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Inhalation of human insulin is associated with improved insulin action compared with subcutaneous injection and endogenous secretion in dogs

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Nonstandard abbreviations used: AUC, area under the curve; INH; insulin human (rDNA origin) inhalation powder; SC; subcutaneous; IVC, inferior vena cava.

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ABSTRACT

This study compared the effects of endogenous (portal) insulin secretion vs. peripheral insulin administration with subcutaneous (SC) or inhaled human insulin (INH; Exubera® (insulin human [rDNA origin]) Inhalation Powder) on glucose disposal in fasted dogs. In the control group, glucose was infused into the portal vein (Endo; $n = 6$). In 2 other groups, glucose was infused portally, while insulin was administered peripherally by inhalation (INH; $n = 13$) or SC injection (SC; $n = 6$) with somatostatin and basal glucagon. In the Endo group, over the first 3 h, the arterial insulin concentration was twice that of the peripheral groups, whereas hepatic sinusoidal insulin levels were half as much. Although net hepatic glucose uptake was greatest in the Endo group, the peripheral groups demonstrated larger increases in nonhepatic glucose uptake so that total glucose disposal was greater in the latter groups. Compared with SC insulin action, glucose excursions were smaller and briefer and insulin action was at least twice as great following INH. Thus, at the glucose dose and insulin levels chosen, peripheral insulin delivery was associated with greater whole body glucose disposal than endogenous (portal) insulin secretion; INH administration resulted in increased insulin sensitivity in nonhepatic, but not in hepatic tissues compared with SC delivery.

INTRODUCTION

Most patients with diabetes requiring insulin treatment rely on daily injections of insulin. Various alternatives to injectable insulin have been investigated, including insulin patches, pumps, oral formulations, and inhalation. Inhaled human insulin (INH; Exubera® (insulin human [rDNA origin]) Inhalation Powder) appears to be a promising alternative for effective, noninvasive insulin delivery to injection. It is approved in the United States and European Union for treatment of hyperglycemia in adults with diabetes mellitus.

Insulin delivered by inhalation or SC administration enters directly into the peripheral circulation, exposing muscle, fat, and other nonhepatic tissues to relatively higher insulin concentrations than the liver (Cherrington et al, 2004). Endogenously secreted insulin, on the other hand, enters into the portal vein, exposing the liver to the highest concentrations of insulin. Since insulin has discrete effects on hepatic and nonhepatic tissues, it is important to understand how differences in the route of insulin appearance may affect glucose metabolism, both at the liver and other tissues. One purpose of this study, therefore, was to compare the effect of portal (endogenous) insulin delivery to the effect of insulin delivered peripherally (by inhalation or SC injection), on hepatic and nonhepatic glucose metabolism during a simulated oral glucose load.

In addition, in clinical trials comparing INH with SC (Humulin®) insulin, INH reduced the postprandial glucose rise (0–3 h) at least as effectively as Humulin®, with less late (4–5 h) glucose reduction below baseline (Skyler, 2005). Remarkably, compared to SC administration, insulin inhalation also reduced fasting plasma glucose in patients with diabetes by as much as 40 mg/dL, a finding observed with dry powder (Garg, 2006; Quattrin et al, 2004; Skyler et al, 2005; Hollander et al, 2004) and liquid (Hermansen, 2004) formulations. When insulin inhalation was compared to SC injection in the dog, despite similar overall arterial and hepatic insulin area under the curves (AUCs), the glucose required to maintain euglycemia was 20% greater after inhalation (Cherrington et al, 2004). These observations raise the question as to whether there is a unique glucose-lowering effect associated with insulin delivered by inhalation, although that study was not specifically designed to test the bioefficacy of insulin delivered by inhalation compared to SC injection. Recently, we demonstrated that enhanced nonhepatic glucose clearance is indeed associated with the pulmonary route of insulin administration when compared to intravenous insulin infusion (Edgerton et al, 2005). Therefore, the second aim of the

present study was to further investigate the apparent unique effect of insulin inhalation on increased insulin sensitivity.

METHODS

Experiments were conducted on 25 healthy conscious 18 h fasted male beagle dogs (8–10 kg). Prior to the study they were fed a standard chow diet once a day, and water was provided ad libitum. The surgical facility met the standards published by the American Association for the Accreditation of Laboratory Animal Care, and the Lovelace Respiratory Research Institute Institutional Animal Care and Use Committee approved the protocols prior to the start of the study. All dogs underwent a laparotomy 3 weeks before the experiment to implant infusion catheters into the jejunal and splenic veins and the inferior vena cava, and sampling catheters into the femoral artery and portal and hepatic veins. Transonic flow probes (Transonic Systems Inc., Ithaca, NY) were placed around the hepatic artery and portal vein, as described elsewhere (Edgerton et al, 2001). Intraportal catheters (splenic and jejunal) were used for the infusion of glucose (50% Dextrose, Baxter Healthcare Corporation, Deerfield, IL). Each animal was used only one time.

Inhaled human insulin (INH; Exubera® (insulin human [rDNA origin]) Inhalation Powder; Pfizer Inc, New York, NY), a regular human insulin of recombinant origin and specially formulated for intrapulmonary administration (Nektar Therapeutics, San Carlos, CA), was used for inhalation. The insulin was packaged in a foil blister pack with each blister containing 1.0 mg of human insulin. The total weight of powder in the blister was 1.7 mg. Humulin® R (recombinant human insulin, Eli Lilly, Indianapolis, IN) was used for SC insulin injection. It is assumed that the biological activity of the insulin in both preparations was identical.

On the day of exposure, all dogs were briefly anesthetized. They were then given 0.2 ml of acepromazine SC and approximately 15 min later 5% isofluorane was administered by inhalation until palpebral and pedal reflexes disappeared. The dogs were then intubated with an endotracheal tube, and anesthesia was maintained with 1–2% isofluorane in oxygen. Intravenous catheters were placed into the cephalic and/or saphenous veins to allow for somatostatin (Bachem California, Torrance, CA), glucagon (Eli Lilly, Indianapolis, IN), and glucose delivery, when required. Insulin was then administered by inhalation in the Exubera® group and SC injection in the SC group. No insulin was given in the endogenous group. The INH and SC group experiments were fully randomized. Although the Endo group experiments were performed within a few months of the other groups, the location of the study, animal source, diet, and

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animal care were the same for all groups, however, minimizing the possibility of non-randomization effects.

Insulin Inhalation (INH group; Exubera®): Data from this group (n = 13) were previously published (Edgerton et al, 2005) and are included here for ease of comparison. The animals were exposed to the contents of a 1 mg (~2.8 units/kg) blister of Exubera® using a modified P2.3 device (Nektar Therapeutics, San Carlos, CA) for administration (Edgerton et al, 2005). The time of the end of the inhalation exposure was considered t = 0. No adverse clinical signs related to the inhalation of insulin were observed during the study.

Subcutaneous Insulin Injection (SC group; Humulin®): After sham inhalation exposure, Humulin® R was administered by SC injection at a dose of either 0.18 units/kg (n = 2) or 0.24 units/kg (n = 4) in the left flank region. The dose was adjusted to allow the SC and INH group insulin levels to be more closely matched. No significant differences in glucose response between doses were observed. The time of injection was considered t = 0.

Endogenous Insulin (Endo group): Sham inhalation exposure occurred at t = 0 in this group (n = 6).

Following insulin or sham inhalation, all animals were placed into slings and allowed to recover from the anesthesia, which occurred rapidly, ~5–10 min post t = 0. At t = 5, intravenous infusions of somatostatin (to inhibit endogenous insulin secretion; 0.8 µg/kg per min) and peripheral glucagon (to replace basal glucagon secretion; 0.5 ng/kg per min) were started in the INH and SC groups. These infusions were continued throughout the remainder of the experiment. Glucagon and somatostatin were not administered in the Endo group, allowing normal endogenous insulin secretion to occur. In line with a recent report (Fery et al, 2005), we performed preliminary studies, which demonstrated that somatostatin limits gut glucose absorption in the beagle; therefore intraportal (rather than intraduodenal) glucose infusion (50% Dextrose) was started at t = 5 in all groups, using an algorithm designed to mimic glucose absorption from the gut after oral administration (Table 1). At t = 170 the intraportal glucose infusion was stopped, and thereafter glucose was infused into a leg vein to maintain euglycemia, if required.

Blood sampling and analytical procedures. Blood samples of approximately 4 ml were collected from the aorta (femoral artery), and 2 ml samples were collected from the portal and hepatic veins at the following time points: prior to acepromazine administration (jugular only),

post anesthesia (arterial only), *t* = 0 (arterial only), 5, 10 (arterial only), 15 (arterial only), 20, 35, 50 (arterial only), 65, 80 (arterial only), 95, 125, 155, 185, 215 (arterial only), 245, 305, 335, and 365 min.

Arterial blood glucose was measured in duplicate with a Glucometer Elite® and Glucometer Elite® blood glucose test strips (Bayer Corporation, Pittsburgh, PA) for adjustment of the glucose infusion rate (to maintain euglycemia after *t* = 170). Hematocrit, plasma glucose, glucagon, insulin and C-peptide concentrations were determined as previously described (Edgerton et al, 2001). An antibody which binds to human and canine insulin to the same extent was used in the insulin radioimmunoassay. Hepatic blood flow was measured using implanted transonic flow probes as described elsewhere (Edgerton et al, 2001).

Data analysis. Net hepatic balance (NHB) was calculated with the A-V difference method using the formula: NHB = $Load_{out}$ – $Load_{in}$ where $Load_{out}$ = [H]*HF and $Load_{in}$ = ([A]*AF) + ([P]*PF) and where [H], [A], and [P] are the substrate concentrations in hepatic vein, femoral artery, and portal vein blood or plasma, respectively, and HF, AF, and PF are the blood flow in the hepatic vein, hepatic artery, and portal vein, as determined by the ultrasonic flow probes. Using this calculation, a positive value represents net output by the liver, while a negative value represents net hepatic uptake. Arterial plasma glucose values were multiplied by 0.74 and portal and hepatic plasma glucose were multiplied by 0.73 to convert them to blood glucose values. These correction factors are based on extensive historical data in the mongrel dog (Moore et al, 1991) and unpublished data, which indicates that these factors are appropriate for use in the beagle (Kathryn Stettler, Vanderbilt University Medical Center, 2003). The approximate substrate levels in plasma entering the liver sinusoids were calculated using the formula ([A] * %AF) + ([P] * %PF) where [A] and [P] are arterial and portal vein hormone concentrations, respectively, and %AF and %PF are the respective percent contributions of arterial and portal flow to total hepatic blood flow.

Nonhepatic glucose uptake was estimated by adding glucose infusion rate to net hepatic glucose balance. Changes in glucose mass were accounted for when deviations from steady state were present. Nonhepatic glucose clearance was calculated by dividing nonhepatic glucose uptake by the arterial glucose concentration. Change from basal was calculated by subtracting the basal period value from the value at each time point. AUC was calculated using the

trapezoidal rule, where the mean of the values between time points was multiplied by the number of minutes between time points.

The distribution of the portally infused glucose to muscle and fat was determined by subtracting the glucose taken up by the liver and by nonhepatic tissues, other than muscle and fat, from the total amount of glucose given during the portal glucose infusion period. This estimate is based on the assumption that uptake in non-insulin-dependent tissues (i.e., the central nervous system and formed elements of the blood) saturates at a glucose level near the fasting plasma glucose concentration, that during basal conditions 75% of glucose production is taken up by tissues other than liver/muscle/fat, and that this amount was unaffected by the prevailing insulin and glucose levels (Cherrington, 1999).

Statistical analysis. All data are presented as means ± SEM. Time course data was analyzed with repeated measures analysis of variance (ANOVA), and univariate *F* tests were used for post hoc comparisons (SigmaStat, SPSS Inc.). One-way ANOVA was used for comparisons of mean data and AUC. Statistical significance was accepted at $P<0.05$ relative to the Endo group, unless otherwise noted.

RESULTS

Following initiation of somatostatin infusion in the INH and SC groups, the arterial C-peptide levels dropped rapidly to concentrations near the minimum level of detection of the assay, indicating that endogenous insulin secretion was quickly and effectively suppressed ($P<0.05$; Table 2). Somatostatin was not infused in the Endo group; therefore the arterial C-peptide level rose in concert with the glucose induced increase in insulin secretion (Table 2). The arterial and liver sinusoidal glucagon levels in the 3 groups were close to the expected basal concentrations and were not different from each other throughout the experiment (Table 2).

The arterial plasma insulin level rose to peak levels of 68 ± 9 μU/ml (20 min), 44 ± 7 μU/ml (65 min), and 34 ± 8 μU/ml (35 min) in the INH, SC, and Endo groups, respectively (Fig. 1). The hepatic sinusoidal plasma insulin levels peaked at 55 ± 7, 38 ± 7, and 83 ± 12 μU/ml, respectively (Fig. 1). The 3 h AUCs for arterial plasma insulin in the INH, SC, and Endo groups were 5945 ± 564, 6068 ± 981, and 3284 ± 325 (μU/ml)*min, respectively, and the 3 h AUCs for hepatic sinusoidal plasma insulin were 4247 ± 401, 4279 ± 839, and 8087 ± 888 (μU/ml)*min, respectively (Fig. 2). Thus, compared to the Endo group, the INH and SC groups had 3 h arterial

plasma insulin AUCs that were twice as large ($P<0.05$), and hepatic sinusoidal insulin AUCs that were half as great ($P<0.05$).

Portal glucose infusion (Table 1) caused the arterial plasma glucose levels to rise in the 3 groups (from baseline by 26 mg/dl, 61 mg/dl, and 75 mg/dl at 35 min in the INH, SC, and Endo groups, respectively; Fig. 3; $P<0.05$ for INH vs. SC & Endo). The delta arterial plasma glucose level AUCs during the first 3 h were –554 ± 1417, 2646 ± 2977, and 8018 ± 1499 (mg/dl)*min in the 3 groups, respectively (Fig. 3; $P<0.05$ for INH vs. SC & Endo). The peripheral glucose infusion rate (GIR) required to maintain euglycemia after the end of the portal glucose infusion period (170 min) was 2.1 ± 0.7 and 3.0 ± 1.1 mg/kg per min in the INH and SC groups, respectively, while no glucose was required in the Endo group. At 365 min the GIR was 0.47 ± 0.33 and 1.91 ± 1.08 mg/kg per min in the 2 groups, respectively ($P<0.05$ for INH & SC vs. Endo). During the last 3 h there was a steady rise in the mean plasma glucose level in the INH group as a result of insulinopenia, which occurred in the presence of basal glucagon.

As a result of portal glucose delivery and the subsequent increase in arterial glucose level, hepatic glucose load increased—initially to 40, 45, and 53 mg/kg per min in the INH, SC, and Endo groups, respectively (Fig. 4; $P<0.05$ Endo vs. INH and SC). Due to the increase in hepatic sinusoidal insulin level and glucose load in each group, there was a switch from net hepatic glucose output to uptake. In the INH and SC groups, net hepatic glucose uptake (NHGU) peaked at 1.50 ± 0.73 and 2.12 ± 1.27 mg/kg per min at 35 min, respectively. It was greatest in the Endo group, averaging 3.75 mg/kg per min during the first hour (Fig. 4; $P<0.05$ Endo vs. INH and SC). Net hepatic glucose output was present again in all groups by 185 min.

The clearance and uptake of glucose by nonhepatic tissues increased rapidly after the rise in arterial plasma insulin and glucose. Nonhepatic glucose clearance (an index of glucose uptake independent of the arterial glucose level) peaked at 17.15 ± 1.71, 13.84 ± 3.12, and 7.69 ± 0.63 ml/kg per min in the INH, SC, and Endo groups, respectively (Fig. 5; $P<0.05$ between each group). Because of the difference in arterial glucose levels among the 3 groups the rates of nonhepatic uptake were more similar (Fig. 5). The ratio of AUC nonhepatic glucose clearance to AUC arterial plasma insulin provides an index of non-hepatic insulin sensitivity. The ratio was similar in all 3 groups during the first hour, and about twice as great in the INH group compared to the SC and Endo groups during the second and third hours (Fig. 6; $P<0.05$ INH vs. SC and Endo).

The total glucose mass delivered by portal glucose infusion (between 5–170 min) was 17.3 ± 0.4, 18.2 ± 0.6, and 16.6 ± 0.5 g in the INH, SC, and Endo groups, respectively (1.5 g/kg). The mass of glucose taken up by the liver was 1.5 ± 0.4 (8%), 2.4 ± 0.9 (13%), and 3.6 ± 0.4 g (22%) in the 3 groups, respectively ($P<0.05$ INH vs. Endo), and nonhepatic glucose uptake accounted for 15.8 ± 0.4 (92%), 15.8 ± 0.9 (87%), and 13.0 ± 0.7 g (78%) ($P<0.05$ INH and SC vs. Endo). More specifically, muscle and fat glucose uptake accounted for 13.3 ± 0.7 (78%), 12.4 ± 1.9 (69%), and 9.5 ± 0.9 g (57%) of the nonhepatic uptake ($P<0.05$ INH vs. Endo).

DISCUSSION

The present study compared the efficacy of peripherally administered insulin (via the lung or subcutaneously injected) vs. endogenous (portal) insulin secretion on glucose disposal. In addition, it investigated the ability of inhalation of insulin to enhance glucose disposal relative to SC insulin administration. Compared to endogenous insulin release in response to the glucose load, peripheral insulin delivery resulted in twice the arterial and half the hepatic sinusoidal plasma insulin exposure during the first 3 h after insulin administration (Fig. 2). Since hepatic insulin extraction is ~50% (Sindelar et al, 1996; Ader and Bergman, 1990), the average rates of insulin appearance in plasma during the first 180 min were therefore similar in the 3 groups.

Interestingly, the response of the body to the glucose challenge was such that the plasma glucose level was kept below the renal threshold for glucose and such that within 3 hours of the start of glucose administration euglycemia was re-established. Clearly, when an equal amount of insulin entered the blood from the lungs or a subcutaneous depot it improved glycemic control in the first 2 to 3 hours, but this was at the cost of later hypoglycemia in the SC group (a GIR of 1.9 mg/kg per min was required to maintain euglycemia 6 hours post injection), and progressive hyperglycemia in the INH group after 3 hours.

In addition, the eventual tissue distribution of portally delivered glucose is determined by the size of the glucose load reaching the liver, the portal glucose signal (hepatic artery/portal vein glucose gradient), and the insulin concentration within the liver sinusoids (Moore et al, 1991; Cherrington, 1999). Previously, when conscious dogs were given an oral glucose load of 1.63 g/kg, approximately 24% of the absorbed glucose was taken up by the liver (Abumrad et al, 1982). In the present study, 22% of the glucose delivered via the portal vein (5–185 min) in the Endo group was taken up by the liver, similar to other previously published reports (Abumrad et

al, 1982; Barrett et al, 1985; Bergman et al, 1982). In contrast, hepatic glucose uptake in the INH and SC groups accounted for only 8 and 13% of the infused glucose mass, respectively, despite improved glycemic control (Fig. 3). This difference in liver response was due to the 2-fold higher hepatic insulin level and greater hepatic glucose load in the Endo group since the portal signal was equal in all 3 groups. On the other hand, the increase in nonhepatic glucose uptake was 40% and 30% greater in the INH and SC groups compared to the Endo group, respectively, with an estimated 78, 69, and 57% of the portal glucose infusion distributed to muscle and fat in the INH, SC, and Endo groups, respectively. Thus, peripheral insulin delivery resulted in preferential disposal of glucose by muscle rather than the liver in accord with higher insulin levels in the arterial blood.

Somatostatin was infused in the INH and SC groups to prevent endogenous insulin secretion during hyperglycemia. Since α-cell secretion is also inhibited by somatostatin, glucagon was infused to prevent hypoglucagonemia relative to the Endo group. In this way the glucagon levels were similar in the 3 groups. Had the study design not required somatostatin, however, changes in α-cell secretion relative to the arterial insulin level (i.e. a small decrease in plasma glucagon) could have caused a small increase in net hepatic glucose uptake in the INH and SC groups.

In the SC group, although liver disposal was 9% less than in the Endo group, glucose uptake by muscle and fat was 12% greater. Liver glucose disposal was 14% less in the INH vs. the Endo group, while glucose uptake at muscle and fat was 21% greater, despite lower arterial glucose levels. These differences in flux allowed euglycemia to be achieved by 60 and 120 min in the INH and SC groups, respectively, compared to 180 min in the Endo group (Fig. 3). Although net hepatic glucose balance is exquisitely sensitive to small changes in insulin (Edgerton et al, 2001; Sindelar et al, 1996), it is clear from these data that the role of nonhepatic tissues becomes more prominent at higher insulin and glucose concentrations. Thus, at the glucose dose and insulin levels that were chosen, the 2-fold higher insulin concentration in the artery had a greater impact on glucose disposal than the 2-fold greater insulin level at the liver. This is presumably because the effect of insulin on hepatic glucose uptake nears saturation at lower insulin levels than muscle glucose uptake, as well as much greater muscle mass compared to the liver. Had the dose of insulin been lower, the liver would have played a greater role in whole body glucose disposal.

Previously, it was demonstrated that inhalation of insulin is associated with enhanced glucose disposal, an effect that is independent of the arterial or hepatic insulin pharmacokinetics (Edgerton et al, 2005). Comparison of the INH and SC groups in the present study provides further evidence of this effect. Although the PK profiles were different, the arterial and hepatic 180 min insulin AUCs were closely matched (Fig. 2). During the simulated oral glucose load, a large excursion in arterial plasma glucose level was prevented by insulin inhalation, with an AUC change from basal of near zero –554 (mg/dl)*min in the INH group during the first 3 h. On the other hand, during this period the arterial glucose AUCs were 2646 and 8018 (mg/dl)*min in the SC and Endo groups, respectively. In addition, euglycemia was achieved 1 h earlier in the INH compared to SC group (Fig. 3). Thus, despite matched glucose appearance in the 3 groups, whole body glucose disposal was greater following insulin inhalation compared to both SC insulin administration (where the arterial and hepatic insulin AUCs were similar but the route of delivery and PK profiles were different) and endogenous insulin release (where the arterial and hepatic insulin AUCs were inverted).

Rates of muscle and fat glucose uptake depend on circulating glucose and insulin levels, neither of which was matched between groups. Nonhepatic glucose clearance provides a measurement of glucose uptake by tissues other than the liver which is largely independent of the arterial glucose level. Therefore insulin sensitivity can be determined by expressing the rate of nonhepatic glucose clearance relative to the arterial plasma insulin level. While this ratio was similar between the SC and Endo groups, it was about twice as great during the 2^{nd} and 3^{rd} hours in the INH group compared to the SC and Endo groups (Fig. 6). These results support a previous study comparing inhalation and SC delivery (2 mg vs. 0.36 units/kg, respectively), where 20% more glucose was required to maintain euglycemia for 6 h following inhalation despite similar arterial insulin AUCs (Cherrington et al, 2004). The greater nonhepatic insulin action with inhalation of insulin is probably not explained by the difference in PKs between groups since a similar difference in insulin sensitivity occurred in a previous study even when the arterial insulin PKs were matched (Edgerton et al, 2005). As observed here, in that study the greatest difference in glucose uptake between groups was observed between 60 and 180 min.

Possible mechanisms of increased nonhepatic insulin sensitivity associated with insulin inhalation have been postulated, including a nitric oxide (NO) mediated event (Edgerton et al, 2005). Another potential mechanism is blockade of the renin-angiotensin system through

inhibition of angiotensin-converting enzyme (ACE), which has been shown clinically to increase insulin sensitivity (Damas et al, 2004; Henriksen and Jacob, 2003; Jandelit-Dahm et al, 2005). In addition to its stimulatory effects on NO synthase, insulin also decreases ACE activity, including in the lung (Krulewitz et al, 1984; Erman et al, 1998; Sharifi et al, 2004), thus increasing kinin levels and decreasing angiotensin II. Binding of bradykinin to the B_2 receptor directly increases NO levels, enhances insulin signaling (via IRS-1 phosphorylation and PI3-kinase activity), increases GLUT-4 glucose transporter translocation in skeletal muscle, and has been shown to be a potent insulin-dependent enhancer of glucose uptake in muscle and fat (Damas et al, 2004; Henriksen and Jacob, 2003). Studies also suggest that reduced binding of angiotensin II to the AT_1 receptor may also increase insulin sensitivity (Henriksen et al, 2001). However, further studies will be required to identify the specific site and mechanism of increased nonhepatic insulin sensitivity which is associated with insulin inhalation.

In summary, at the insulin and glucose levels chosen in this study, peripheral insulin delivery was associated with greater whole body glucose disposal compared to endogenous (portal) insulin delivery. In addition, inhalation of insulin resulted in increased insulin sensitivity in nonhepatic but not hepatic tissues compared to SC insulin delivery.

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FOOTNOTES

Lovelace Respiratory Research Institute received funding from Pfizer Inc to conduct this study.

LEGENDS FOR FIGURES

Fig. 1. Arterial and hepatic sinusoidal plasma insulin levels for the INH, SC, and Endo groups (n=13, 6, 6, respectively; mean ± SEM. For arterial plasma insulin $P<0.05$ between INH vs. SC from 5-50 and 95-155 min, between INH vs. Endo from 5-65 min, and between SC vs. Endo from 50-80 and at 125 min. For hepatic sinusoidal plasma insulin $P<0.05$ between INH vs. SC from 5-35 min, between INH vs. Endo from 5-125 min, and between SC vs. Endo from 5-95 min).

Fig. 2. AUC arterial and hepatic sinusoidal plasma insulin levels (5–185 min) for the INH, SC, and Endo groups (n=13, 6, 6, respectively; mean ± SEM; *$P<0.05$ Endo vs. INH and SC).

Fig. 3. Arterial plasma glucose level and 5–185 min AUC change from basal plasma glucose levels for the INH, SC, and Endo groups (n=13, 6, 6, respectively; mean ± SEM. For arterial plasma glucose $P<0.05$ between INH vs. SC from 35-80 and 305-365 min, between INH vs. Endo from 20-125 and 305-365 min, and between SC vs. Endo from 80-125 min. For AUC change from basal plasma glucose *$P<0.05$ INH vs. Endo).

Fig. 4. Hepatic glucose load and net hepatic glucose balance for the INH, SC, and Endo groups (n=13, 6, 6, respectively; mean ± SEM; For hepatic glucose load $P<0.05$ between INH vs. Endo from 20-125 min and between SC vs. Endo at 20 and from 65-155 min. For net hepatic glucose balance $P<0.05$ between INH vs. SC at 305 min, between INH vs. Endo at 20 and from 65-95 min, and between SC vs. Endo at 20 and 65 min).

Fig. 5. Nonhepatic glucose clearance and uptake for the INH, SC, and Endo groups (n=13, 6, 6, respectively; mean ± SEM. For non-hepatic glucose clearance $P<0.05$ between INH vs. SC at 35 min, between INH vs. Endo from 20-155 min, and between SC vs. Endo at 20 and 95 min. For non-hepatic glucose uptake $P<0.05$ between INH vs. SC at 35 min, between INH vs. Endo from 20-95 min, and between SC vs. Endo at 20 and 95 min).

Fig. 6. Ratio of AUC nonhepatic glucose clearance and AUC arterial plasma insulin during the 1^{st}, 2^{nd} and 3^{rd} hours for the INH, SC and Endo groups (n=13, 6, 6, respectively; mean ± SEM; *P<0.05 vs. INH group).

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Table 1: Algorithm for Intraportal Glucose Infusion Rate in the INH, SC and Endo groups

Time (min)	**GIR** (mg/kg per min)
5	12
95	10
110	8
125	6
140	4
155	2
170	0

TABLE 2: Arterial plasma C-peptide levels and arterial plasma and hepatic sinusoidal glucagon levels in overnight fasted conscious dogs during portal glucose infusion where endogenous insulin secretion was allowed to occur (n=6) or insulin was administered by inhalation (n=13) or subcutaneous injection (n=6).

	5	10	15	20	35	50	65	95	125	185	245	305	365
Arterial plasma C-peptide level (ng/ml)													
Endo	0.28 ± 0.10	0.51 ± 0.14	1.23 ± 0.17	1.56 ± 1.53	1.51 ± 0.25	1.18 ± 0.18	1.19 ± 0.18	1.23 ± 0.10	1.14 ± 0.30	0.24 ± 0.07	0.25 ± 0.05	0.20 ± 0.04	0.27 ± 0.05
INH	0.37 ± 0.07	0.19 ± 0.03	0.12 ± 0.02*	0.09 ± 0.02*	0.06 ± 0.02*	0.06 ± 0.01*	0.07 ± 0.02*	0.08 ± 0.02*	0.06 ± 0.02*	0.06 ± 0.01*	0.06 ± 0.01*	0.07 ± 0.02	0.08 ± 0.02
SC	0.47 ± 0.06	0.24 ± 0.03	0.19 ± 0.02*	0.17 ± 0.03*	0.16 ± 0.05*	0.14 ± 0.04*	0.10 ± 0.04*	0.14 ± 0.03*	0.13 ± 0.04*	0.12 ± 0.04*	0.18 ± 0.03*	0.14 ± 0.04	0.13 ± 0.03
Arterial plasma Glucagon Level (pg/ml)													
Endo	46 ± 3	49 4	42 ± 4	44 ± 3	40 ± 3	41 ± 3	35 ± 3	33 ± 3	35 ± 4	35 ± 3	37 ± 4	27 ± 3	41 ± 11
INH	40 ± 3	45 ± 3	42 ± 3	43 ± 3	41 ± 3	37 ± 2	35 ± 3	35 ± 2	32 ± 2	32 ± 3	29 ± 2	32 ± 2	32 ± 2
SC	38 ± 7	47 4	44 ± 3	44 ± 4	41 ± 3	39 ± 2	40 ± 2	32 ± 5	35 ± 5	34 ± 5	35 ± 2	33 ± 6	33 ± 5
Hepatic Sinusoidal Glucagon Level (pg/ml)													
Endo	50 ± 2			53 ± 2	46 ± 4		41 ± 2	36 ± 2	32 ± 3	44 ± 5	44 ± 7	36 ± 5	45 ± 10
INH	44 ± 4			44 ± 3	38 ± 2		37 ± 2	34 ± 2	29 ± 2	30 ± 3*	28 ± 3*	32 ± 3	31 ± 2*
SC	48 ± 7			43 ± 4	38 ± 4		35 ± 5	32 ± 6	35 ± 5	34 ± 4	33 ± 4	32 ± 6	31 ± 5*

Mean ± SEM; *P < 0.05; INH or SC versus Endo.

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Figure 1

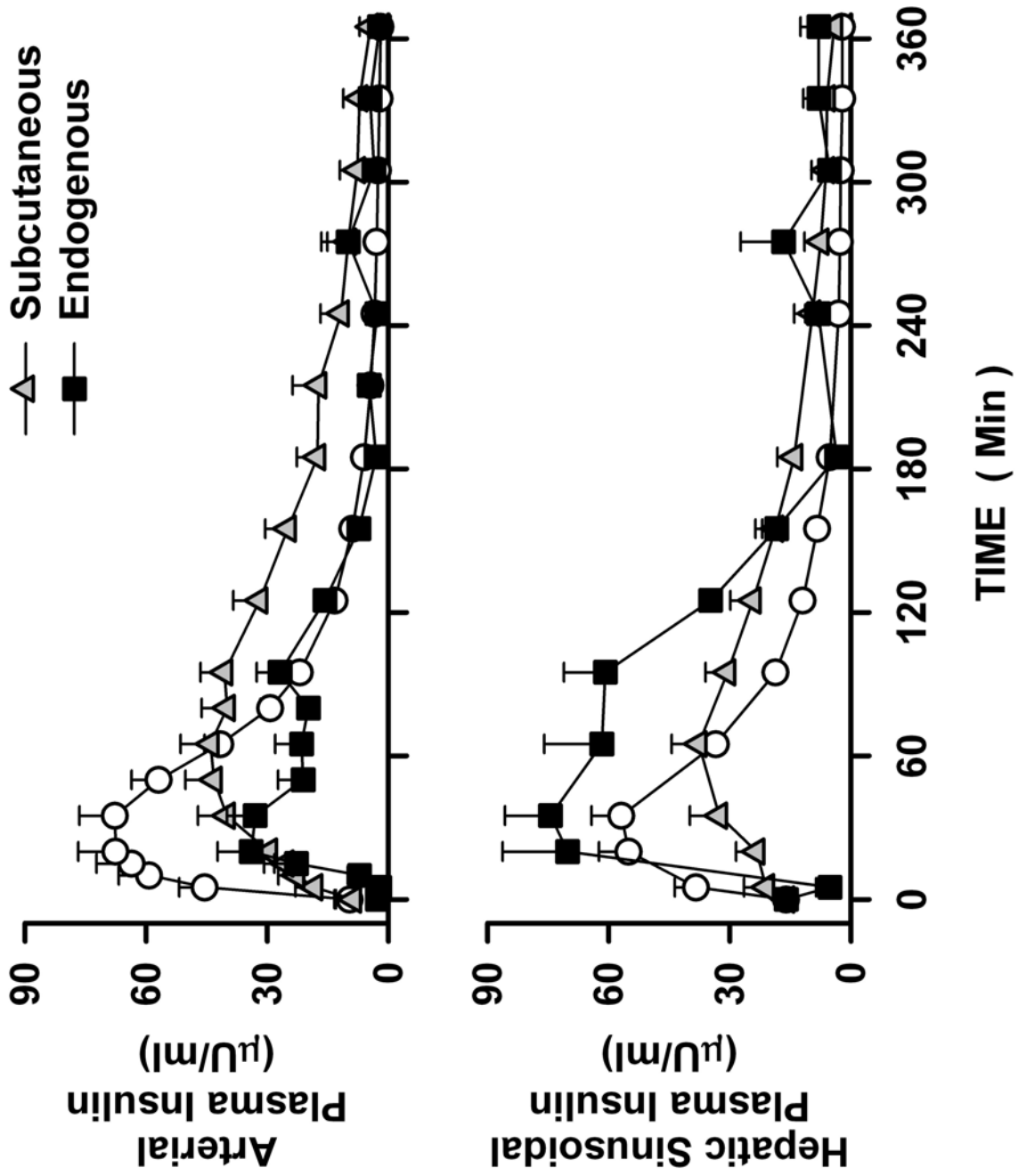

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Figure 2

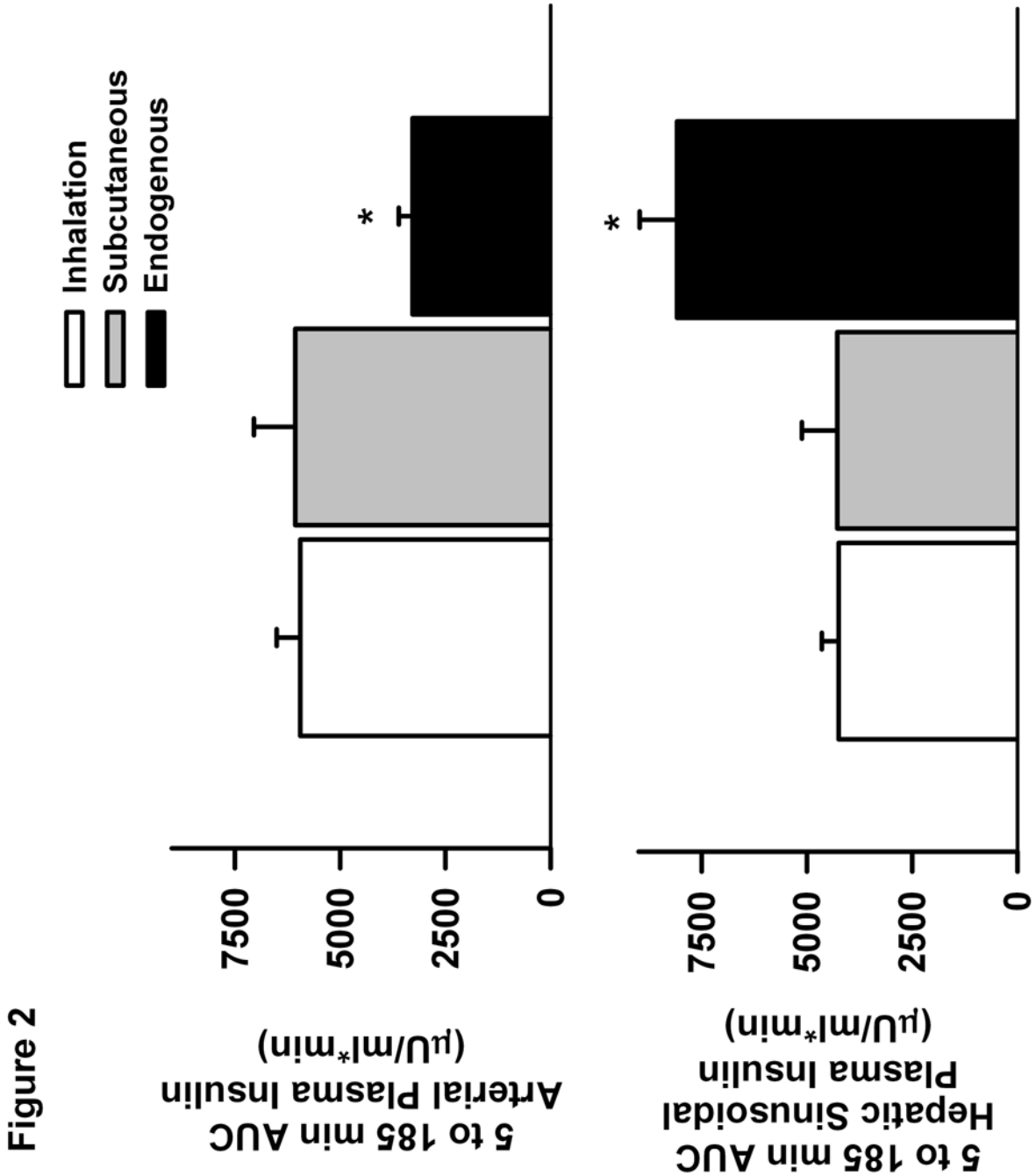
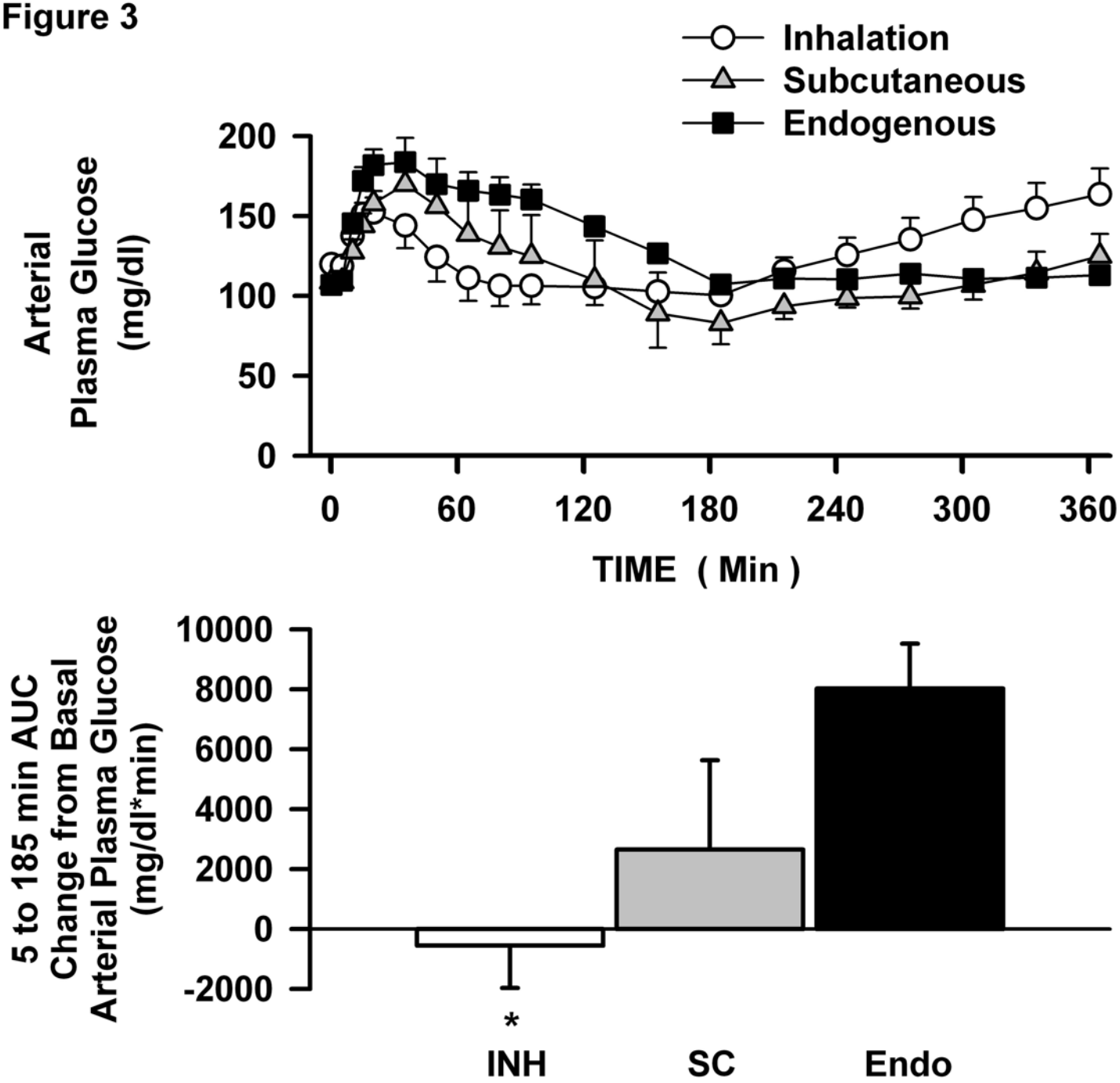


Figure 3



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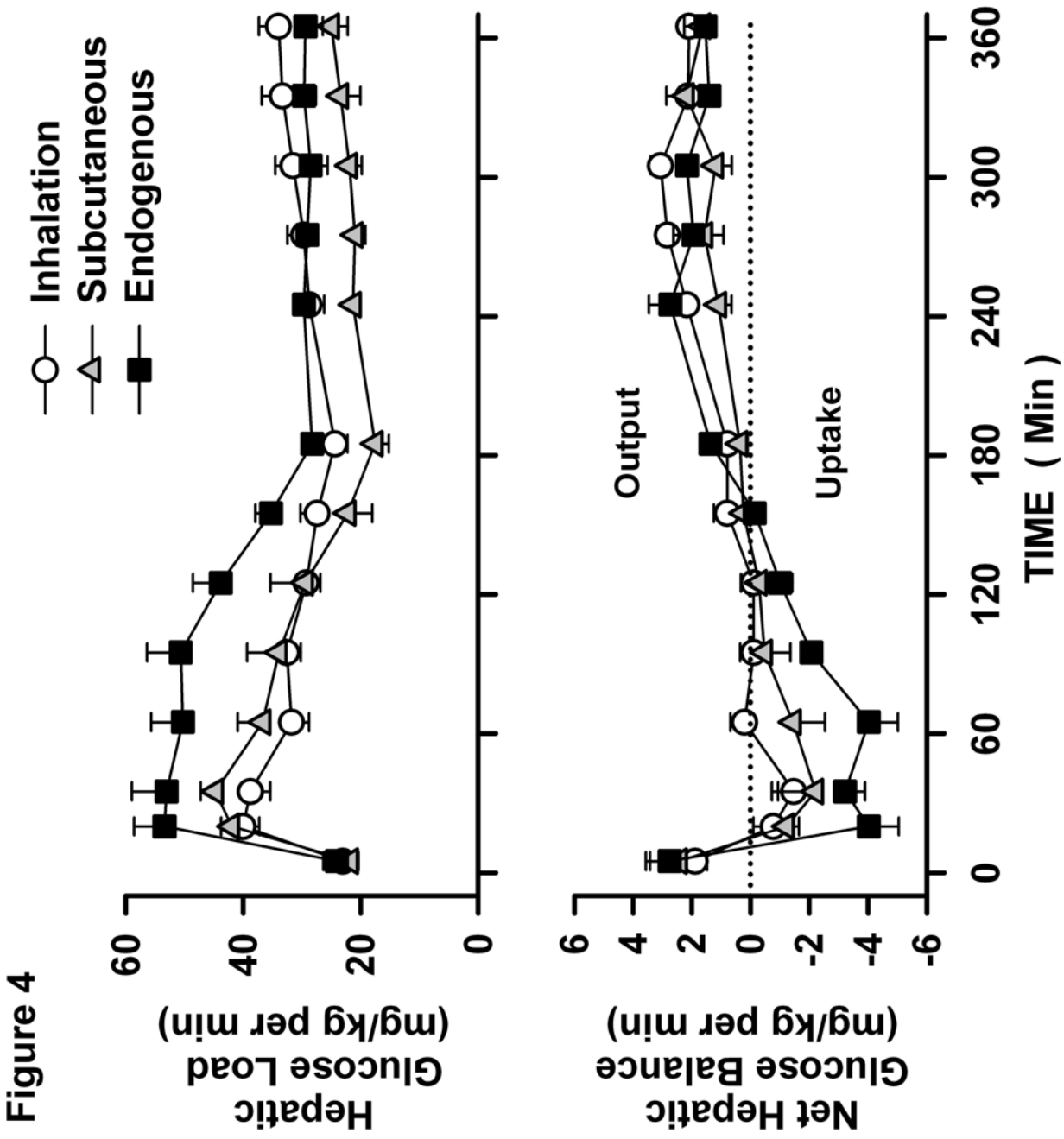

Figure 4

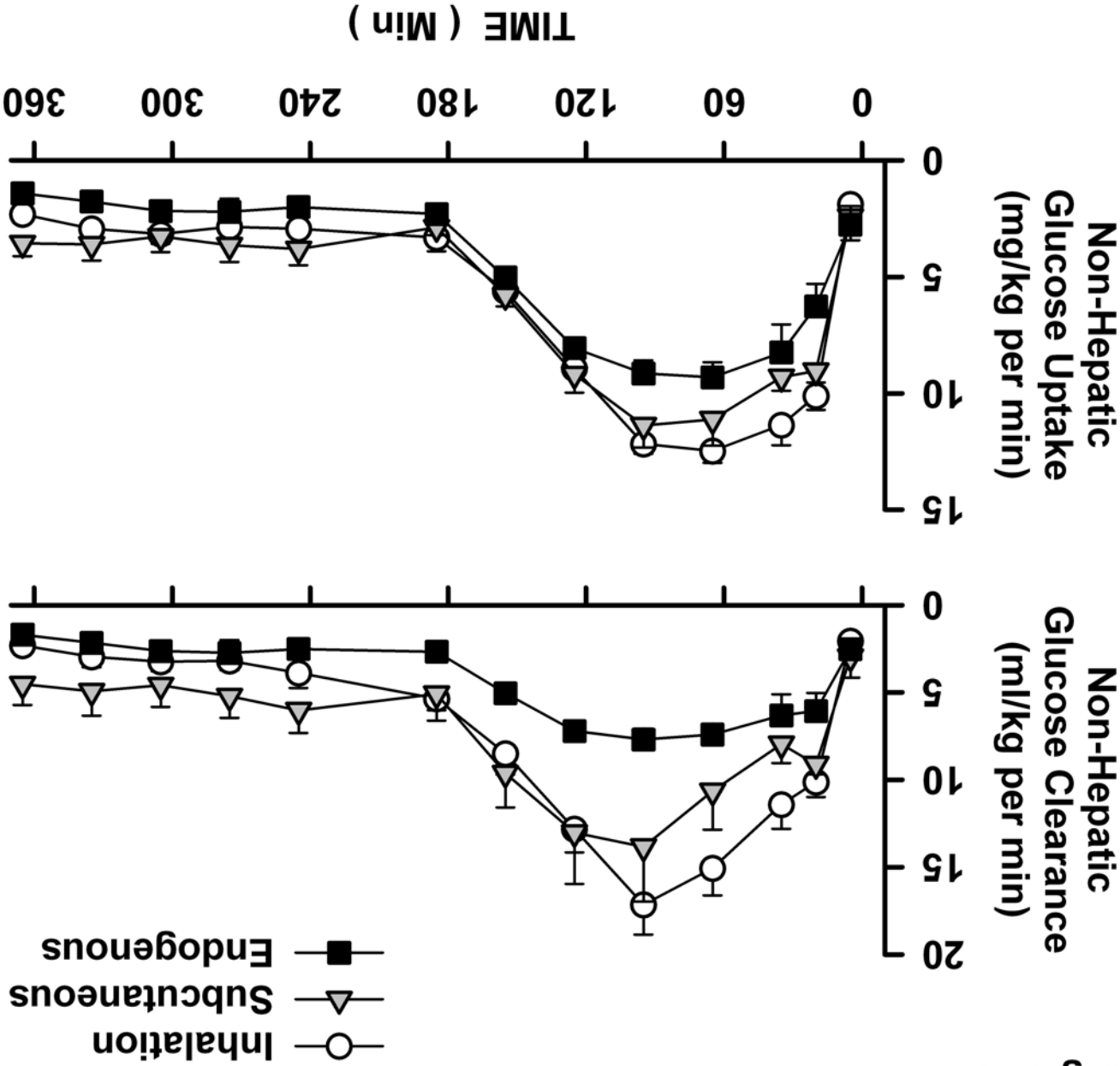

Figure 5

Figure 6