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CA 2762121 A1 2001/08/09

(21) 2 762 121

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(22) Date de dépôt/Filing Date: 2001/02/01

(41) Mise à la disp. pub./Open to Public Insp.: 2001/08/09

(62) Demande originale/Original Application: 2 398 445

(30) Priorité/Priority: 2000/02/01 (DK PA 2000 00163)

(51) Cl.Int./Int.Cl. *A61K 31/19* (2006.01), *A61P 3/10* (2006.01), *A23L 1/30* (2006.01)

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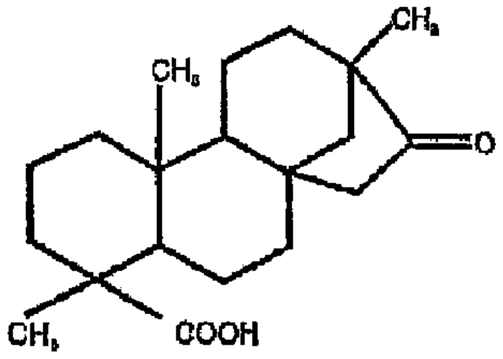
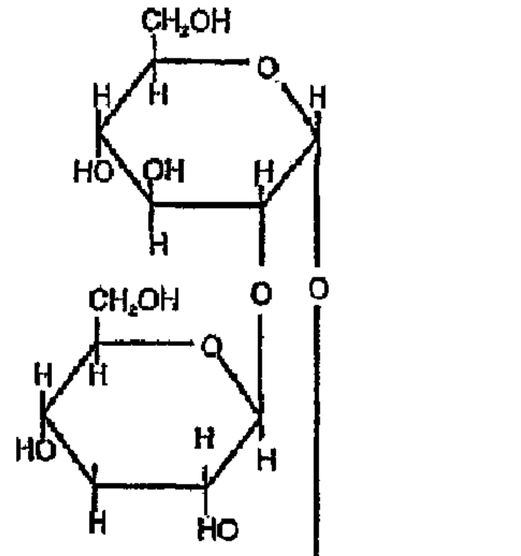
(54) Titre : SUBSTANCE S'UTILISANT DANS UNE SUPPLEMENTATION ALIMENTAIRE OU DANS LA PREPARATION D'UN MEDICAMENT POUR LE TRAITEMENT DU DIABETE NON INSULINO-DEPENDANT, DE L'HYPERTENSION ET/OU DU SYNDROME METABOLIQUE

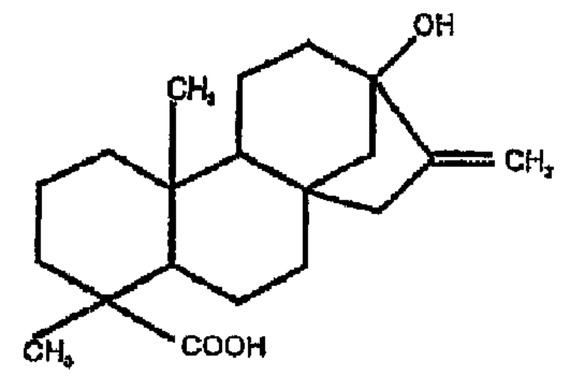
(54) Title: A SUBSTANCE FOR THE USE IN A DIETARY SUPPLEMENTATION OR FOR THE PREPARATION OF A MEDICAMENT FOR THE TREATMENT OF NON-INSULIN DEPENDENT DIABETES MELLITUS, HYPERTENSION AND/OR THE METABOLIC SYNDROME



Isosteviol



Stevioside



Steviol

(57) Abrégé/Abstract:
A substance including the chemical structures of bicyclo[3.2.1]octan or the chemical structures of kaurene for the use in a dietary supplementation or as a constituent in a medicament for the treatment of non-insulin dependent diabetes mellitus, hypertension

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(57) Abrégé(suite)/Abstract(continued):
and/or the metabolic syndrome. The unique chemical structures of bicyclo[3.2.1]octan alone or in a kaurene structure provides the substances, such as e.g. steviol, isosteviol and stevioside with the capability of enhancing or potentiating the secretion of insulin in a plasma glucose dependent manner. The substances including these unique chemical structures also have the capability of reducing the glucagon concentration in the blood and/or lowering the blood pressure thereby providing a self-regulatory treatment system for non-insulin dependent diabetes mellitus and/or hypertension. In a combination drug which also comprise a soy protein, and/or soy fiber and/or at least one isoflavone these substances act synergistically and such combination drugs are highly useful both prophylacticly or directly in the treatment of e.g. the metabolic syndrome and obesity and has due to the self-regulatory effect a widespread applicability as a dietary supplementation.

ABSTRACT

A substance including the chemical structures of bicyclo[3.2.1]octan or the chemical structures of kaurene for the use in a dietary supplementation or as a constituent in a medicament for the treatment of non-insulin dependent diabetes mellitus, hypertension and/or the metabolic syndrome. The unique chemical structures of bicyclo[3.2.1]octan alone or in a kaurene structure provides the substances, such as e.g. steviol, isosteviol and stevioside with the capability of enhancing or potentiating the secretion of insulin in a plasma glucose dependent manner. The substances including these unique chemical structures also have the capability of reducing the glucagon concentration in the blood and/or lowering the blood pressure thereby providing a self-regulatory treatment system for non-insulin dependent diabetes mellitus and/or hypertension. In a combination drug which also comprise a soy protein, and/or soy fiber and/or at least one isoflavone these substances act synergistically and such combination drugs are highly useful both prophylacticly or directly in the treatment of e.g. the metabolic syndrome and obesity and has due to the self-regulatory effect a widespread applicability as a dietary supplementation.

A substance for the use in a dietary supplementation or for the preparation of a medicament for the treatment of non-insulin dependent diabetes mellitus, hypertension and/or the metabolic syndrome

The present invention relates to a new medicament for the treatment of non-insulin dependent diabetes mellitus, hypertension and/or the metabolic syndrome.

Diabetes is a common disease that has a prevalence of 2-4% in the population. Non-insulin dependent diabetes mellitus comprises about 85 % of diabetes most commonly occurring at the age above 40 years. The incidence of non-insulin dependent diabetes mellitus is increasing and is at a global level expected to surpass 200 mill. subjects at year 2010.

Diabetes is associated with increased morbidity and a 2-4-fold increase in mortality primarily due to cardiovascular diseases and strokes.

Non-insulin dependent diabetes mellitus develops especially in subjects with insulin resistance and a cluster of cardiovascular risk factors such as obesity, hypertension and dyslipidemia, a syndrome which first recently has been recognised and is named "The metabolic syndrome" (Alberti K.G., Zimmet P.Z.; Definition, diagnosis and classification of diabetes mellitus and its complications". Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet. Med. 1998 Jul;15(7), p. 539-53).

A patient has in accordance with the WHO-definition (www.idi.org.au/whoreport.htm) the metabolic syndrome if insulin resistance and/or glucose intolerance is present together with two or more of the following components

- reduced glucose tolerance or diabetes

- insulin sensivity (under hyperinsulinaemic, euglycaemic conditions corresponding to a glucose uptake below the lower quartile for the background population)
- increased blood pressure (≥ 140/90 mmHg)
- increased plasma triglyceride (≥ 1,7 mmol/l) and/or low HDL cholesterol (< 0,9 mmol/l for men; < 1,0 mmol/l for women)
- central adipositas (waist/hip ratio for men: > 0,90 and for women > 0,85) and/or Body Mass Index > 30 kg/m^2)
- micro albuminuria (urine albumin excretion: ≥ 20µg min^{-1} or albumin/creatinine ratio ≥ 2,0 mg/mmol.

It has become increasingly evident that the treatment should aim at simultaneously normalising blood glucose, blood pressure, lipids and body weight to reduce the morbidity and mortality. Diet treatment, exercise and avoiding smoking is the first treatment modalities that should be started. However, it will often be necessary to add pharmacological therapy but until today no single drug that simultaneously attack hyperglycaemia, hypertension and dyslipidemia are available for patients with the metabolic syndrome. Instead, these patients may be treated with a combination of several different drugs in addition to e.g. diet. This type of treatment is difficult to adjust and administer to the patient and such treatment may result in many unwanted adverse effects which in themselves may need medical treatment.

Consequently there is a long felt need for a new and combined medicament for the treatment of the metabolic syndrome thereby also preventing an increase in the number of persons developing the non-insulin dependent diabetes mellitus.

Existing oral antidiabetic medicaments to be used in such treatment include the classic insulinotropic agents sulphonylureas (Lebovitz H.E. 1997. "The oral hypoglycemic agents". In: Ellenberg and Rifkin's Diabetes Mellitus. D.J.

Porte and R.S. Sherwin, Editors: Appleton and Lange p. 761-788).

They act primarily by stimulating the sulphonylurea-receptor on the insulin producing beta-cells via closure of the K^+_{ATP}-sensitive channels.

However if such an action also affects the myocytes in the heart, an increased risk of cardiac arrhytmias might be present.

Also, it is well know in the art that sulphonylureas can cause severe and lifethreatening hypoglycemia, due to their continuous action as long as they are present in the blood.

Consumption of soy protein rather than animal protein has been found to lower blood cholesterol (Anderson J.W., Johnstone B.M., Cook-Newell M.E.: Meta-analysis of the effects of soy protein intake on serum lipids. N. Engl. J. Med. 1995; 333; p. 276-282). In addition to this knowledge, recent research also provides evidence that soy protein and/or isoflavones may improve endothelial function and attenuate events leading to both lesion and thrombus formation (Anderson J.W., Johnstone B.M., Cook-Newell M.E.: "Meta-analysis of the effects of soy protein intake on serum lipids"; N. Engl. J. Med. 1995; 333; p. 276-282; Potter S.M., Soy protein and cardiovascular disease: "The impact of bioactive components in soy". Nutrition Reviews 1998;56, p. 231-235).

Several attempts to develop new antidiabetic agents and drugs for the treatment or prophylactic treatment of the syndrome not having the adverse effects mentioned above, e.g. hypoglycemia and potential harmful actions on the heart functions have been made over the years.

For this purpose, plants provide a vast resource of compounds with the potential to become new antidiabetic agents.

For instance extracts of the leaves of *Stevia rebaudiana Bertoni*, a herbaceous member of the Compositae family, have been used for many years in the treatment of diabetes among Indians in Paraguay and Brazil (Sakaguschi M., Kan P Aspesquisas japonesas com Stevia rebaudiana (Bert) Bertoni e o estevioside. Cienc. Cultur. 34; p. 235-248,1982; Oviedo C.A., Franciani G., Moreno R., et al. "Action hipoglucemiante de la Stevia Rebaudiana Bertoni (Kaa-he-e)". Excerpt. Med. 209, p. 92,1979; Curi R., Alvarez M., Bazotte R.B., et al. Effect of Stevia rebaudiana on glucose tolerance in normal adult humans. Braz. J. Med. Biol. Res., 19, p. 771-774,1986; Hansson J.R., Oliveira B.H., "Stevioside and related sweet diterpenoid glycoside". Nat. Prod. Rep. 21, p.301-309,1993).

Also, an antihyperglycemic effect has been found in rats when supplementing the diet with dried *S. rebaudiana* leaves (Oviedo C.A., Franciani G., Moreno R., et al. "Action hipoglucemiante de la Stevia Rebaudiana Bertoni (Kaa-he-e)". Excerpt. Med. 209:92,1979).

Curi et al. found a slight suppression of plasma glucose when extracts of *Stevia rebaudiana* leaves were taken orally during a 3-day period. Furthermore, Oviedo et al. reported that tea prepared from the leaves caused a 35% reduction in blood glucose in man.

A number of *Stevia* species have been examined and shown to contain labdanes, clerodanes, kaurenes and beyerenes (Hansson J.R., Oliveira B.H., "Stevioside and related sweet diterpenoid glycoside". Nat. Prod. Rep. 21, p. 301-309,1993). Any of these substances as well as many others unidentified substances in the leaves could be responsible for the reduction in blood glucose in man.

In the work of Malaisse W.J. et al (Malaisse W.J., Vanonderbergen A., Louchami K, Jijakli H. and Malaisse-Lagae F., "Effects of Artificial Sweeteners on Insulin Release and

Cationic Fluxes in Rat Pancreatic Islets", Cell. Signal. Vol 10, No. 10, p. 727-733, 1998) the effect of several artificial sweeteners, including stevioside, on insulin release from isolated normal pancreatic rat islets were studied. In this study it was reported that in the presence of 7 mmol/l D-glucose, stevioside in a concentration of 1,0 mmol/l caused a significant increase in insulin output. Also the control group demonstrated a significant increase in insulin output of about 16 times above the basal release value in the presence of 20 mmol/l D-glucose increase. It is therefore uncertain whether the insulin releasing effect is due to the increased glucose level or the presence of stevioside. No diabetic islet cells were studied and the skilled person within the art will know that the mechanism for stimulating normal pancreatic islet cells either not functions at its optimum or not functions at all in the diabetic pancreatic cells, and that the study provided no certain indication of the possible use of stevioside in the treatment of non-insulin dependent diabetes mellitus, hypertension and/or the metabolic syndrome.

In a Chinese study (Lin Qi-Xian, Cao Hai-Xing, Xie Dong, Li Xing-Ming, Shang Ting-Lan, Chen Ya-Sen, Ju Rui-Fen, Dong Li-Li, Wang Ye-Wen, Quian Bao-Gong, " Experiment of Extraction of Stevioside", Chinese Journal og Pharmaceuticals 1991, No. 22, p 389-390) is indicated a method for extracting stevioside from stevioside leafs from the origin of Bingzzhou in the Hunan Province. The content of stevioside in the extract was determined using HPLC although the article is silent of the purity of the extract. The produced stevioside tablets were for no apparent reason and medical indication applied to patients in the Wuhan Second Hospital. No data on the influence of stevioside on blood glucose, insulin and/or blood pressure is revealed. It is stated that the tablets were effective to diabetes and hypertension during preliminary clinical observations. However, total lack of data on blood glucose, insulin and/or blood pressure i.e. lack of support by test results and the missing information of which types of

diabetes that were treated makes this an unsupported and unconfirmed assertion.

Any detailed information of which substance or substances in the leaves that might cause a possible anti-hyperglycemic effect has not yet been disclosed for certainty, and the mechanism of how and to which extent the plasma glucose is reduced is unknown.

The above mentioned articles and studies are concerned with the initial discovery of the effects and provide no evidence of which specific component(s) in the leaves that might be the active one(s).

The effect of intravenous stevioside on the blood pressure was studied in spontaneously hypertensive rats ("The Effect of Stevioside on Blood Pressure and Plasma Catecholamines in Spontaneously Hypertensive Rats", Paul Chan, De-Yi Xu, Ju-Chi Liu, Yi-Jen Chen, Brian Tomlinson, Wen-Pin Huang, Juei-Tang Cheng, Life Science, Vol. 63, No. 19, 1998, p. 1679-1684). The study showed that during an intravenously administration of stevioside of 200 mg/kg the hypotensive effect was at a maximum, but although reported as being significantly the fall in the systolic blood pressure was only small. Neither the heart rate nor the plasma catecholamines were significantly changed during the observation period. This study indicated that stevioside advantageously could be used for treating hypertension.

No reports of an effect on plasma glucagon level have previously been reported. Glucagon, a pancreatic islet hormone, acts as a diabetogenic hormone by increasing the hepatic glucose output thereby elevating blood glucose.

Recent studies and tests made by the present inventors have focused on especially the diterpenoid glycoside stevioside which is a major constituent found in the leaves of *Stevia*

rebaudiana where it may occur in amounts of up to about 10 % (Hansson J.R., Oliveira B.H., "Stevioside and related sweet diterpenoid glycoside". Nat. Prod. Rep. 21, p.301-309,1993; Bridel M., Lavielle R., Physiologie Vegetale: "Sur le principe sucre'du Kaa' he'e (Stevia rebaudiana Bertoni): II Les produits d'hydrolyse diastasique du stevioside, glucose et steviol". Acad. Sci. Paris 192, p. 1123-1125,1931; Soejarto D.D., Kinghorn A.D., Farnsworth N.R., Potential sweetening agent of plant origin. III: "Organoleptic evaluation of Stevia leaf herbarium samples for sweetness". J. Nat. Prod. 45, p. 590-598,1983; Mossettig E., Nes W.E. Stevioside. II: "The structure of the aglucone"; J. Org. Chem. 20, p. 884-899,1955; Kohda H., Hasai R., Yamasaki K. et al. "New sweet diterpene glucosides from Stevia rebaudiana". Phytochemistry 15, p. 981-983,1976).

Also, its aglycone, steviol, has been found to be contained in the leaves of *Stevia rebaudiana* as well as other sweet-tasting glycosides e.g. Steviolbioside, Rebaudioside A,B,C,D and E, and Dulcoside (Bridel M., Lavielle R., Physiologie Vegetale: "Sur le principe sucre'du Kaa' he'e (Stevia rebaudiana Bertoni): II Les produits d'hydrolyse diastasique du stevioside, glucose et steviol". Acad. Sci. Paris 192, p. 1123-1125,1931; Soejarto D.D., Kinghorn A.D., Farnsworth N.R., Potential sweetening agent of plant origin. III: "Organoleptic evaluation of Stevia leaf herbarium samples for sweetness". J. Nat. Prod. 45, p. 590-598,1983; Mossettig E., Nes W.E. Stevioside. II: "The structure of the aglucone"; J. Org. Chem. 20, p. 884-899,1955; Mossettig E., Nes W.E. Stevioside. II: "The structure of the aglucone"; J. Org. Chem. 20, p. 884-899,1955; Kohda H., Hasai R., Yamasaki K. et al. "New sweet diterpene glucosides from Stevia rebaudiana". Phytochemistry 15, p. 981-983,1976).

The inventors of the present invention have successfully proved that both stevioside and steviol have an anti-hyperglycemic, glucagonostatic and insulinotropic effect when

administered intravenously to rats and a stimulatory effect on the insulin secretion from mouse islets *in vitro*.

No well defined, chemical stable, non-toxic, reliable and without adverse effects alternative to the sulphonylureas for the treatment of non-insulin dependent diabetes mellitus is available today and these findings have given rise to further studies and tests of analogues and derivates of these substances in order to find improved and alternative drugs for a self-regulatory treatment of diabetes, hypertension and especially the metabolic syndrome in mammals, preferably man.

In order to prevent sequelae or to delay the developing in man of a number of the above-mentioned metabolic and functional disorders it is also aimed to provide new and beneficial dietary supplementations or new self-administrable non-prescription drugs for prophylaxis.

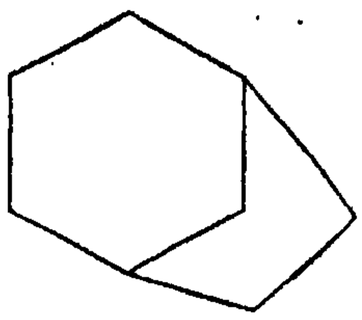
According to one aspect of the invention, there is provided a substance or a composition of substances, wherein the substance(s) includes a bicyclo[3.2.1]octan of the structural formula I shown in claim 1 or a kaurene structure of the structural formula II shown in claim 2 for the preparation of a medicament for the use in the treatment of non-insulin dependent diabetes mellitus.

According to another aspect of the invention, there is provided a substance or a composition of substances, wherein the substance(s) includes a bicyclo[3.2.1]octan of the structural formula I shown in claim 1 or a kaurene structure of the structural formula II shown in claim 2 for the preparation of a medicament for the use in the simultaneous treatment of non-insulin dependent diabetes mellitus and hypertension.

According to a third aspect of the invention, there is provided a substance or a composition of substances, wherein

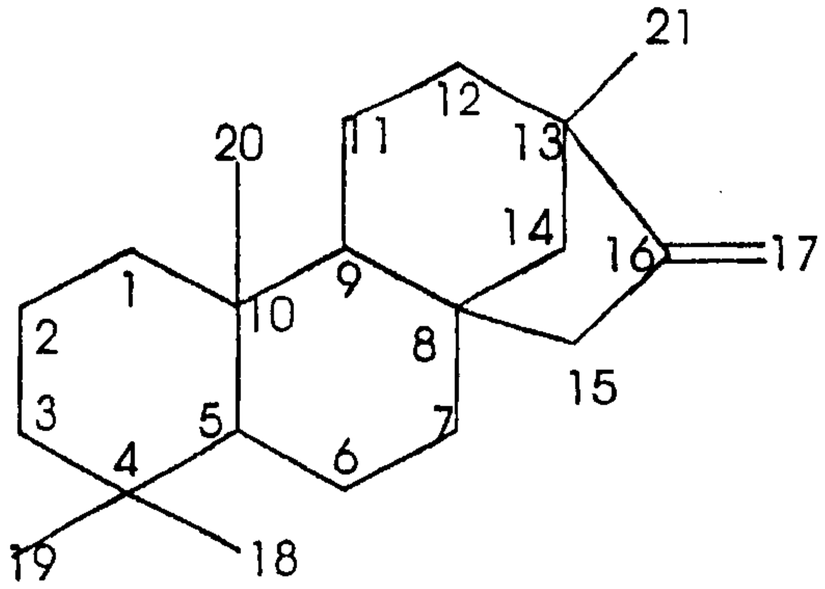
the substance(s) include a bicyclo[3.2.1]octan of the structural formula I shown in claim 1 or a kaurene structure of the structural formula II shown in claim 2 in combination with at least one soy protein and at least one isoflavone for the preparation of a medicament for the use in the treatment of the metabolic syndrome.

Careful structural chemistry studies by the inventors have revealed that all potential substances for stimulating the insulin secretion extracted from the leaves of *Stevia rebaudiana* share the common unique skeletal structure of bicyclo[3.2.1]octan of the formula I:



This bicyclo[3.2.1]octan can be found in e.g. steviol, isosteviol and in stevioside. The formula I structure has also been recognised in glucosilsteviol, gymnemic acid, steviolbioside, Rebaudioside A, Rebaudioside B, Rebaudioside C, Rebaudioside D, Rebaudioside E and Dulcoside A.

All these substances also share the common structure of formula II:



which is the basic structure in kaur-16-en-18-oic acid.

These specific structures of the formula I or II are recognised in several chemical compounds, which have been shown to have a highly potent insulin stimulating effect on isolated mouse pancreatic β-cell, and these structures of formula I and II are evidently the active parts of the molecules in causing the stimulating task.

This assumption is further confirmed by the fact that tests have shown that steviol having the smallest skeletal structure stimulate the insulin secretion to a greater extent than e.g. the glycoside stevioside having a much larger skeletal structure. Also, the inventors of the present invention have succeeded in purifying the different Rebaudiosides from *Stevia rebaudiana* and preclinical animal studies indicate the same stimulatory effect on insulin secretion.

Consequently this indicate that other compounds including the structures of the formula I or II, such as e.g. analogues, derivates and metabolites of the compounds mentioned above can be used alternatively.

Studies and tests on rats have disclosed that the insulin stimulating effect of these substances is dependent on the concentration of the plasma glucose.

The substances comprising the chemical structures, which includes the formula I or II, did not cause an insulin release as long as the plasma glucose concentration was below approximately 6 mmol/l. At plasma glucose concentration above 6 mmol/l, the stimulating effect of the compounds provided an elevated plasma insulin concentration resulting in an immediate suppression of plasma glucose concentration thereby keeping this at a normal level.

In addition to the above findings, the present inventors have surprisingly found that the substances comprising the chemical structures including the formula I or II also have the

capabilities of reducing the glucagon concentration in the blood.

This characteristic nature and qualities of the said substances make them an obvious choice as a component in a medicament for the treatment of especially non-insulin dependent diabetes mellitus (NIDDM).

The finding that e.g. intravenously administered stevioside inhibited blood glucose responses to intravenous glucose in NIDDM rats (GK rats) but not in normal rats supports this fact. This finding is new and surprisingly has neither been expected nor demonstrated in earlier studies which has only been concerned with normal pancreatic islet cells.

As a further example of the unique action of the substances according to the invention, stevioside infusion at normal blood glucose did not cause any hypoglycemia irrespective of it being given as a bolus or at a constant intravenous infusion.

Due to the insulin secretory stimulating effect induced by a slightly elevated plasma glucose concentration, the simultaneous plasma glucagon reducing effect and the inhibited blood glucose response, these substances are able to control, regulate and adjust the plasma glucose concentration of a NIDDM patient to a normal level.

As a consequence of the glucose-dependency the substances only act when needed, e.g. after the patient has increased blood glucose after having eaten. In NIDDM patients treated with medicaments including these substances hypoglycemia will not occur and hypoglycemia will be counteracted.

Therefore, the substances provide a self-regulatory system responding only at elevated plasma glucose concentration.

The substances are preferably used in medicaments for oral medication. When taken orally, the glycosylated substances can be partially metabolised but the basic skeletal structure of the formula I or II will not be changed and the different characteristic effects mentioned above will be preserved.

The treatment with a medicament including these substances provides an attractive alternative to different types of drugs available and presently used today for the treatment of NIDDM, such drugs being drugs for stimulating the insulin secretion (sulphonylureas or repaglinide), drugs for improving the insulin sensivity (biguanides and thiazolidinediones) or drugs for retarding gastrointestinal carbohydrate absorption (α-glucosidase inhibitors).

The potential of these new substances has for the first time also been tested in human NIDDM studies and the beneficial and advantageously combined multiple effects in humans of a single substance according to the invention has been demonstrated and will be described later in the present description and the examples.

The above-mentioned human tests has been conducted by orally administrating the substances, but within the scope of the invention the substances can optionally be used in the preparation of medicaments for intravenous, subcutaneous or intramuscular medication.

The substances further bring along the blood pressure reducing effect. In long-term experiments stevioside acutely suppresses blood pressure in diabetic rat. This important discovery is of the benefit to the diabetic patients that have developed hypertension in relation to or besides their disease.

When at least one of the substances according to the invention is combined in a medicament also comprising at least one soy protein alone or in combination with at least one isoflavone,

it is possible to manufacture a combined preparation of a drug for the treatment of patients with the metabolic syndrome in accordance with the previously definition. Such a medicament may advantageously be used in prophylactic treatment of patient in a risk group. For example, a slow-release drug on the basis composition mentioned above provides a convenient treatment for the patient with the metabolic syndrome.

The inventors of the present invention have demonstrated that the combination of the substances according to the invention and at least one soy protein have a new unexpected and surprisingly synergistic effect surpassing the additive effect of the single components of the medicament thereby providing a completely new and very important medicament for therapeutic or prophylactic treatment of the metabolic syndrome.

The inventors of the present invention has used the combination of the substances according to the invention and at least one soy protein as a dietary supplementation in human studies. The test results significantly proved, as will be seen in the following examples, that such combination has a beneficial impact on cardiovascular risk markers in type II diabetic subjects.

Stevioside at a dose as high as 15 g/kg body weight was not lethal to either mice, rats or hamsters (Toskulkao C., Chaturat L., Temcharoen P., Glinsukon T. "Acute toxicity of stevioside, a natural sweetener, and its metabolite steviol, in several animal species". Drug Chem. Toxicol. 1997 Feb-May;20(1-2), p. 31-44). In rats and mice, LD_{50} values of steviol were higher than 15g/kg body weight while the LD_{50} for hamsters were 5-6 g/kg body weight. The latter was accompanied with degeneration of the proximal tubular cells, which correlated to increases in blood urea nitrogen and creatinine. Stevioside is excreted by the urine (Melis M.S. "Renal excretion of stevioside in rats". J. Nat. Prod. 1992 May;55(5), p. 688-90) and is not metabolised in the isolated

perfused rat liver (Ishii-Iwamoto E.L., Bracht A. "Stevioside is not metabolised in the isolated perfused rat liver". Res. Commun. Mol. Pathol. Pharmacol. 1995 Feb;87(2), p. 167-75).

Stevioside and steviol showed no mutagenic effect on a number of *Salmonella typhimurium* strains (Klongpanichpak S., Temcharoen P., Toskulkao C., Apibal S., Glinsukon T. "Lack of mutagenicity of stevioside and steviol in *Salmonella typhimurium* TA 98 and TA 100". J. Med. Assoc. Thai 1997 Sep;80 Suppl. 1, p. 121-128; Suttajit M., Vinitketkaumnuen U., Meevatee U., Buddhasukh D. "Mutagenicity and human chromosomal effect of stevioside, a sweetener from Stevia rebaudiana Bertoni". Environ Health Perspect 1993 Oct.;101 Suppl. 3, p. 53-56). In another study, it was confirmed that stevioside was not mutagenic whereas steviol, however, produced dose-related positive responses in some mutagenicity test (Matsui M., Matsui K., Kawasaki Y., Oda Y., Noguchi T., Kitagawa Y., Sawada M., Hayashi M., Nohmi T., Yoshihira K., Ishidate M. Jr., Sofuni T. "Evaluation of the genotoxicity of stevioside and steviol using six *in vitro* and one *in vivo* mutagenicity assays". Mutagenesis 1996 Nov.;11(6), p. 573-579).

Stevioside is not carcinogenic in F344 rats (Toyoda K., Matsui H., Shoda T., Uneyama C., Takada K., Takahashi M. "Assessment of the carcinogenicity of stevioside in F344 rats". Food Chem. Toxicol. 1997 Jun.;35(6), p. 597-603). Doses as high as 2.5 g/kg body weight/day had no effect on growth or reproduction in hamsters (Yodyingyuad V., Bunyawong S. "Effect of stevioside on growth and reproduction". Hum. Reprod. 1991 Jan.;6(1), p. 158-165).

To the knowledge of the inventors, no observations or reports showing potential toxic effects in humans have been published.

It will be obvious to the person skilled in the art that rearranged structures of the formula II are within the scope of the invention, and such rearrangements might occur

naturally in the gastro intestinal tract. As example can be mentioned that rearrangement may occur at the C16 forming a double bond to the C15 and thereby leaving a single bond open for substitution at position 17. A COOH group at position 18 is open for a number of reactions such as reaction with alcohol, as well as a number of substituents can be provided at any point of the formula II structure. Also, other substituents such as e.g. saccharides, at the various C-atoms and the structures may be anticipated.

The invention is further illustrated by means of the following examples and the accompanying drawing, wherein:

Fig. 1 shows the chemical structure of steviol, isosteviol and stevioside,

Fig. 2a shows the effect of stevioside on blood glucose during i.v. glucose tolerance test in normal Wistar rats,

Fig. 2b shows the effect of stevioside on blood glucose during i.v. glucose tolerance test in GK rats,

Fig. 3a shows the effect of stevioside on glucose-induced release during i.v. glucose tolerance test in normal Wistar rats,

Fig. 3b shows the effect of stevioside on glucose-induced release during i.v. glucose tolerance test in GK rats,

Fig. 4a shows the effect of stevioside on glucose-stimulated insulin secretion from isolated mouse islets,

Fig. 4b shows the effect of steviol on glucose-stimulated insulin secretion from isolated mouse islets,

Fig. 5a shows the effect of an i.v. bolus injection of glucose on plasma glucagon levels during an intravenous glucose tolerance test in GK rats,

Fig. 5b shows the effect of an i.v. bolus injection of glucose and stevioside on plasma glucagon levels during a glucose tolerance test in GK rats,

Fig. 6a shows the systolic blood pressure during 6 weeks treatment of GK rats with stevioside,
Fig. 6b shows the diastolic blood pressure in GK rats treated with stevioside.

Fig. 7a shows the effect of 10^{-3} mmol/l stevioside on the insulin secretion from isolated mouse islets in the presence of glucose ranging between 0 and 16,7 mmol/l,

Fig. 7b shows the effect of 10^{-6} mmol/l steviol on the insulin secretion from isolated mouse islets in the presence of glucose ranging between 0 and 16,7 mmol/l,

Fig. 8 a-d shows the acute effects of stevioside in type II diabetic patients,

Fig. 9a-g shows the effects of the action of the combination of stevioside and soy based dietary supplementation i diabetic GK-rats.

EXAMPLES

In the following examples, the type II diabetic Goto-Kakizaki (GK) rats originated from Takeda Chemical Ind., Tokyo, Japan and were bred locally.

The normal Wistar rats and the NMRI mice were available from Bomholtgård Breeding and Research Centre Ltd., Ry, Denmark.

The rats had a weight of 300-350 g and the mice a weight of 22-25 g. The animals were kept on a standard pellet diet and tap water *ad libitum*.

The stevioside is obtained from the Japanese company WAKO-TriCHEM.

The abbreviation IAUC means Incremental Area Under the Curve (above basal).

Example 1:

As examples of the effects of a compound including the chemical formulas II, stevioside was tested on normal Wistar rats and on GK rats. 2.0 g glucose/kg body weight and 0.2 g stevioside/kg body weight were dissolved in 0.9% saline and infused intravenously. The plasma glucose and insulin levels were measured over a period of 2 hours.

The results are shown in figs. 2a, 2b, 3a and 3b, were the O-O series (n=6 for Wistar and n=14 for GK) illustrate glucose infused alone and the ●-● series (n=6 for Wistar and n=12 for GK) illustrate the combined glucose and stevioside infusion. Data are given as mean±SEM.

After administration of the glucose load, plasma glucose raised immediately and plasma insulin raised abruptly. When stevioside was added together with the glucose, a diminished glucose response was found in the GK-rat and a significant decrease was observed already after 30 min. In the GK rat, stevioside caused a pronounced increase in the insulin response compared to the Wistar rat. The stevioside-induced insulin response was delayed and increased throughout the whole test. The insulin response was monophasic.

This discovery of stevioside having a blood glucose reducing effect in the type II diabetic rat indicates that stevioside

and compounds having a similar chemical structure can be used in a medicament for the treatment of NIDDM in man.

Example 2:

Islet from 6-10 NMRI mice were isolated and incubated in the presence of 16.7 mmol/l and 10^{-9}-10^{-3} mol/l stevioside or 10^{-9}-10^{-3} mol/l steviol.

The results of these tests are illustrated in figs. 4a and 4b where each column represents mean±SEM from 24 incubations of single islets. Black bars in fig. 4a indicate that stevioside is present and hatched bars indicate that stevioside is absent.

Black bars in fig. 4b indicate that steviol is present and hatched bars indicate that steviol is absent.

The figures show that stevioside and steviol are capable of potentiating glucose-stimulated insulin secretion. Further tests confirmed that a stimulatory effect was found already at a very low concentration (above 0.1 nM).

Example 3

During a glucose tolerance test, an intravenous bolus of stevioside of 0.2 g/kg body weight was injected in GK rats (the ●-● serie (n=6)). GK rats receiving 0.9 % saline intravenously served as controls (the O-O serie (n=6)). Glucose 2.0 g/kg body weight was administered as a bolus at timepoint 0 min. The plasma glucagon responses are shown as mean±SEM in figs. 5a (control) and 5b (GK). The plasma glucagon was suppressed in the stevioside treated GK rat.

Example 4

GK rats were treated with stevioside 0.025 g/kg body weight/24h for 6 weeks. Stevioside was administered in the drinking water. GK rats receiving drinking water with 0.111 g

D-glucose/kg body weight/24h served as controls. Systolic (fig. 6a, control: O-O series, stevioside-treated: ●-● series) and diastolic (fig. 6b, control: O-O series, stevioside-treated: ●-● series) blood pressures were measured on the tail.

The figures show a 10-15% decrease in the blood pressure detectable after 2 weeks of treatment and the effect hereafter was stable and consistent during the study period.

Examples 5

The influence of the maximal stimulatory doses of 10^{-3} mol/l stevioside and 10^{-6} mol/l steviol was studied in NMRI mouse islets over a range between 0 and 16.7 mmol/l glucose. Both stevioside (fig. 7a) and steviol (fig. 7b) potentiated insulin secretion at and above 8.3 mmol/l and indicated that the initiating level for stimulating insulin secretion was between 3.3 mmol/l and 8.3 mmol/l of glucose. Black bars in fig. 7a indicate that stevioside is present and hatched bars indicate that stevioside is absent. Black bars in fig. 7b indicate that steviol is present and hatched bars indicate that steviol is absent.

Examples 6

Twenty type II diabetic patients (6 female/14 males) with a mean age of 63.6±7.5 years participated in a controlled randomised double blind crossover trial. They were supplemented for 6 weeks with soy protein for (50g/day) with high levels of isoflavones (minimum 165 mg/day) and cotyledon fibers (20g/day) or placebo (casein 50g/day) and cellulose (20 g/day) separated by a 3 week wash-out period.

This dietary supplement significantly reduced LDL-Cholesterol by 10% ($p<0.05$), LDL/HDL ratio by 12% ($p<0,05$), Apo B-100 by 30% ($p<0,01$), triglycerides by 22% ($p<0,05$) and homocystein by 14 % ($p<0,01$). No change was observed in HDL-Cholesterol,

Factor VIIc, von Willebrandt factor, fibrinogen, PAI-1, HbA1c or 24 hour blood pressure.

The results indicate beneficial effects of dietary supplementation with soy protein on cardiovascular risk markers in type II diabetic subjects. The improvement is also seen in individuals with near-normal lipid values. Ingestion of soy product has been shown to further improve the effectiveness of low-fat diets in non-diabetic subjects and the dietary supplementation in type II diabetic patients may provide an acceptable and effective option for blood lipid control, thereby postponing or even preventing drug therapy.

Examples 7

Twelve type II diabetic patients (4 female/8 males) with a mean age of 65.8±1.6 years, a diabetes duration of 6.0±1.3 years, a mean body mass index of 28.5±1.0, and a mean glycated hemoglobin HbA1c of 7.4±0.4 percent were included in the study.

The experiment was an acute, paired, cross-over study in which two test meals were served during the experiments (A: Standard meal supplemented with 1 g of stevioside given orally; B: Standard meal given together with 1 g of gelatine (placebo) given orally. The total energy content of the test meals was 1725 kJ (protein 16 E%, fat 30 E%, carbohydrate 54 E%).

Blood samples were drawn from an antecubital vein 30 minutes before and 240 minutes after ingestion of the test meal. The arterial blood pressure was continuously monitored during the experiment. Students paired t-test was used for comparing the effects of stevioside with placebo on the parameters measured. Data are given as mean ± SEM.

Stevioside reduced the postprandial blood glucose response by 18±5% ($p<0.004$) compared to placebo (absolute IAUC 638±55 vs. 522±64 mmol/l x 240 min; $p<0.02$) as seen in fig. 8a. Stevioside

tended to stimulate the insulin response in type II diabetic patients (enhance the area under the insulin response curve (IAUC)), however the difference did not reach statistical significance (p=0.09) (fig. 8b).

Stevioside significantly reduced the postprandial glucagon levels compared to placebo (348±46 vs. 281±33; p=0.02) (fig. 8c).

Stevioside significantly reduced the postprandial glucagon like peptide-1 (GLP-1) levels compared to placebo (2208±253 vs. 1529±296; p<0.045) (fig.8d).

Examples 7

Four test diets (A: Standard carbohydrate rich laboratory animal diet (Altromin); n=12 (Alt). B: Altromin supplemented with stevioside (Altromin+Stevioside); n=12; (Alt+Ste). C: Soy plus 20% Altromin; n=12; (Soy). D: Soy plus 20% Altromin plus stevioside; n=12; (Soy+Ste)) were administered for four weeks to four groups of adult rats. Each experimental group consisted of twelve female Goto-Kakizaki with an age of 9 weeks. The rats received the stevioside (0.025 g/kg body weight/day) with the drinking water. By the end of the third experimental week intra-arterial catheters were implanted into the carotid artery thereby enabling blood sampling during a 240 minutes glucose-tolerance test which was carried out by the end of the experiment at week 4. Blood samples were drawn after a bolus infusion of 2,0 g D-glucose/kg body weight. Plasma concentrations of glucose, insulin, and glucagon were measured during the glucose tolerance test. Immediately before the glucose tolerance test fasting levels of triglycerides and cholesterol were determined. Concomitantly, the systolic blood pressure was measured using a tail cuff.

Effects on plasma-glucose:

As seen at fig. 8 and in Table I below stevioside reduced the incremental area (IAUC) under the glucose response curve during the glucose tolerance testing both in the Altromin ($p<0.05$) and in the soy + 20% Altromin group (Soy) ($p<0.001$). The relative effect of stevioside was more pronounced in the group receiving soy + 20% Altromin group compared to the group receiving Altromin. The combination of soy and stevioside synergistically reduced the area under the glucose response curve compared to the Altromin group ($p<0.0001$) (fig 9a.).

[Plasma glucose was measured using MPR 3, 166 391, Glucose/GOD-PAP Method from Boehringer Mannheim]

Effects on plasma insulin:

The group receiving soy + stevioside (Soy+Ste) has reduced incremental area under the insulin response curve compared to the Altromin + stevioside group (Alt+Ste) as seen at fig 9 and in Table I below. Considering the concomitant blood glucose responses this indicates that soy increases the insulin sensitivity. Stevioside did not alter the insulin responses in the Altromin and soy diets when studying the total response curve from 0 to 240 minutes. However, in both groups supplementation of the diets with stevioside significantly improved the first phase insulin responses - which is subdued as a characteristic feature of type II diabetes. The combination of soy + stevioside synergistically improved the first phase insulin response ($p<0.05$) (fig 9b).

[Plasma insulin was measured using Sensitive Rat Insulin RIA, Cat # SRI-13K from Linco]

Effects on plasma glucagon:

Stevioside significantly reduced the area under the plasma-glucagon response curve during the glucose tolerance test in both the groups receiving Altromin ($p<0.003$) and soy ($p<0.01$) (see fig. 9c and Table I below).

[Plasma glucagon was measured using Glucagon RIA, Cat # GL-32K from Linco]

Effects on blood pressure:

A marked significant suppression of the systolic blood pressure ($p<0.05$) (Table I) is elicited by stevioside in combination with either Altromin (Δ = -28 mmHg) or soy (Δ= -21 mmHg) as depicted in fig. 9d.

[Blood pressure was measured using TSE Non-Invasive Blood Pressure Monitoring System from Technical Scientific Equipment GmbH]

Effects on body weight:

The initial weights in the four groups did not differ (Fig 5). Apparently the combination of soy and stevioside prevented weight gain as seen in fig. 9e.

Effects on triglyceride and cholesterol.

Stevioside causes a significant suppression of the fasting triglyceride levels in combination with either Altromin ($p<0.05$) or soy ($p<0.02$) (Table I). Soy significantly reduced the fasting triglyceride levels with or without supplementation of stevioside ($p<0.05$ and $p<0.002$, respectively) (Table I). Stevioside given in combination with soy synergistically reduced the fasting total cholesterol levels compared to diets containing Altromin alone ($p<0.0001$). Soy alone also reduced the total cholesterol levels compared to Altromin alone ($p<0.002$) (fig 9f. and fig.9g) (Table I).

[Plasma cholesterol was measured GOD-PAP from Roche and triglycerides was measured using GHOD-PAP from Roche]

Stevioside exerts beneficial effects in type II diabetes i.e. reduces blood glucose, suppresses glucagon and improve first phase insulin secretion. The results also indicates that soy

improves insulin sensitivity, a characteristic feature of the metabolic syndrome. Stevioside exerts a pronounced blood pressure reduction both with as well as without the presence of soy. The combination of stevioside and soy has a synergistic suppressive effect on blood glucose levels, enhances first phase insulin secretion, supresses fasting plasma triglyceride and total cholesterol and the combination of soy and stevioside seems to prevent weight gain. The combination of stevioside and soy appears to possess the potential of an effective treatment of a number of the characteristic features of the metabolic syndrome i.e. type II diabetes, hypertension, dyslipidemia and obesity.

Group	IAUC p-glucose (mM x 240 min)	IAUC p-insulin (ng/ml x 240 min)	IAUC p-insulin (ng/ml x 30 min)	IAUC p-glucagon (pg/ml x 240 min)	Change in blood pressure (mmHg) From week 0 to 4	Triglycerides (mM)	Cholesterol (mM)
Altromin	991±96	317±55	11±4	21918±1467	5±4	0.72±0.10	2.51±007
Altromin + Stevioside	757±53	375±42	19±4	17023±1449	-23±6	0.50±0.04	2.28±0.18
Soy + 20% Altromin	820±75	218±22	9±2	26200±2410	8±3	0.49±0.04	2.13±0.08
Soy + 20% Altromin + Stevioside	439±56	248±27	24±5	17229±1819	-13±5	0.37±0.02	1.84±0.06

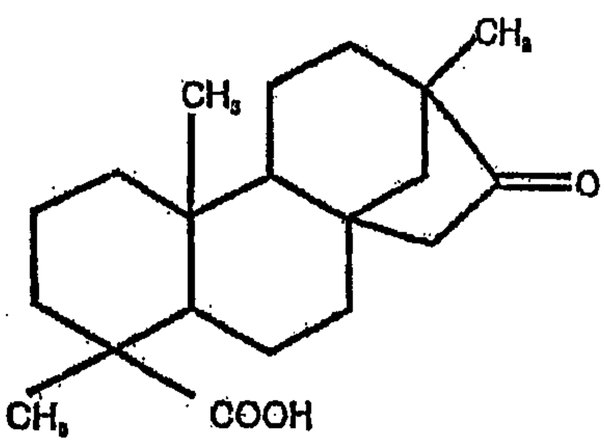
Table I: Areas under the p-glucose, -insulin and -glucagon response curves during the glucose tolerance test in the four experimental groups. Change in systolic blood pressure at start and at end of the study period. Fasting plasma- triglyceride and -total cholesterol concentrations by the end of the study.

CA 02762121 2011-12-20

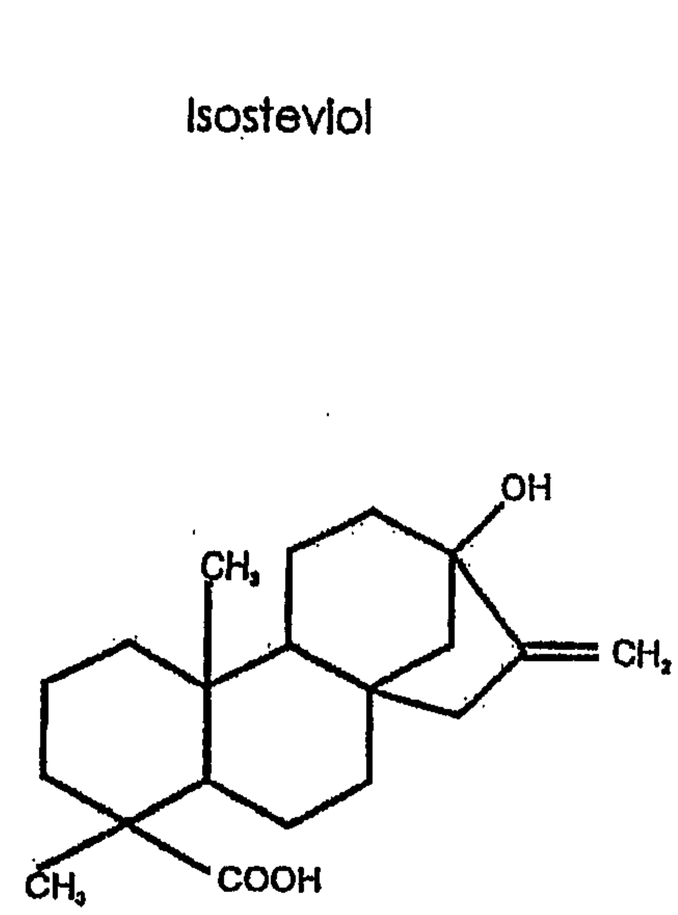
What is claimed is:

1. A medicament comprising a substance selected from steviol and isosteviol for oral administration.

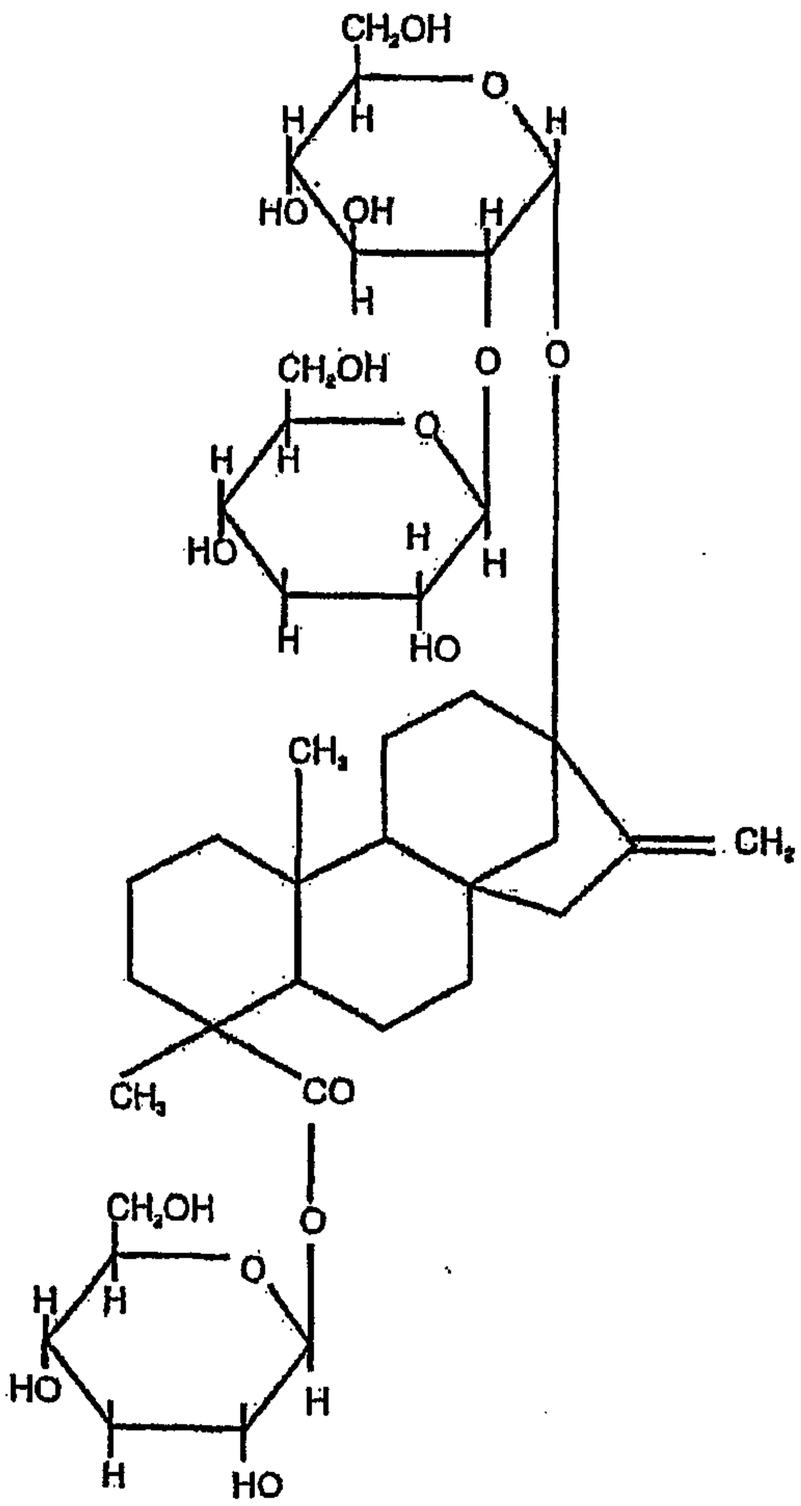
2. The medicament according to claim 1, wherein the substance is isosteviol.



Isosteviol



Steviol



Steviolside

Fig. 1

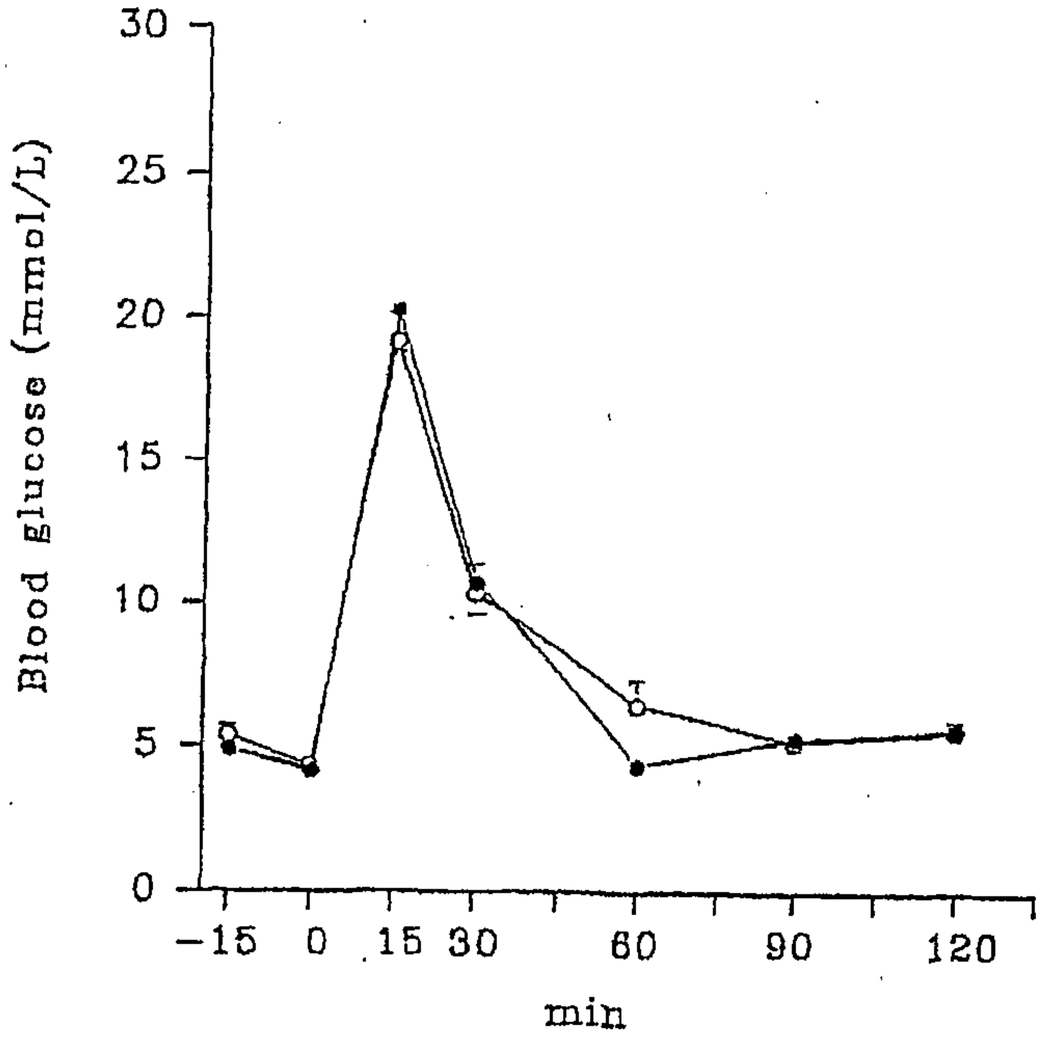
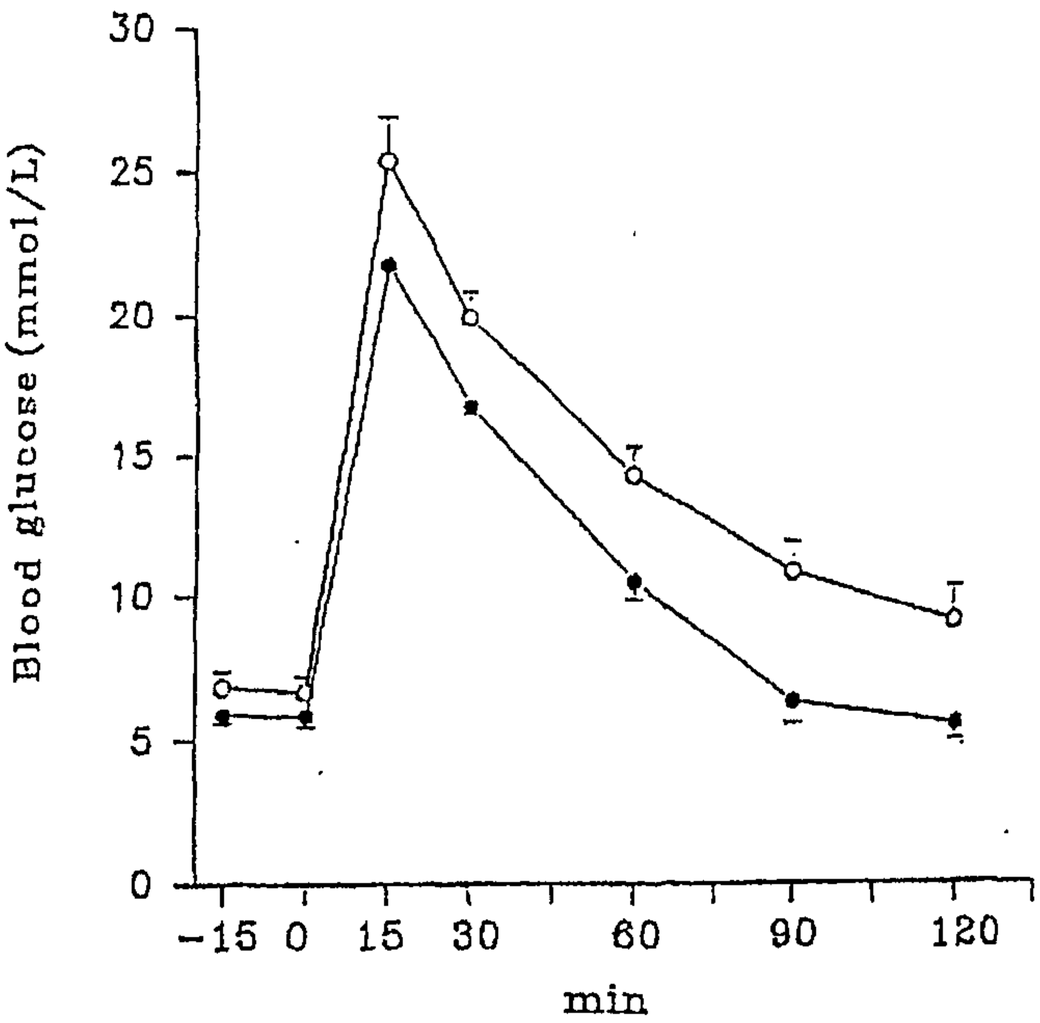

Blood glucose (mmol/L)
30
25
20
15
10
5
0
-15
0
15
30
60
90
120
min

Fig. 2a


Blood glucose (mmol/L)
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25
20
15
10
5
0
-15
0
15
30
60
90
120
min

Fig. 2b

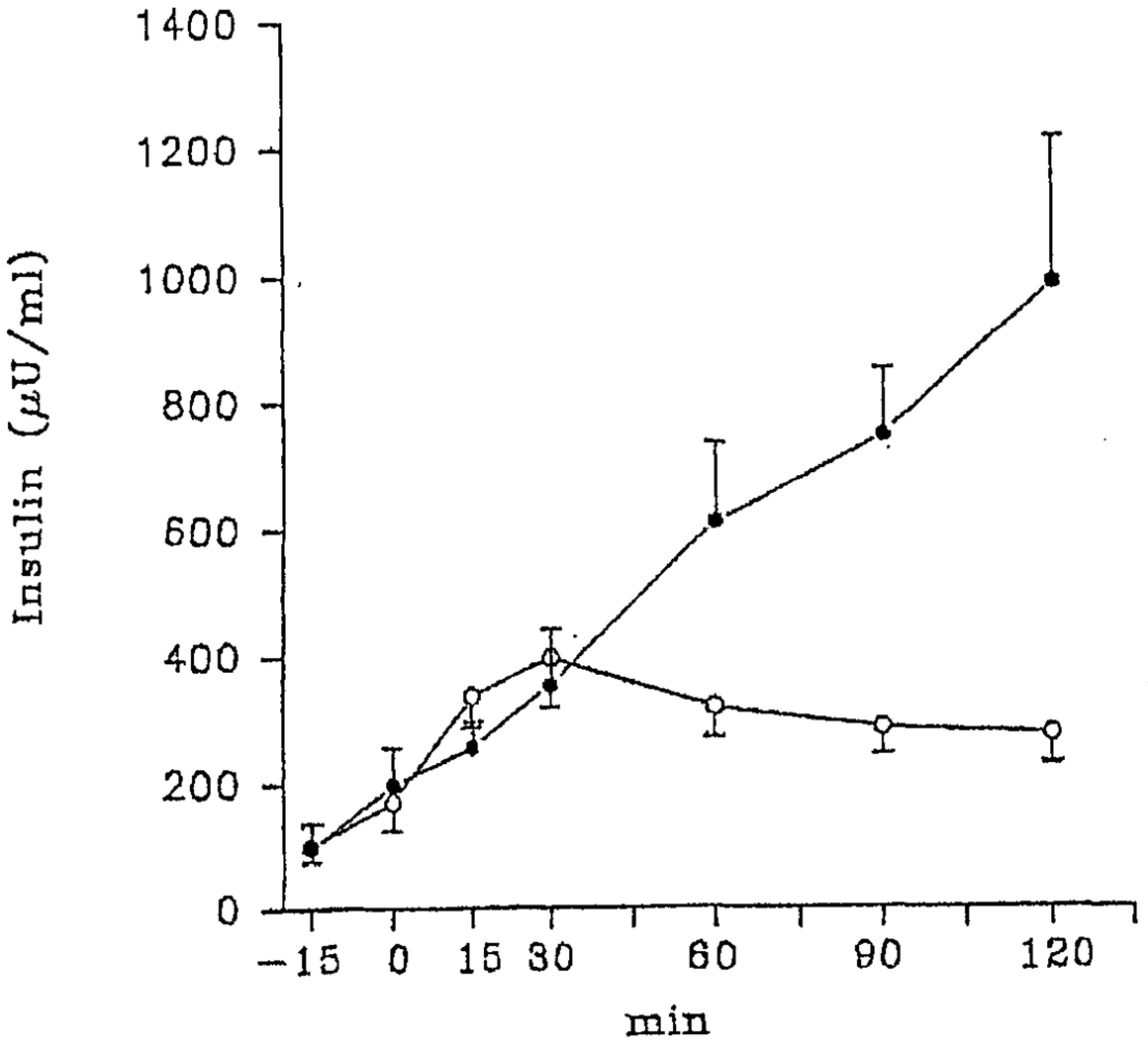

1400
1200
1000
800
600
400
200
0
Insulin (µU/ml)
-15
0
15
30
60
90
120
min

Fig. 3a

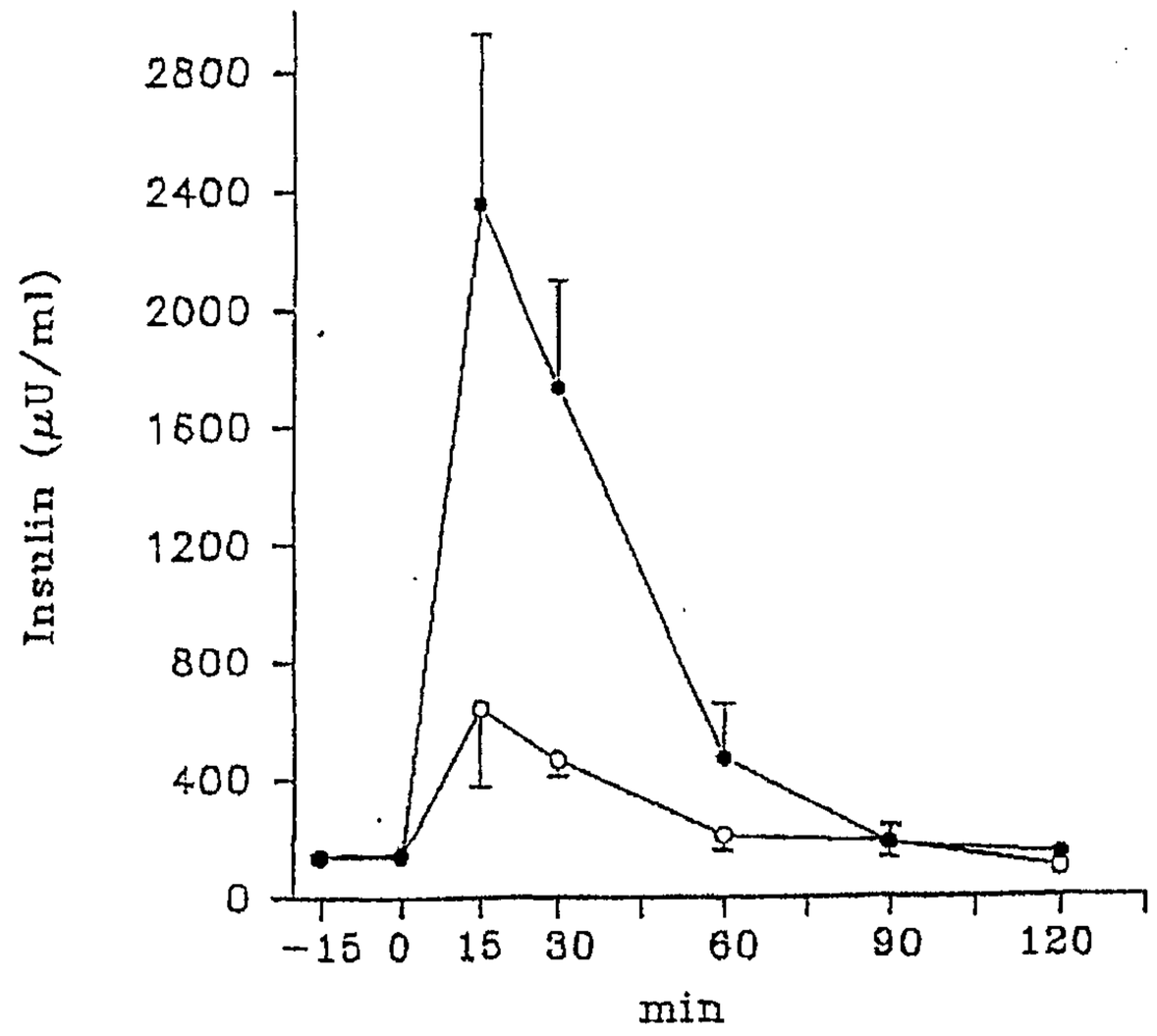

2800
2400
2000
1600
1200
800
400
0
Insulin (µU/ml)
-15
0
15
30
60
90
120
min

Fig. 3b

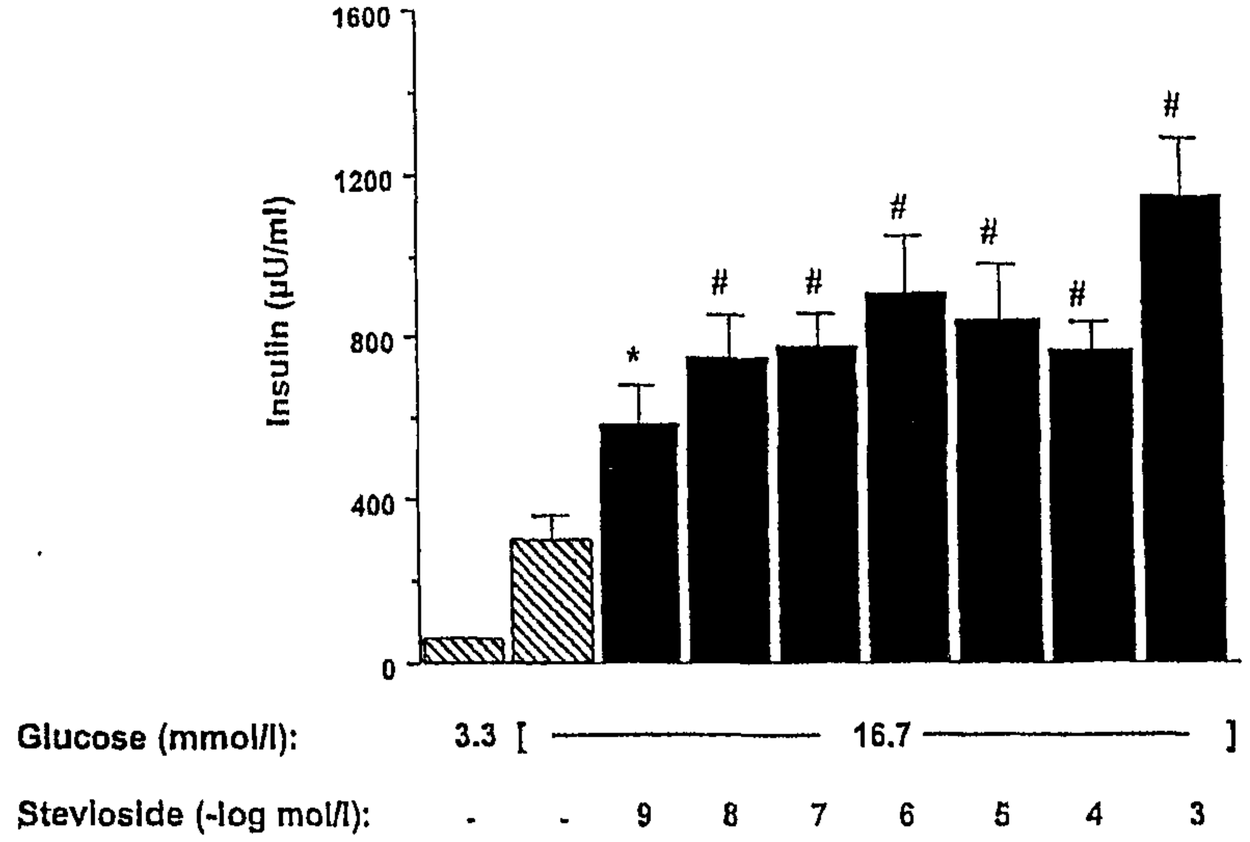

Insulin (µU/ml)
1600
1200
800
400
0
*
#
Glucose (mmol/l): 3.3 [16.7]
Stevioside (-log mol/l): - - 9 8 7 6 5 4 3

Fig. 4a

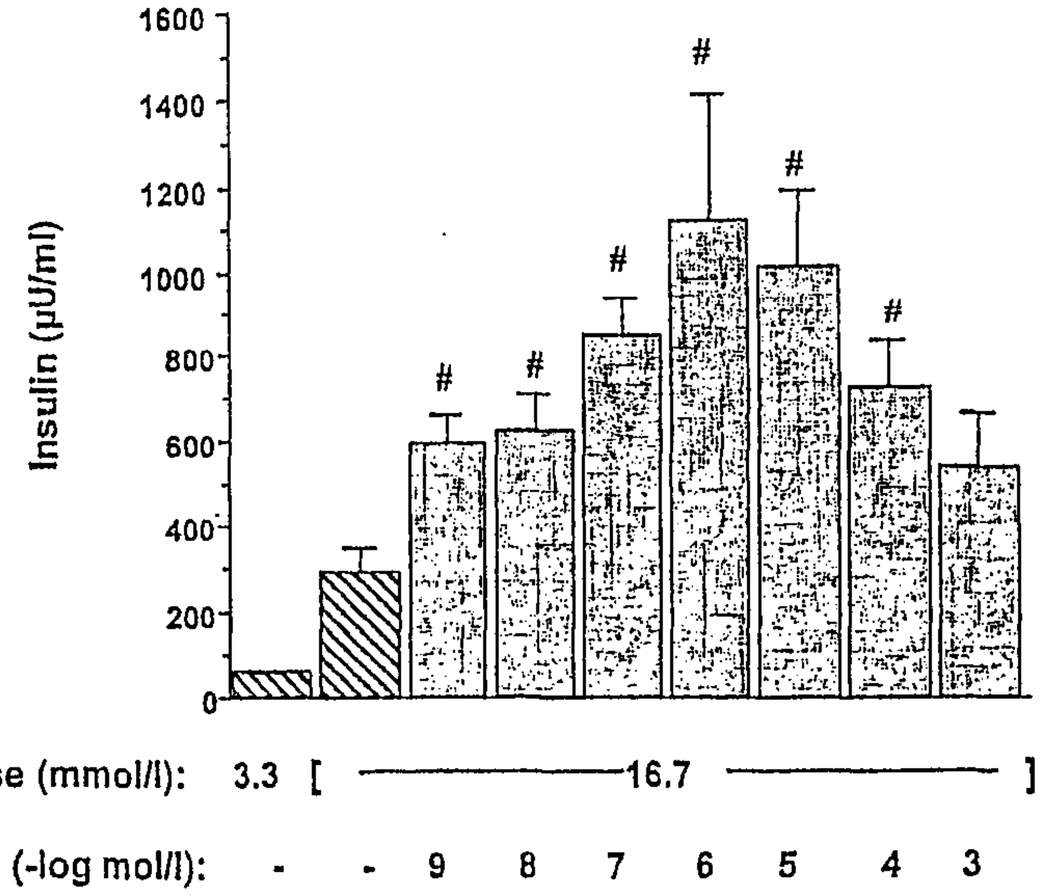

Insulin (µU/ml)
1600
1400
1200
1000
800
600
400
200
0
#
Glucose (mmol/l): 3.3 [16.7]
Steviol (-log mol/l): - - 9 8 7 6 5 4 3

Fig. 4b

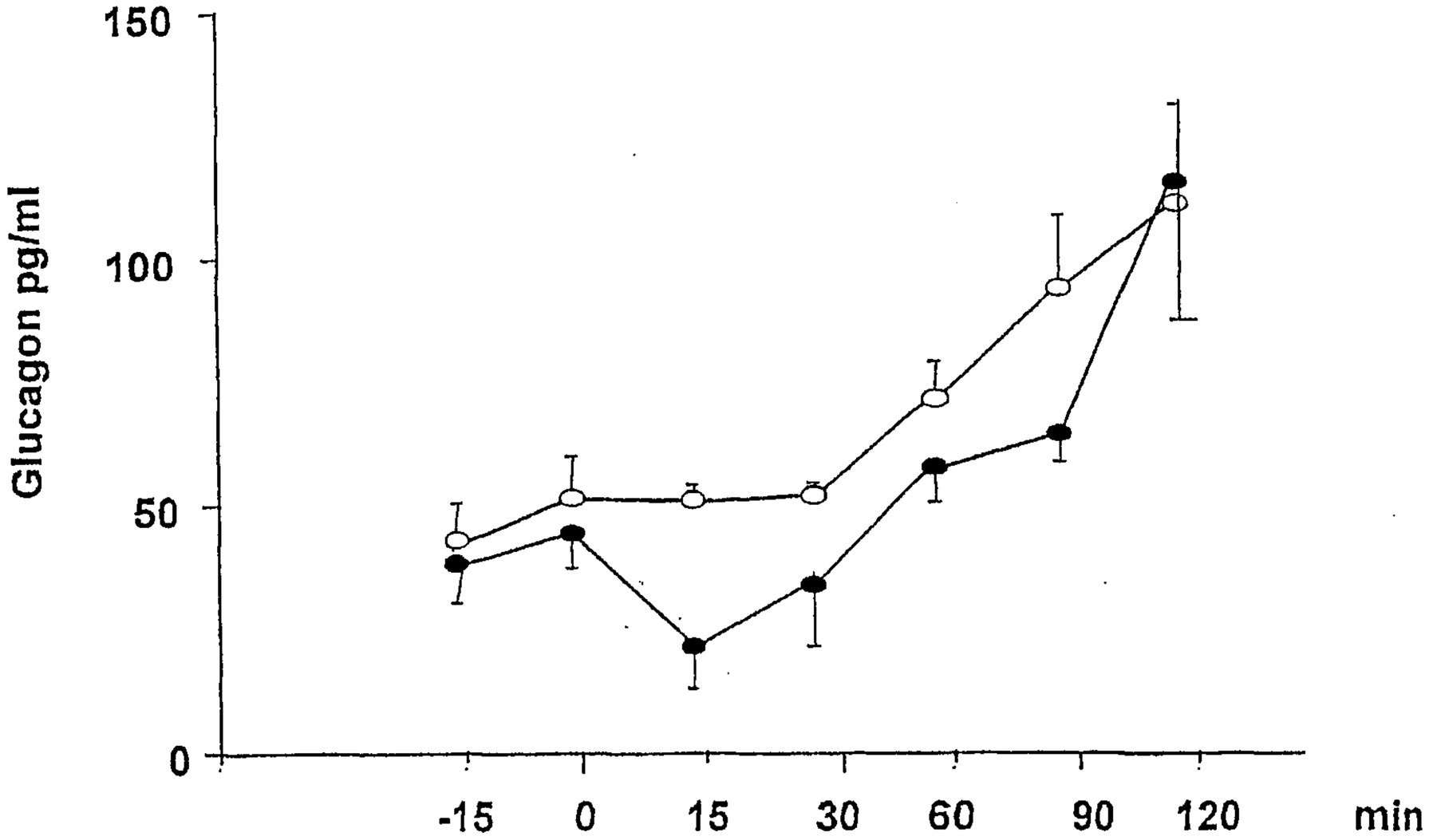

150
100
50
0
Glucagon pg/ml
-15
0
15
30
60
90
120
min

Fig. 5a

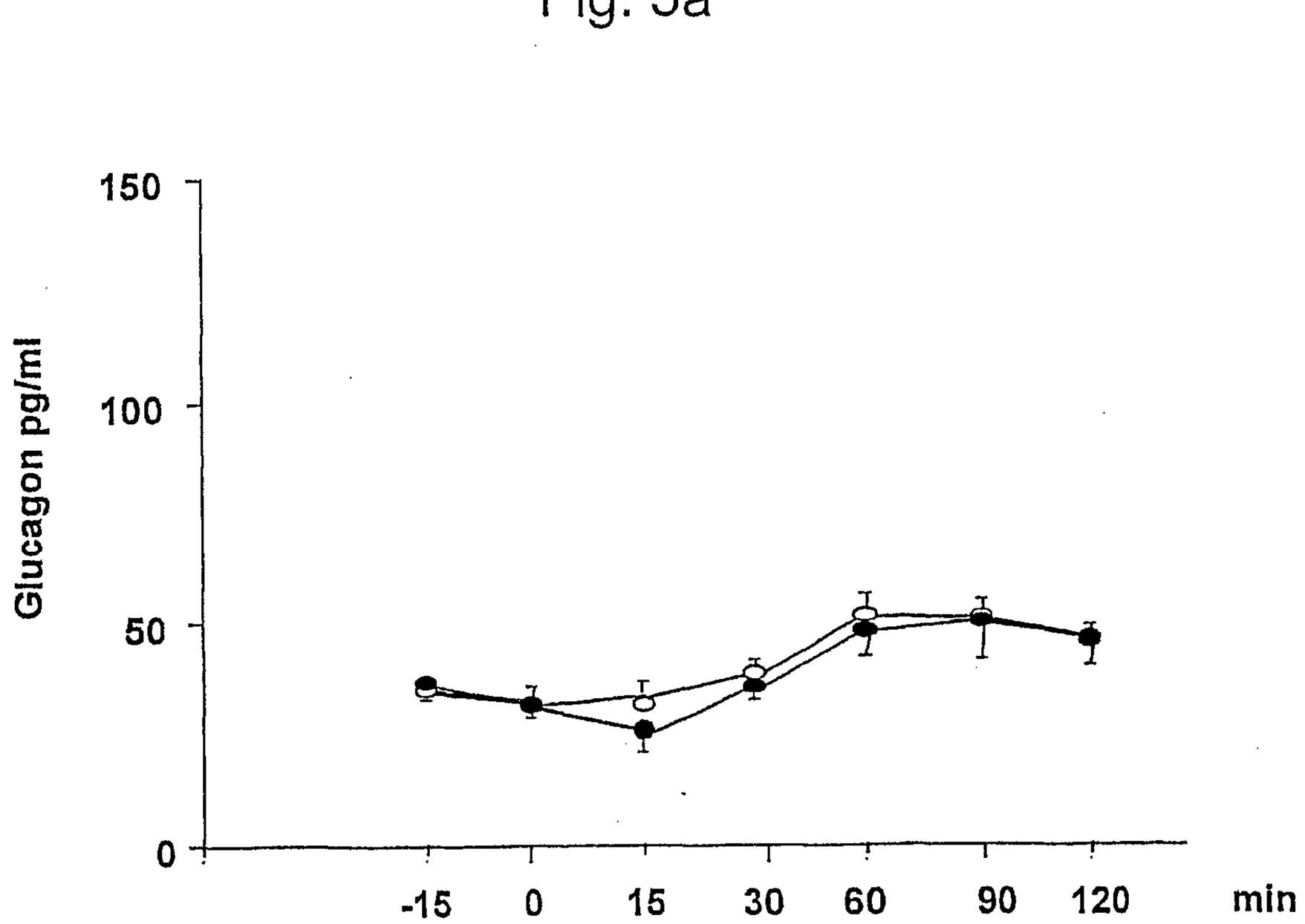

150
100
50
0
Glucagon pg/ml
-15
0
15
30
60
90
120
min

Fig. 5b

In vivo stevioside experiment

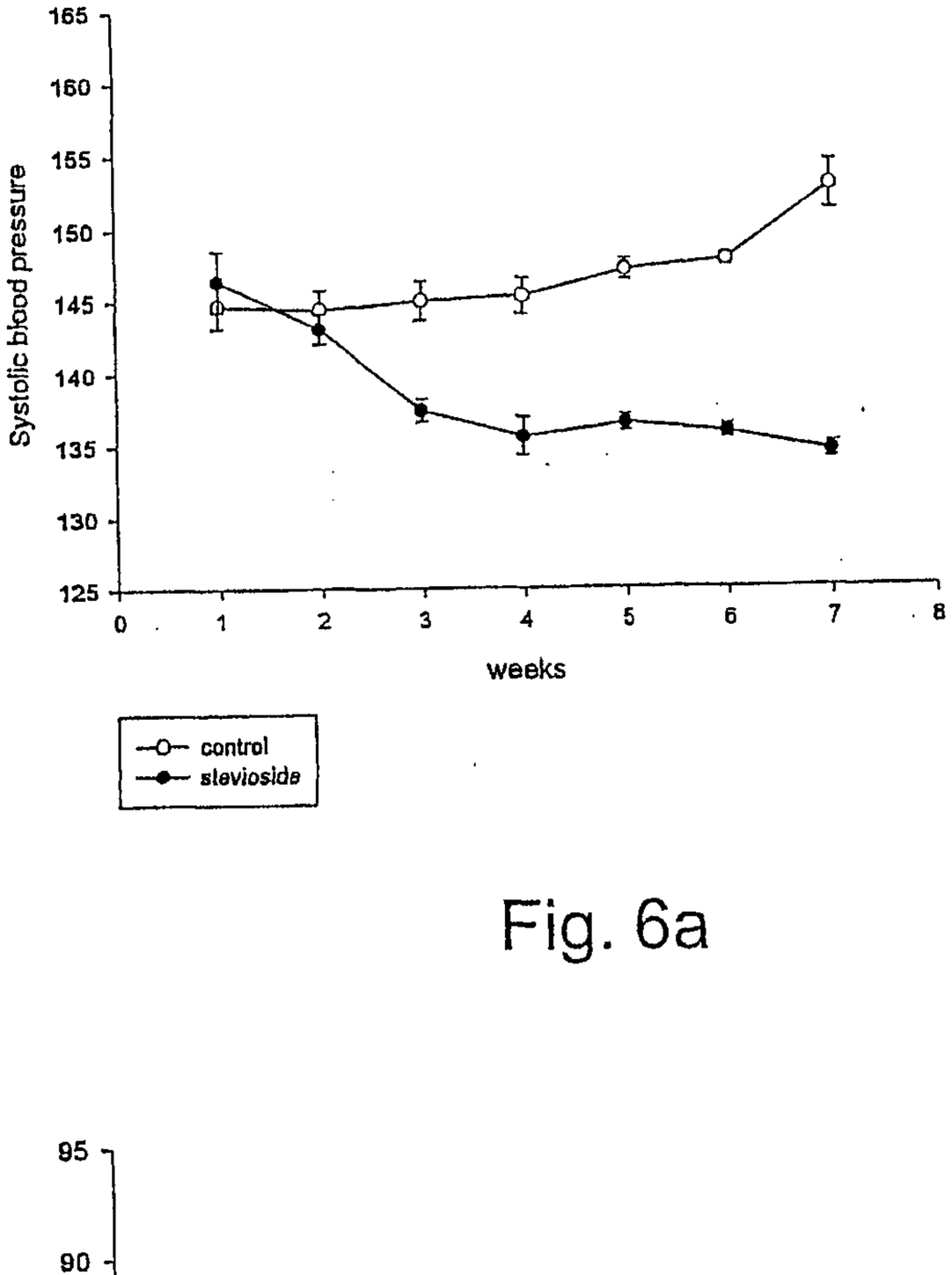


Fig. 6a

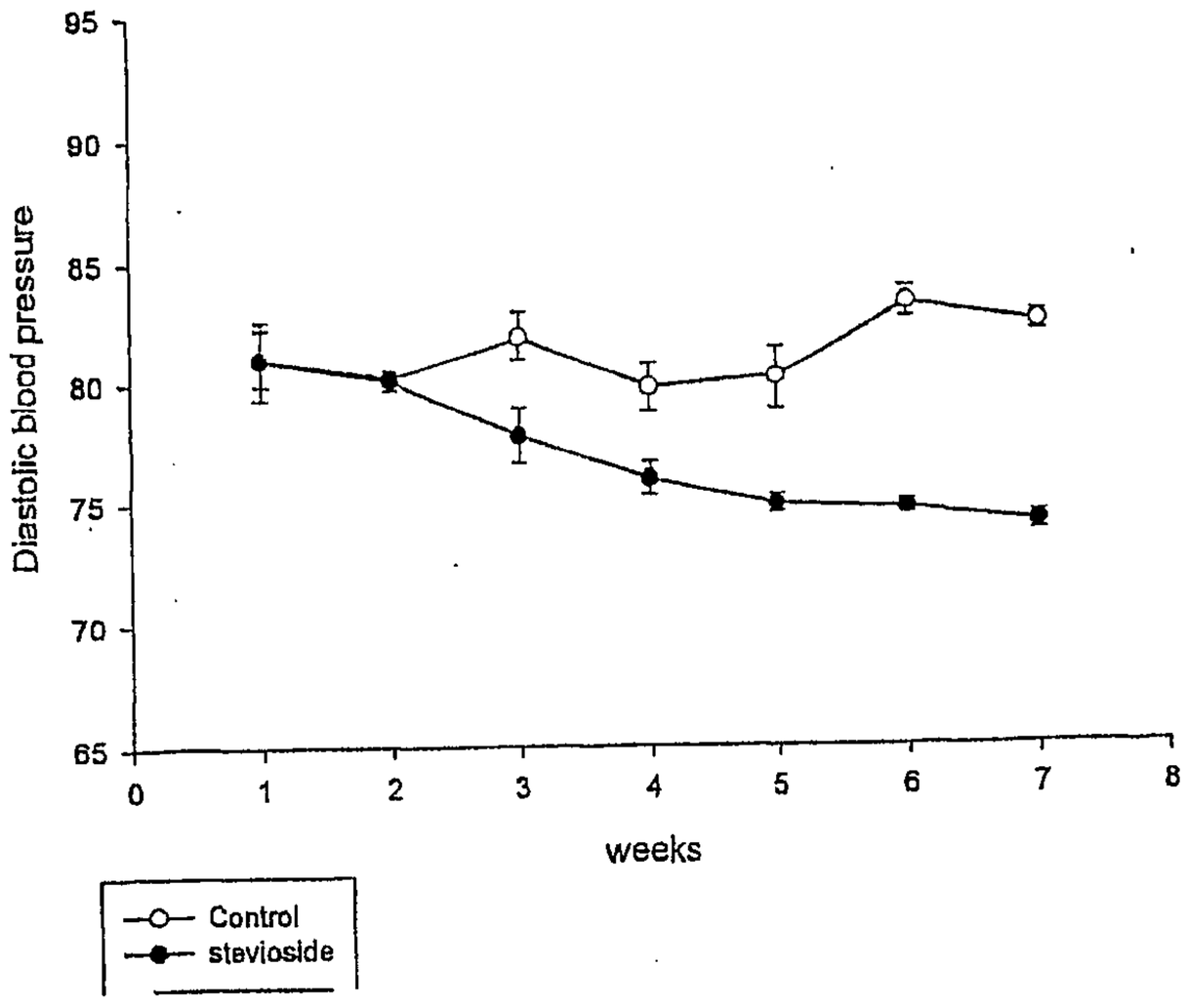


Fig. 6b

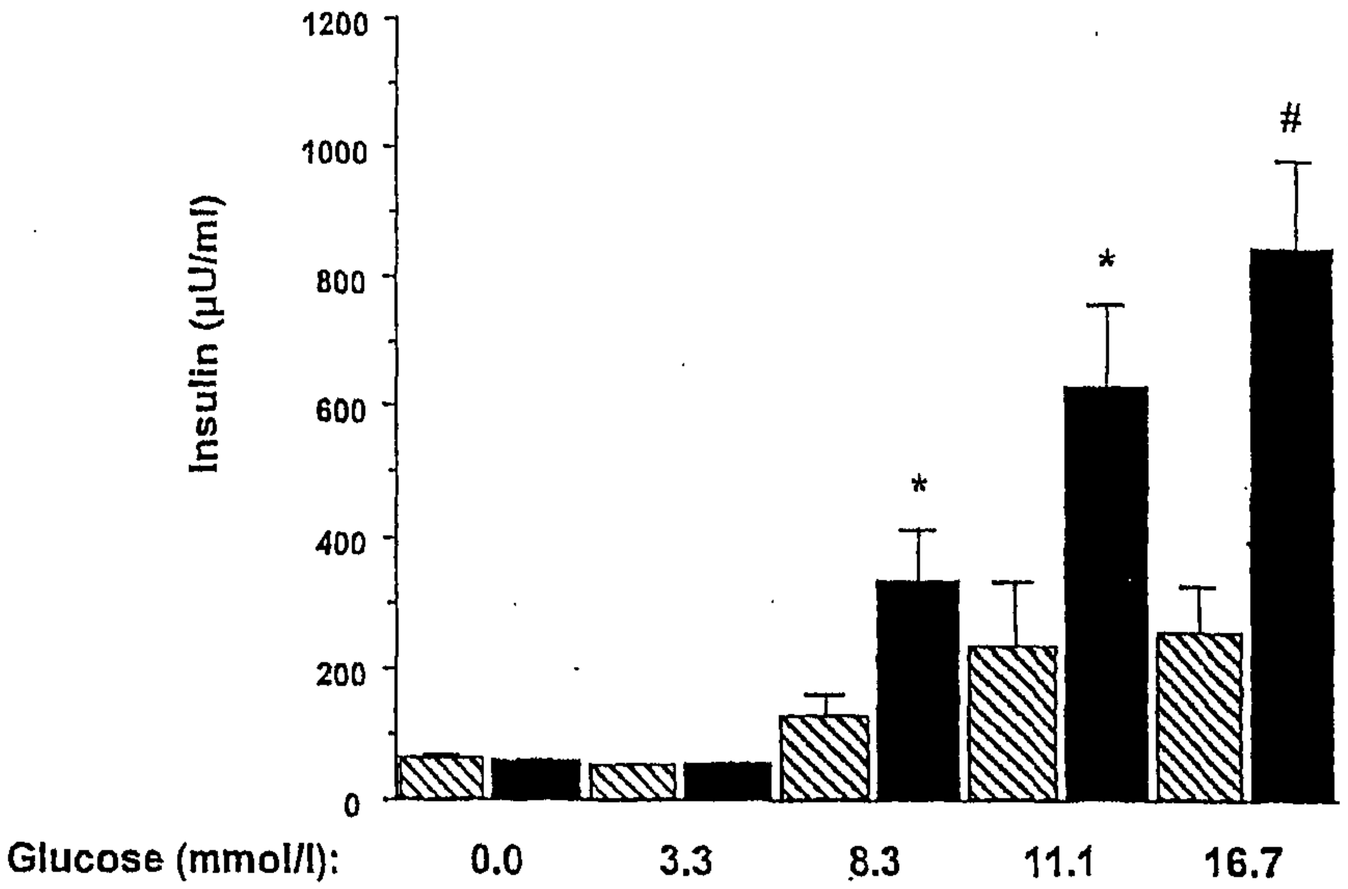

1200
1000
800
600
400
200
0
Insulin (µU/ml)
*
*
#
Glucose (mmol/l):
0.0
3.3
8.3
11.1
16.7

Fig. 7a

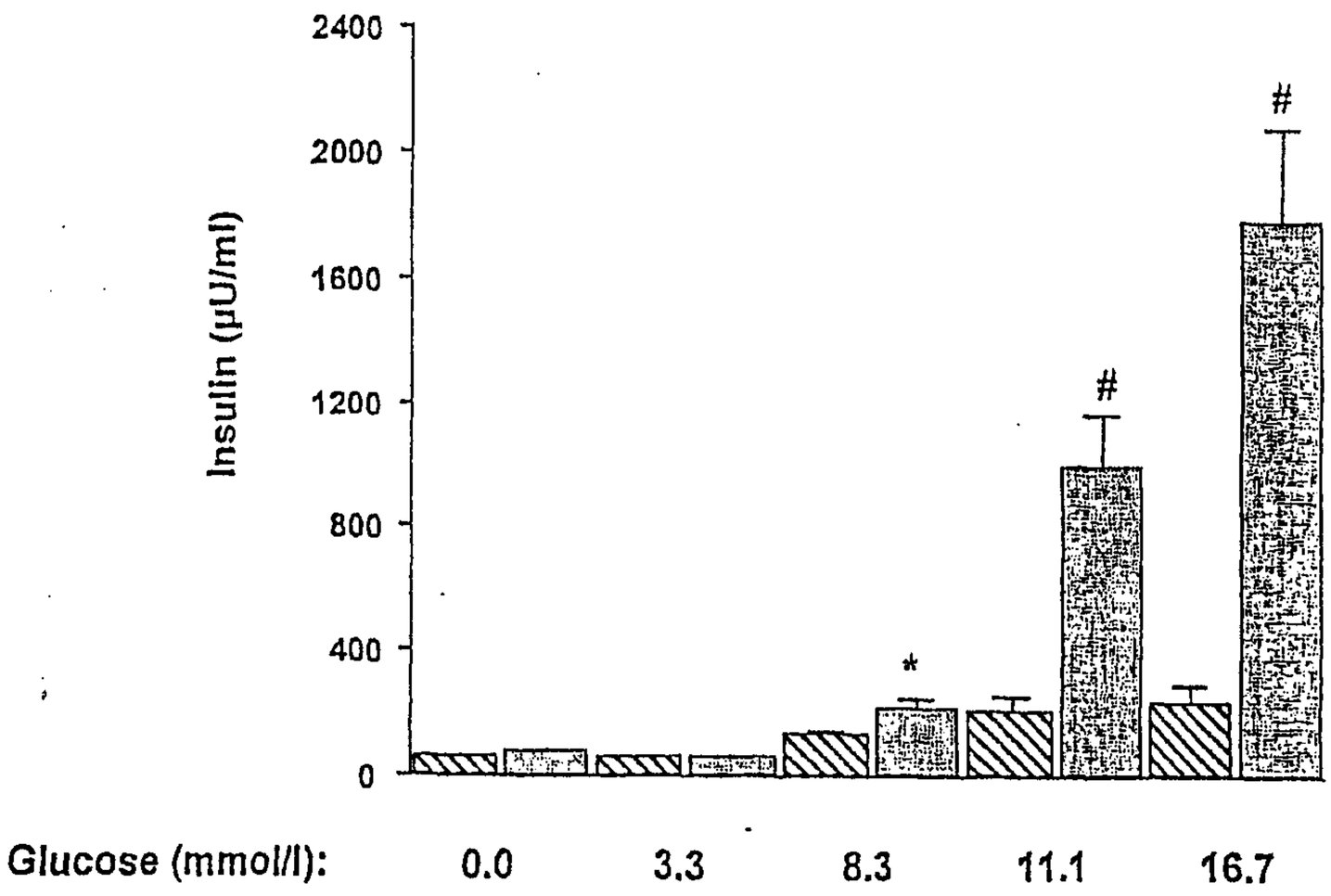

2400
2000
1600
1200
800
400
0
Insulin (µU/ml)
*
#
#
Glucose (mmol/l):
0.0
3.3
8.3
11.1
16.7

Fig. 7b

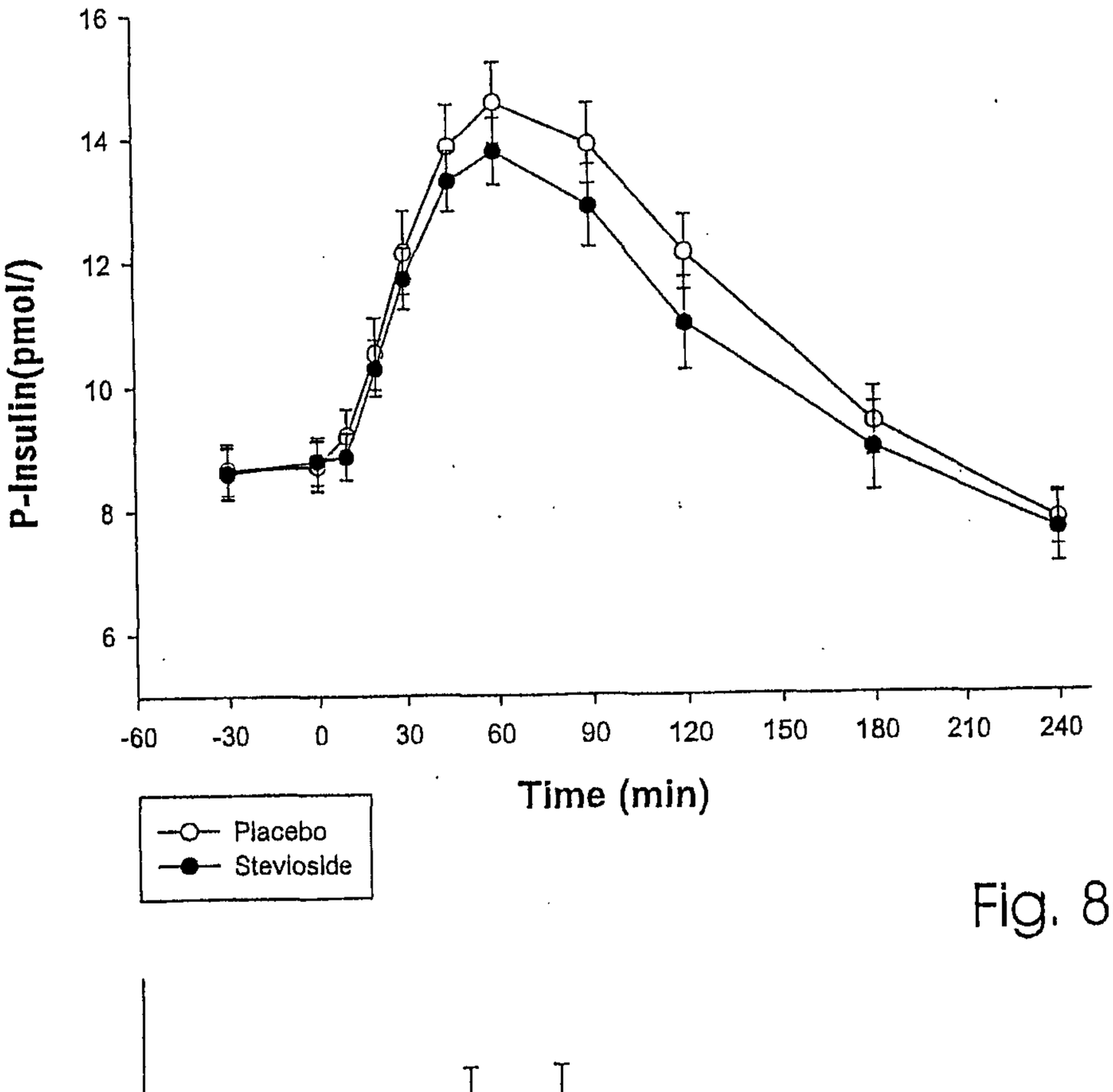

16
14
12
10
8
6
P-Insulin(pmol/)
-60
-30
0
30
60
90
120
150
180
210
240
Time (min)
Placebo
Stevioside

Fig. 8a

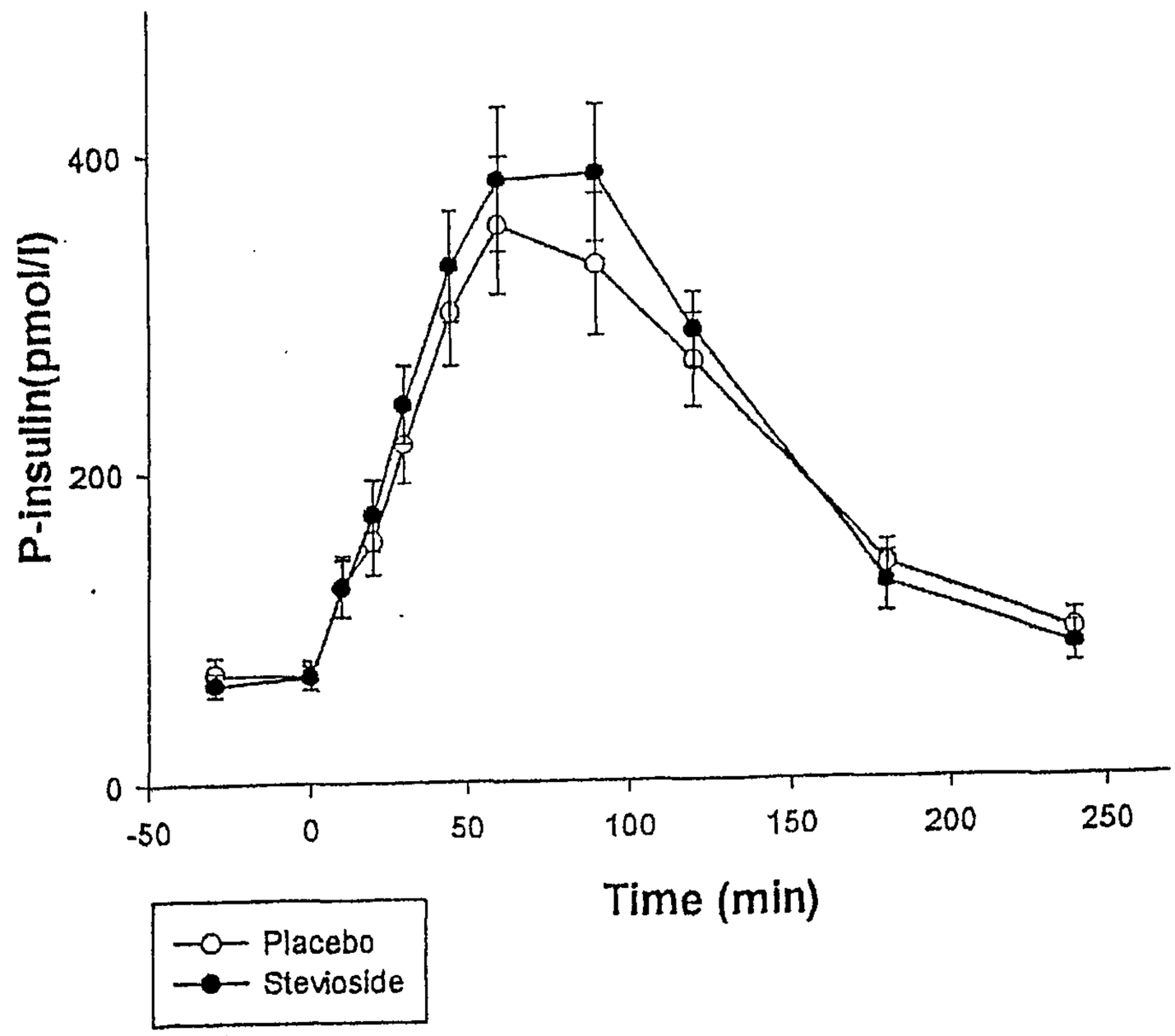

400
200
0
P-insulin(pmol/l)
-50
0
50
100
150
200
250
Time (min)
Placebo
Stevioside

Fig. 8b

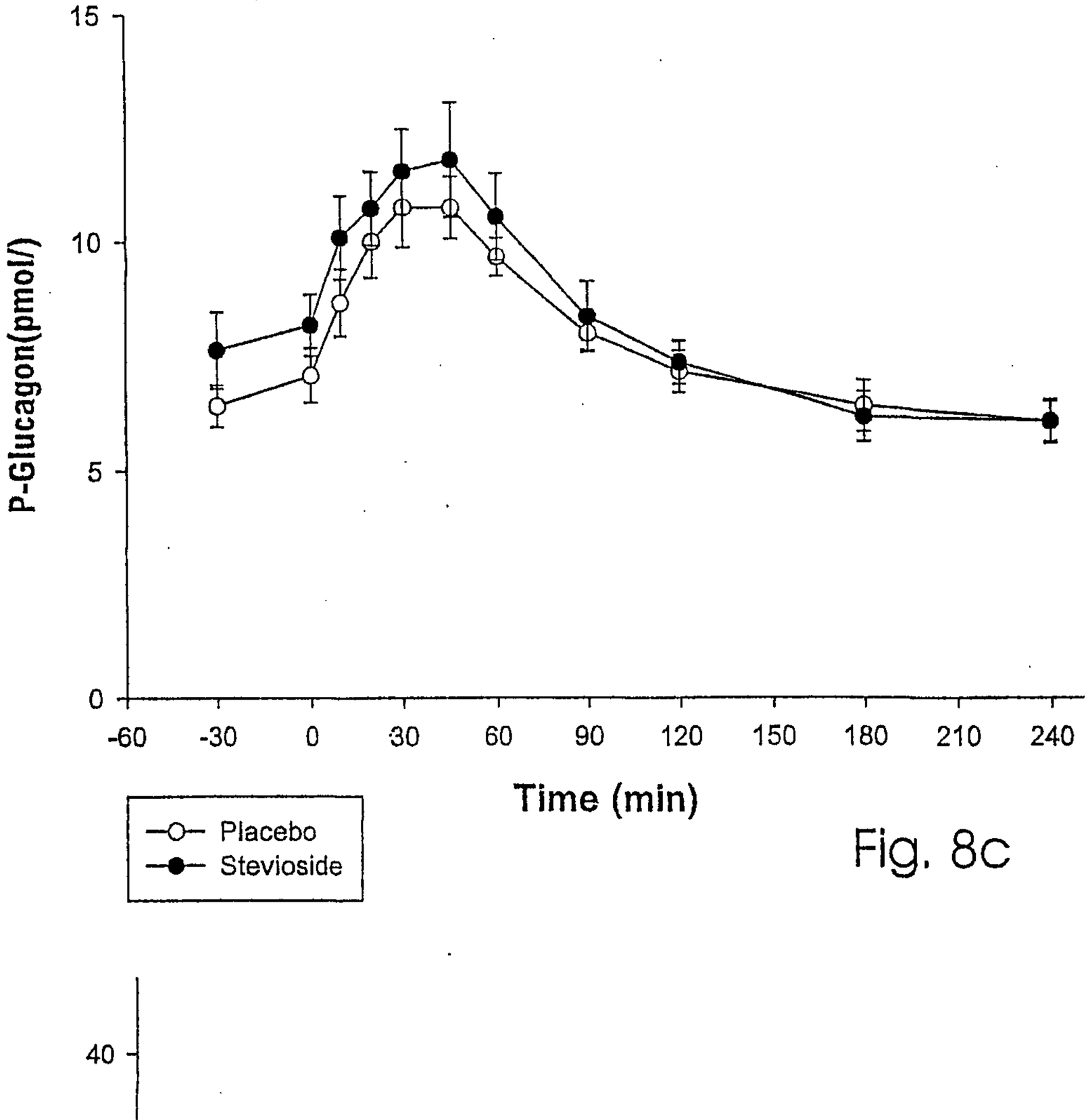

15
10
5
0
P-Glucagon(pmol/)
-60
-30
0
30
60
90
120
150
180
210
240
Time (min)
Placebo
Stevioside

Fig. 8c

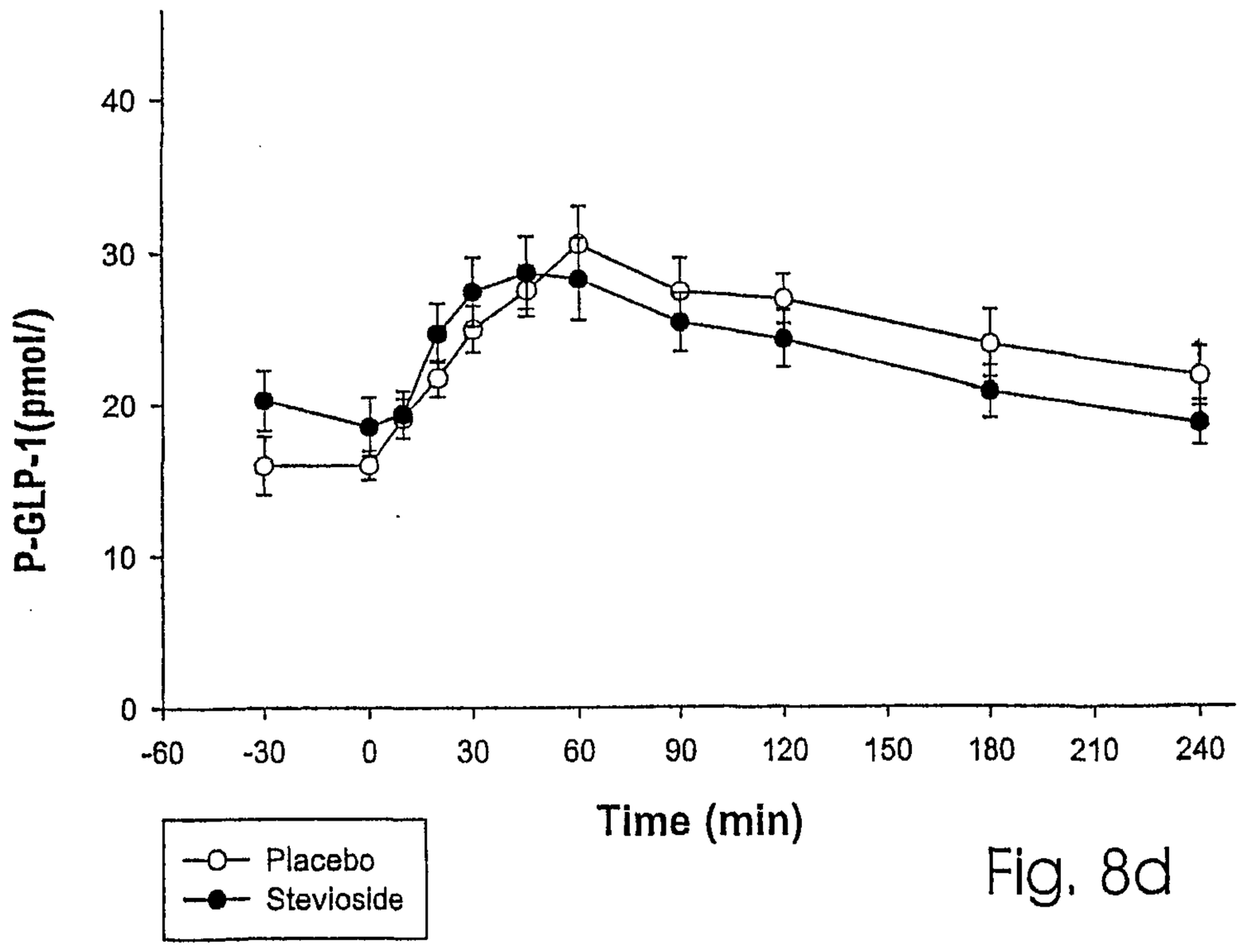

40
30
20
10
0
P-GLP-1(pmol/)
-60
-30
0
30
60
90
120
150
180
210
240
Time (min)
Placebo
Stevioside

Fig. 8d

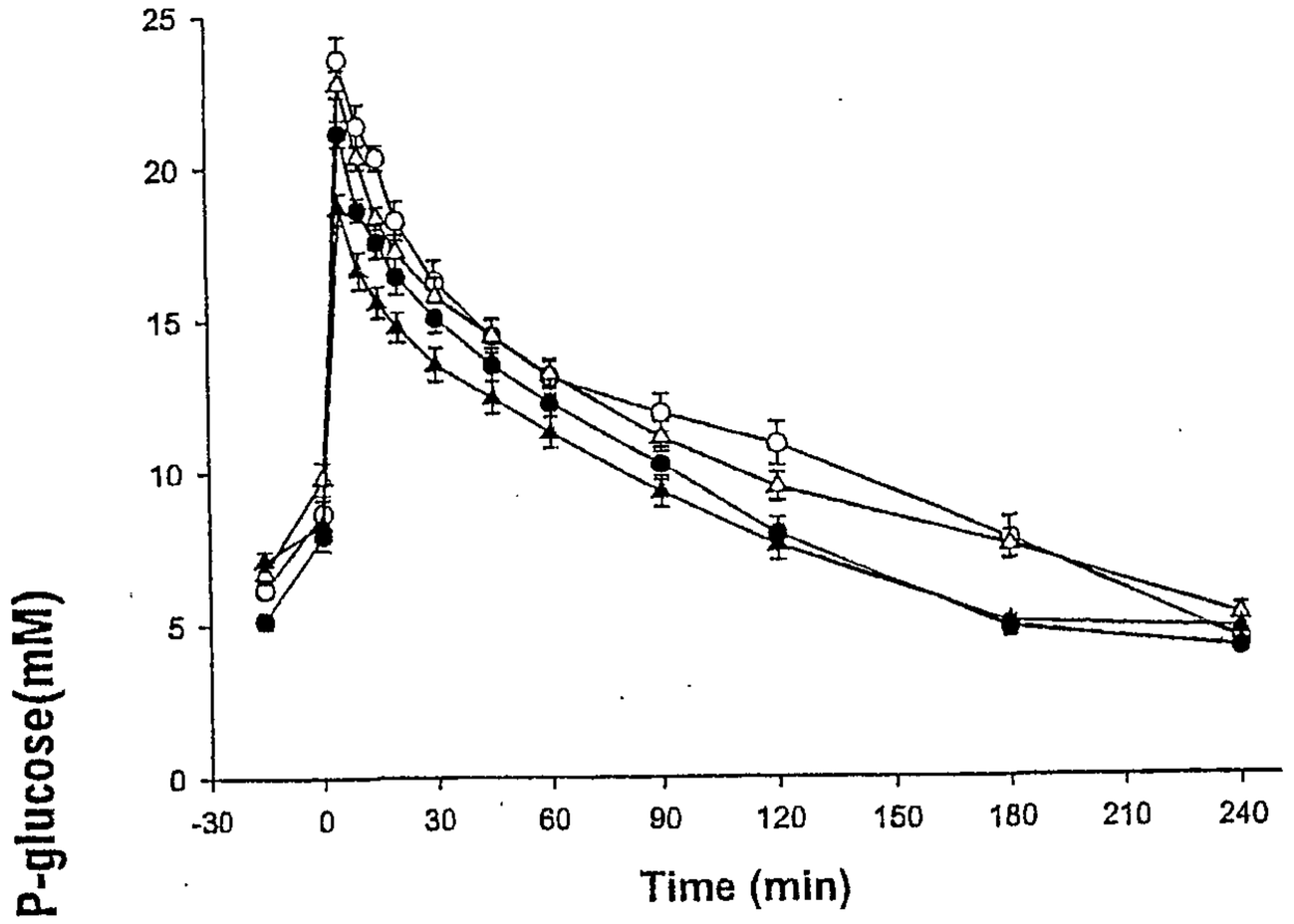

P-glucose(mM)
0
5
10
15
20
25
-30
0
30
60
90
120
150
180
210
240
Time (min)
Alt
Alt + Ste
Soy
Soy + Ste

Fig. 9a

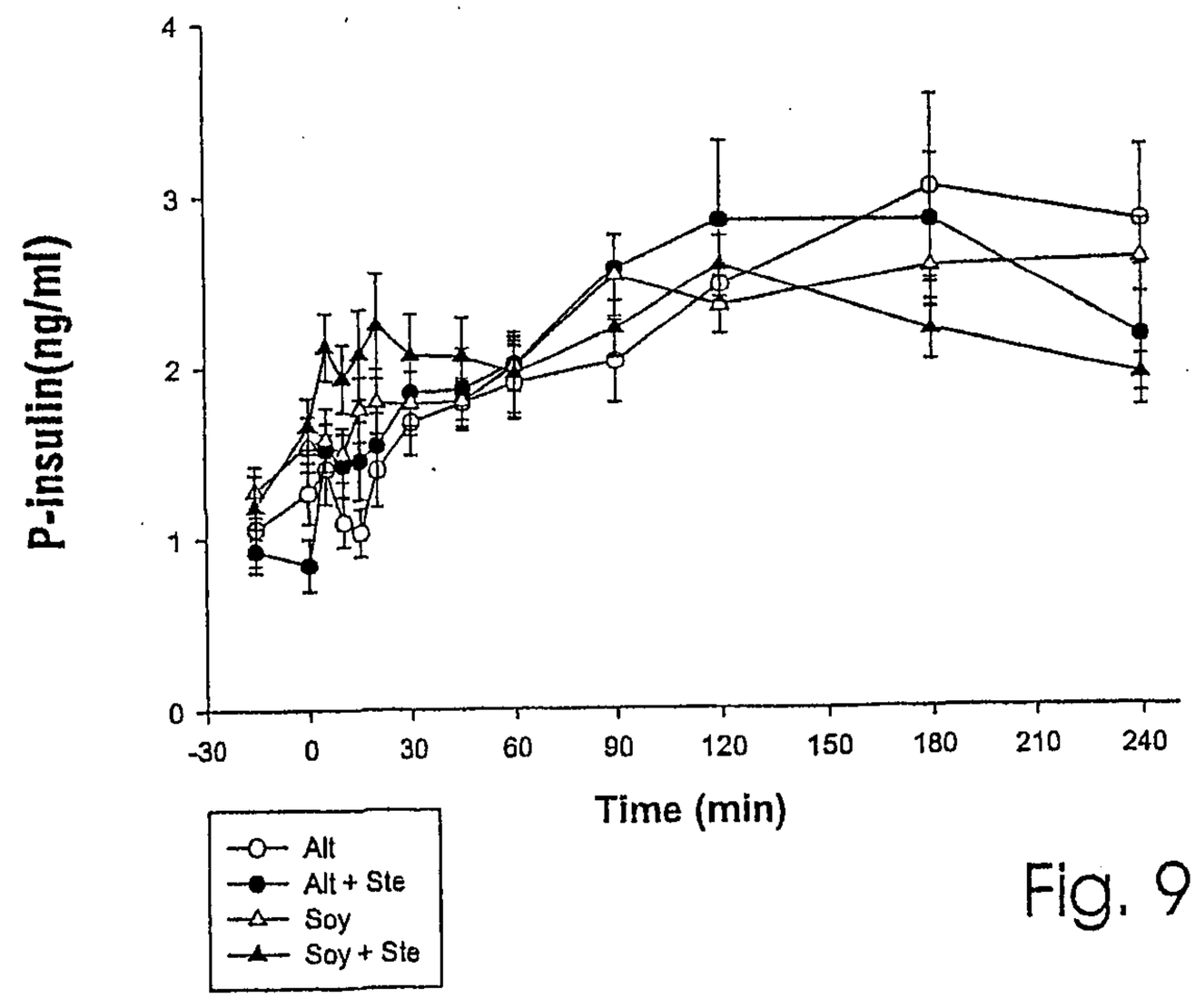

P-insulin(ng/ml)
0
1
2
3
4
-30
0
30
60
90
120
150
180
210
240
Time (min)
Alt
Alt + Ste
Soy
Soy + Ste

Fig. 9b

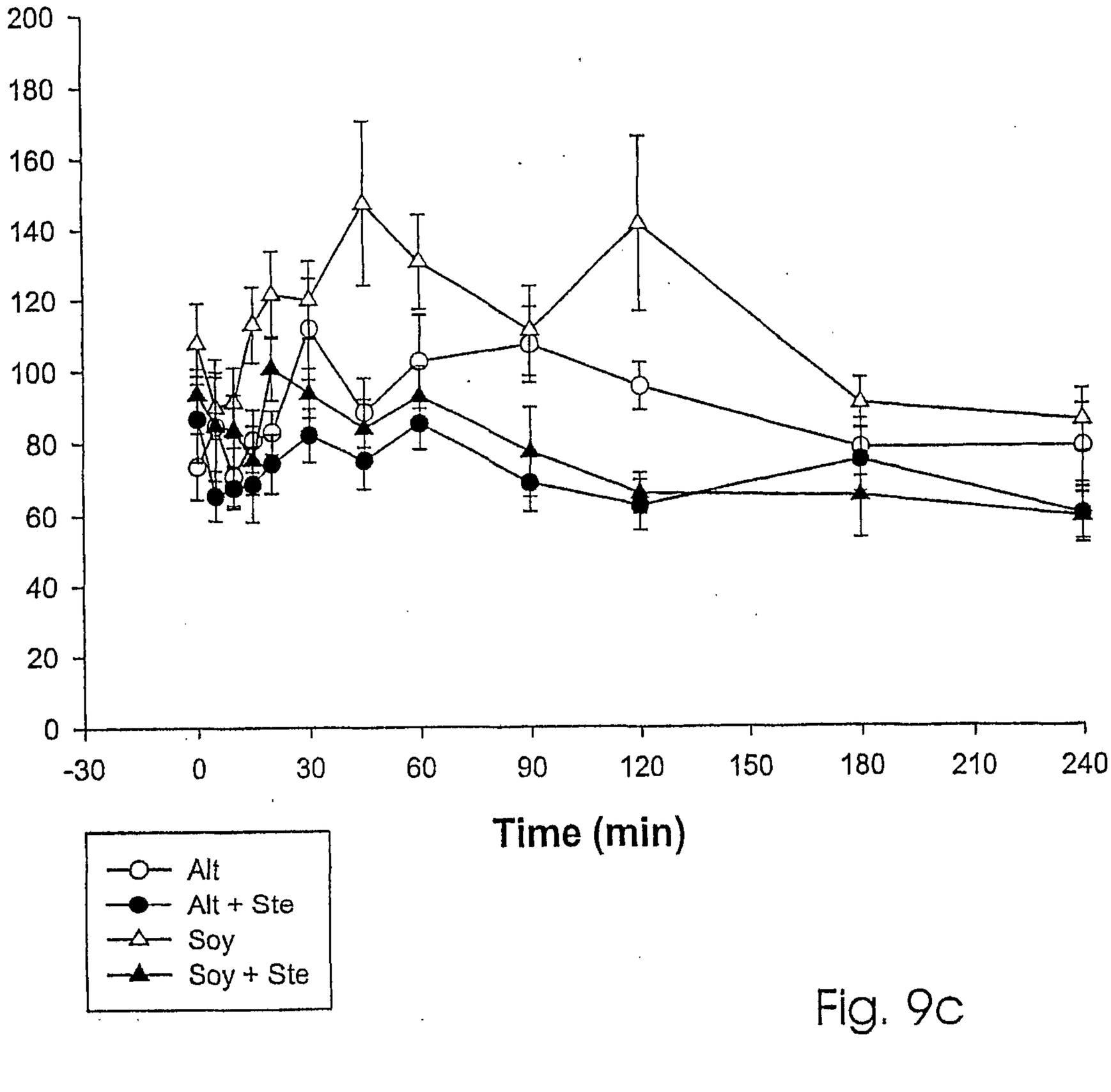

200
180
160
140
120
100
80
60
40
20
0
-30
0
30
60
90
120
150
180
210
240
Time (min)
Alt
Alt + Ste
Soy
Soy + Ste

Fig. 9c

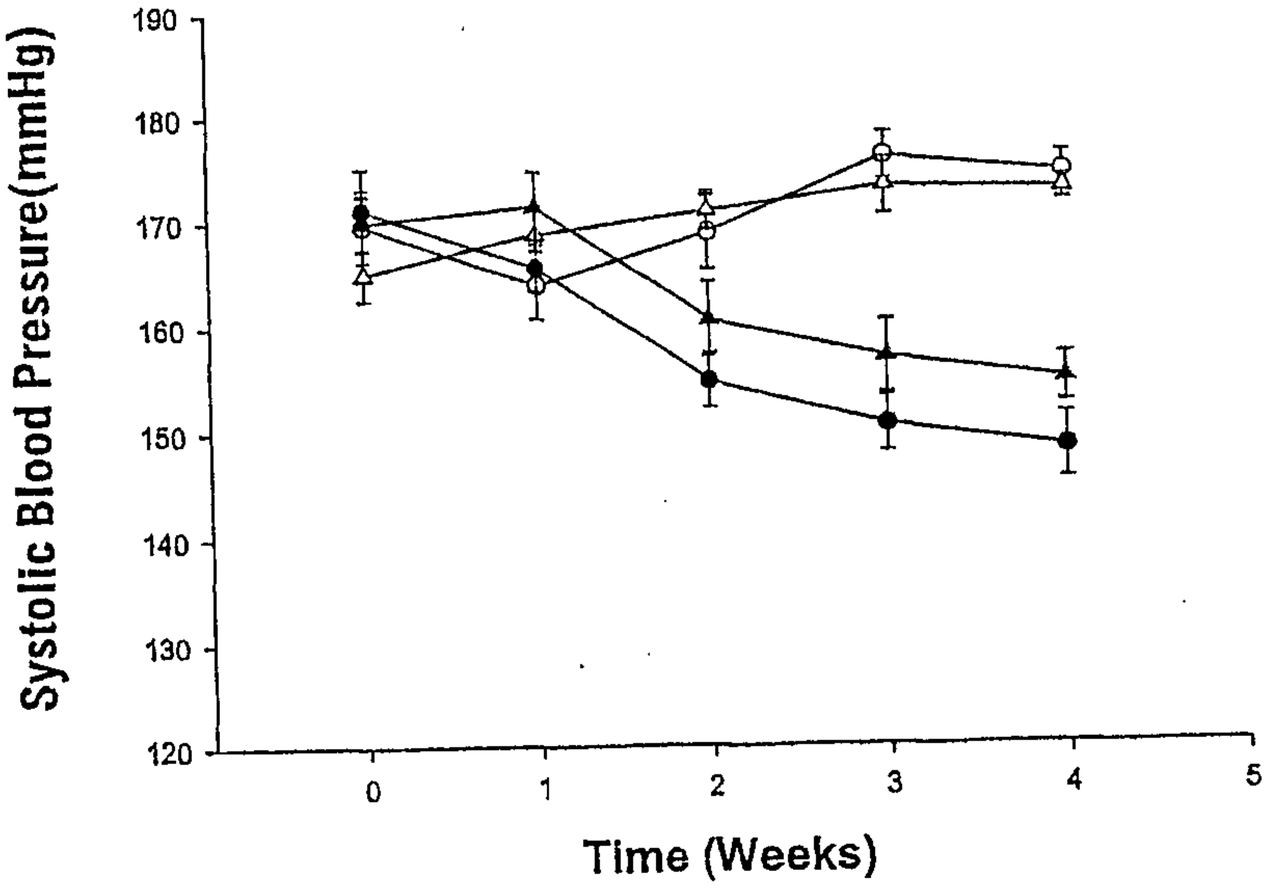

Systolic Blood Pressure(mmHg)
190
180
170
160
150
140
130
120
0
1
2
3
4
5
Time (Weeks)
Alt
Alt + ste
Soy
Soy + ste

Fig. 9d

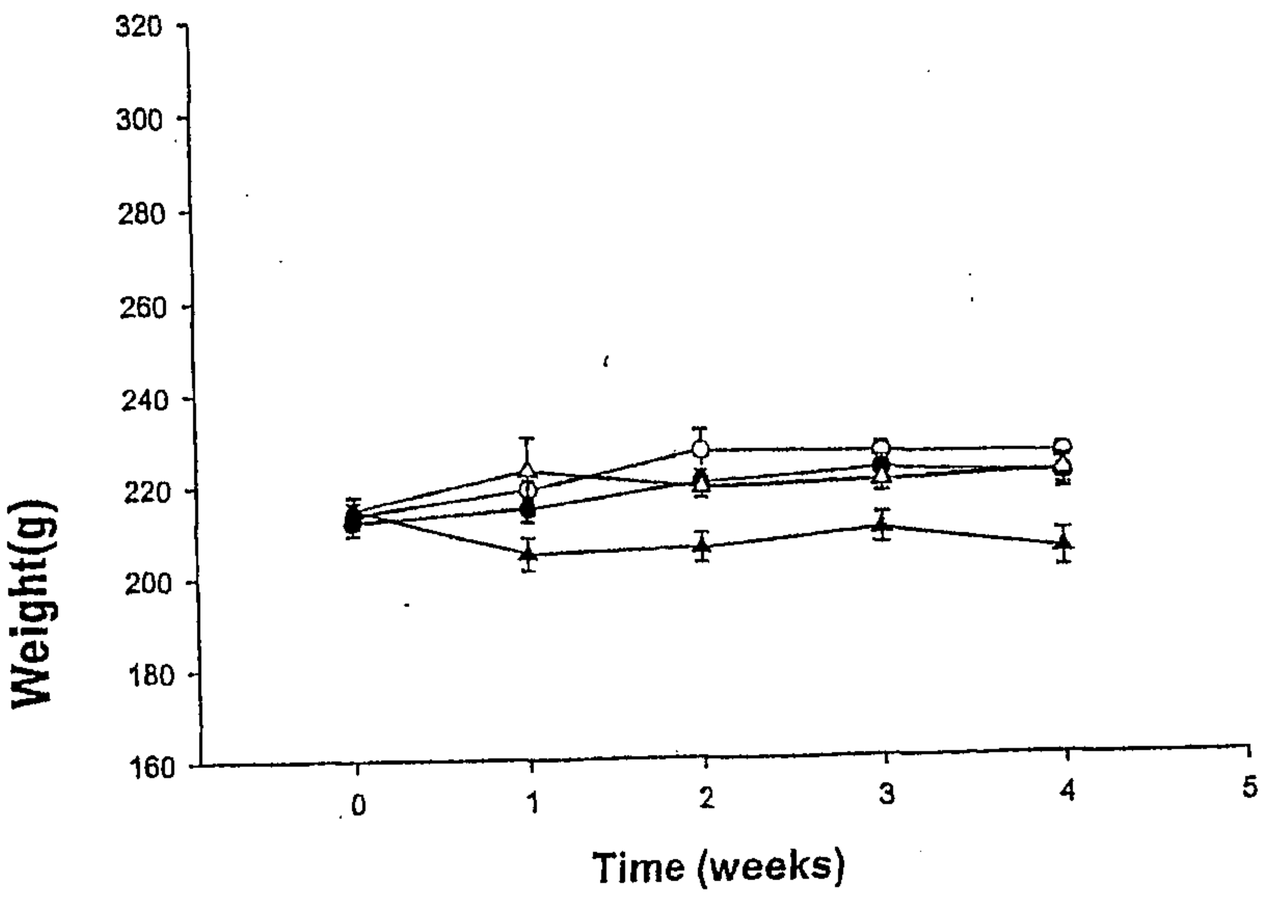

Weight(g)
320
300
280
260
240
220
200
180
160
0
1
2
3
4
5
Time (weeks)
Alt
Alt + ste
Soy
Soy + ste

Fig. 9e

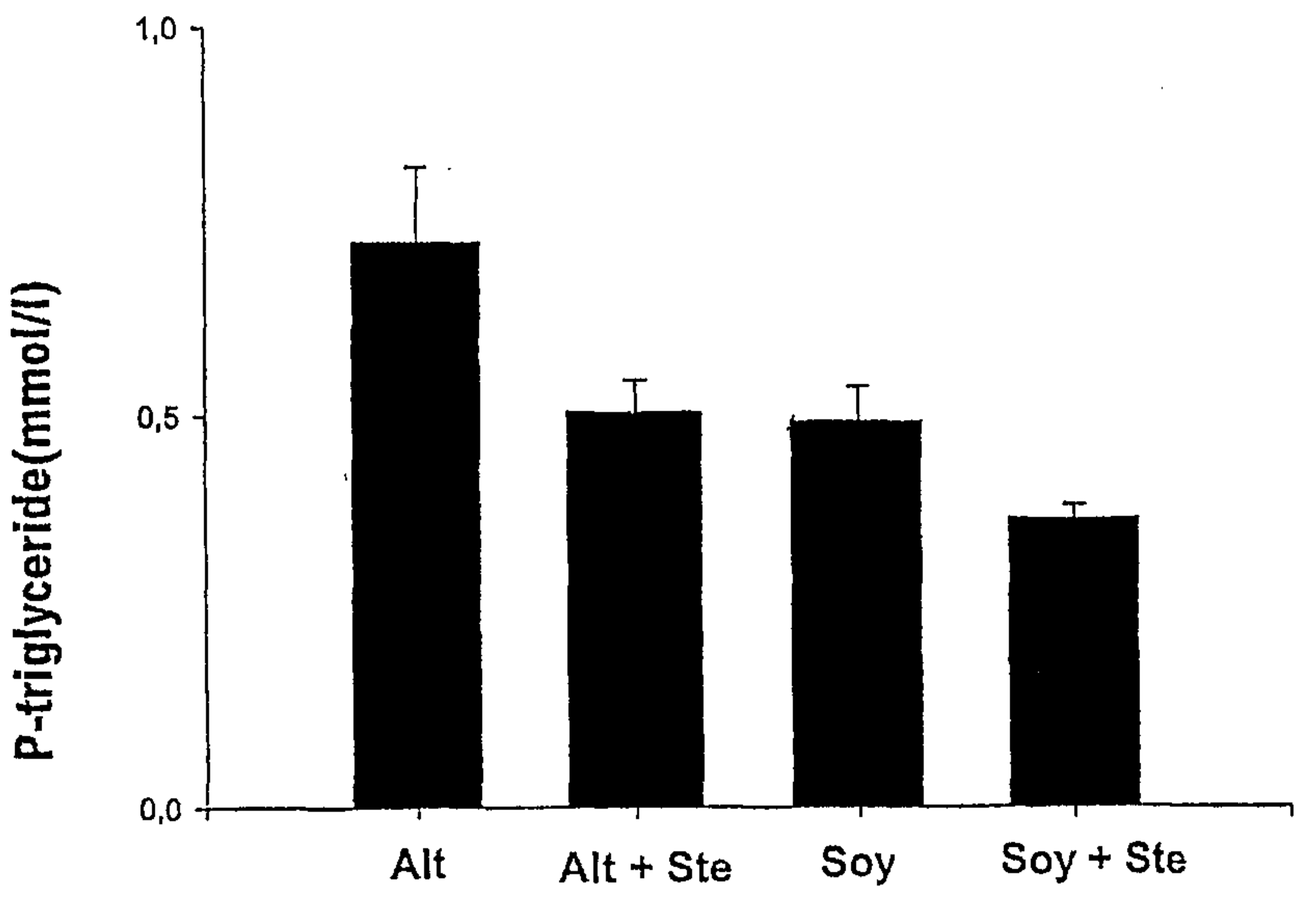

1,0
0,5
0,0
P-triglyceride(mmol/l)
Alt
Alt + Ste
Soy
Soy + Ste

Fig. 9f

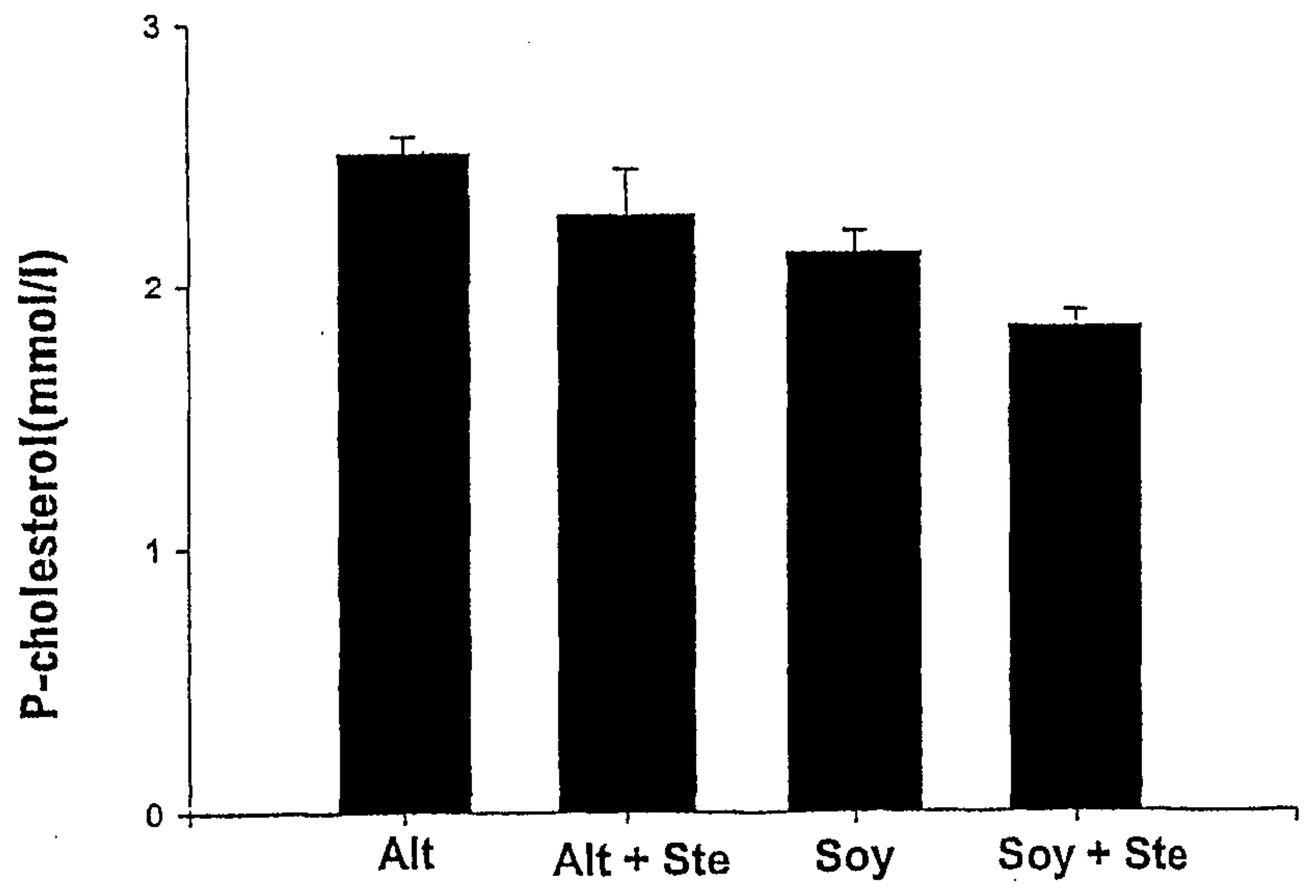
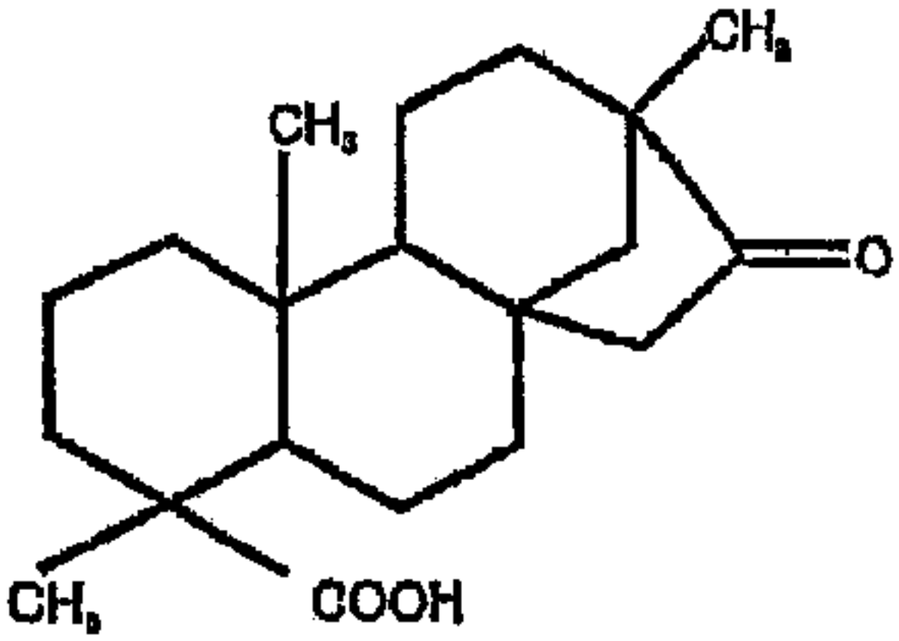
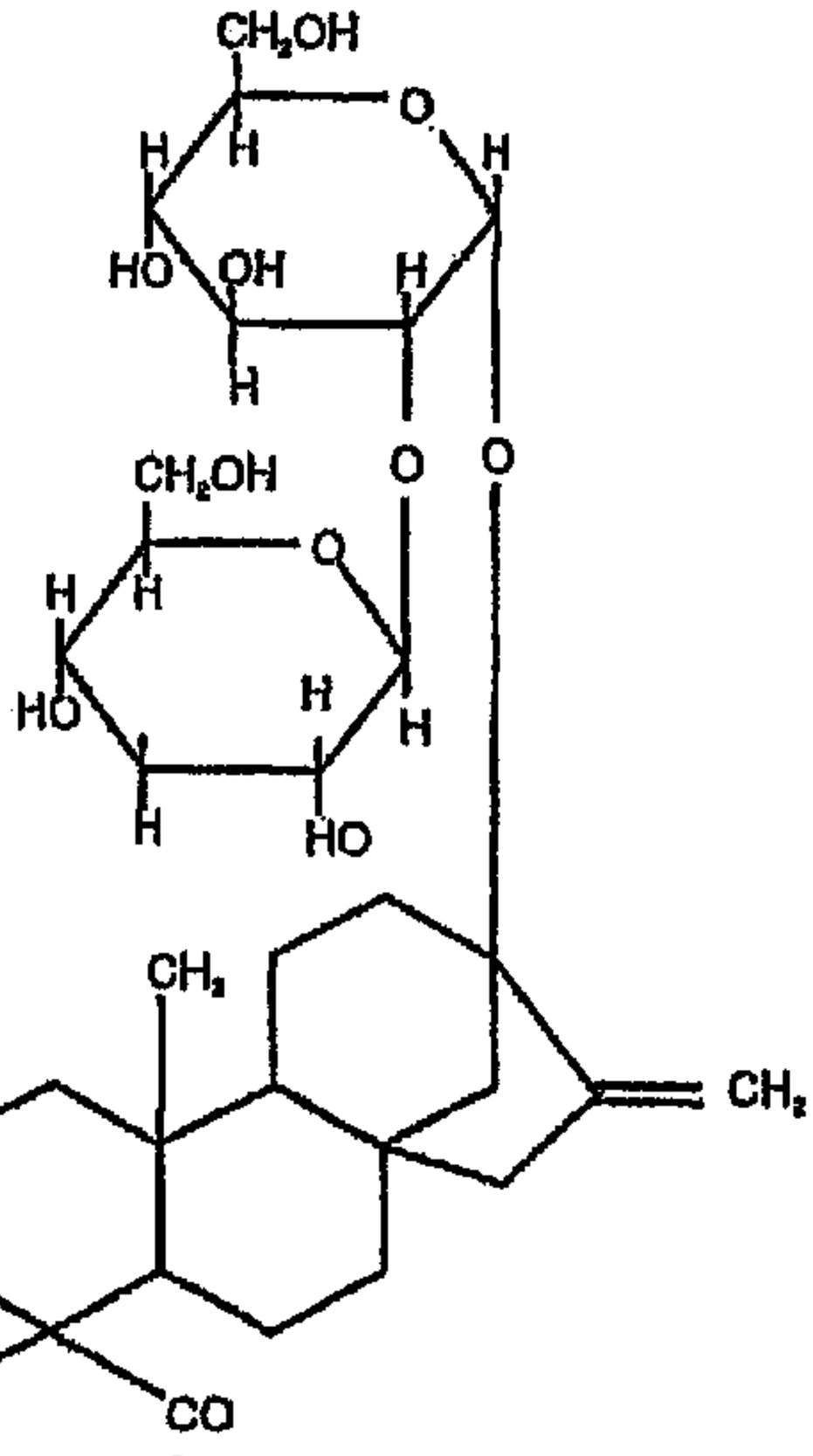

3
2
1
0
P-cholesterol(mmol/l)
Alt
Alt + Ste
Soy
Soy + Ste

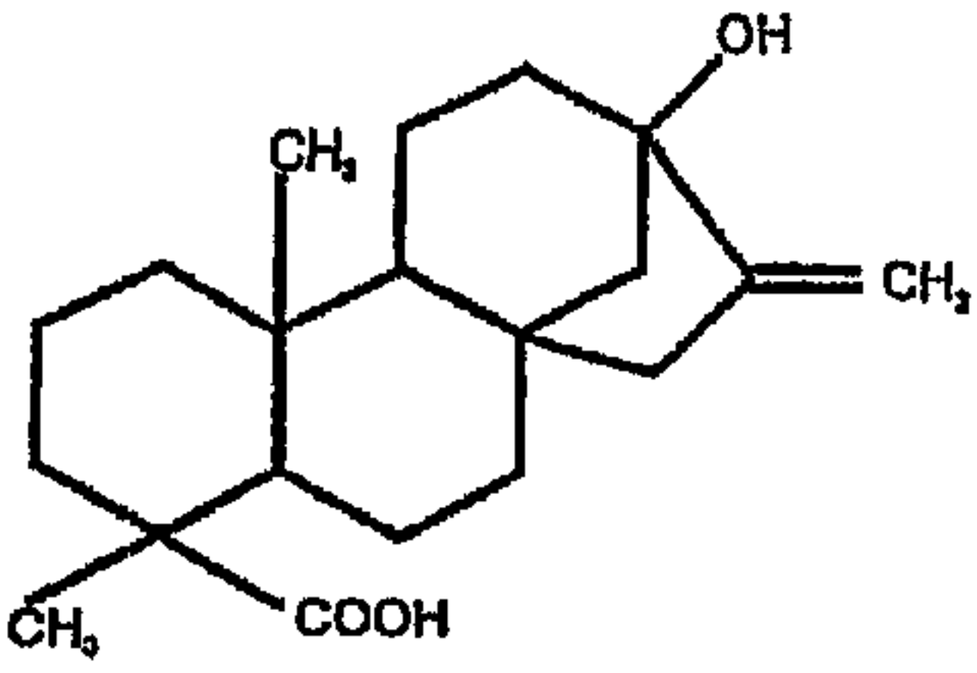
Fig. 9g



Isosteviol



Stevioside



Steviol