# Colonic Absorption of Insulin-like Growth Factor I *in Vitro*

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Colonic absorption of recombinant human insulin-like growth factor I (rhIGF-I) was measured *in vitro* using both rat and minipig colon. The permeability coefficients were 8.03 ± 1.03 and 4.75 ± 0.43 × $10^{-8}$ cm $sec^{-1}$ in the rat and minipig, respectively. The steady-state flux in rat colon was linearly related to the donor concentration over the range 1 to 10 mg/mL. rhIGF-I was metabolically stable in contact with both mucosal and serosal surfaces of washed colon for 5 hr. The amount of IGF-I permeating through the tissue was quantitated by radioimmunoassay and the identity and integrity of the permeating species were confirmed by reverse-phase HPLC, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and Western blotting. In all cases the permeant was identical to authentic rhIGF-I. The integrity of the colonic tissue *in vitro* was demonstrated by the maintenance of electrophysiological parameters, a secretory response to serosal theophylline, and the ability of sodium azide, a metabolic inhibitor, to abolish the barrier properties and cause a large increase in flux.

**KEY WORDS:** insulin-like growth factor I; colon; drug delivery; protein; absorption.

## INTRODUCTION

Insulin-like growth factor I (IGF-I) is a 7649-dalton (Da) protein consisting of 70 amino acid residues in a single chain connected by three disulfide bridges. It shows considerable homology with proinsulin (1) but exerts its biological actions through specific IGF-I receptors (2). It is produced predominantly in the liver for release into the circulation, but it is also produced locally in many tissues where it exerts a paracrine effect (3). IGF-I circulates bound to specific binding proteins which serve to protect IGF-I from rapid metabolism and to modulate its physiological effects (4). The half-life of free IGF-I is 4 to 20 min, compared to 4 to 18 hr for bound IGF-I (5,6).

In clinical studies, IGF-I was found to lower blood glucose in insulin-resistant diabetic patients (7) and may have an important therapeutic role in these patients who have poorly controlled blood glucose levels. It may also offer therapeutic advantages over insulin in terms of lipid metabolism (8). Many of the long-term complications seen in diabetes may be related to hyperinsulinemia and the use of IGF-I may obviate these effects, particularly since the IGF-I binding proteins (BP) serve to modulate plasma levels of IGF-I. Other potential therapeutic uses include catabolic states (9) in which patients have a negative nitrogen balance, for example, following burns or major surgery, and osteoporosis, in which IGF-I may stimulate bone growth and thus reverse bone loss (10).

Therapeutic administration of large peptides and proteins is generally by either subcutaneous or intravenous injection. Oral delivery is the preferred route of administration for low molecular weight drugs, but proteins are subject to extensive degradation and denaturation in the upper gastrointestinal (GI) tract. Additionally, the epithelial membrane offers a barrier to their absorption since they are large and frequently hydrophilic. Recently, attention has focused on the colon as a potential site for the delivery and absorption of proteins due to its lower proteolytic activity (11) and long residence time. About 80% of the total GI transit time is spent in the colon (12,13), with the time varying between 10 and 20 hr on average. The colon is generally less permeable than the small intestine (14), but the increase in residence time and decreased proteolytic activity may compensate for this.

There is evidence that small amounts of proteins are absorbed intact from the GI tract (15). Bovine serum albumin (MW 68 kD) was absorbed to a greater extent from the jejunum than the colon in the rat (1.3 and 0.13% of the dose, respectively, 16). Recently, colonic absorption of calcitonin was reported in the rat, with a bioavailability of 0.2 to 0.9% (17). Colonic absorption of insulin was increased in the pig *in vivo* by the addition of aprotinin, a protease inhibitor, and 5-methoxysalicylic acid, an absorption enhancer (18).

The present study describes permeation studies using rat and minipig colonic mucosa *in vitro* to measure the absorption of recombinant human IGF-I (rhIGF-I) by this route. The *in vitro* colonic system utilized in these studies has been described previously (19). The use of the Ussing chamber also provides preliminary information on tissue metabolism of the compound.

## MATERIALS AND METHODS

### Materials

rhIGF-I was obtained from Physical and Analytical Chemistry, Ciba-Geigy Corporation (Suffern, NY) and was used without further purification. IGF-I/SmC reagent packs and blotting detection kits were purchased from Amersham Corporation (Arlington Heights, IL). The blotting kits contained biotinylated anti-rabbit immunoglobulin from donkey, streptavidin–alkaline phosphatase conjugate, and an alkaline phosphatase substrate system consisting of nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indoyl phosphate (BCIP). Sigmacote and bovine serum albumin (Fraction V) were obtained from Sigma Chemical Company (St. Louis, MO); Norit A charcoal, HPLC-grade acetonitrile and certified trifluoroacetic acid (TFA) were from Fisher Scientific (Springfield, NJ); and dextran T-70 was from Pharmacia (Piscataway, NJ). All other reagents were of analytical grade.

Gel electrophoresis was performed on a Pharmacia

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Model GE-2/4LS apparatus using precast gradient gels (17–27%) from Integrated Separation Systems, Inc. (Hyde Park, MA). Western blotting was done on nitrocellulose membranes (0.2 μm) obtained from Bio-Rad (Richmond, CA). Proteins were electrotransferred from gels to nitrocellulose membranes using the Bio-Rad Trans-Blot Electrophoretic Transfer Cell Model 170-3910.

### Tissue Preparation

Male Sprague–Dawley rats (400–600 g; Charles River Laboratories, Wilmington, MA) were deprived of food overnight but allowed free access to water. The descending colon was removed following anesthetization with an intraperitoneal injection of a 1:10 mixture of acepromazine (10 mg/mL):ketamine (100 mg/mL) at a dose of 1 mL/kg. The colon was stripped of underlying muscle by the method of Parsons and Paterson (20) and was mounted in modified Ussing chambers (WPI, Sarasota, FL). Pig colon was obtained from one male Yucatan minipig (35 kg; Charles River Laboratories), sacrificed by an intravenous overdose of Nembutal. The descending colon (about 5–12 cm above the anus) was removed, opened along the mesentery, cleaned of its contents, stripped of the underlying muscle layer, and mounted as described above.

### Experimental Procedure

The chambers were connected to water-jacketed glass reservoirs to maintain the temperature of the tissue at 37°C and 4 mL of Krebs–Henseleit buffer, containing 0.1% bovine serum albumin (BSA), and 10 m*M* glucose was added to each side of the reservoir. The buffer was gassed with 95% $O_2$/5% $CO_2$ to maintain tissue viability, control the buffer pH at 7.4, and circulate the fluid past the surface of the tissue. All glassware used in these experiments was treated with Sigmacote, a silanizing agent, in order to prevent adsorption of IGF-I to the surface of the glassware. The area of exposed tissue was 0.69 $cm^2$. Transepithelial potential difference (p.d.) and short-circuit current ($I_{sc}$) were measured as previously described (19).

After a 30-min equilibration period to allow stabilization of the electrical parameters, fluid was drained from both sides of the chamber and replaced with rhIGF-I donor solution in buffer on the mucosal side and fresh buffer on the serosal side. Samples (500 μL) were removed from the serosal side at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 hr and the volume was replaced with an equal volume of buffer. Some incubations were carried out in buffer with no exogenous rhIGF-I to measure the release of endogenous IGF-I from the tissue, and samples were taken from the serosal side at the same times as those detailed above. Electrophysiological measurements were recorded at the same time as samples were taken. Samples were collected in microfuge tubes on ice and assayed for immunoreactive IGF-I (*vide infra*) immediately following the study. At the end of the experimental period, 10 m*M* theophylline was added to the serosal surface of the colon and the $I_{sc}$ monitored to assess tissue viability. The steady-state flux ($J_{ss}$) was calculated from the linear portion of the cumulative flux-vs-time curve and the time lag ($T_L$) was obtained from the intercept on the abscissa. The permeability coefficient ($P$) was calculated as shown below:

$$P = \frac{J_{ss}}{C}$$

where $C$ is the initial donor concentration.

### Radioimmunoassay for IGF-I

The polyclonal antiserum and 3-[$^{125}$I]iodotyrosyl IGF-I were reconstituted in the assay buffer provided in the kit and stored in plastic containers at 4°C. rhIGF-I standards (1–500 ng/mL) were prepared by serial dilution in assay buffer from a stock solution of 0.2 mg/mL. All samples were assayed in duplicate in polystyrene tubes. Briefly, 10–100 μL of sample (the volume was adjusted with Krebs–Henseleit buffer to give a total of 100 μL) or standard (100 μL) was added to the tubes. Assay buffer (100 μL) was added to the sample tubes and Krebs buffer (100 μL) was added to standard tubes to ensure the same matrix in all tubes. One hundred microliters of [$^{125}$I]IGF-I was added, followed by 100 μL of antiserum, and the samples were vortexed and incubated at 2–8°C for 24 hr. Antibody-bound IGF-I was separated from free IGF-I by the addition of 0.5 mL of dextran-coated charcoal (1.6% Norit A charcoal/0.16% dextran T-70 in 0.25 *M* sodium phosphate buffer, pH 7.2) followed by centrifugation at 1500*g* and 4–8°C for 15 min. The supernatant was decanted and counted for bound radioactivity in a Minaxi Auto-Gamma 5000 (Packard Instrument Co., Sterling, VA). The amount of IGF-I in the samples was then calculated from the standard curve obtained by a logit–log transformation of the data. The coefficient of variation for quality control samples was 10.6 to 20.9% over the range of the standard curve.

The results are expressed as the mean ± 1 SE.

### High-Performance Liquid Chromatography (HPLC)

rhIGF-I was separated by reverse-phase HPLC on a Nucleosil 5 C-18 column (Macherey-Nagel, 20.0 cm × 4 mm) with detection at 214 nm (484 Detector; Waters Associates, Milford, MA). Samples (20 to 200 μL) were injected automatically by an UltraWisp 715 (Waters Associates) and the mobile phase was pumped at 0.8 mL/min using a gradient controller (600E; Waters Associates). The gradient system consisted of mobile phase A (0.1% TFA in water) and mobile phase B [0.08% TFA in acetonitrile/water (80/20)]. The proportion of mobile phase B was increased from 30 to 38% over 57 min.

### Gel Electrophoresis and Western Blotting

Samples from flux studies and standard rhIGF-I (0.0125–5 μg) were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing conditions. Precast gradient gels (17–27%) were run at a constant current of between 25 and 40 mA per gel. Gels were either stained with Coomassie brilliant blue or electrotransferred to nitrocellulose membranes and probed with antiserum to IGF-I. The antigen–antibody complex was detected using a biotinylated anti-rabbit immunoglobulin obtained from the donkey. The blots were then incubated

with a streptavidin–alkaline phosphatase conjugate which binds to the biotinylated complexes. The bands were visualized by the addition of an alkaline phosphatase substrate system.

## RESULTS

Release of endogenous IGF-I from rat colon was low (<2 ng/mL) and constant over the experimental period. The steady-state fluxes of rhIGF-I were 0.31 ± 0.08, 1.6 ± 0.5, 2.6 ± 0.5 μg/cm²/hr from donor solutions of 1, 5, and 10 mg/mL (Fig. 1), with time lags to steady state of 66 ± 20, 40 ± 18, and 58 ± 17 min, respectively ($n$ = 5 to 7). There were no significant differences among the time lags for the different donor concentrations. The steady-state flux was linearly related to the donor concentration (Fig. 2; $r$ = 0.99). The addition of sodium azide, a metabolic inhibitor that abolishes the potential difference across colonic tissue, caused a four- to fivefold increase in flux. Release of immunoreactive endogenous IGF-I was considerably higher from pig colon (13–80 ng/mL) than from rat colon. The steady-state flux of IGF-I from a donor solution of 10 mg/mL was 1.7 ± 0.2 μg/cm²/hr, with a time lag of 46 ± 6 min ($n$ = 3). Sodium azide caused a fourfold increase in flux. The flux data are summarized in Table I.

Donor solutions were analyzed by reverse-phase HPLC (RP-HPLC) before exposure to the mucosal surface of colon and after 5 hr of exposure. The HPLC profiles were almost identical, indicating that essentially no metabolism of rhIGF-I had occurred during exposure to the luminal surface of the colon (Fig. 3). The rhIGF-I accounted for 99.3% of the peak areas before exposure to the tissue and 98.4% after 5 hr of incubation with colon.

Although the presence of immunoreactive IGF-I was detected in the serosal fluid from flux studies, confirmation of the integrity of the molecule was sought by other techniques. The amount of IGF-I in the earlier samples during the time course was below the limit of detection by RP-HPLC, but analysis of serosal samples obtained after longer transport times yielded a main peak with the same retention time as authentic rhIGF-I. This was confirmed by analysis of serosal fluid by SDS-PAGE and staining or electrotransfer onto nitrocellulose and reaction with IGF-I antiserum, which showed that the serosal material was identical to authentic rhIGF-I that had been similarly treated.

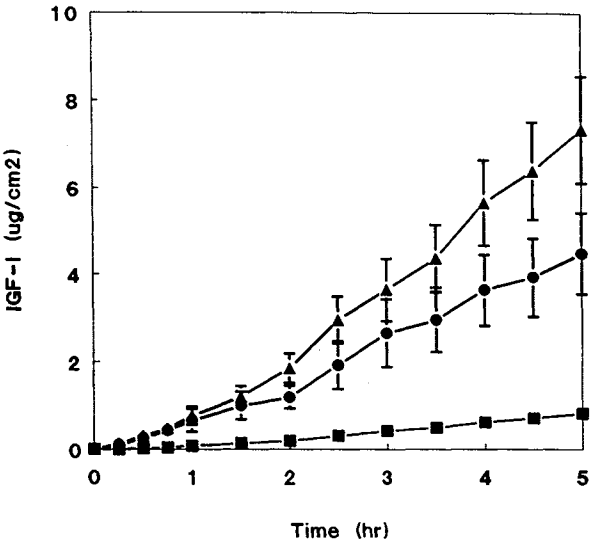


Fig. 1. Cumulative absorption of immunoreactive IGF-I across rat colon from rhIGF-I donor solutions of 1, 5, and 10 mg/mL. Squares, 1 mg/mL; circles, 5 mg/mL; triangles, 10 mg/mL.

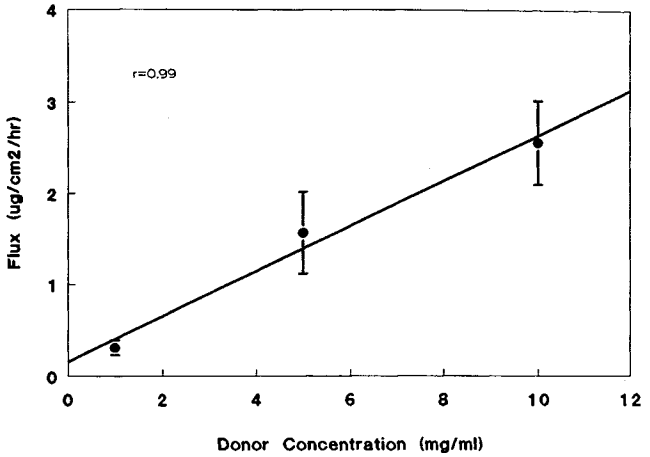


Fig. 2. Linear relationship between the steady-state flux of IGF-I ($J_{ss}$) and the donor concentration in rat colon.

Electrophysiological parameters (p.d., $I_{sc}$, resistance, and conductance) were monitored to assess the viability of the colon. Based on earlier experiments which indicated an exponential increase in flux of a low molecular weight compound with decreasing resistance (19), only tissue with a mean resistance greater than 100 Ω cm² was utilized in this study. All tissue also demonstrated at least a threefold increase in $I_{sc}$ in response to the addition of serosal theophylline. A positive response to theophylline is a reflection of cellular viability. The electrophysiological parameters are summarized in Table II. The addition of rhIGF-I caused a transient increase in conductance in rat colon at all concentrations. However, the mean conductance was not significantly different from control values and the increase was not related to the concentration of rhIGF-I.

In minipig colon, the p.d. decreased significantly after a 3-hr incubation period in both control and rhIGF-I-treated colon, suggesting a loss of tissue viability and potentially of barrier properties. Electrical parameters were constant over 3 hr and the flux studies were analyzed only over that time period.

Table I. Steady-State Flux ($J_{ss}$), Time Lag ($T_L$), and Permeability Coefficients ($P$) for IGF-I in Rat and Minipig Colon[a]

| Tissue | Donor (mg/mL) | $J_{ss}$ (μg/cm²/hr) | $T_L$ (min) | $P$ (cm/sec) × $10^{-8}$ |
|---|---|---|---|---|
| Rat colon | 1 | 0.31 ± 0.08 (5) | 66 ± 20 | 8.62 ± 2.11 |
| | 5 | 1.6 ± 0.5 (5) | 40 ± 18 | 8.73 ± 2.52 |
| | 10 | 2.6 ± 0.5 (7) | 58 ± 17 | 7.12 ± 1.28 |
| Pig colon | 10 | 1.7 ± 0.5 (3) | 46 ± 6 (3) | 4.75 ± 0.43 |

[a] Results are the mean ± 1 SE; the number of experiments is given in parentheses.

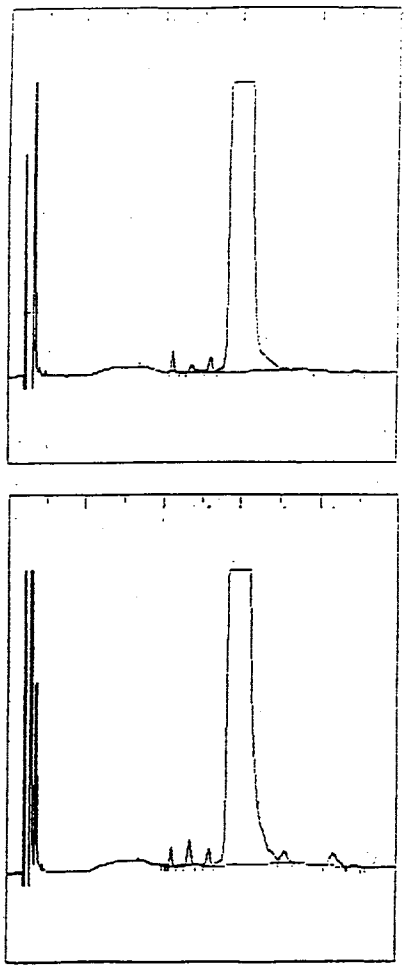

Fig. 3. RP-HPLC profile for the rhIGF-I donor solution from rat colon flux studies. Top, preincubation; bottom, after 5 hr in contact with the mucosal surface of the colon in a modified Ussing chamber. Injection volume, 100 μL.

## DISCUSSION

Steady-state flux of IGF-I was demonstrated *in vitro* through isolated colon from rat and minipig. In the rat, the flux was linearly related to donor concentration over the range 1 to 10 mg/mL. The permeating species was quantitated by radioimmunoassay (RIA) using a polyclonal antibody. However, this method may not distinguish between intact IGF-I and fragments containing the epitopes recognized by the antiserum. RP-HPLC and SDS-PAGE were used to confirm the identity and integrity of the permeating species, which behaved in the same way as authentic rhIGF-I. The permeability coefficients for IGF-I flux through rat and minipig colon were 8.03 ± 1.04 and 4.75 ± 0.43 × $10^{-8}$ cm $sec^{-1}$, respectively ($n$ = 17 and 3, $P < 0.02$). For comparison, the permeability coefficients for insulin in everted sacs of rat small intestine ranged from 0.72 to 7.8 × $10^{-7}$ cm $sec^{-1}$ (21). The integrity of the mucosal barrier was monitored in several ways: the maintenance of electrophysiological properties, the secretory response to serosal theophylline, and the abolition of potential difference and concomitant increase in flux in response to sodium azide. Tissue resistance declined minimally over the 5-hr incubation period and was 78.9, 85.0, 109.1, and 83.9% of the initial resistance in control colon and colon exposed to 1, 5, and 10 mg/mL, respectively. All tissue demonstrated at least a threefold increase in $I_{sc}$ in response to theophylline added at the end of the flux studies.

Table II. Electrophysiological Parameters in Rat Colon[a]

| IGF-I (mg/mL) | p.d. (mV) | $I_{sc}$ (μA/cm²) | Resistance (Ω cm²) | Conductance (mS/cm²) |
|---|---|---|---|---|
| — | 5.9 ± 1.1 | 48 ± 13 | 136.9 ± 15.1 | 7.7 ± 0.8 |
| 1 | 4.9 ± 2.3 | 28 ± 11 | 155.9 ± 10.4 | 6.8 ± 0.4 |
| 5 | 4.5 ± 1.1 | 25 ± 6 | 184.4 ± 13.2 | 5.8 ± 0.4 |
| 10 | 5.6 ± 1.6 | 40 ± 13 | 148.0 ± 8.1 | 7.3 ± 0.3 |

[a] Results are the mean ± 1 SE; $n$ = 5–7.

The studies reported here were carried out using concentrated solutions of rhIGF-I in contact with the mucosal surface. In the physiological state, rhIGF-I would be diluted in the GI contents and subject to metabolism by luminal and brush border enzymes. The tissue was washed before the addition of rhIGF-I and there was no evidence of metabolism of rhIGF-I in either the donor or the receiver solutions in contact with the colon for up to 5 hr. A similar lack of metabolism was reported for insulin and 8-L-arginine vasopressin in contact with washed small intestine (21,22), although metabolism was extensive in the latter case in ileal luminal contents. Metabolism, although reduced in the colon, is still fairly extensive (23) and colonic contents may be sufficiently solid to interfere with the dosage form and impair drug release and diffusion to the membrane.

rhIGF-I added to the mucosal surface caused a slight, statistically insignificant increase in conductance in rat colon that occurred over the first 1 to 2 hr of the study. The change in conductance was not seen in minipig colon. IGF-I receptors have been identified in the GI tract in both the rat and the rabbit (24,25), suggesting the importance of IGF-I as a local mediator of intestinal growth and metabolism. Both insulin and IGF-I have been shown to regulate the paracellular pathway in intestinal cells in culture (26,27). The increase in paracellular permeability in the latter studies was manifested as a decrease in electrical resistance and an increase in flux of mannitol, a paracellular marker, over a 3- to 4-day period. Both insulin and IGF-I exerted their effects only when added to the basolateral surface and not the apical surface. IGF-I and epidermal growth factor (EGF) have also been reported to increase the flux of horseradish peroxidase (MW 40 kD) across conjunctiva *in vitro* (28). The proposed mechanism was by interaction with specific receptors and stimulation of fluid phase transcytosis, since the flux of paracellular markers was unchanged. In the current study, the time course and the site of application of the IGF-I were different from those seen in intestinal cells in culture, and it is unlikely that the flux was due to an increase in conductance of the epithelial membrane.

The current studies demonstrate that IGF-I was absorbed intact across rat colonic mucosa *in vitro* as determined by RP-HPLC, SDS-PAGE, and Western blotting. Attempts may be made to extrapolate from these observations to their significance for drug delivery in the clinical setting. Assuming 500 mg of IGF-I could be protected from the en-

vironment of the upper gastrointestinal tract and delivered into the colon, a drug concentration of 5 mg/mL would be obtained if 100 mL of fluid were present in the ascending colon. The flux from a 5 mg/mL donor solution was 1.6 $\mu g/cm^2/hr$ (*vide supra*), and exposure of the drug solution to the entire length of the ascending colon could result in the absorption of 8 mg of IGF-I over an 8-hr period. This is potentially a clinically significant amount. However, these calculations ignore the effects of metabolism, which may be as great as 20 mg of protein hydrolyzed/g wet wt of GI contents/hr (11). The relative significance of proteolysis and permeation through the intestinal wall must be assessed for each protein.

## REFERENCES


1. E. Rinderknecht and R. E. Humbel. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J. Biol. Chem.* 253:2769–2776 (1978).
2. S. Gammeltoft. Insulin-like growth factors and insulin: Gene expression, receptors, and biological actions. In J. Martinez (ed.), *Peptide Hormones as Prohormones: Processing, Biological Activity, Pharmacology,* Halsted Press, New York, 1989.
3. V. R. Sara and K. Hall. Insulin-like growth factors and their binding proteins. *Physiol. Rev.* 70:591–614 (1990).
4. S. Hardouin, P. Hossenlopp, B. Segouia, D. Seurin, G. Portolan, C. Lassame, and M. Binoux. Heterogeneity of insulin-like growth factor binding proteins and relationships between structure and affinity. I. Circulating forms in man. *Eur. J. Biochem.* 170:121–132 (1987).
5. P. E. Walton, R. Gopinath, B. D. Burleigh, and T. D. Etherton. Administration of recombinant human insulin-like growth factor I to pigs: Determination of circulating half-lives and chromatographic profiles. *Horm. Res.* 31:138–142 (1989).
6. S. C. Hodgkinson, S. R. Davis, B. D. Burleigh, H. V. Henderson, and P. D. Gluckman. Metabolic clearance rate of insulin-like growth factor-I in fed and starved sheep. *J. Endocr.* 115:233–240 (1987).
7. D. S. Schalch, N. J. Turman, V. Marcsisin, and H. P. Guler. Short-term metabolic effects of recombinant human insulin-like growth factor I (rhIGF-I) in type II diabetes mellitus. 2nd International IGF Symposium on Insulin-Like Growth Factors/Somatomedins, 1991.
8. A. Giacca, R. Gupta, S. Efendic, K. Hall, A. Skottner, L. Lickley, and M. Vranic. Differential effects of IGF-I and insulin on glucoregulation and fat metabolism in depancreatized dogs. *Diabetes* 39:340–347 (1990).
9. F. M. Tomas, S. E. Knowles, P. C. Owens, L. C. Read, C. S. Chandler, S. E. Gargosky, and F. J. Ballard. Increased weight gain, nitrogen retention and muscle protein synthesis following treatment of diabetic rats with insulin-like growth factor (IGF-I) and des(1-3)IGF-I. *Biochem. J.* 276:547–554 (1992).
10. E. M. Spencer, C. C. Liu, E. C. C. Si, and G. A. Howard, *In vivo* actions of insulin-like growth factor-I (IGF-I) on bone formation and resorption in rats. *Bone* 12:21–26 (1991).
11. S. A. W. Gibson, C. McFarlan, S. Hay, and G. T. MacFarlane. Significance of microflora in proteolysis in the colon. *Appl. Env. Microbiol.* 55:679–683 (1989).
12. M. Proano, M. Camilleri, S. F. Phillips, M. L. Brown, and G. M. Thomforde. Transit of solids through the human colon: Regional quantification in the unprepared bowel. *Am. J. Physiol.* 258:G856–G862 (1990).
13. B. Krevsky, L. S. Malmud, F. D'Ercole, A. H. Maurer, and R. S. Fisher. Colonic transit scintigraphy. A physiological approach to the quantitative measurement of colonic transit in humans. *Gastroenterology* 91:1102–1112 (1986).
14. V. S. Chadwick, S. F. Phillips, and A. F. Hofmann. Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). II. Application to normal and abnormal permeability states in man and animals. *Gastroenterology* 73:247–251 (1977).
15. M. L. G. Gardner. Gastrointestinal absorption of intact proteins. *Annu. Rev. Nutr.* 8:329–350 (1988).
16. A. L. Warshaw, C. A. Bellini, and W. A. Walker. The intestinal mucosal barrier to intact antigenic protein. Difference between colon and small intestine. *Am. J. Surg.* 133:55–58 (1977).
17. J. Hastewell, S. Lynch, I. Williamson, R. Fox, and M. Mackay. Absorption of human calcitonin across the rat colon *in vivo*. *Clin. Sci.* 82:589–594 (1992).
18. A. N. Elias, I. Gordon, N. D. Vaziri, G. Chune, M. R. Pandian, G. Gwinup, and R. Wesley. Effective portal insulin delivery with enzyme-protected capsules in pancreatectomized pigs. *Gen. Pharmac.* 23:55–59 (1992).
19. E. Quadros, J. Cassidy, K. Gniecko, and S. LeRoy. Buccal and colonic absorption of CGS 16617, a novel ACE inhibitor. *J. Control. Release* 19:77–86 (1991).
20. G. Parsons and C. R. Paterson. Fluid and solute transport across rat colonic mucosa. *Q. J. Exp. Physiol.* 50:220–231 (1965).
21. R. J. Schilling and A. K. Mitra. Intestinal transports of insulin. *Int. J. Pharm.* 62:53–64 (1990).
22. B. Matuszewska, G. G. Liversidge, F. Ryan, J. Dent, and P. L. Smith. *In vitro* study of intestinal absorption and metabolism of 8-L-arginine vasopressin and its analogues. *Int. J. Pharm.* 46:111–120 (1988).
23. N. M. Landzert, K. Gniecko, and E. Quadros. Protein digestion in the gastrointestinal tract. *Pharm. Res.* 9:S179 (1992).
24. M. Laburthe, C. Rouyer-Fessard, and S. Gammeltoft. Receptors for insulin-like growth factors I and II in rat gastrointestinal epithelium. *Am. J. Physiol.* 254:G457–G462 (1988).
25. B. Termanini, R. V. Nardi, T. M. Finan, I. Parikh, and L. Y. Korman. Insulin-like growth factor I receptors in rabbit gastrointestinal tract. *Gastroenterology* 99:51–60 (1990).
26. J. A. McRoberts, R. Aranda, N. Riley, and H. Kang. Insulin regulates the paracellular permeability of cultured intestinal epithelial cell monolayers. *J. Clin. Invest.* 85:1127–1134 (1990).
27. J. A. McRoberts, N. Riley, R. Aranda, and H. Kang. Regulation of colonic epithelial cell tight junction permeability by insulin-like growth factors. 2nd International Symposium on Insulin-like Growth Factors/Somatomedins, 1991.
28. M. Narawane and V. H. L. Lee. Growth factors as penetration enhancers for transepithelial protein transport. *Pharm. Res.* 9:S-242 (1992).