Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 356 (2008) 231–238
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmacokinetics and pharmacodynamics of exenatide following alternate routes of administration

Bronislava R. Gedulin, Pamela A. Smith, Carolyn M. Jodka, Kim Chen, Sunil Bhavsar, Loretta L. Nielsen [1], David G. Parkes, Andrew A. Young *

Amylin Pharmaceuticals, Inc. 9360 Towne Centre Dr, Ste 110, San Diego, CA 92121, United States

Received 18 September 2007; received in revised form 22 December 2007; accepted 9 January 2008
Available online 17 January 2008

Abstract

Exenatide is a 39-amino acid peptide incretin mimetic approved for adjunctive treatment of type 2 diabetes. It shares several glucoregulatory activities with the mammalian hormone, glucagon-like peptide-1 (GLP-1). In clinical use, subcutaneous exenatide injections demonstrate glucoregulatory and weight loss effects with sustained plasma concentrations in the 50–100 pM range. We investigated the pharmacokinetics of exenatide in normoglycemic rats and biological activity in diabetic *db/db* mice after delivery to various epithelial surfaces of the intestinal and respiratory tracts. In rats, elimination kinetics were similar for all routes of administration (median k_e 0.017 min^{-1}). Bioavailability (versus intravenous administration) and C_{max} per unit dose differed markedly. For gastrointestinal administration, sublingual administration invoked the highest bioavailability (0.37%); in *db/db* mice, potentially therapeutic concentrations were obtainable. In contrast, intraduodenal bioavailability was low (0.0053%). In regard to respiratory surfaces, bioavailability of intratracheal exenatide was up to 13.6%, and for nasal administration, 1.68%. Both routes of administration produced therapeutic plasma concentrations and glucose-lowering in *db/db* mice. At high doses, aerosolized exenatide also achieved effective concentrations and glucose-lowering. In summary, the intestinal tract seems to have limited potential as a route of exenatide administration, with sublingual being most promising. In contrast, the respiratory tract appears to be more viable, comparing favorably with the clinically approved subcutaneous route. Despite little optimization of the delivery formulation, exenatide bioavailability compared favorable to that of several commercially available bioactive peptides.

Keywords: Exenatide; Exendin-4; Delivery routes; Type 2 diabetes

1. Introduction

Exenatide (exendin-4) is a 39-amino acid amidated peptide incretin mimetic that shares several glucoregulatory activities with the mammalian incretin hormone, glucagon-like peptide-1 (GLP-1) (Buse et al., 2004; Nauck, 2004; Nielsen, 2005; Kendall et al., 2005; Heine et al., 2005; DeFronzo et al., 2005). These include: glucose-dependent enhancement of insulin secretion, glucose-dependent suppression of inappropriately high glucagon secretion, slowing of gastric emptying to modulate nutrient absorption, reduction of food intake and body weight, and increase of β-cell mass (animal studies) and markers of β-cell function (Young et al., 1999; Buse et al., 2004; Nielsen, 2005; Kendall et al., 2005; Heine et al., 2005; DeFronzo et al., 2005; Gedulin et al., 2005; Mari et al., 2006; Nielsen et al., 2004). Exenatide is a poor substrate for degradation by dipeptidyl peptidase-IV (DPP-IV) *in vitro*, the major route of GLP-1 degradation (Mentlein et al., 1993), so the peptide circulates predominantly as the parent molecule, with filtration at the kidneys being the predominant route of elimination (Parkes et al., 2001a; Copley et al., 2006; Linnebjerg et al., 2007). Exenatide displays prolonged pharmacokinetics relative to GLP-1, and an *in vivo* potency up to 3000-fold higher (Young et al., 1999; Parkes et al., 2001a, 2001b).

Exenatide is approved as adjunctive therapy for patients with type 2 diabetes failing to achieve glycemic control with oral antidiabetic agents (metformin, sulfonylureas, thiazolidine-

* Corresponding author. Tel.: +1 858 642 7122; fax: +1 858 334 1122.
E-mail addresses: lnielsen@amylin.com (L.L. Nielsen), andrew.young@amylin.com (A.A. Young).
[1] Tel.: +858 642 7203; fax: +858 334 1203.

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.01.015

diones) when administered twice daily before meals in the morning and evening by subcutaneous injection in the abdomen, thigh, or arm (Buse et al., 2004; Calara et al., 2005; Heine et al., 2005; DeFronzo et al., 2005; Zinman et al., 2007).

Because injection may impose barriers to acceptance of therapy, we investigated in rodents the pharmacokinetics and biological activity of exenatide when it was delivered via non-injected routes. Potential benefits of alternate delivery routes include favorable drug absorption, lower pre-systemic metabolism and higher patient convenience and compliance. Several routes were identified that could present a feasible alternative to injection.

2. Methods

2.1. Animals

Male Harlan–Sprague–Dawley rats (HSD) were used to study pharmacokinetics of exenatide. Rats (Indianapolis IN) weighing 315–350 g were housed at 22.8 ± 0.8 °C in a 12:12 h light:dark cycle (all experiments are performed during the light cycle) and were fed and watered *ad libitum* (diet LM-485 Teklad, Madison, Wisconsin, USA). The rats were fasted overnight (approximately 18 h) before each experiment, unless otherwise specified.

For some routes of administration, changes in plasma glucose in mice were used to indicate the appearance of bioactive amounts of exenatide. Male diabetic *db/db* mice weighing 45–60 g were fasted for 2 h. All animals remained anesthetized throughout blood collection.

2.2. Intraduodenal delivery

Rats were anesthetized with 5% halothane, maintained at 2% halothane during surgery, and then 1% halothane thereafter. Tracheotomy and cannulation of the right femoral artery were performed and body temperature was controlled with a thermoregulator (YSI model 73A, Yellow Springs, OH, USA) on a heated operating table. Thereafter, a midline laparotomy was done to expose the stomach and duodenum, and the pylorus region of the stomach was ligated. The bile duct was cannulated and flow was directed outside the abdominal cavity. Exenatide (1 mg/250 μL saline) was administered 1.5 h after completion of surgery using a 0.5 mL syringe with a 28 gauge needle that was inserted into the duodenum, parallel to the intestinal wall, about 3.0 cm from the ligated pylorus and about 1.5 cm distal to the sphincter of Oddi.

2.3. Sublingual delivery

Rats were anesthetized with halothane (5% induction, 2% during surgery, 0.7% maintenance dose), then tracheotomized and the femoral artery cannulated. Five microliters of saline containing 210 μg exenatide ($N = 4$) was administered sublingually at $t = 0$.

Diabetic mice were anesthetized with methoxyflurane and either saline or 100 μg exenatide in 3 μL saline was administered sublingually via a Hamilton syringe. Blood samples were collected from the orbital sinus at 0 and 60 min for determination of plasma exenatide and glucose concentrations.

2.4. Intranasal delivery

Fed rats were restrained by hand, then 100 μg exenatide in 3 μL saline ($N = 4$) was administered into one nostril. Blood samples from anesthetized tail tips were collected for determination of exenatide plasma concentrations.

2.5. Intratracheal delivery

Rats were anesthetized with halothane (5% in O_2 for induction, 2% for surgery, 0.7% for maintenance). Each animal was cannulated in the trachea and femoral artery under anesthesia. At $t = 0$ min, each rat received 30 μL saline vehicle with or without exenatide into the trachea. Plasma samples were collected from the femoral artery and blood pressure was recorded manually at each time of blood sampling.

Diabetic *db/db* mice were intubated through the trachea under anesthesia. Each animal received 20 μL saline with or without 1 μg exenatide into the trachea. Plasma samples were collected from the orbital sinus for glucose (baseline and 1.5 h) and exenatide (baseline and 4.5 h).

2.6. Pulmonary aerosol delivery

Rats were placed in a 4.5 L chamber and exposed to aerosolized exenatide for 10 min. Exenatide (2 mg in 2 mL) was nebulized at a rate of 0.2 mg/min in an air flow of 5 L/min. The concentration of aerosolized exenatide was estimated from samples of chamber atmosphere drawn during the course of the experiment.

Diabetic mice were fasted for 2 h, then placed in a 4.5 L chamber and exposed to aerosolized exenatide (2 mg) for 10 min as described above. Plasma samples were collected at −15 and 60 min after commencement of aerosol administration.

2.7. Intravenous delivery

Anesthesia was induced in rats with 5% halothane and maintained at 2% during surgery and 1–1.5% thereafter. Rats underwent tracheotomy and cannulation of the right saphenous vein for intravenous injection, and of the right femoral artery for sampling. Heparinized saline (2 U/mL) was infused via the arterial line at an infusion rate of 3–4.5 mL/h from $t = -1$ h. Mean arterial pressure was monitored via the arterial line (Spectramed P23XL transducer, 13-4615-58 Universal amplifier, Gould, Valley View, Ohio). Colonic temperature was measured and controlled using a thermistor probe/controller (YSI model 73A, Yellow Springs, OH).

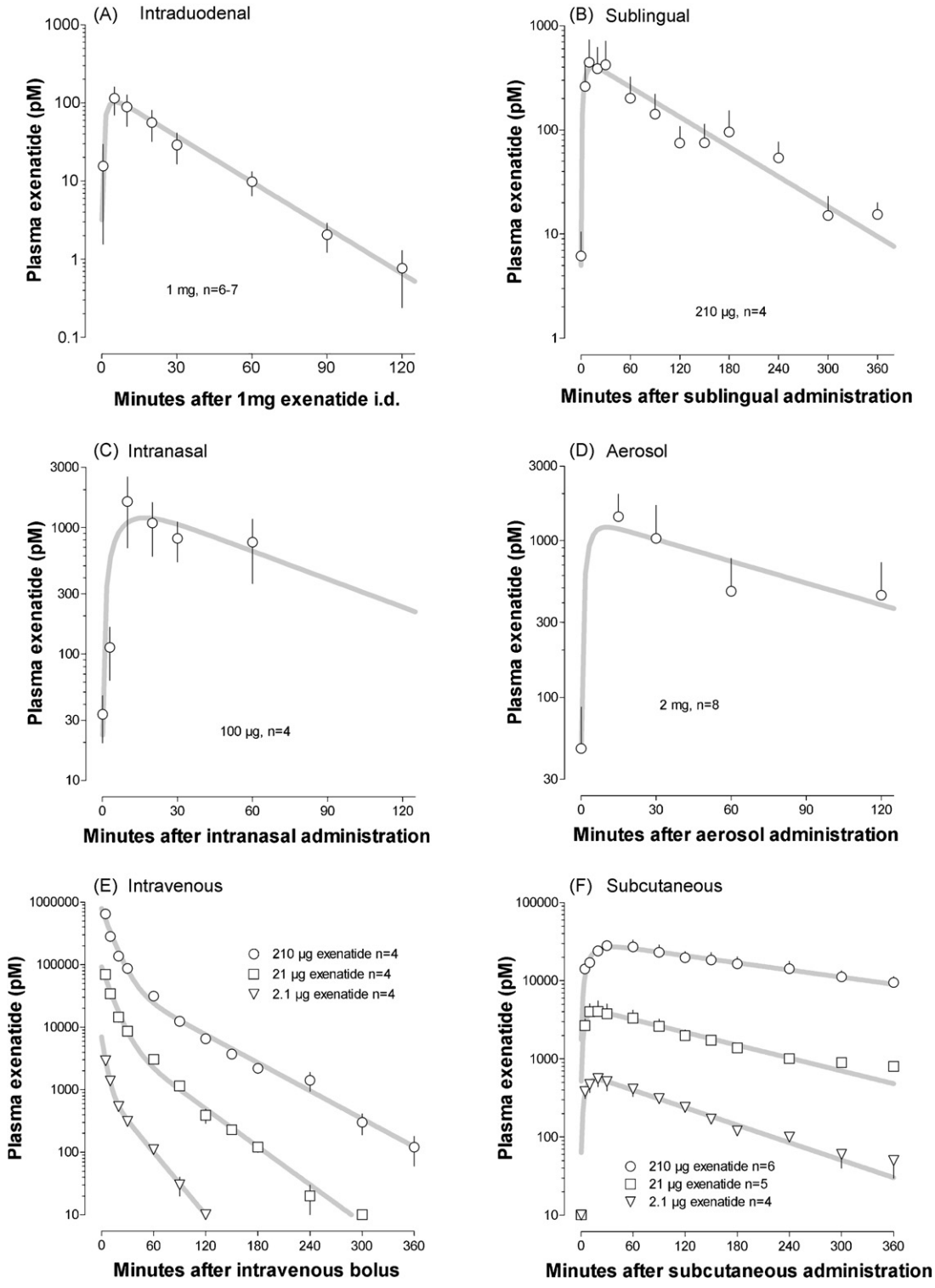

Fig. 1. Exenatide pharmacokinetic profiles in rats. (A) Intraduodenal administration of 1 mg. $N = 6$–7. (B) Sublingual administration of 210 µg. $N = 4$. (C) Intranasal administration of 100 µg. $N = 4$. (D) Pulmonary administration of 2 mg by aerosol. $N = 8$. (E) Bolus intravenous administration. $N = 3$–4 per group. (F) Subcutaneous administration. $N = 4$–6 per group. Symbols are means ± S.E.M. in this and subsequent figures.

2.8. Subcutaneous delivery

Rats prepared as above, for intravenous injection, were administered exenatide in saline by subcutaneous injection with no additional experimental manipulations.

2.9. Analytical methods

Glucose concentrations were determined by a glucose oxidase method on a YSI 2300 Stat Plus analyzer (YSI, Yellow Springs, OH). Insulin concentrations were determined by

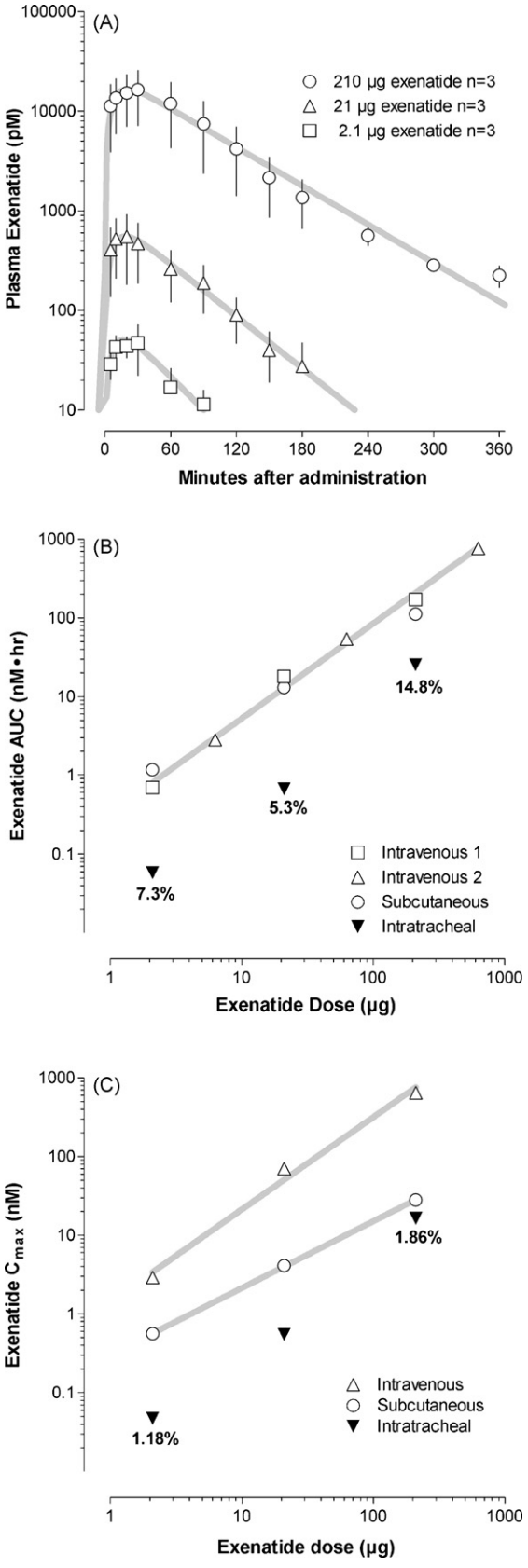

Fig. 2. Intratracheal administration of exenatide in rats. (A) Pharmacokinetic profile. $N = 3$ per group. (B and C) Bioavailability in rats, assessed by AUC and C_{max}, compared to subcutaneous and intravenous administration. (See Fig. 1E and F for related data).

radioimmunoassay (LINCO Research, St. Charles, MO). Exenatide concentrations were determined by IRMA as previously described (Parkes et al., 2001a).

2.10. Statistical analyses

Data were analyzed using one-way ANOVA or Students' *t* test (Prism v5, Graphpad Software, Inc., San Diego, CA). Results are reported as mean ± S.E.M. unless otherwise indicated. The kinetics of plasma concentration decay after intravenous administration was modeled with a 2-compartment exponential decay. Kinetics of all other modes of administration were modelled to single-exponential absorption and decay (Prism v5, Graphpad Software, Inc., San Diego, CA), fitted to the equation

$$\text{concentration} = \frac{(F \times \text{dose} \times k_a)}{V(k_e - k_a)}(e^{-k_a t} - e^{-k_e t})$$

where k_a and k_e are time constants for absorption and elimination, respectively, and t is time. AUC for each mode of administration was estimated as the area under the best fitting curve, integrated for 8 h (Prism v5, Graphpad Software, Inc., San Diego, CA). Bioavailability for each mode of exenatide delivery was related to intravenous route using the equation $(AUC/AUC_{i.v.})/(dose/dose_{i.v.})$ (Rowland and Tozer, 1989).

3. Results

3.1. Plasma concentrations in rats

Exenatide was detectable in rat plasma after intraduodenal administration of a large 1 mg bolus (Fig. 1A). However, the peak concentration was only 115 pM.

Sublingual administration of 210 µg exenatide to rats (Fig. 1B) resulted in a peak plasma concentration of 443 pM, and at 0.37% [versus i.v.; Fig. 1E], was 18-fold higher in bioavailability than the intraduodenal route.

Intranasal administration of 100 µg exenatide to rats resulted in a peak plasma concentration of 1606 pM (Fig. 1C), and at 1.68%, was a further 4.5-fold more bioavailable than sublingual.

Peak mean plasma concentration was 1427 pM, following administration of 2 mg nebulized into a mist (Fig. 1D). The fraction of the aerosolized dose of exenatide that appeared in the plasma of rats exposed for 10 min was 0.09%. This low fraction contrasted with that for exenatide administered by intratracheal infusion (below) where bioavailability was the highest of any non-injected route. With the aerosol system, the actual exenatide dose delivered to the animal was unknown, but estimable. Air sampled from the chamber contained ~8 ng exenatide/mL. Typical resting (daytime) ventilation rates for adult rats are reported as ~600–750 mL/kg min (Seifert et al., 2000). The inspired dose was thereby calculated to be ~20 µg, or 1% of that added to the nebulizer. Adjusting for this 99% "wastage", the fraction of the dose actually delivered to the animal that appeared in plasma was estimated at ~9–10%.

Fig. 2A shows exenatide pharmacokinetic profiles for three doses of exenatide (2, 21, 210 µg) given intratracheally to rats. Peak plasma concentrations (58 pM, 667 pM, and 25 nM for 2,

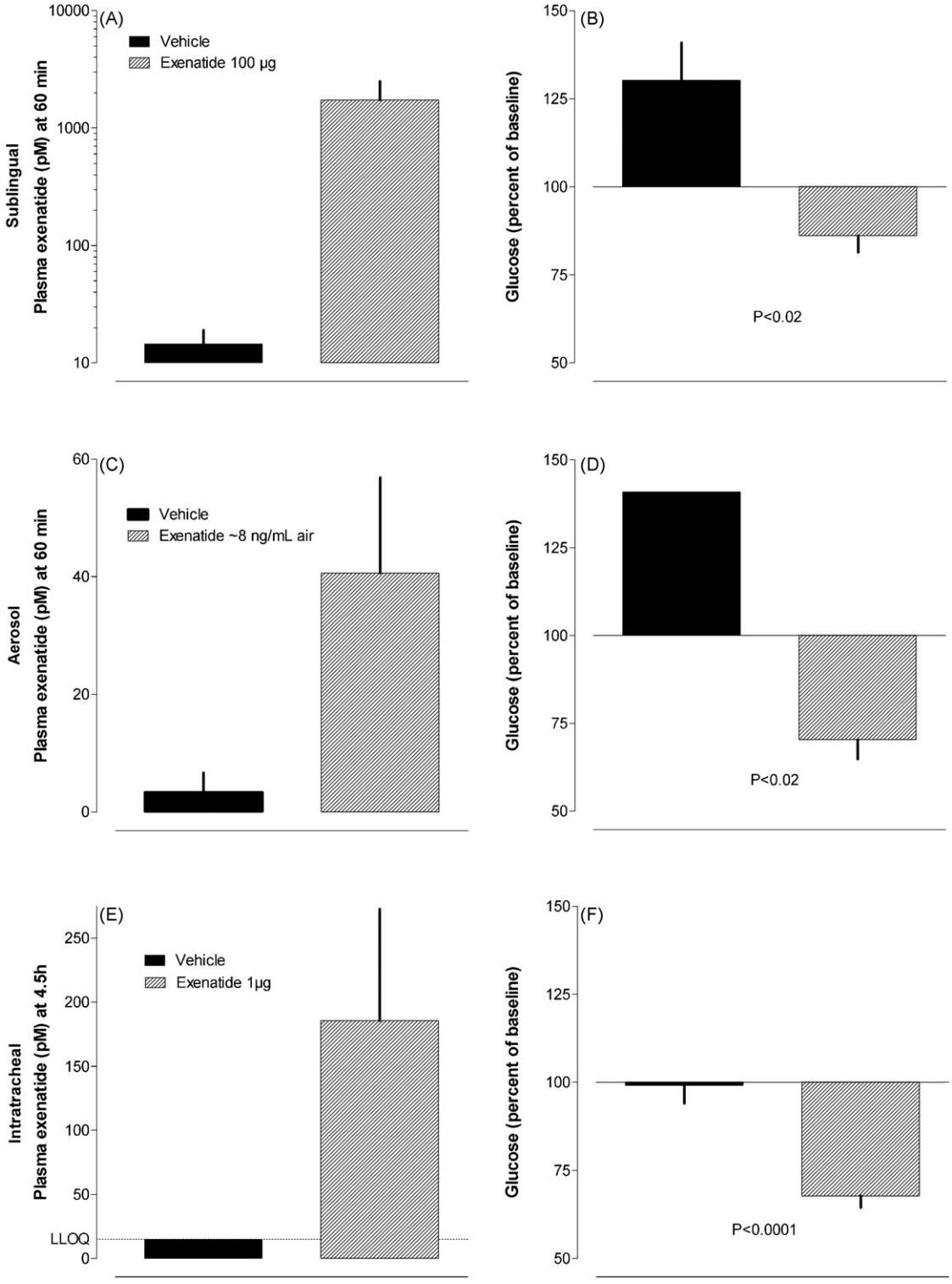

Fig. 3. Plasma concentrations of exenatide (A, C and E), and change in plasma glucose (B, D and F) in diabetic *db*/*db* mice. (A and B) Sublingual administration of 100 μg exenatide. $N = 3$–4 per group. (C and D) Pulmonary delivery via aerosol. $N = 10$–11 per group. (E and F) Intratracheal administration of 1 μg exenatide. Exenatide was measured at 4.5 h, glucose at 1.5 h. $N = 3$–4 per group. Glycemic response was otherwise shown as glucose after 1 h as a percent of the baseline value. Bars are means ± S.E.M.

Table 1
AUC (0–6 h) for each route, derived from each best-fit curve, is normalized per unit dose, and then per the intravenous result, to obtain the tabulated relative bioavailability

	k_a (min^{-1})	k_e (min^{-1})	AUC_{480}	Dose (μg)	AUC/dose (pM min/μg)	Rel BA (%)	C_{max} (pM/μg)
Intraduodenal	0.49	0.045	2895	1000	2.89	0.0053	0.115
Sublingual	0.21	0.011	42737	210	203.5	0.37	2.11
Intranasal	0.15	0.017	92100	100	921	1.68	16.06
Aerosol	0.41	0.017	100285	2000	50.1	0.092	0.71
Intravenous	0.091	0.017	11482000	210	54600	100	3757
Subcutaneous	0.099	0.0035	7080240	210	33715	61.7	134.3
Intratracheal	0.12	0.014	1556000	210	7410	13.6	327.1

21, and 210 μg, respectively) were dose-dependent, with half-lives ranging from 50 to 90 min. Sixty-one to 74% of peak plasma exenatide concentrations were observed within 5 min post-dose. T_{max} occurred between 20 and 30 min post-dose. The mean EC_{50} for the species-specific pressor effect of exenatide was 28 pM. The bioavailability of intratracheal, compared to intravenous (Fig. 1A) or subcutaneous (Fig. 1F), exenatide administration ranged from 7.3% to almost 15%, with corresponding C_{max} values of 1.2–1.9% (Fig. 2B and C).

The plasma concentration profiles following different routes of administration were modeled to single-component absorption and decay, represented as the gray lines in Figs. 1 and 2. Pharmacokinetic parameters k_a and k_e, and derived estimates of bioavailability versus intravenous administration are shown in Table 1.

3.2. Effects in db/db mice

Diabetic mice administered 100 μg exenatide sublingually had a mean exenatide plasma concentration of 1740 pM 1 h post-dose (Fig. 3A) accompanied by significant reductions in plasma glucose concentrations (Fig. 3B).

Aerosolized exenatide was detectable in the plasma of diabetic mice 1 h after 10 min exposure (Fig. 3C), and was accompanied by significant suppression of plasma glucose concentration ($P < 0.0001$; Fig. 3D) and a 4-fold elevation of plasma insulin concentration ($P = 0.011$; data not shown) without evidence of respiratory distress.

In diabetic mice, 1 μg intratracheal exenatide resulted in a mean plasma exenatide concentration of 186 pM 4.5 h after dosing (Fig. 3E) and was associated with significant reduction in plasma glucose concentration ($P = 0.019$; Fig. 3F).

4. Discussion

To circumvent their enteric degradation, therapeutic peptides are generally delivered parenterally, typically via subcutaneous injection or continuous infusion. However, even with microfine [essentially painless] needles, there appear to be barriers to the initiation or acceptance of injection as a mode of delivery. This situation may adversely affect patient compliance. The peptide incretin mimetic, exenatide, is currently administered by twice daily subcutaneous injection as adjunctive therapy for type 2 diabetes. Because many patients may prefer alternate routes of delivering exenatide, the feasibility of this was evaluated for two epithelial surfaces, the intestinal tract and the respiratory tract. Systemic absorption of exenatide was assessed in rats and mice via pharmacokinetic modeling of plasma concentration profiles. The pharmacodynamic capacity to deliver an effective antidiabetic dose was assessed from fall in plasma glucose concentration in diabetic *db*/*db* mice.

A growing number of bioactive peptides administered by nontraditional routes are commercially available or are under study for therapeutic efficacy. These include calcitonin, insulin, growth hormone, parathyroid hormone (PTH), somatostatin, follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), vasopressin, detrirelix and cetrorelix (luteinizing hormone-releasing hormone antagonists) (Agu et al., 2001; Owens et al., 2003; Munoz-Torres et al., 2004; Ugwoke et al., 2005; Thompson PDR Electronic Library™, 2006). Bioavailability of these peptide agents range from 0.3% to 45% compared to parenteral administration in preclinical models. Bioavailability is highly dependent on formulation excipients, mechanical delivery device, and physical state (e.g., powder) to facilitate membrane transduction. However, it has generally been in the lower part of this range in human clinical trials (Agu et al., 2001; Owens et al., 2003; Munoz-Torres et al., 2004; Mahesh Kumar and Misra, 2004).

Previous studies in patients with type 2 diabetes have demonstrated glucoregulatory effects from subcutaneous exenatide treatment when plasma concentrations reach 50–100 pM for sustained periods (Taylor et al., 2005). Although intraduodenal administration of a large bolus dose of exenatide to normoglycemic rats produced a spike in plasma exenatide concentration of this magnitude [115 pM] within 10 min, levels became undetectable within an hour, and no physiologic effects were detected. We consider the opportunity for enteral [intraduodenal] delivery exenatide to be low relative to other routes.

Sublingual administration of 210 μg exenatide to normoglycemic rats resulted in a 443 pM spike in plasma concentration within 5 min, followed by a 30 min plateau and an exponential type decay. Calculated bioavailability was 0.65% (versus subcutaneous) in the absence of permeability-enhancing excipients. Given the potency of exenatide, bioavailability in this range may not be limiting. Sublingual administration of 100 μg to *db*/*db* mice resulted in therapeutic concentrations (1740 pM at 1 h) and 34% reduction in plasma glucose relative to vehicle controls. Although sublingual bioavailability of insulin is reportedly low (Aungst et al., 1988), a buccal spray formulation (Ora-Lyn®) is sold in some countries.

Clinically used intranasal salmon calcitonin formulations have pharmacological bioequivalences up to 40% compared to parenteral delivery (Munoz-Torres et al., 2004). Marketed versions of intranasal calcitonin have a wide range of bioavailability [0.3–30%; mean 3%] compared to intramuscular injection (Thompson PDR Electronic Library™, 2006), but part of this range may be attributable to varied assay quantitation. Intranasal salmon calcitonin bioavailability was 1.6% in healthy human volunteers in one report (Lee et al., 1994), while an oral formulation of calcitonin containing a caprylic acid derivative as a carrier had a bioavailability range of 0.5–1.4% depending on dose (Buclin et al., 2002). In addition to assay variability, vehicle excipients such as dimethyl β-cyclodextrin have a direct effect on the nasal membrane and may enhance drug absorption by binding with the membrane components that serve as a barrier to calcitonin transport (Mahesh Kumar and Misra, 2004). This molecule transiently opens epithelial tight junctions via extraction of membrane cholesterol. Furthermore, it protects calcitonin from enzymatic degradation by molecular encapsulation and by causing the release of membrane-bound proteins resulting in inactivation of the proteolytic enzymes (Marttin et al., 1998).

Intranasal administration of exenatide (100 μg) into normoglycemic rats produced a rapid increase in plasma exenatide concentrations to 1606 pM within 10 min and maintenance for at least 60 min, indicating the potential of intranasal administration. Although bioavailability of intranasal insulin between 4% and 15% has been attained with permeation enhancers in humans (Pontiroli et al., 1987; Drejer et al., 1992; Jacobs et al., 1993), these are irritants, and the variation in bioavailability is an issue with insulin, where the therapeutic index demands dosing precision (Harris, 1993). Given the glucose-dependence of its effects, its wide therapeutic index, and its potency, the 1.7% bioavailability of exenatide [observed here without permeation enhancers], if attained in the clinic, may not be limiting.

Alternatives to injected insulin have received widespread scrutiny. Bioavailability of intratracheally-administered insulin was reported to be 13% in Sprague–Dawley rats (Okumura et al., 1992). Insulin bioavailability in rats was dependent on the pH of the insulin solution and was ∼42% relative to subcutaneous insulin injection. The bioavailability of aerosolized insulin has approached that of subcutaneous insulin, and in one rabbit study was ∼50% (Sakr, 1992). In 17 healthy human volunteers (Rave et al., 2005), relative bioefficacy over an entire glucose clamp period of 10 h was 10% for inhaled regular human insulin, and was 11% versus 18 U subcutaneously injected insulin lispro. In the first hour after administration, relative bioefficacy was 15%, and bioavailability 18% versus regular human insulin; total bioavailability over 10 h was 9%. In patients with type 1 diabetes, the same inhaled formulation was estimated to be 5–6% bioavailable versus subcutaneously injected insulin (Owens et al., 2003). Low acceptance of the device necessary for powdered inhalation has led to its market withdrawal. Other ultra-low density dry insulin formulations, where the drug penetrates deeper into the lung, have bioavailability of ∼77% (Rave et al., 2007) and may not be subject to the necessity for such a device.

The present dose-ranging study of intratracheal exenatide in rats demonstrated a rapid increase in plasma concentrations followed by decay with plasma half-lives typical of injected peptide. Intratracheal exenatide had up to ∼15% bioavailability compared to injection, and in diabetic *db*/*db* mice, reduced plasma glucose by 30% (1.5 h post-dose). At 186 pM, mean plasma concentration was well above the bioactive range (Taylor et al., 2005) 4.5 h after administration. The mode of delivery of aerosolized exenatide to normoglycemic rats was calculably inefficient, with 99% of drug being wasted. Nonetheless, exposure for 10 min resulted in a peak in plasma concentration within 5 min with no apparent respiratory distress, and was accompanied in diabetic *db*/*db* mice by reduction in plasma glucose. These data collectively identify the intra-pulmonary route of exenatide administration as a possible alternate to injection. If, for example, observed bioavailabilities prevailed in the clinic, antidiabetic efficacy could be attained without need for titration with fixed daily doses of less than 0.15 mg, comparing favorably with the ∼10–15 mg of inhaled insulin recommended for a similar antidiabetic effect (NDA 21-868/EXUBERA® US Package Insert, 2006).

In summary, the intestinal tract may have limited potential as a route of exenatide administration, with sublingual being the most promising. In contrast, the respiratory tract, represented by nasal and intra-pulmonary routes, appears to be more viable for exenatide treatment, comparing favorably in animal models with the clinically approved subcutaneous route. Despite the lack of any optimization of exenatide-delivery formulation, exenatide bioavailability was comparable to the bioavailability of several commercially available bioactive peptides.

References

Thompson PDR Electronic Library™, 2006. Accessed Nov 28, 2006.

NDA 21-868/EXUBERA® US Package Insert. Jan 27, 2006. Accessed Dec 21, 2007.

Agu, R.U., Ugwoke, M.I., Armand, M., Kinget, R., Verbeke, N., 2001. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir. Res. 2, 198–209.

Aungst, B.J., Rogers, N.J., Shefter, E., 1988. Comparison of nasal, rectal, buccal, sublingual and intramuscular insulin efficacy and the effects of a bile salt absorption promoter. J. Pharmacol. Exp. Ther. 244, 23–27.

Buclin, T., Cosma Rochat, M., Burckhardt, P., Azria, M., Attinger, M., 2002. Bioavailability and biological efficacy of a new oral formulation of salmon calcitonin in healthy volunteers. J. Bone Miner. Res. 17, 1478–1485.

Buse, J.B., Henry, R.R., Han, J., Kim, D.D., Fineman, M.S., Baron, A.D., 2004. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. Diabetes Care 27, 2628–2635.

Calara, F., Taylor, K., Han, J., Zabala, E., Carr, E.H., Wintle, M., Fineman, M., 2005. A randomized, open-label, crossover study examining the effect of injection site on bioavailability of exenatide (synthetic exendin-4). Clin. Ther. 27, 210–215.

Copley, K., McCowen, K., Hiles, R., Nielsen, L.L., Young, A., Parkes, D.G., 2006. Investigation of exenatide elimination and its in vivo and in vitro degradation. Curr. Drug Metab. 7, 367–374.

DeFronzo, R.A., Ratner, R.E., Han, J., Kim, D.D., Fineman, M.S., Baron, A.D., 2005. Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. Diabetes Care 28, 1092–1100.

Drejer, K., Vaag, A., Bech, K., Hansen, P., Sorensen, A.R., Mygind, N., 1992. Intranasal administration of insulin with phospholipid as absorption enhancer: pharmacokinetics in normal subjects. Diabet. Med. 9, 335–340.

Gedulin, B.R., Nikoulina, S.E., Smith, P.A., Gedulin, G., Nielsen, L.L., Baron, A.D., Parkes, D.G., Young, A.A., 2005. Exenatide (exendin-4) improves insulin sensitivity and ß-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. Endocrinology 146, 2069–2076.

Harris, A.S., 1993. Review: clinical opportunities provided by the nasal administration of peptides. J. Drug Target 1, 101–116.

Heine, R.J., Van Gaal, L.F., Johns, D., Mihm, M.J., Widel, M.H., Brodows, R.G., 2005. Exenatide versus insulin glargine in patients with suboptimally controlled type 2 diabetes: a randomized trial. Ann. Intern. Med. 143, 559–569.

Jacobs, M.A., Schreuder, R.H., Jap-A-Joe, K., Nauta, J.J., Andersen, P.M., Heine, R.J., 1993. The pharmacodynamics and activity of intranasally administered insulin in healthy male volunteers. Diabetes 42, 1649–1655.

Kendall, D.M., Riddle, M.C., Rosenstock, J., Zhuang, D., Kim, D.D., Fineman, M.S., Baron, A.D., 2005. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. Diabetes Care 28, 1083–1091.

Lee, W.A., Ennis, R.D., Longenecker, J.P., Bengtsson, P., 1994. The bioavailability of intranasal salmon calcitonin in healthy volunteers with and without a permeation enhancer. Pharm. Res. 11, 747–750.

Linnebjerg, H., Kothare, P.A., Park, S., Mace, K., Reddy, S., Mitchell, M., Lins, R., 2007. Effect of renal impairment on the pharmacokinetics of exenatide. Br. J. Clin. Pharmacol. 64, 317–327.

Mahesh Kumar, T., Misra, A., 2004. Pulmonary absorption enhancement of salmon calcitonin. J. Drug Target 12, 135–144.

Mari, A., Nielsen, L.L., Nanayakkara, N., DeFronzo, R.A., Ferrannini, E., Halseth, A., 2006. Mathematical modeling shows exenatide improved beta-cell function in patients with type 2 diabetes treated with metformin or metformin and a sulfonylurea. Horm. Metab. Res. 38, 838–844.

Marttin, E., Verhoef, J.C., Merkus, F.W., 1998. Efficacy, safety and mechanism of cyclodextrins as absorption enhancers in nasal delivery of peptide and protein drugs. J. Drug Target 6, 17–36.

Mentlein, R., Gallwitz, B., Schmidt, W.E., 1993. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur. J. Biochem. 214, 829–835.

Munoz-Torres, M., Alonso, G., Raya, M.P., 2004. Calcitonin therapy in osteoporosis. Treat. Endocrinol. 3, 117–132.

Nauck, M.A., 2004. Glucagon-like peptide 1 (GLP-1) in the treatment of diabetes. Horm. Metab. Res. 36, 852–858.

Nielsen, L.L., 2005. Incretin mimetics and DPP-IV inhibitors for the treatment of type 2 diabetes. Drug Discov. Today 10, 703–710.

Nielsen, L.L., Young, A.A., Parkes, D.G., 2004. Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. Regul. Pept. 117, 77–88.

Okumura, K., Iwakawa, S., Yoshida, T., Seki, T., Komada, F., 1992. Intratracheal delivery of insulin: absorption from solution and aerosol by rat lung. Int. J. Pharm. 88, 63–73.

Owens, D.R., Zinman, B., Bolli, G., 2003. Alternative routes of insulin delivery. Diabet. Med. 20, 886–898.

Parkes, D., Jodka, C., Smith, P., Nayak, S., Rinehart, L., Gingerich, R., Chen, K., Young, A., 2001a. Pharmacokinetic actions of exendin-4 in the rat: comparison with glucagon-like peptide-1. Drug Dev. Res. 53, 260–267.

Parkes, D.G., Pittner, R., Jodka, C., Smith, P., Young, A., 2001b. Insulinotropic actions of exendin-4 and glucagon-like peptide-1 in vivo and in vitro. Metabolism 50, 583–589.

Pontiroli, A.E., Alberetto, M., Pajetta, E., Calderara, A., Pozza, G., 1987. Human insulin plus sodium glycocholate in a nasal spray formulation: improved bioavailability and effectiveness in normal subjects. Diabet. Metab. 13, 441–443.

Rave, K., Bott, S., Heinemann, L., Sha, S., Becker, R.H., Willavize, S.A., Heise, T., 2005. Time-action profile of inhaled insulin in comparison with subcutaneously injected insulin lispro and regular human insulin. Diabetes Care 28, 1077–1082.

Rave, K., de la Pena, A., Tibaldi, F.S., Zhang, L., Silverman, B., Hausmann, M., Heinemann, L., Muchmore, D.B., 2007. AIR inhaled insulin in subjects with chronic obstructive pulmonary disease: pharmacokinetics, glucodynamics, safety, and tolerability. Diabetes Care 30, 1777–1782.

Rowland, M., Tozer, T.N., 1989. Clinical Pharmacokinetics: Concepts and Applications. Lea and Febiger, Philadelphia.

Sakr, F.M., 1992. A new approach for insulin delivery via the pulmonary route: design and pharmacokinetics in non-diabetic rabbits. Int. J. Pharm. 86, 1–7.

Seifert, E.L., Knowles, J., Mortola, J.P., 2000. Continuous circadian measurements of ventilation in behaving adult rats. Respir. Physiol. 120, 179–183.

Taylor, K., Kim, D., Nielsen, L.L., Aisporna, M., Baron, A.D., Fineman, M.S., 2005. Day-long subcutaneous infusion of exenatide lowers glycemia in patients with type 2 diabetes. Horm. Metab. Res. 37, 627–632.

Young, A.A., Gedulin, B.R., Bhavsar, S., Bodkin, N., Jodka, C., Hansen, B., Denaro, M., 1999. Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (*ob/ob,* d*b*/d*b*) mice, diabetic Fatty Zucker rats and diabetic rhesus monkeys (*Macaca mulatta*). Diabetes 48, 1026–1034.

Zinman, B., Hoogwerf, B.J., Duran Garcia, S., Milton, D.R., Giaconia, J.M., Kim, D.D., Trautmann, M.E., Brodows, R.G., 2007. The effect of adding exenatide to a thiazolidinedione in suboptimally controlled type 2 diabetes. Ann. Intern. Med. 146, 477–485.