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# Oral absorption of insulin encapsulated in artificial chyles of bile salts, palmitic acid and α-tocopherol dispersions

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**Abstract**

The hypoglycemic effect of orally given insulin was studied on rabbits, using different bile salts as absorption promoters, in two different carriers to form an artificial chyloform system ready to be absorbed by intestinal mucosa. The rank order of enhancement by bile salts in the presence of 1% ethanol was deoxycholate > cholate > glycocholate > glycodeoxycholate > taurodeoxycholate > no bile salts. The dose response studies with increased insulin loaded in the chyle showed a greater corresponding hypoglycemic effect with the system of cholate–palmitic-α-tocopherol dispersions than the cholate–palmitic acid dispersions. A more effective hypoglycemic effect was achieved using lower doses of the deoxycholate–palmitate–tocopherol–chyle dispersions.


*Keywords:* Insulin; Fatty acids; Tocopherol; Bile salts; Chyles

## 1. Introduction

Significant improvements of oral insulin absorption were achieved by masking the hydrophilic surface of its molecule (Meisha and El-Bitar, 1980; Schilling and Mitra, 1990), or through chemical modification of the insulin molecule by using fatty acids (Hashizume et al., 1992, and Muranushi et al., 1993). Optimizing the fatty acid chain length in a previous study lead to the choice of palmitic acid (Mesiha et al., 1994) as the best carrier for oral absorption enhancement of insulin. However, the bile salt of choice had to be investigated. Therefore, a new technique for the preparation of insulin lipophilic co-precipitation for oral dosage, using palmitic acid and bile salts of different chemical moieties is reported.

## 2. Materials and methods

### 2.1. Materials

Human insulin of recombinant DNA origin, Humulin®-R, Eli Lilly Co. was used. It had a claimed activity of 100 units/ml. The palmitic acid

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Table 1
Hypoglycemic effect of oral insulin given to rabbits, 10 U/Kg in chyles prepared by different bile salts and palmitic acid in aqueous system

| Bile salt used | Percent of blood glucose level relative to initial level after time (hours) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 |
| No bile control | 94±5.6 | 95±2.8 | 95±4.2 | 97±6.3 | 90±4.9 | 93±4.9 | 93±4.8 | 94±5.6 |
| Glycocholate | 81±11[b] | 75±21[b] | 83±12[b] | 83±14[b] | 88±2.0 | 85±8.6[a] | 89±4.3 | 88±6.4 |
| Cholate | 80±1.5[b] | 94±2.0 | 101±4.3 | 105±2.0 | 104±2.1 | 108±1.0 | 112±3.0 | 111±1.5 |
| Deoxycholate | 93±6.0 | 95±3.7 | 99±8.0 | 98±6.6 | 99±8.6 | 94±2.0 | 106±8.0 | 101±3.7 |
| Taurodeoxcholate | 109±9.2 | 103±4.9 | 105±5.1 | 106±2.5 | 107±6.5 | 104±10 | 111±7.7 | 111±8.7 |
| Glycodeoxycholate | 94±2.3 | 91±2.8 | 93±3.6 | 97±3.4 | 99±2.5 | 100±3.0 | 102±2.5 | 101±3.5 |

Data represent the mean±standard deviation ($n = 6$).
[a] $P < 0.05$.
[b] $P < 0.01$ compared with the no insulin control data at similar time interval.

and sodium salts of cholic acid, glycocholic acid, deoxycholic acid, glycodeoxycholic acid, and taurodeoxycholic acid were purchased from Sigma Chemical Co. (St. Louis, MO). α-Tocopherol (vitamin E) and other chemicals used were of pharmaceutical or analytically pure grade.

### 2.2. Animals

Male white New Zealand rabbits, weighing 2500±240 g, were purchased from Hare Marland, Hewitt, NJ. They were kept to meet IACUC approved protocol, with normal day/night time variation; fasted overnight before experiments, but water was provided ad libitum.

### 2.3. Equipment

The Accu-check® IIm blood glucose monitor (Boehringer Mannheim Diagnostics, Indianapolis, IN) was used to measure blood glucose content using standardized glucose oxidase strips.

### 2.4. Formulations

Two different dispersions one containing purified water and the other ethyl alcohol (1% v/v) as dispersion media were prepared for the study. All the preparations were in 1:3 mM ratio of fatty acid to bile salt composition. Palmitic acid equivalent to 1 mM was dissolved in 1.0 ml of 0.1 N NaOH solution. Three times the mM amount of respec-

Table 2
Hypoglycemic effect of oral insulin given to rabbits, 10 U/Kg in chyles prepared by different bile salts and palmitic acid in hydroalcoholic system

| Bile salt used | Percent of blood glucose level relative to initial level after time (hours) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 |
| No Bile Control | 113±5.6 | 104±4.2 | 108±2.8 | 105±5.6 | 90±4.9 | 99±2.8 | 103±1.4 | 92±4.2 |
| Glycocholate | 93±2.0[a] | 79±14[b] | 90±14.6 | 97±17[a] | 97±6.0 | 88±4.9[a] | 103±9.5 | 102±5 |
| Cholate | 82±4.6[b] | 88±5.0[a] | 96±4.3 | 95±8.3 | 91±6.0 | 91±6.0 | 90±11.0 | 88±11.5 |
| Deoxycholate | 81±6.0[b] | 64±3.1[b] | 86±3.4[b] | 85±2.3[b] | 75±4.6[b] | 87±1.5[a] | 86±4.9[b] | 88±3.0 |
| Taurodeoxycholate | 83±7.7[b] | 94±8.8 | 91±8.5 | 93±6.6 | 88±6.4[a] | 100±2.5 | 85±5.5[b] | 96±8.0 |
| Glycodeoxycholate | 93±5.7 | 90±5.8 | 87±4.5[b] | 77±8.5[b] | 78±3.7[b] | 91±7.0 | 93±6.1 | 94±6.1 |

Data represent the mean±standard deviation ($n = 6$).
[a] $P < 0.05$.
[b] $P < 0.01$ compared with the control data at similar time interval.

Table 3
Hypoglycemic effect of oral insulin given to rabbits, 10 U/Kg in chyles prepared by different bile salts and palmitic acid in α-tocopherol system.

| Bile salt used | Percent of blood glucose level relative to initial level after time (hours) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 |
| Control | 95±0.5 | 97±1.4 | 98±0.7 | 96±2.8 | 96±6.3 | 93±0.7 | 92±1.4 | 94±5.6 |
| Glycocholate | 95±4.7 | 92±1.0[b] | 94±1.5[a] | 97±3.2 | 98±6.1 | 99±5.6 | 100±4.7 | 102±5.1 |
| Cholate | 80±5.6[b] | 85±9.8[b] | 81±12[b] | 80±12[b] | 86±2.8 [b] | 87±7.1[b] | 87±2.8[b] | 88±1.4 |
| Control | 102±0.7 | 107±0.7 | 106±0.7 | 101±0.7 | 104±0.7 | 95±0.7 | 97±0.7 | 96±0.7 |
| Deoxycholate | 82±9.4[b] | 83±10[b] | 80±9.8[b] | 83±10[b] | 86±1.5[b] | 91±5.5[a] | 96±3.0 | 101±2.8 |
| Taurodeoxycholate | 83±14[a] | 94±7.7 | 94±4.2[a] | 96±7.0 | 97±0.7 | 98±6.3 | 94±4.3 | 102±2.8 |

Data represent the mean±standard deviation ($n = 5$). Top three systems, including the control are aqueous; while the remaining are in hydro-alcoholic systems.

[a] $P < 0.05$.

[b] $P < 0.01$ compared with the control data at similar time interval.

tive bile salt to be tested was dissolved in 1.0 ml of water or 3.5% v/v ethanol in case of the hydro alcoholic dispersions. The insulin, 50 U/0.5 ml solution was measured and mixed with 1.0 ml of 0.1 N HCl. The palmitate solution was first mixed thoroughly with the bile salt solution. A phase separation with pH change was effected, when insulin solution in the equivalent amount of HCl was added. The resultant opaque dispersion containing 50 U of insulin, 3 mM bile salt and 1 mM palmitic acid was formed.

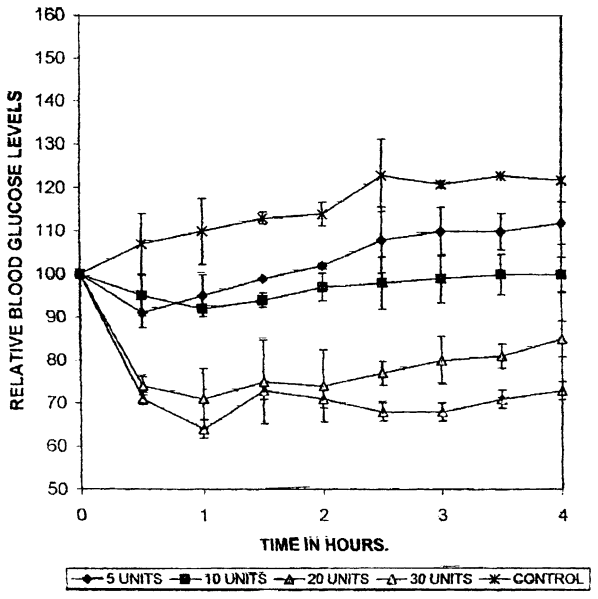


Fig. 1. Hypoglycemic effect of different doses of insulin given to rabbits orally in glycocholate palmitic acid aqueous chyle dispersion system.

The system with ethyl alcohol, bile salts were dissolved in 1.0 ml 3.5% ethanol to bring the final alcohol concentration to 1% by volume.

Tocopherol 20% w/w in soybean oil mixed with 1 g palmitic acid was sonicated for 10 min and used to test the effect of tocopherol on the absorption of insulin.

All the dispersions were homogenized and used immediately after preparation. Doses of insulin were measured using micro-syringe, assuming no significant loss has occurred. No further analysis in the dispersion was performed.

Control preparations: Controls were prepared using similar methods.

Control I (without insulin): The preparation contained palmitic acid and bile salt under testing.

Control II (without Bile salt): The preparation contained insulin 50 U/1 mM palmitic acid.

Control III (without fatty acid): The preparation contained insulin 50 U and 3 mM bile salt (data shown for the deoxycholate).

### 2.5. Hypoglycemic effect

The rabbits were fasted overnight and were given water ad libitum. Each animal was weighed

and was given the preparation under investigation or controls. The preparation was accurately measured into dry stomach tube and flushed into the animal's gut with 5 ml of water through a syringe attached to the tube. Blood samples were taken from the marginal ear vein at 30-min intervals over four-hour periods. Oral doses ranging from 5 to 30 U/Kg were tried. The dose administered was 10 U/Kg unless otherwise indicated. The rabbits were used in-groups of three using a Latin square crossover design. Analysis of variance (Anova) test was used for the statistical analysis of potential differences from the control test.

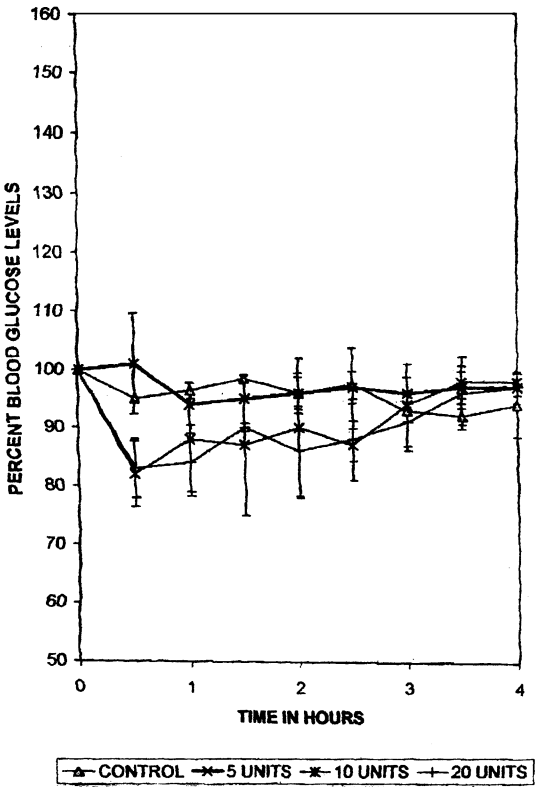


Fig. 2. Hypoglycemic effect of different doses of insulin given to rabbits orally in cholate–palmitic acid–α-tocopherol aqueous dispersion system.

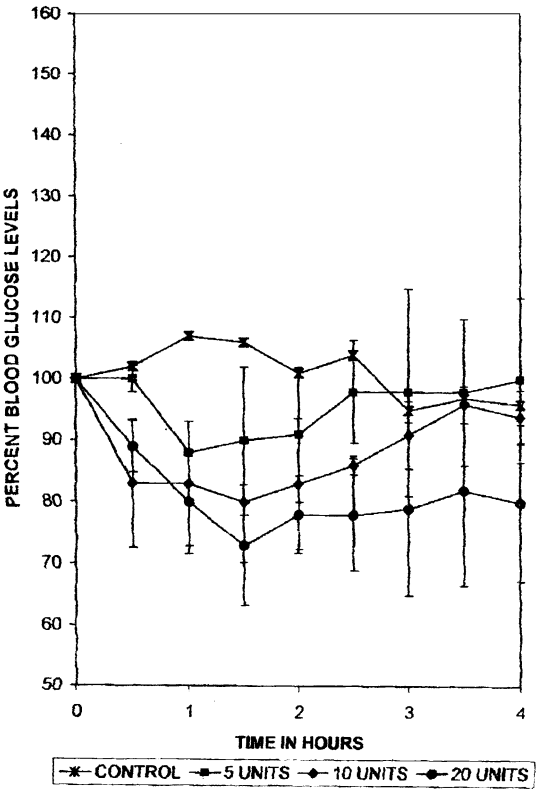


Fig. 3. Hypoglycemic effect of different doses of insulin given to rabbits orally in deoxycholate–palmitic acid–α-tocopherol hydroalcoholic dispersion system.

## 3. Results and discussion

Control experiments devoid of insulin did not show noticeable differences from normal blood glucose-time profiles of fasted animals under similar conditions. The inclusion of insulin (10 U/kg) with either palmitic acid-in control II or bile salt-in control III alone did not result in a significant reduction in blood glucose levels ($P > 0.05$). Control II data are shown in Table 1, while the hypoglycemic effect of orally given Control III after 0.5, 1, 2, 3, and 4 h intervals ranged between 97 and 101 mg/dl ($\pm 5.4$, $n = 6$). Significant hypoglycemic effects were achieved when insulin was given with palmitic acid combined with the bile salt in the form of aqueous fatty acid dispersions or chyle system. The order of hypoglycemic enhancement effect as illustrated in Table 1, was as follows: glycocholate > cholate > no bile palmi-

tic acid dispersions. A smaller hypoglycemic effect was recorded with insulin systems when the taurodeoxycholate or the glycodeoxycholate were used in the aqueous medium.

The inclusion of minimum amounts of ethanol while building up the chyle structure resulted in a positive effect on promoting insulin absorption. All the bile salts showed significant ($P \leqslant 0.05$, Anova) enhancement of oral insulin given to the rabbits 10 U/Kg in palmitic acid aqueous dispersions containing 1% alcohol by volume (Table 2). A possible explanation of the greater enhancement effect is the in-chyle entrapment of insulin from water. Addition of more alcohol was avoided, since it would result in a sort of protein solvent destability (Timasheff et al., 1993). The rank order of the potency of bile salts species in enhancing insulin oral glucose lowering data as shown in Table 2; was sodium deoxycholate > sodium cholate > sodium glycocholate > sodium glycodeoxycholate > sodium taurodeoxycholate > no bile salt (one way Anova, $P > 0.05$).

The study of insulin in the presence of sodium glycocholate in aqueous medium showed a dose dependence when using rabbits with increasing doses from 5 to 30 U/Kg (Fig. 1). The positive correlation of increasing enhancement effect of insulin absorption both by increasing the hydrophobicity of the bile salt used and by dose-increase may suggest a passive mechanism of drug enhancement absorption. The term passive here does not necessarily mean simple diffusion, but a dose related mechanism. However, considering the less solubility of insulin in presence of alcohol and higher hydrophobicity acquired by the chyle structure, other mechanisms could actually enhance its absorption by increasing the thermodynamic activity of the drug (Pouton et al., 2000).

The hypoglycemic effect of oral insulin dispersions prepared with α-tocopherol achieved limited measures without the presence of bile salts. Addition of sodium cholate to the α-tocopherol system in aqueous medium resulted in easier dispersion of the tocopherol and more significant hypoglycemic effects (Table 3). The animal studies with cholate-tocopherol palmitic acid chyles of the hormone in the aqueous system (Fig. 2) revealed smaller response to dose changes on lowering blood glucose levels, than the similar experiments with deoxycholate–tocopherol–palmitic acid system in hydroalcoholic dispersion, as shown in Fig. 3.

In conclusion, an artificial chyle of insulin is suggested which is easily be prepared in the presence of either ethanol 1% or tocopherol. The deoxycholate palmitate chyle system is the recommended one.

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