSTAT3/STAT3 in the liver, compared with glargine infusion. There was no differential effect of detemir vs glargine on protein phosphorylation in the hypothalamus. Recently insulin detemir has been reported not to cross the blood-brain barrier of mice [47], although

other investigators observed that the concentrations of insulin detemir in total mouse brain homogenates were higher than those of human insulin when the two insulin preparations were injected via peripheral IV [5]. Moreover, when injected IV in mice, insulin detemir had a greater and more rapid effect on hypothalamic insulin receptor phosphorylation than human insulin did [5]. The current findings, together with the controversies raised by the murine data, justify further examination of the central effects of insulin detemir.

In conclusion, during acute administration, there were no significant differences observed between insulin detemir and insulin glargine on hepatic or whole body glucose metabolism. However, there were indications that insulin detemir might have greater indirect effects on hepatic glucose metabolism than insulin glargine. Specifically, low-dose insulin detemir suppressed circulating NEFA concentrations more than insulin glargine. Moreover, hepatic Akt and STAT3 phosphorylation was enhanced by head infusion of insulin detemir compared with glargine. Insulin's acute effects on hepatic glucose production are primarily exerted via suppression of glycogenolysis [8, 36, 48]. In vitro data clearly point to an effect of insulin on gluconeogenesis [49], however, and in vivo data also show that insulin can suppress gluconeogenesis [50]. The effects of insulin detemir on NEFA and STAT3 signaling would be anticipated to be associated with suppression of gluconeogenesis. The effects of insulin detemir on hepatic glucose metabolism warrant further investigation, particularly during chronic administration of the analogue.

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Abbreviations

A artery

AF	hepatic artery blood or plasma flow
Akt	also known as protein kinase B (PKB)
CNS	central nervous system
Det	insulin detemir
DetH, DetPo and DetIV	groups in which Det was delivered via the carotid and vertebral arteries, hepatic portal vein and peripheral vein, respectively
EGP	endogenous glucose production
GIR	glucose infusion rate
Glar	insulin glargine
GlarH, GlarPo and GlarIV	groups in which Glar was delivered via the carotid and vertebral arteries, the hepatic portal vein and peripheral vein, respectively
H	hepatic vein
HBV	total hepatic blood flow
NEFA	nonesterified fatty acids
NHGO	net hepatic glucose output
NHGU	net hepatic glucose uptake
P	portal vein
P1	Period 1 of the clamp study (0-300 min)
P2	Period 2 of the clamp study (300-420 min)
PF	portal vein blood or plasma flow
R_d	rate of glucose disposal
STAT3	signal transducer and activator of transcription 3

Figure 1.

Arterial and hepatic sinusoidal insulin concentrations during the basal period (-30 to 0 min) were endogenous canine insulin, and they did not differ among the six groups. During 0-420 min, the concentrations were for insulin glargine (left panels) and insulin detemir (right panels).

Figure 2.

There were no differences between GlarPo vs DetPo, GlarIV vs DetIV, or GlarH vs DetH in regard to arterial plasma glucose, net hepatic glucose balance, endogenous glucose production (EGP), glucose disappearance (R_d), or glucose infusion rate. Data are mean $\pm$ SEM; Low Insulin and High Insulin values are the mean of the data during the last h of each insulin infusion period.

Figure 3.

Delivery of insulin detemir into the portal vein or a peripheral vein suppressed arterial plasma NEFA significantly more than administration of insulin glargine. Data are mean $\pm$ SEM; Low Insulin and High Insulin values are the mean of the data during the last h of each insulin infusion period. * $P < 0.05$ vs insulin glargine via the same route

Figure 4.

Infusion of insulin detemir into the carotid and jugular arteries significantly enhanced phosphorylation of Akt and STAT3 in the liver. Graphs show ELISA for pAKT and Western blots of pAkt and pSTAT3 relative to total Akt and STAT3, respectively. Representative gels are shown for the Western blots. Tissues were taken at the end of the High Insulin period. Neg and Pos refer to negative and positive control samples taken in the post-absorptive state and under clamp conditions with 8× basal intraportal infusion of regular human insulin, respectively. Data are mean ± SEM; all data are normalized to the GlarPo group. * $P < 0.05$ vs GlarH

Table 1
Plasma hormone concentrations

Parameter and group	Basal period	Low Insulin	High Insulin
Insulin concentrations (pM)*			
<i>Arterial</i>			
Glargine groups			
Po	60 ± 12	402 ± 103	1353 ± 286
IV	60 ± 18	659 ± 196	1618 ± 343
Head	48 ± 8	651 ± 73	1961 ± 282
Detemir groups			
Po	54 ± 13	1051 ± 250	2201 ± 586
IV	36 ± 9	1132 ± 214	2580 ± 251
Head	50 ± 9	959 ± 249	2570 ± 189
<i>Hepatic Sinusoidal</i>			
Glargine groups			
Po	155 ± 38	1191 ± 303	4371 ± 1081
IV	131 ± 22	562 ± 158	1300 ± 298
Head	105 ± 12	509 ± 49	1590 ± 256
Detemir groups			
Po	132 ± 36	1292 ± 304	2658 ± 705
IV	77 ± 13	1131 ± 198	2615 ± 190
Head	110 ± 13	930 ± 236	2548 ± 215
<i>Jugular vein</i>			
GlarH	41 ± 10	1402 ± 204	2779 ± 304
DetH	43 ± 7	927 ± 268	2580 ± 294
Glucagon concentrations (ng/l)			
<i>Arterial</i>			
Glargine groups			
Po	44 ± 5	46 ± 4	36 ± 4
IV	38 ± 4	36 ± 3	34 ± 2
Head	36 ± 4	35 ± 3	31 ± 1
Detemir groups			
Po	36 ± 2	37 ± 3	33 ± 3
IV	42 ± 6	43 ± 5	39 ± 5
Head	37 ± 5	35 ± 4	34 ± 3
<i>Hepatic Sinusoidal</i>			
Glargine groups			
Po	53 ± 8	58 ± 4	60 ± 3
IV	40 ± 5	46 ± 7	43 ± 9
Head	45 ± 6	45 ± 4	46 ± 2
Detemir groups			
Po	44 ± 4	49 ± 4	44 ± 4

Parameter and group	Basal period	Low Insulin	High Insulin
IV	45 ± 7	53 ± 4	48 ± 3
Head	41 ± 5	47 ± 6	46 ± 2

Data are mean ± SEM; Low Insulin and High Insulin values are the mean of the data during the last h of each insulin infusion period.

* Insulin concentrations during the basal period are endogenous canine insulin. The concentrations during the Low and High Insulin periods are those of the appropriate analogue; C-peptide concentrations were below the limits of detection during those periods. These insulin data are summaries of those presented in Figure 1.

There were no significant differences in glucagon between groups for any route of insulin infusion, nor were there any significant differences in basal insulin concentrations. Differences in analogue concentrations between Glar and Det during the Low and High Insulin periods are related to the nature and clearance characteristics of the analogues; no comparison between groups is possible.

Table 2
Blood lactate concentrations and net hepatic balance of lactate and glycerol

Parameter and group	Basal period	Low Insulin	High Insulin
Arterial lactate ($\mu\text{mol/l}$)			
Portal insulin infusion			
Glar	496 $\pm$ 133	454 $\pm$ 114	502 $\pm$ 90
Det	521 $\pm$ 78	585 $\pm$ 132	609 $\pm$ 111
IV insulin infusion			
Glar	508 $\pm$ 126	517 $\pm$ 70	410 $\pm$ 117
Det	390 $\pm$ 69	506 $\pm$ 70	556 $\pm$ 64
Head insulin infusion			
Glar	450 $\pm$ 73	629 $\pm$ 122	607 $\pm$ 67
Det	537 $\pm$ 89	564 $\pm$ 85	554 $\pm$ 83
Net hepatic lactate balance ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)			
Portal insulin infusion			
Glar	-1.1 $\pm$ 2.2	1.1 $\pm$ 0.6	0.4 $\pm$ 0.9
Det	-1.9 $\pm$ 1.9	2.9 $\pm$ 1.6	-0.6 $\pm$ 0.7
IV insulin infusion			
Glar	-2.4 $\pm$ 2.2	2.7 $\pm$ 2.7	1.5 $\pm$ 2.5
Det	-4.4 $\pm$ 1.6	1.9 $\pm$ 1.7	-1.0 $\pm$ 1.7
Head insulin infusion			
Glar	-0.6 $\pm$ 2.3	4.5 $\pm$ 1.8	2.0 $\pm$ 1.0
Det	0.3 $\pm$ 2.0	5.2 $\pm$ 1.7	2.2 $\pm$ 1.0
Net hepatic glycerol uptake ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)			
Portal insulin infusion			
Glar	1.6 $\pm$ 0.3	0.9 $\pm$ 0.2	0.6 $\pm$ 0.3
Det	1.4 $\pm$ 0.2	0.7 $\pm$ 0.2	0.9 $\pm$ 0.2
IV insulin infusion			
Glar	1.5 $\pm$ 0.4	0.7 $\pm$ 0.5	0.5 $\pm$ 0.4
Det	1.8 $\pm$ 0.2	0.8 $\pm$ 0.2	0.7 $\pm$ 0.1
Head insulin infusion			
Glar	1.8 $\pm$ 0.5	0.7 $\pm$ 0.2	0.5 $\pm$ 0.2
Det	1.9 $\pm$ 0.3	1.3 $\pm$ 0.5	1.3 $\pm$ 0.5

Data are mean $\pm$ SEM; Low Insulin and High Insulin values are the mean of the data during the last h of each insulin infusion period. Negative values for hepatic balance indicate net hepatic uptake. There were no significant differences between analogues for any delivery route.

Table 3
Relative STAT3 and Akt phosphorylation, expressed as mean (95% confidence limits), with all data normalized to GlarPo

Analogue and delivery route	Liver		Hypothalamus			
	Western blot		Western blot		ELISA	
	pSTAT3/STAT3	pAkt/Akt	pSTAT3/STAT3	pAkt/Akt	Akt (% Phos.)	Akt (% Phos.)
Portal						
Glar	1.0 (0.8-1.2)	1.0 (0.4-1.6)	1.0 (0.9-1.1)	1.0 (0.5-1.5)	1.0 (0.7-1.3)	1.0 (0.7-1.3)
Det	1.3 (0.7-1.9)	1.2 (0.6-1.8)	0.8 (0.7-1.0)	1.4 (0.6-2.2)	0.9 (0.7-1.1)	0.9 (0.7-1.1)
IV						
Glar	1.1 (0.7-1.5)	1.0 (0.6-1.4)	1.0 (0.6-1.5)	1.0 (0.7-1.2)	1.0 (0.5-1.4)	1.0 (0.5-1.4)
Det	1.2 (0.9-1.5)	1.0 (-0.2-2.3)	0.9 (0.8-1.0)	1.1 (0.4-1.9)	1.0 (0.6-1.5)	1.0 (0.6-1.5)
Head						
Glar	0.9 (0.7-1.1)	1.0 (0.6-1.4)	0.9 (0.8-1.1)	1.1 (0.4-1.8)	1.1 (0.5-1.7)	1.1 (0.5-1.7)
Det	1.4 (0.9-1.9)	1.7 (1.2-2.2)	1.0 (0.8-1.1)	0.9 (0.5-1.3)	1.0 (0.6-1.4)	1.0 (0.6-1.4)

n=6, 6, 5, 5, 6 for GlarPo, DetPo, GlarIV, DetIV, GlarH and DetH except DetIV=4 for hepatic ELISA, and GlarH=4 for hypothalamic ELISA.