(11) Application No. AU 2005204378 B2

(19) AUSTRALIAN PATENT OFFICE

(54) Title

A method of reducing serum proinsulin levels in type 2 diabetics

(51)[6] International Patent Classification(s)

A61K 38/28 (2006.01) 20060101AFI2005100

A61K 38/28 8BMEP

PCT/US2005/000596

(21) Application No: 2005204378 (22) Application Date: 2005.01.10

(87) WIPO No: WO05/067964

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/535,945	2004.01.12	US

(43) Publication Date : 2005.07.28

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(56) Related Art

Cefalu W. et al. Annals of Internal Medicine, February 2001, Vol 134(3): pages 203-207

Engelau, M., Diabetes Care, 2000. Vol 23(10): pages 1563-1580

WO 2001/000654 A3

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 July 2005 (28.07.2005)

PCT

(10) International Publication Number
WO 2005/067964 A1

(51) International Patent Classification[7]: A61K 38/28

(21) International Application Number:
PCT/US2005/000596

(22) International Filing Date: 10 January 2005 (10.01.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/535,945 12 January 2004 (12.01.2004) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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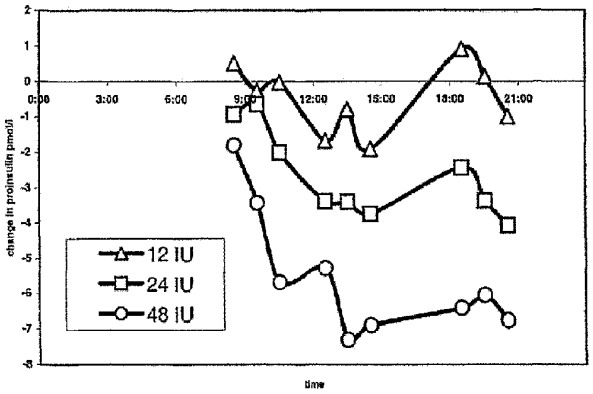
(54) Title: A METHOD OF REDUCING SERUM PROINSULIN LEVELS IN TYPE 2 DIABETICS



(57) Abstract: Methods are provided for reducing serum proinsulin levels, lessening post-prandial pancreatic stress, and reducing risk factors for atherosclerosis in subjects with diabetes mellitus, type 2. The method includes administration of insulin in a manner that mimics the meal-related first phase insulin response, using a dose sufficient to reduce serum levels of proinsulin. In some embodiments of the method insulin administration is commenced early in the course of the disease. Mimicking first phase kinetics, peak serum insulin levels can be reached within about 18 minutes of administration. In increasingly preferred embodiments peak serum insulin levels can be reached within about 15, 12, or 10 minutes of administration. Serum insulin levels return to baseline within about two hours of administration.

WO 2005/067964 A1

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

A METHOD OF REDUCING SERUM PROINSULIN LEVELS IN TYPE 2 DIABETICS

This application claims priority to U.S. Serial No. 60/535,945 filed in the U.S. Patent and Trademark Office on January 12, 2004.

FIELD OF THE INVENTION

This invention is generally in the field of treatment of diabetes mellitus, type 2 and related sequela using prandial insulin substitution regimens that mimic the meal-related first phase insulin response. In particular it relates to the reduction of serum proinsulin levels, pancreatic stress, and atherogenic factors in type 2 diabetics.

BACKGROUND OF THE INVENTION

Diabetes mellitus is present in 17 million Americans. Its prevalence is growing at a rate of 4.5% per year, particularly diabetes mellitus type 2, also variously called adult-onset and insulin-resistant diabetes. The paradigmatic defect in diabetes is the mis-regulation of serum glucose levels. In addition to the deleterious effects of hyperglycemia, inability to properly respond to high serum glucose levels creates stress on the pancreas that can accelerate progression of the disease in type 2 diabetes. Also, the diagnosis of diabetes carries a 2 to 4 times greater risk of stroke and heart attack in individuals with the disorder. The presence of diabetes in an individual without known heart disease places their risk of new myocardial infarction at the same level as a person who has already had a myocardial infarction but who does not have diabetes. Thus there are important therapeutic goals in the treatment of type 2 diabetes in addition to hyperglycemia per se that are not adequately addressed by currently available treatments.

It is therefore an object of the present invention to provide alternative treatments, especially treatments for type 2 diabetes.

SUMMARY OF THE INVENTION

Methods are provided for reducing serum proinsulin levels, lessening post-prandial pancreatic stress, and reducing risk factors for atherosclerosis in subjects with diabetes mellitus, type 2. The method includes

administration of insulin in a manner that mimics the meal-related first phase insulin response, using a dose sufficient to reduce serum levels of proinsulin. In some embodiments of the method insulin administration is commenced early in the course of the disease. Mimicking first phase kinetics, peak serum insulin levels can be reached within about 18 minutes of administration. In increasingly preferred embodiments peak serum insulin levels can be reached within about 15, 12, or 10 minutes of administration. Serum insulin levels return to baseline within about two hours of administration. In one embodiment, insulin is administered within one hour after the start of a meal. In a preferred embodiment, insulin is administered within about 10 minutes after starting a meal.

In a further aspect, the present invention provides a method of reducing a risk factor of atherosclerosis in an individual with type 2 diabetes, comprising the steps of:

a) selecting a type 2 diabetic individual in the early stage of type 2 diabetes wherein said individual's serum proinsulin level is elevated, and

b) administering to the type 2 diabetic individual in the early stage of type 2 diabetes an insulin composition in a prandial manner and dose that mimics a physiologic meal-related first phase insulin response;

wherein said risk factor of atherosclerosis is reduced in said individual.

Diketopiperazine microparticle drug delivery systems are used in various embodiments of the method. In further embodiments of the method, the insulin is administered by pulmonary delivery using synthetic biodegradable polymeric or diketopiperazine microparticles incorporating the insulin. In preferred embodiments, delivery is achieved by inhalation of a dry powder. In aspects of the method utilizing diketopiperazine microparticles, fumaryl diketopiperazine is a preferred type of diketopiperazine, the insulin is dimeric or monomeric, and preferred dosages are in the range of about 15 to 90 IU or greater than 24 IU of insulin. In preferred embodiments, inhalation of the dry powder is facilitated by use of a unit dose inhaler. Embodiments of the method reducing risk factors for atherosclerosis include ones wherein the risk factor is LDL particle size and LDL particle size is increased; and wherein the risk factor is plasminogen activator inhibitor type-1 (PAI-1), and PAI-1 expression is reduced, using the method of administration and formulations described herein.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a graph of the changes in proinsulin levels over time, following pulmonary administration of diketopiperazine/insulin particles.

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DETAILED DESCRIPTION OF THE INVENTION

The development of pulmonary insulin formulations is designed to provide new and effective alternatives for meal-related (prandial) insulin

substitution in diabetic patients. The ideal kinetics of insulin formulations for prandial substitution include a rapid and early onset of action and a duration of action long enough to cover meal-related glucose absorption. One problem with existing formulations of insulin for subcutaneous (s.c.) injections has been the unpredictable variability of absorption, exceeding 50% in some cases, and the relatively slow rise in serum insulin levels compared to physiologic meal-related first phase insulin response, in which serum insulin levels can peak by about 6 minutes.

Meal-related first phase insulin originates from storage vesicles in the beta cells of the islets of Langerhans of the pancreas, where proinsulin undergoes enzymatic cleavage into insulin and C-peptide. Type 2 diabetes, as distinct from type 1, is characterized by a loss of the meal-related first phase insulin response. This loss occurs early in the disease process. Type 2 diabetes, again as distinct from type 1, is further characterized by elevated levels of serum proinsulin. Such circulating intact proinsulin (iPi) likely signifies that insulin requirements exceed beta cell capacity, causing pancreatic stress leading to premature release of the storage vesicles.

The rat model of type 2 diabetes has been used to see what happens if insulin is administered versus sham injections to determine how fast the cohort develop type 2 diabetes. In those animals treated with small injections of insulin, it has been shown that they take as much as twice as long to develop the prevalence of diabetes as the sham treated animals. Also, when they are sacrificed, those receiving the insulin injections have a higher number of viable beta cells in their pancreas. The accepted interpretation is that the injections of insulin take stress off the pancreas and that something about stressing the pancreas makes the beta cells die off faster. Thus serum proinsulin is a useful indicator of pancreatic stress and relief of this stress is observable as reduction in serum proinsulin levels.

Type 2 diabetes is typified by elevated serum levels of proinsulin from early points in the progression of the disease. In addition to signifying pancreatic stress, serum proinsulin can be detrimental in its own right. Serum proinsulin is positively associated with an increased risk of atherosclerotic

cardiovascular disease in humans (Haffner et al., Stroke.29:1498-1503, 1998; Hanley et al., Diabetes Care 24:1240-1247, 2001; Zethelius et al., Circulation. 105:2153, 2002). It is also associated with known atherogenic risk factors such as reduced LDL particle size (Festa et al. Diabetes Care 22:1688-1693, 1999) and increased plasminogen activator inhibitor type-1 (PAI-1) expression (Schneider et al., Diabetes, 41:890-895, 1992). Administration of proinsulin to humans in clinical trials in the 1980s resulted in an increased incidence of myocardial infarction and death in subjects receiving the agent. Thus reduction of serum proinsulin levels is an additional therapeutic goal, and one that is not addressed by the current therapies used in the earlier stages of the disease that focus on serum glucose level.

Insulin Formulations

Insulin is commercially available, in either monomeric or dimeric form.

Useful carriers are also available, or can be made using published technology. Pulmonary insulin delivered using diketopiperazine microparticles is rapidly absorbed reaching peak serum levels in about 10 to 15 minutes. This is fast enough to mimic the kinetics of the physiologic meal-related first phase insulin response, as evidenced by the shutoff of gluconeogenesis that is observed. Such treatment also leads to reduced levels of serum proinsulin, which is not seen with slower acting insulin preparations. The relative ease of administration via this mode of treatment also facilitates treatment of type 2 diabetes much earlier in the course of the disease than has been traditionally practiced. Thus by using an insulin delivery that mimics first phase kinetics, serum proinsulin levels can be reduced and the literature indicates that this will be accompanied by similar reductions in atherogenic risk factors. By commencing insulin therapy early in the course of the disease, reduction in pancreatic stress can slow progression of the disease itself.

Diketopiperazine microparticle drug delivery systems and associated methods are described in U.S. Patents 5,352,461 and 5,503,852 entitled Self

Assembling Diketopiperazine Drug Delivery System and Method for Making Self Assembling Diketopiperazine Drug Delivery System, respectively. The use of diketopiperazine and biodegradable polymer microparticles in pulmonary delivery is described in U.S. Patents 6,428,771 and 6,071,497 entitled Method for Drug Delivery to the Pulmonary System, and Microparticles for Lung Delivery Comprising Diketopiperazine, respectively. Details regarding various aspects of possible formulation and manufacturing processes can be found in U.S. Patents 6,444,226 and 6,652,885 both entitled Purification and Stabilization of Peptide and Protein Pharmaceutical Agents, and in U.S. Patent 6,440,463 entitled Methods for Fine Powder Formation. The properties and design of a preferred breath-powered dry powder inhaler system is disclosed in PCT/US00/40454 and PCT/US2004/028699.

Other formulations can consist solely of drug particles, drug plus surfactant particles, and polymer drug particles, such as particles of poly(lactic acid-co-glycolic acid) encapsulating the drug to be administered.

Method of Administration

Methods are provided for reducing serum proinsulin levels, lessening post-prandial pancreatic stress, and reducing risk factors for atherosclerosis in subjects with diabetes mellitus, type 2, by administering insulin in a manner that mimics the meal-related first phase insulin response, using a dose sufficient to reduce serum levels of proinsulin. In a preferred embodiment, the insulin administration is commenced early in the course of the disease. Mimicking first phase kinetics, peak serum insulin levels can be reached within about 18 minutes of administration. Formulations and methods of administration, preferably by pulmonary administration, are selected so that peak serum insulin levels can be reached within about 15, 12, or 10 minutes of administration. Serum insulin levels return to baseline within about two hours of administration. In one embodiment, insulin is administered within one hour after the start of a meal. In a preferred embodiment, insulin is administered within about 10 minutes after starting a meal. Embodiments of the method reducing risk factors for atherosclerosis

include ones wherein the risk factor is LDL particle size and LDL particle size is increased; and wherein the risk factor is plasminogen activator inhibitor type-1 (PAI-1), and PAI-1 expression is reduced, using the method of administration and formulations described herein.

In aspects of the method utilizing diketopiperazine microparticles, fumaryl diketopiperazine is a preferred type of diketopiperazine, the insulin is dimeric or monomeric, and preferred dosages are in the range of about 15 to 90 IU or greater than 24 IU of insulin. In preferred embodiments, inhalation of the dry powder is facilitated by use of a unit dose inhaler.

The present invention will be further understood by reference to the following non-limiting examples. The following examples make use of Technosphere®/insulin, a proprietary product composed of insulin complexed with fumaryl diketopiperazine microparticles administered as a dry powder aerosol by inhalation.

Example 1. Pulmonary Delivery of Technosphere®/Insulin to Rats Results in Rapid Absorption.

The pharmacokinetic (PK) profile of pulmonary Technosphere®/insulin particles administered as a dry powder aerosol was compared to the PK profile of human insulin delivered by subcutaneous (s.c.) injection in the rat. A flow-past, nose-only inhalation exposure system was used to administer the aerosols. In the first experiment, all animals received the same formulation (9.1% insulin) but the duration of dosing was adjusted to deliver doses of approximately 1 IU and 3 IU per rat (200 g body weight). A linear dose-dependent response was observed: the maximal serum insulin concentration (C_{MAX}) was 76 ± 12 μIU/mL after a 0.9 IU dose of Technosphere®/insulin and 240 ± 49 μIU/mL after a 2.7 IU dose. The maximum serum insulin levels were obtained in samples taken immediately after the dosing was completed, indicating rapid absorption of Technosphere®/insulin into the systemic circulation. The time to C_{MAX} (T_{MAX}) following inhalation of 0.9 IU Technosphere®/insulin was less than the mean exposure time of 14.5 minutes while the T_{MAX} was 20 minutes for s.c. injection of 1.5 IU. In addition, inhaled Technosphere®/insulin

demonstrated a high relative bioavailability of 50 – 70%, compared to s.c. insulin.

In a further experiment, the exposure time was held constant while the insulin content of the Technosphere®/insulin was varied from 2.9 to 11.4% to deliver insulin doses of approximately 0.8 IU, 1.5 IU, and 3 IU. Again, a dose-dependent increase in serum insulin was observed in all groups indicating that the rate of absorption is insensitive to the exact composition of the Technosphere®/insulin powder over this range.

In summary, the precise loading of insulin onto Technosphere® fumaryl diketopiperazine particles and the accurate pulmonary delivery of insulin makes Technosphere®/insulin a non-invasive therapeutic option in the management of diabetes mellitus.

Example 2. Technosphere® Fumaryl Diketopiperazine Particles Facilitate the Absorption of Insulin in a Primary Cell Culture Model of Alveolar Epithelium Without Evidence of Cytotoxicity.

To investigate the mechanism by which Technosphere®/Insulin product crosses the epithelial barrier of the deep lung, experiments were conducted using monolayers of rat alveolar epithelium in primary culture. Alveolar type II cells were isolated and cultured on semi-permeable polycarbonate membranes until tight monolayers with high trans-epithelial electrical resistance (TEER) were formed. Insulin transport experiments with the Technosphere®/Insulin product and an un-formulated insulin control were then conducted across these monolayers in the apical to basolateral direction at 37°C. Insulin demonstrated an apparent permeability (P_{app}) of $1.90 \pm 0.34 \times 10^{-8}$ cm/s, while the Technosphere®/Insulin product demonstrated a P_{app} that was ten-fold higher at $2.08 \pm 0.82 \times 10^{-7}$ cm/s. The TEER did not change appreciably between these two groups, or the naïve (untreated) control, indicating that Technosphere® particles do not facilitate the absorption of insulin by disrupting the intercellular tight junctions as calcium chelators do. Apical (donor) well samples were also analyzed for the release of lactate dehydrogenase (LDH), which is a well-established assay for cytotoxicity. LDH activity in the apical media of all groups was

less than that of the naïve controls (spontaneous LDH release), indicating that Technosphere® particles do not facilitate the absorption of insulin by permeabilizing the cell membrane as non-ionic surfactants and bile salts do. These data indicate that Technosphere®/Insulin product greatly increases the absorption of insulin across the alveolar epithelium without exhibiting any deleterious effects on either intercellular tight junctions or cell membrane integrity.

Example 3. Treatment of humans with Pulmonary Insulin Reduces Serum Proinsulin Levels.

Inhalation of Technosphere®/Insulin (TI) provides a rise in serum insulin, comparable to the first phase response. This study investigated the pharmacodynamics of TI and its impact on intact proinsulin release, iPi release. Twenty-four patients with Type 2 diabetes received doses of Technosphere® base with 4 different loadings of insulin, either 0, 12 IU, 24 IU or 48 IU of recombinant regular human insulin, five minutes after start of standardized meals, on separate study days. Blood glucose (BG), serum insulin and serum iPi were measured before (0 min), 60 and 120 min after initiation of each meal.

TI lowered postprandial BG levels in a dose-dependent manner. Sixty minutes after lunch, BG (mg/dl) (± SD) was 183.2 (± 44.4) for placebo; 170.8(± 30.5) for 12 IU ($p = 0.266$); 156.3(± 31.9) for 24 IU, ($p = 0.020$) and 132.6(± 29.1) for 48 IU, ($p < 0.001$). All doses caused an increase in serum insulin at 60 minutes ($p<0.05$), but not at 120 minutes following inhalation. Administration of TI with 24 IU and 48 IU insulin load doses suppressed iPi levels at all time points throughout the day ($p < 0.05$) (Fig 1).

The use of inhaled TI to mimic the rapid onset and short duration of the first phase insulin response therefore should reduce postprandial stress on the beta cell population. This can improve general beta cell function and endogenous glucose homeostasis

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Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not to the exclusion of any other element, integer or step, or group of elements, integers or steps.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

All publications mentioned in this specification are herein incorporated by reference. Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of the application.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of reducing a risk factor of atherosclerosis in an individual with type 2 diabetes, comprising the steps of:

a) selecting a type 2 diabetic individual in the early stage of type 2 diabetes wherein said individual's serum proinsulin level is elevated, and

b) administering to the type 2 diabetic individual in the early stage of type 2 diabetes an insulin composition in a prandial manner and dose that mimics a physiologic meal-related first phase insulin response;

wherein said risk factor of atherosclerosis is reduced in said individual.

2. The method of claim 1, wherein the dose is sufficient to control blood glucose levels and reduce serum levels of proinsulin.

3. The method of claim 1, wherein step (b) is accomplished by pulmonary administration of said insulin composition.

4. The method of claim 1, comprising administering the insulin in a manner that mimics a physiologic meal-related first phase insulin response, in a dose sufficient to control blood glucose levels and reduce serum levels of proinsulin, whereby pancreatic stress is attenuated.

5. The method of claim 1, comprising administering insulin in a manner that mimics a physiologic meal-related first phase insulin response, in a dose sufficient to control blood glucose levels and reduce serum levels of proinsulin.

6. The method of claim 1, wherein the risk factor is serum proinsulin levels, whereby serum proinsulin levels are reduced.

7. The method of claim 1, wherein the risk factor is LDL particle size, whereby LDL particle size is increased.

8. The method of claim 1, wherein the risk factor is plasminogen activator inhibitor type-1 (PAI-1), whereby PAI-1 expression is reduced.

9. The method of claim 1 comprising administering insulin in a manner that mimics a physiologic meal-related first phase insulin response in a dose sufficient to shut off gluconeogenesis.

10. The method of any one of claims 1 to 9 wherein the insulin in step (b) is administered within about 10 minutes after starting a meal.

11. The method of any one of claims 1 to 9 wherein the insulin in step (b) is administered as a pulmonary or dry powder formulation.

12. The method of claim 11, wherein the dry powder formulation comprises a diketopiperazine.

13. The method of claim 12 wherein the diketopiperazine is fumaryl diketopiperazine.

14. The method of claim 11 wherein the insulin is administered by pulmonary delivery as biodegradable polymeric or surfactant microparticles incorporating the insulin.

15. The method of any one of claims 1 to 14 wherein the insulin is dimeric or monomeric.

16. The method of any one of claims 1 to 15 wherein the dose of the insulin is between about 15 IU and 90 IU.

17. The method of claim 16 wherein the dose is between about 24 IU and 48 IU.

18. The method of any one of claims 1 to 17 wherein serum insulin levels peak within about 18 minutes of administration.

19. The method of any one of claims 1 to 17 wherein serum insulin levels return to baseline within about 2 hours of administration.

20. The method of any one of claims 1 to 19 wherein the insulin is administered within about one hour after starting a meal.

21. The method of claim 1 substantially as hereinbefore described with reference to any one of the Examples or Figure 1.

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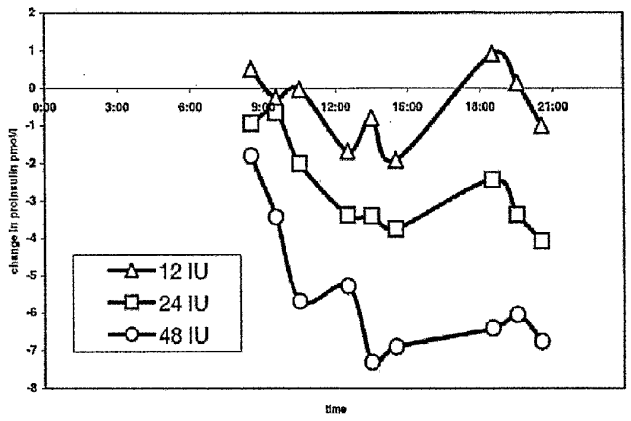
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18:00
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change in pre-insulin pmol/l
time
12 IU
24 IU
48 IU

Figure 1