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# Enhanced oral absorption of insulin from desolvated fatty acid-sodium glycocholate emulsions

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**Abstract**

Lipoidal dispersions of insulin in fatty acids were prepared using a bile salt as an emulsifier and as absorption promoter. The orally administered desolvated emulsions containing insulin-sodium glycocholate combinations showed different hypoglycemic effects depending on the fatty acid used. A palmitic acid system, containing 5 U insulin/50 mg dispersion per kg rabbit weight, resulted in the reduction of blood glucose from 105 to 75 mg/dl in 30 min. The effect extended over 150 min with statistically significant difference from non-fatty acid systems. Unsaturated fatty acids did not show the same enhancing effect as saturated fatty acids of the same carbon chain length.

*Keywords:* Peptide transport; Insulin; Fatty acid dispersion; Sodium glycocholate; Desolvated emulsion

## 1. Introduction

A significant improvement in the oral absorption of insulin can be achieved by rendering the protein hormone more lipophilic, through either liposome encapsulation (Patel and Ryman, 1976), by solid dispersion in stearic acid (Mesiha and El-Bitar, 1981) and some other fatty dispersions (Mesiha, 1981), also by embedding in water in oil microemulsion (Cho and Flynn, 1989), and even by chemical modification with palmitic acid (Hashizume et al., 1992).

Oral insulin provides much more than just a convenient route of administration, it offers a means of improving portal levels of insulin, and curtails the peripheral hyperinsulinaemia associated with other insulin regimens which may be an important factor in the development of artherosclerosis (Gwinup et al., 1990; Kennedy, 1991).

The objective of this study was to screen the fatty acids as they offered a good potential for enhancing the oral absorption of insulin (Mesiha, 1981), using a new technique to prepare the in-

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Table 1
Fatty acids used in the study and their characteristics

| Fatty acid | No. of carbon atoms | Melting point (°C) | Structure |
|---|---|---|---|
| Saturated | | | |
| Lauric | 12 | 44 | $CH_3(CH_2)_{10}COOH$ |
| Palmitic | 16 | 63 | $CH_3(CH_2)_{14}COOH$ |
| Stearic | 18 | 70 | $CH_3(CH_2)_{16}COOH$ |
| Unsaturated | | | |
| Palmitoleic | 16 | 32 | $CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH(cis)$ |
| Linoleic | 18 | −5 | $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH(cis,cis)$ |

sulin dispersion in the fatty acids through organic solvent evaporation.

## 2. Materials and methods

### 2.1. Materials

Insulin was obtained as a regular insulin solution of recombinant DNA origin (Humulin® R; produced by Eli Lilly Co). It had a claimed content of 100 U/ml.

The fatty acids used in this study and glycocholic acid, sodium salt (SGC), were all purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). They were at least 99% pure. The fatty acids are listed in Table 1. All other chemicals or reagents were of analytical grade.

### 2.2. Animals

Male white rabbits weighing 2800 ± 150 g were fed with pelleted chow. All animals were fasted overnight before tests, but water was provided ad libitum.

### 2.3. Blood glucose determination

An Accu-Check® IIm apparatus (Boehringer Mannheim Diagnostics, Indianapolis, IN) was used to measure rabbits' blood glucose content through standardized gluco-strips.

### 2.4. Preparation of the desolvated emulsions

Insulin solution equivalent to 50 U was used to dissolve 200 mg of SGC. An ethereal solution of the fatty acid under investigation was prepared by dissolving 300 mg of the fatty acid in 1 ml of the solvent. The two solutions were triturated in a small mortar to form an emulsion, which was then dried overnight in a desiccator under reduced pressure. The final preparation contained 5 U of insulin per 50 mg of the mixed carriers. A control preparation was prepared with SGC and fatty acid but without insulin. One formulation was prepared with insulin in SGC (5 U/20 mg) and no fatty acid.

### 2.5. Animals' response to insulin

The animals used in this study were healthy rabbits, having a fasting blood glucose level of 65–85 mg/dl. The tested animals were subjected to a random insulin-response test by injecting the insulin subcutaneously to three rabbits (0.5 U/kg) and measuring blood glucose every 30 min for 3 h.

### 2.6. Hypoglycemic effect of insulin dispersions

The oral absorption of intact, physiologically active insulin was monitored by measuring the hypoglycemic effect of the preparations. 12 rabbits were used in groups of three using a Latin square crossover design. Animals were fasted overnight before testing. Preparations were weighed into dry flexible tubes and 10 ml water was injected to flush them into the stomach. Blood samples were taken from the marginal ear vein at 30 min intervals over 3 h, then analyzed for glucose content by using the Accu-Check IIm automatic blood glucose monitor. Analysis of

variance (ANOVA) test was used for the statistical analysis of data for potential differences from the control test. Rabbits were given insulin desolvated dispersions, equivalent to 5 U per kg animal weight, to compare the different fatty acids. A stearic acid dispersion of insulin was given at two additional dose levels of 10 and 20 U/50 mg carrier/kg animal weight.

## 3. Results and discussion

The tested animals responded to the injected insulin fairly rapidly, reaching a blood glucose level of $29 \pm 3$ mg/dl in 1 h after injection. The animals were immediately injected with an i.v. glucose solution to prevent potential hypoglycemic coma. This was carried out in order to check the sensitivity of these rabbits to the insulin used. The normal blood glucose level of the animals after no treatment did not differ from the control data shown in Fig. 1. Insulin-sodium glycocholate combination without the fatty acids resulted in a measureable reduction in blood glucose level (Fig. 2). The use of SGC as an absorption promoter for insulin has been reported for the intranasal route (Moses et al., 1983). The latter route requires large amounts of insulin (up to 500 U/ml) and is also associated with nasal

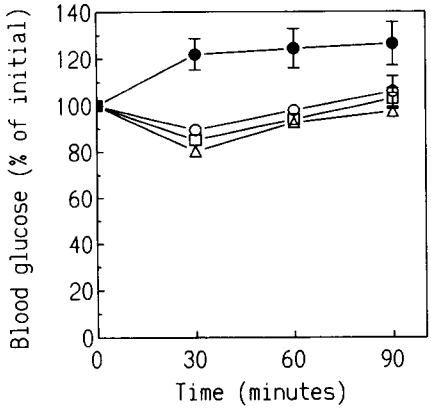


Fig. 1. Hypoglycemic effect of stearic acid desolvated emulsions, containing different doses of insulin. Insulin dose: (●) control, (○) 5 U, (□) 10 U, (△) 20 U.

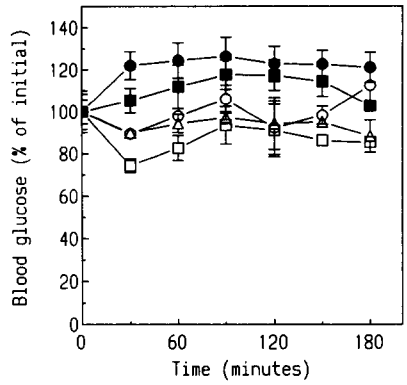


Fig. 2. Hypoglycemic effect of insulin desolvated emulsions prepared using different saturated fatty acids and sodium glycocholate (5 U/50 mg/kg rabbit weight). (●) Control, no insulin; (■) insulin, no fatty acids; (△) lauric acid; (□) palmitic acid; (○) stearic acid.

congestion and rhinorrhoea (Salzman et al., 1985; Kennedy, 1991). SGC increased the efficacy of insulin by other routes as well, the rank order being nasal > rectal > buccal > sulingual (Banga and Chien, 1988).

The combined enhancement effect of SGC and stearic acid was preliminarily tested on rabbits over a period of 90 min at three differnt doses. The hypoglycemic effect of orally administered insulin was significantly greater when the bile salt and fatty acid were combined (Fig. 1). The response was slightly affected by the insulin dose given. Stearic acid was previously reported (Mesiha and Hussein, 1980) to potentiate the enhancing effect of different surfactants, when their melted mixture was used as an oral insulin carrier. SGC, as a bile salt, is expected to facilitate the oral absorption of stearic acid. Thus, the inclusion of this bile salt offered a readily absorbable form of the fatty acid, encapsulating the dispersed insulin in a reversed-phase micellar or macromicellar form.

Comparison of the hypoglycemic effect of oral insulin using SGC and fatty acids with three different chain lengths (Fig. 2) revealed a significant effect of the carrier on the responses. There was a significant reduction in blood sugar ($p < 0.005$) within 30 min. Statistical analysis of the

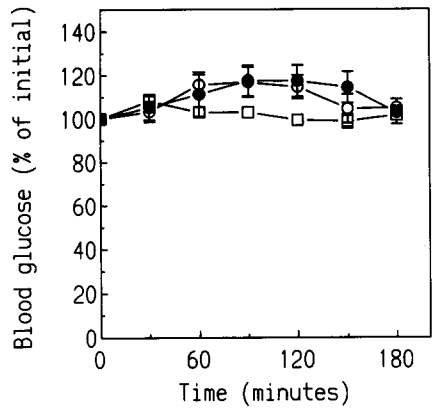


Fig. 3. Hypoglycemic effect of insulin desolvated emulsions prepared using different unsaturated fatty acids and sodium glycocholate (5 U/50 mg/kg rabbit weight). (●) Control, no insulin; (□) palmitoleic acid; (△) linoleic acid.

data showed that palmitic acid significantly promoted the absorption activity of SGC more effectively than did stearic and lauric acids.

A recent report (Hashizume et al., 1992) suggested chemical modification of insulin with palmitic acid to improve its large intestinal absorption. The present solvent evaporation procedure was adopted as a much simpler method for including insulin in the palmitic acid.

Fatty acids of similar number of carbon atoms, but containing one or more double bonds, were substituted for the optimum chain length palmitic acid. Palmitoleic and linoleic acids containing the bile salt and insulin resulted in a much less significant enhancing effect on oral insulin absorption compared to palmitic acid (Fig. 3). The lack of a synergistic effect of these low melting points fatty acids may be attributed to a shorter period of contact with the gut mucosa, or to the formation of a less rigid micelle wall.

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