A Moderate Decline in Specific Activity Does Not Lead to an Underestimation of Hepatic Glucose Production During a Glucose Clamp

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We have previously shown that modeling errors lead to underestimation of hepatic glucose production (HGP) during glucose clamps when specific activity (SA) declines markedly. We wished to assess whether the failure to keep SA constant substantially affects calculation of HGP during insulin infusion when glucose requirements to maintain the glucose clamp are moderate. Therefore, 150-minute hyperinsulinemic (5.4 pmol · kg^{-1} · min^{-1}) clamps were performed in depancreatized dogs that were maintained hyperglycemic (~ 10 mmol/L) with either (1) unlabeled glucose infusate (COLD Ginf, n = 5) or (2) labeled glucose infusate (HOT Ginf, n = 6) containing high-performance liquid chromatography (HPLC) purified [6-^{3}H]glucose. Insulinemia and glucagonemia were similar between the two groups. Additionally, glucose infusion rates were equivalent with COLD and HOT Ginf, indicating comparable insulin effects on overall glucose metabolism. The SA decreased a maximum of 32% with COLD Ginf, but remained constant with HOT Ginf. HGP was suppressed equally with COLD or HOT Ginf treatments at each time point during the clamp (mean suppression during last hour of clamp, 69% ± 4% and 69% ± 5%, P = NS, COLD and HOT Ginf, respectively). We conclude that when glucose requirements are moderate and SA changes slowly, as in the diabetic dog, it is not necessary to keep SA perfectly constant to avoid significant modeling errors when calculating HPG during hyperinsulinemic clamps.

DUE TO ITS SIMPLICITY, Steele's fixed-pool, one-compartment model of glucose kinetics[1] has been extensively used to calculate non–steady-state glucose fluxes. However, this model is associated with errors particularly evident when fluxes are calculated during a glucose clamp, where a model-associated underestimation of glucose turnover can result in negative values for hepatic glucose production (HGP). Finegood et al[2,3] and subsequently others[4-9] have demonstrated that such underestimation results mainly from decreasing plasma glucose specific activity (SA) and can be corrected with labeled glucose infused to maintain SA constant (HOT Ginf method). Also, during moderate exercise, a step increase in the rate of tracer infusion can limit the changes in SA, which improves the calculation of HGP, particularly at the start of exercise when changes in SA occur rapidly.[10] The inadequacies of Steele's model to calculate glucose turnover accurately when glucose requirements are high and consequently SA is decreasing rapidly are well documented.[6,7,11] It is less clear whether Steele's model also leads to substantial underestimations of glucose turnover (1) when changes in glucose turnover are moderate, such as during low-dose insulin infusions or under conditions of insulin resistance, (2) when approaching the steady-state portion of a glucose clamp, or (3) when the HOT Ginf procedure is used but the decrease in SA is not completely prevented. It is important to ascertain an acceptable limit for the decline in SA during a clamp, inasmuch as it would not induce a significant underestimation of HGP. Additionally, it is important to determine whether, under these conditions of a moderate decline in SA, previous measurements of hepatic insulin sensitivity need to be reevaluated.

In the present study, we wished to determine how important it is to use the HOT Ginf procedure to evaluate glucose turnover during clamps when glucose requirements are low and SA changes slowly, such as in the diabetic dog.

MATERIALS AND METHODS

Animals

Adult male mongrel dogs weighing between 20 and 30 kg were selected for pancreatectomy. During surgery, three Silastic (Dow Corning, Midland, MI) catheters were inserted into the superior vena cava via the external jugular vein for infusions of tracer, glucose, and insulin. A fourth catheter was inserted into the portal vein via the splenic vein for basal insulin infusion. A sampling catheter was inserted into the carotid artery. All catheters were tunneled subcutaneously and exteriorized at the back of the neck. All procedures were in accordance with Canadian Council on Animal Care standards and were approved by the Animal Care Committee of the University of Toronto.

After surgery, the dogs were gradually fed until they consumed a diet of 350 g dry chow (Purina Mills, St Louis, MO) mixed with 550 g canned meat (Dr. Ballard-Champion; Friskies, Toronto, Ontario). Pancreatic enzymes were supplemented (Cotazym; Organon Canada, Toronto, Ontario). Diabetes was treated with daily subcutaneous injections of regular and NPH porcine insulin (Lilly, Indianapolis, IN). Porcine insulin does not induce formation of antiinsulin antibodies for at least 2 months,[12] thus allowing accurate measurements of plasma insulin levels. The diabetic dog was used as the experimental model because of the small changes in glucose fluxes induced by insulin in depancreatized dogs, due to insulin resistance.[13] In this model, the changes in SA mimic the moderate SA changes observed when the HOT Ginf method is used but the decrease in SA is only partially prevented, such as during high-dose insulin infusions in nondiabetic subjects.

Only healthy dogs with at least 5 days of relatively well-

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controlled diabetes (glycosuria <1%) and an absence of diarrhea and visible steatorrhea were used for experimental studies. The average daily NPH and regular insulin dose for 5 days before the day of experiment was 21.3 ± 2.2 U HOT and 18.6 ± 2.8 U COLD NPH insulin (P = NS) and 10.0 ± 2.7 U HOT and 9.4 ± 3.4 U COLD regular insulin (P = NS). Each dog's last meal was given 24 hours before the start of the experiment, thus ensuring a postabsorptive state during each study. At that time, the dog received the last subcutaneous injection of regular insulin (HOT, 13 ± 1 U; COLD, 10 ± 1 U) and NPH insulin (HOT, 15 ± 2 U; COLD, 11 ± 1 U). Plasma glucose levels were approximately 20 to 30 mmol/L the following morning (HOT, 23.7 ± 1.1; COLD, 24.1 ± 0.3; P = NS). The two groups of dogs were matched for insulin sensitivity based on the glucose infusion rates during the hyperinsulinemic clamp.

An additional protocol was performed in some of the animals previously studied (n = 3). We were concerned that (1) the 120-minute tracer equilibration period might not be long enough to reach a steady state, (2) the prolongation of fasting might contribute to the decrease in HGP over the course of the clamp, and (3) these two sources of variability might confound our results comparing HOT and COLD Ginf protocols. This additional protocol therefore served as a control study to show the time course of SA and glucose turnover during basal intraportal insulin replacement (ie, no hyperinsulinemic clamp).

Experimental Design

At the beginning of the experiment, regular porcine insulin (Iletin II) was infused intraportally according to the dog's initial blood glucose. Initially, a maximum dose of 20 pmol · kg^{-1} · min^{-1} was administered, and then the infusion rate was gradually reduced over the course of 3.5 hours until glucose levels reached approximately 10 mmol/L. These glycemic levels (9.4 to 11.1 mmol/L) were achieved with cumulative insulin doses of 32.8 ± 4.8 μmol over 219 ± 10 minutes for HOT Ginf and 33.0 ± 4.9 μmmol over 213 ± 9 minutes for COLD Ginf groups (P = NS). Glycemia was subsequently maintained with a constant basal intraportal insulin infusion (1.70 ± 0.30 and 1.61 ± 0.27 pmol · kg^{-1} · min^{-1} for COLD and HOT Ginf protocols, respectively, P = NS). This constant basal intraportal insulin infusion was maintained throughout the experiment. A primed (35 μCi) infusion (0.24 μCi/min) of high-performance liquid chromatography (HPLC)-purified [6-^{3}H]glucose tracer (New England Nuclear, Boston, MA) was started and continued for at least 120 minutes (130 ± 4 minutes) for tracer equilibration before the 30-minute basal blood sampling period. HPLC-purified tracer was used to avoid any potential errors in the evaluation of glucose turnover associated with impure tracers.[14] After tracer equilibration, blood samples for 6-^{3}H and glucose determinations were taken at −30, −20, −10, 0, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 130, 140, and 150 minutes, and blood samples for hormone determinations were taken at −20, 0, 10, 30, 60, 90, 120, and 150 minutes. At time 0, an infusion of insulin prepared in a saline vehicle containing approximately 4% (vol/vol) of the dog's own plasma was administered peripherally (54 pmol · kg^{-1} bolus and 5.4 pmol · kg^{-1} · min^{-1}) and continued for 150 minutes. Plasma glucose was clamped at the initial hyperglycemic levels by stepwise adjustment of the glucose infusion (Dextrose 50% wt/vol); Abbott, Montreal, Quebec, Canada) based on the results of plasma glucose determinations obtained every 5 minutes. The glucose infusate (Ginf) contained either unlabeled glucose (COLD Ginf, n = 5) or HPLC-purified [6-^{3}H]glucose (HOT Ginf, n = 6) mixed together with the 50% dextrose solution to maintain plasma SA constant throughout the experiment.[2,3] The SA of the Ginf ([SA_{Ginf}] disintegrations per minute per micromole) was calculated based on estimations of the parameters in the formula reported by Finegood et al,[2] modified to allow for incomplete suppression of HGP[13]:

$$SA_{Ginf} = \frac{I \times \left[\frac{Ginf_{ss}}{HGP_b} - F\right]}{Ginf_{ss} \times BW},$$

where I is the constant tracer infusion rate (dpm · min^{-1}), $Ginf_{SS}$ is the steady-state Ginf (μmol · kg^{-1} · min^{-1}), HGP_b is the basal HGP (μmol · kg^{-1} · min^{-1}), BW is body weight (kg), HGP_{SS} is the steady-state HGP (μmol · kg^{-1} · min^{-1}), and F (fractional suppression) is (HGP_b − HGP_{SS})/HGP_b.

We based our initial estimates on data previously obtained using [6-^{3}H]glucose. The following initial estimates were used: HGP_b, 19 μmol · kg^{-1} · min^{-1}; HGP_{SS}, 0.25 · HGP_b; and $Ginf_{SS}$, 28 μmol · kg^{-1} · min^{-1}. HGP_b was the mean basal HGP under identical basal conditions,[13] and HGP_{SS} and $Ginf_{SS}$ were taken from means obtained in experiments using a similar protocol.[13] The concentration of glucose was determined in each commercial batch of the unlabeled Ginf and recalculated after addition of the tracer solution.

As described earlier, three additional experiments were conducted on the same group of dogs. In these control experiments, at time 0, instead of insulin and glucose, saline was infused with no attempt made to clamp glycemia or SA. All infusions were performed under sterile conditions.

Analytic Procedures

Plasma glucose concentration was measured using a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA). Insulin was assayed by radioimmunoassay.[15] Immunoreactive glucagon was determined using O4A antiserum obtained from R. Unger (Dallas, TX).[16] The interassay coefficient of variation as determined on reference plasma was 13% for both radioimmunoassays.

For determination of SA, centrifuged plasma was deproteinized with $Ba(OH)_2$ and $ZnSO_4$, passed through anion- and cation-exchange resins (Ag 2-X8 and Ag 50W-X8; Bio-Rad Laboratories, Richmond, CA) to remove labeled glucose metabolites, evaporated, and counted in a β-scintillation counter. Aliquots of the [6-^{3}H]glucose and of the labeled Ginf were assayed together with the plasma samples.

Data Analysis

To calculate glucose turnover, raw data for SA and plasma glucose concentration were smoothed according to the optimal-segments method,[17] which yielded estimates of dSA/dt from slopes between smoothed successive time points (ie, $\Delta SA/\Delta t$) in disintegrations per minute per micromole. The total rate of appearance (Ra) and rate of disappearance (Rd) of glucose were calculated with Steele's equation[1] as modified by Finegood et al[2] to account for the added source of radiolabeled glucose infusion. The total space for glucose distribution (V) was fixed at 25% of the dog's body weight, and the pool fraction was fixed at 0.65 as determined by Cowan and Hetenyi[18] and validated by Radziuk et al.[19] With the HOT Ginf method, the monocompartmental assumption becomes minor because the non–steady-state component of Steele's equation is close to zero. Although glycosuria was not assessed, since plasma glucose was clamped well below the canine renal threshold for glucose, previously determined to be 16 mmol/L,[20] total glucose uptake was equivalent to calculated Rd. Exogenous glucose infusion (ie, Ginf) was subtracted from the total glucose Ra to determine HGP.[2]

Statistical analysis was performed with the Statistical Analysis System (SAS Institute, Cary, NC) package on an IBM-compatible personal computer. One-way ANOVA for repeated measures was

used to test for differences between experimental groups (HOT and COLD Ginf) during two experimental periods (basal, −30 to 0 minutes; clamp, 0 to 150 minutes). In addition, where indicated, values for the two treatment groups are compared during the last hour of the clamp. ANOVA was also used to test for differences between experimental periods (basal and clamp) for each of the two treatments. Additionally, ANOVA comparisons were determined between treatments at individual time points throughout the basal period and during the clamp. The data are expressed as the mean ± SEM in all tests, and unless otherwise noted, significance was presumed at P less than .05.

RESULTS

Hormones

Basal plasma insulin levels were similar in both protocols (59 ± 9 and 52 ± 7 pmol/L, COLD and HOT Ginf protocols, respectively, P = NS; Fig 1). During the clamp, peripheral insulin infusions (54 pmol · kg^{-1} bolus + 5.4 pmol · kg^{-1} · min^{-1}) increased plasma insulin levels in both protocols similarly, to 300 ± 19 and 312 ± 8 pmol/L (COLD and HOT Ginf respectively, P = NS). Immunoreactive glucagon derived from the gastric mucosa in these depancreatized dogs was not significantly different between the two groups during the basal period (120 ± 23 *v* 161 ± 28 ng/L, COLD and HOT treatments, respectively, P = NS). During the clamp, glucagon levels did not change significantly from basal and remained comparable in the two groups (121 ± 10 *v* 142 ± 13 ng/L, COLD and HOT treatments, respectively, P = NS; Fig 1).

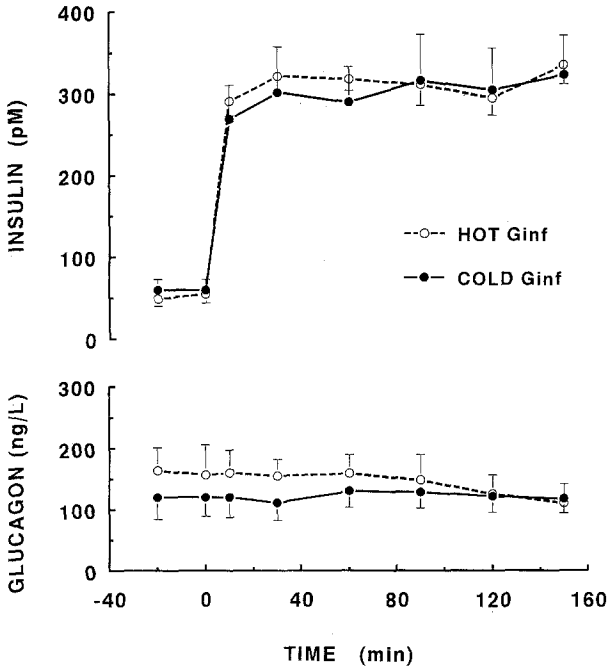

Fig 1. Insulin and glucagon levels (mean ± SEM) before and during a hyperinsulinemic clamp (5.4 pmol · kg^{-1} · min^{-1}) in depancreatized dogs maintained hyperglycemic with HOT (n = 6) and COLD (n = 5) Ginf. A fixed, basal intraportal insulin infusion (1.7 ± 0.3 and 1.6 ± 0.3 pmol · kg^{-1} · min^{-1}, COLD and HOT Ginf, respectively) maintaining moderate hyperglycemia in the basal period was continued throughout the clamp.

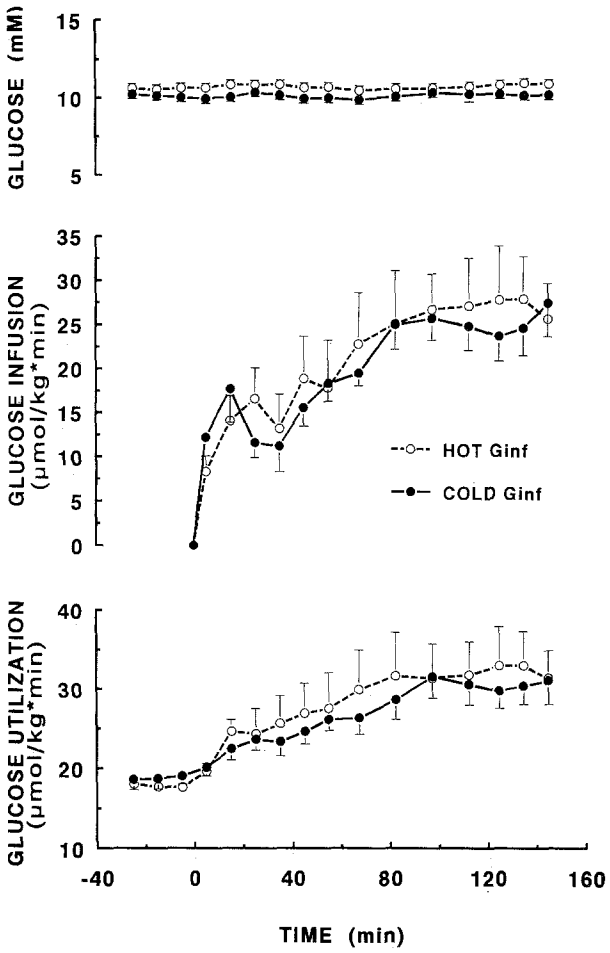

Fig 2. Glucose concentration, rate of glucose infusion, and glucose utilization (mean ± SEM) before and during a hyperinsulinemic clamp (5.4 pmol · kg^{-1} · $^{-1}$) in depancreatized dogs maintained hyperglycemic with HOT; (n = 6) and COLD (n = 5) Ginf.

Glucose Turnover

Plasma glucose levels in the basal period were 10.1 ± 0.1 and 10.6 ± 0.1 mmol/L for the COLD and HOT treatments, respectively (P < .05; Fig 2). During the clamp, glucose levels did not deviate significantly from basal. The rate of glucose infusion required to maintain glycemia was equal throughout the clamp and increased to similar values of 25.2 ± 1.3 and 27.0 ± 2.1 μmol · kg^{-1} · min^{-1} during the last hour of the clamp (COLD and HOT treatments, respectively, P = NS; Fig 2). The equal glucose infusion rates between the groups during the clamp indicate tracer-independent comparable insulin effects on glucose metabolism in the two treatment groups. Glucose utilization (Rd), calculated as the rate of disappearance of [6-^{3}H]glucose, was not different in the basal period (18.8 ± 0.6 and 17.8 ± 0.4 μmol · kg^{-1} · min^{-1}) and increased similarly in the two groups (30.6 ± 1.1 and 32.0 ± 1.8 μmol · kg^{-1} · min^{-1}, COLD and HOT treatments, respectively, P = NS, during the last hour of the clamp).

In the basal period, the SA for [6-^{3}H]glucose was slightly higher in the COLD versus the HOT treatment group

(1.5 ± 0.1 *v* 1.2 ± 0.1 × 10^3 dpm/μmol, $P < .05$). This small difference reflected a minor difference in radioactivity of the tracer infusates. We have therefore expressed the data as percent deviations from the first time point (Fig 3). The SA appeared to increase slightly in the basal period. However, this increase (<4%) was not significant, since there was no difference between the three basal SAs for each treatment group. With HOT Ginf during the clamp, SA remained constant (maximum deviation, <8%). However, with COLD Ginf, SA declined significantly ($P < .01$) during the clamp. By the end of the clamp, COLD Ginf resulted in a maximum SA decrease of 32% ± 5%. Since it is the rate of decrease of SA over time with respect to SA that is used in Steele's equation for the calculation of glucose turnover, the ratio dSA/dt/SA versus time is plotted in Fig 3. During the clamp, dSA/dt/SA for HOT Ginf oscillated around zero, indicating approximate steady-state conditions. However, with COLD Ginf, dSA/dt/SA was significantly lower ($P < .01$) than with HOT Ginf. The ratio dSA/dt/SA with COLD Ginf remained negative throughout the clamp, indicating that a true steady state was not reached.

Basal HGP was similar with COLD and HOT treatments (18.3 ± 0.6 *v* 17.7 ± 0.6 μmol · kg^{-1} · min^{-1}, P = NS; Fig 4). Throughout the clamp, total glucose turnover (Fig 4), which corresponds to Rd under conditions of constant

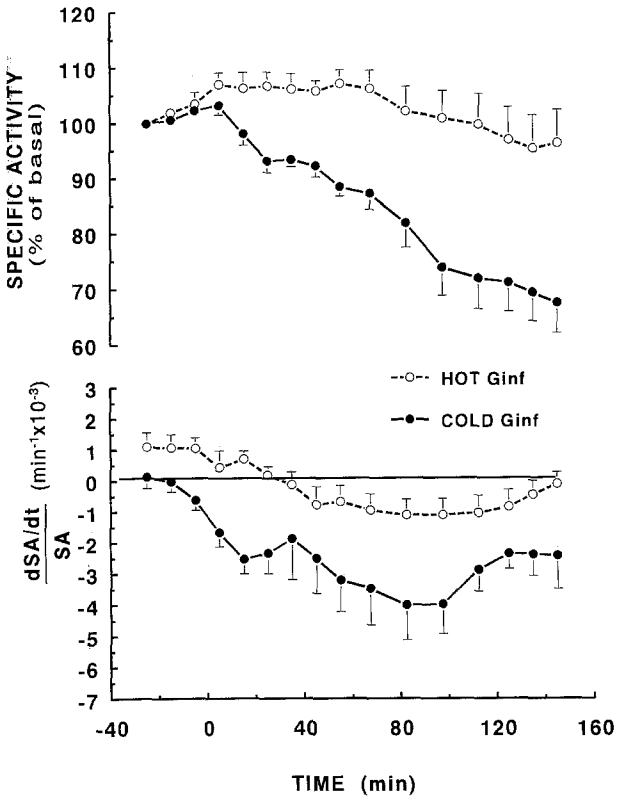

Fig 3. Percent changes in plasma glucose SA from the first time point and rates of change in SA over plasma SA (dSA/dt/SA) (mean ± SEM) before and during a hyperinsulinemic clamp (5.4 pmol·kg^{-1} · min^{-1}) in depancreatized dogs maintained hyperglycemic with HOT (n = 6) and COLD (n = 5) Ginf.

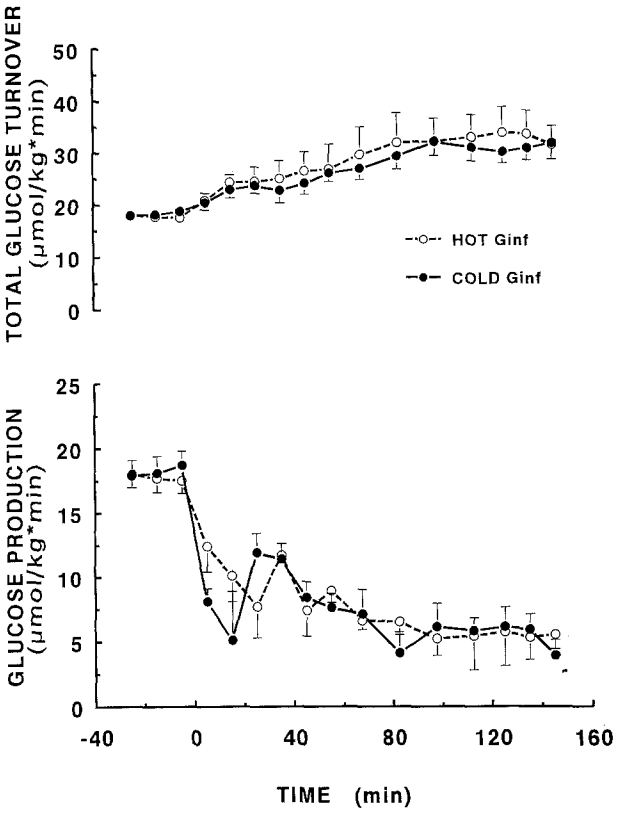

Fig 4. HGP and total glucose turnover (mean ± SEM) before and during a hyperinsulinemic clamp (5.4 pmol · kg^{-1} · min^{-1}) in depancreatized dogs maintained hyperglycemic with HOT; (n = 6) and COLD (n = 5) Ginf.

glycemia, was superimposable with COLD and HOT Ginf, increasing to 30.8 ± 1.1 and 32.4 ± 1.8 μmol · kg^{-1} · min^{-1} (P = NS) during the last hour of the clamp. HGP was similar throughout the clamp. Despite the differences in rates of decline of SA between the two treatments, at no time was HGP significantly different between the two groups. By the last hour of the clamp, HGP was suppressed to 5.6 ± 0.6 versus 5.4 ± 0.8 μmol · kg^{-1} · min^{-1} (COLD and HOT treatments, respectively, P = NS), which represents a suppression of 69% ± 4% and 69% ± 5% (P = NS).

In the additional control protocol (no hyperinsulinemic clamp), glucose levels increased transiently (HGP exceeded Rd) but then stabilized due to a decrease in HGP (Fig 5). SA did not deviate significantly from basal. There were minor oscillations in HGP and Rd. Over 150 minutes, HGP declined minimally from 16 ± 3 to 13 ± 2 μmol · kg^{-1} · min^{-1} and Rd decreased from 15 ± 4 to 14 · 2 μmol · kg^{-1} · min^{-1}.

DISCUSSION

In the present study, all indices of glucose turnover were virtually superimposable, regardless of whether SA declined (COLD Ginf treatment) or remained constant (HOT Ginf treatment) during a glucose clamp at physiologic insulin levels in diabetic dogs.

Insulin resistance in these depancreatized dogs was

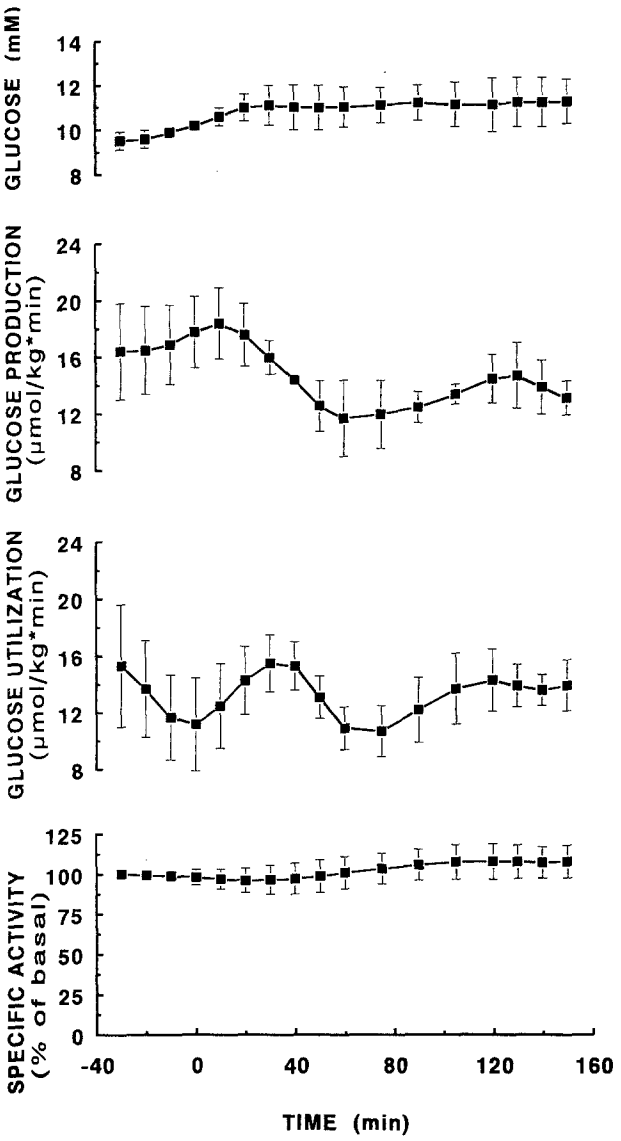

Fig 5. Glucose concentration, production, utilization, and SA (mean ± SEM) for an additional control protocol in the same depancreatized dogs in which basal intraportal insulin infusion was maintained without additional insulin or Ginf at time zero (ie, no hyperinsulinemic clamp).

evidenced by (1) hyperglycemia in the presence of basal normoinsulinemia, (2) incomplete suppression of HGP with insulin levels in the 300-pmol/L range, and (3) a delayed and relatively small increase in glucose infusion and utilization rates in response to suprabasal insulin infusion during the clamp. With the exception of a slight difference in glycemia (0.5 mmol/L), the two groups showed no difference in metabolic parameters. The two groups of dogs were well matched for insulin sensitivity based on (1) similar previous 5-day insulin doses, (2) equal fasting plasma glucose levels on the morning of the experiment, (3) equal insulin doses and time courses necessary to reach similar levels of glycemia immediately before the clamp was initiated, and (4) equal constant basal intraportal insulin infusion rates required to maintain similar levels of glycemia. Furthermore, the glucose infusion rate, which is a tracer-independent variable, was comparable, indicating similar insulin effects on overall glucose metabolism.

During the basal period, SA showed a slow increase, so that the SA of the last sample was (only) 4% higher than that of the first sample, a difference that was not statistically significant. It is possible that, due to the slow fractional turnover rate in diabetic dogs, we did not reach a steady state despite using a tracer prime to infusion ratio of 140:1 and a 120-minute equilibration period before the basal period sampling was started. The additional control protocol, used to verify the time course of SA and glucose turnover during basal insulin replacement, shows an oscillatory pattern indicating that glucoregulation in these diabetic dogs is less well controlled than in normal dogs (Fig 5). Although this is surprising in view of the insulin resistance of these dogs, it is consistent with the loss of glucose-insulin feedback due to the total absence of endogenous insulin secretion in depancreatized dogs. This oscillatory pattern did not obscure a slight tendency for HGP to decrease, which may reflect slow tracer equilibration and/or prolongation of fasting. However, it is unlikely that these two elements of variability of HGP could have confounded our results comparing HOT and COLD Ginf protocols, because during the clamp the effect of a fivefold increment in insulin levels would be expected to override any small intergroup differences in tracer equilibration and HGP response to fasting.

During the clamp, the steady decrease in SA in the COLD Ginf group resulted in constant negative dSA/dt/SA, indicating a non–steady state particularly apparent during the first 90 minutes of the clamp. Consistent with previous studies, it is during the first 90 minutes of the clamp that the most rapid changes in SA occur.[2-5,7] Inspection of the COLD Ginf graph suggests that after 90 minutes, the rate of decrease of SA lessened and dSA/dt/SA approached but did not reach zero (Fig 3). Had we extended our clamp, we speculate, based on other studies, that any underestimation of HGP associated with the failure to keep SA constant would have become even smaller.[2,3,5,7] A nonzero dSA/dt/SA implies a non–steady-state condition and therefore some modeling error in the determination of HGP with Steele's method. However, since at no time point was HGP significantly different with HOT or COLD Ginf, we speculate that such a modeling error was not important in this study, because it was minimal in comparison to experimental variability.

If modeling errors were present, they would cause a proportionally higher degree of HGP underestimation during conditions when HGP is low (ie, during a hyperinsulinemic clamp). In this study, we did not note any significant underestimation of HGP during a moderate decline in SA under conditions when HGP is low. We therefore speculate that modeling errors are unlikely to cause an underestimation of HGP during conditions when HGP increases (such as during stress or exercise), provided the decrease in SA is moderate.

In these experiments, we did not determine validity of the

HOT Ginf methodology, since we have no independent measurement of glucose turnover. Instead, we assessed whether HOT and COLD Ginf produce similar results under conditions of a moderate decrease in SA. Other studies have compared results obtained using HOT Ginf with net hepatic glucose balance obtained by the arteriovenous-difference technique[21] and with glucose turnover obtained by a two-tracer technique.[22] Although each technique has limitations, both studies suggest a good agreement between results obtained with either technique and the HOT Ginf method.

Previously, our laboratory first established that the use of labeled Ginf (HOT Ginf method) avoids underestimation of glucose turnover during high-dose insulin clamps in normal dogs.[2,3] The HOT Ginf method has the advantage over other methods that also avoid this problem of HGP underestimation, such as modeling methods[23] or variable-volume methods,[22] because of greater simplicity, practicality, and computational feasibility. In other studies, the HOT Ginf procedure has been shown to avoid HGP underestimation errors during clamps at physiologic insulin levels, when Ginf was higher than in the present study (50 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Levy et al[4] also indicate that COLD Ginf resulted in significant modeling errors during a constant glucose infusion of 27 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, which is similar to our glucose infusion rates. However, in our HOT Ginf study, SA was kept perfectly constant, whereas it increased by 35% in the HOT Ginf group of Levy et al. Additionally, with constant COLD Ginf, the initial decline in SA was greater than in the present study, wherein Ginf increased gradually. Therefore, the difference in dSA/dt/SA between the COLD and HOT Ginf groups reported by Levy et al was greater than for our COLD and HOT Ginf groups, thus accounting for the greater modeling error in their study versus ours.

It should be noted that in our experimental conditions, changes in glucose turnover were slow due to insulin resistance. Our results do not rule out the possibility that significant modeling errors may exist in normal metabolic states, even with changes in SA within 30% (similar to those in the present study) due to more rapid changes in glucose flux. As indicated earlier, it is not only the percent decline in SA that is important, but also the period over which this decline occurs, as implied by the value dSA/dt/SA. In our case, the peak value was less than 0.004 min^{-1}, which represents a rate of decline of 0.4% per minute. However, studies by Hother-Nielsen et al have suggested that also during high–glucose-turnover clamps in nondiabetic humans, although the HOT Ginf procedure is necessary, precise matching of SA is less critical to avoid significant modeling-related errors in glucose turnover calculations.[8] They could not find significant differences in glucose turnover when Ginfs were labeled to precisely match basal SA or to induce a decline of 25% by the fourth hour of the clamp (20% by the second hour). Thus, both their study[8] and ours indicate that, provided changes in SA are moderate ($< \sim 30\%$), as during HOT Ginf experiments that do not perfectly match basal SA, the residual error in HGP calculations is minimal. This fact is particularly important because when using the HOT Ginf method, SA of the HOT Ginf must be calculated based on estimates of changes in glucose turnover, and therefore a certain deviation of SA from basal is to be expected.

Our study cannot estimate the level above a 30% decrease in SA that is necessary to induce significant modeling errors in glucose turnover assessment. Certainly, all studies in which there was a rapid 70% or greater decrease in SA resulted in significant underestimations of HGP.[2-5,7] In these studies, the peak rate of decrease in SA, when reported, was at least 1% to 2% per minute, which is significantly more than our peak rate of decline of 0.4% per minute. The effects of rapid or slow decreases in SA between 30% and 70% on glucose turnover calculations have not been systematically studied.

In conclusion, the physiologic insulin dose given to our diabetic dogs only perturbed the system slightly, as observed with moderate glucose infusion rates. Thus, COLD Ginf resulted in only a gradual (peak rate of decrease, < 0.004 min^{-1}) but steady decrease in plasma SA of approximately 30%. Under these conditions, HOT and COLD Ginf protocols produced similar results, ie, previously observed errors in the calculation of HGP associated with the failure to maintain plasma SA constant when using Steele's one-compartment model were not evident. These data suggest that (1) it is not necessary to keep SA perfectly constant to avoid significant modeling errors during hyperinsulinemic clamps, but it is sufficient to minimize the SA decline, and (2) most conclusions about glucose turnover measured during glucose clamps that were reached in previous studies before development of the HOT Ginf procedure are not subject to significant underestimation errors associated with the failure to keep SA constant, provided results were obtained in systems not markedly out of steady state and the decline in SA was moderate ($< \sim 30\%$).

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