increases in circulating levels of triglycerides. This is a very likely reason why several studies indicate that hepatic insulin resistance contributes to human dyslipidaemia, hepatic steatosis, cardiovascular vascular dysfunction, and even reduced kidney function observed in people with Type 2 diabetes. In line with this, treatment of insulin resistant subjects with a high concentration of insulin in order to lower blood glucose levels may result in over-stimulation of the non-resistant pathways involved in e.g. de novo lipogenesis. In order to lower blood glucose levels without over-stimulating non-resistant pathways (e.g. the lipogenic pathways), it would be desirable to have access to insulin analogues, which selectively activate the glucose lowering pathways. Such functionally selective insulin analogues would, beyond lowering blood glucose levels, exhibit improved effects on dyslipidaemia, hepatic steatosis, atherosclerosis and cardiovascular diseases (CVD).

WO 2005 054291 allegedly describes single chain insulins with B28K acylation. WO 2009 112583 allegedly describes protease stabilised insulin analogues comprising B28K. WO90/07511, WO96/15804, WO200043034, US2012241356, WO2012015692 and WO9731022 allegedly disclose insulin analogues comprising B28K, some of which allegedly also discloses acylation at the same position.

A great variety of insulin analogues and derivatives have been reported. However they all activate the insulin receptor in quite the same manner, i.e. the downstream effects of the insulin receptor activation are rather similar, regardless of whether the activation results from binding a high-affinity analogue or a low-affinity analogue – only the potency differs.

Thus, there is still a need for insulin analogues and derivatives having functionally selective properties on the resistant and non-resistant pathways, e.g. gluconeogenesis and lipid metabolism pathways.

SUMMARY

In an aspect, there is provided an insulin derivative, wherein said insulin derivative comprises B5Y or B5F and a substituent comprising an acyl group, or a pharmaceutically acceptable salt, amide or ester thereof.

In another aspect, there is provided an intermediate product comprising a backbone selected from the group consisting of:

i. A14E, B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 3 and 4)
ii. A14E, B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 3 and 5)
iii. B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 1 and 7)
iv. B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 1 and 8)

v. B5Y, B28K, desB29-30 (SEQ ID NO: 1 and 9)
vi. A14E, B5F, B26G, B28K, desB29-30 (SEQ ID NO: 3 and 10)
vii. B5F, B28K, desB29-30 (SEQ ID NO: 1 and 11)
viii. B5Y, B26K, desB27-desB30 (SEQ ID 1 and 12)
ix. B5Y, desB30 (SEQ ID 1 and 13)
x. B5Y, B26G, desB30 (SEQ ID 1 and 14)
xi. B5Y, B26A, desB30 (SEQ ID 1 and 15)
xii. B5F, B26A, B28K, desB29-30 (SEQ ID 1 and 6)
xiii. B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 1 and 4)
xiv. B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 1 and 5)
xv. B5F, B26G, B28K, desB29-30 (SEQ ID NO: 1 and 10)
xvi. A14E, B5F, B26A, B28K, desB29-30 (SEQ ID 3 and 6)
xvii. A14E, B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 3 and 7)
xviii. A14E, B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 3 and 8)
xix. A14E, B5Y, B28K, desB29-30 (SEQ ID NO: 3 and 9)
xx. A14E, B5F, B28K, desB29-30 (SEQ ID NO: 3 and 11)
xxi. A14E, B5Y, B26K, desB27-desB30 (SEQ ID 3 and 12)
xxii. A14E, B5Y, desB30 (SEQ ID 3 and 13)
xxiii. A14E, B5Y, B26G, desB30 (SEQ ID 3 and 14)
xxiv. A14E, B5Y, B26A, desB30 (SEQ ID 3 and 15)

In another aspect, there is provided an insulin derivative as defined above, for use as a medicament.

In another aspect, there is provided the use of an insulin derivative as defined above, in the manufacture of a medicament for the prevention or treatment of diabetes, cardiovascular disease, atherosclerosis, and/or endothelial dysfunction and/or for preventing or reducing liver triglyceride content and/or for preventing or reducing body weight gain.

In another aspect, there is provided the use of an insulin derivative as defined above in the manufacture of a medicament for the treatment or prevention of diabetes, diabetes of Type 1, diabetes of Type 2, impaired glucose tolerance, hyperglycemia, dyslipidemia, obesity, metabolic syndrome (metabolic syndrome X, insulin resistance syndrome), hypertension, cognitive disorders, atherosclerosis, myocardial infarction, stroke, cardiovascular disorders, coronary heart disease, stroke, inflammatory bowel syndrome, dyspepsia, hypotension or gastric ulcers.

In another aspect, there is provided a method for determining selectivity of an insulin compound comprising the following steps:

- measuring the maximal AKT phosphorylation induced by said insulin compound relative to human insulin
- measuring the maximal ERK activation induced by said insulin compound relative to human insulin,

wherein the ERK/AKT ratio is less than 1.

The present disclosure relates to an insulin derivative, wherein said insulin derivative comprises B5Y and a substituent comprising an acyl group.

The present disclosure relates to an insulin derivative, wherein said insulin derivative comprises B5F and a substituent comprising an acyl group.

The present disclosure relates to an insulin derivative, wherein said insulin derivative comprises B5Y and B26G or B26A and a substituent comprising an acyl group attached to B28K, B26K or B29K.

The present disclosure relates to an insulin derivative, wherein said insulin derivative comprises B5F and B26G or B26A and a substituent comprising an acyl group attached to B28K, B26K or B29K.

The disclosure provides pharmaceutical compositions comprising the insulin derivative of the disclosure, and one or more pharmaceutically acceptable carriers or diluents.

The disclosure further relates to the use of the insulin derivatives according to the disclosure for the manufacture of a medicament for the treatment or prevention of diabetes, Type 1 diabetes, Type 2 diabetes, impaired glucose tolerance, hyperglycemia, dyslipidemia, obesity, metabolic syndrome (metabolic syndrome X, insulin resistance syndrome), hypertension, cognitive disorders, atherosclerosis, myocardial infarction, stroke, cardiovascular disorders, coronary heart disease, stroke, inflammatory bowel syndrome, dyspepsia, hypotension or gastric ulcers.

Also, or alternatively, the disclosure provides insulin derivatives with improved effects on processes related to obesity, dyslipidemia or cardiovascular complications, such as a lower weight gain, lower increase in body fat mass, lower increase in liver triglycerides or improved endothelial function.

The insulin derivatives of the present disclosure are capable of lowering blood glucose without negatively impacting lipid metabolism.

The insulin derivatives of the present disclosure induce a submaximal insulin receptor phosphorylation, and induce selective signalling, and thus selective cellular response, i.e. give a lower maximal response on lipid metabolism pathways than on glucose lowering pathway, when compared to human insulin.

The insulin derivatives of the present disclosure, besides the glucose lowering, also have lower weight gain, in particular by a lower increase in fat mass.

The disclosure provides insulin derivatives with improved effects on processes related to hepatic dyslipidaemia, hepatic steatosis or non-alcoholic fatty liver disease (NAFLD), in particular by increased lowering of liver triglycerides compared to human insulin.

The disclosure provides insulin derivatives with improved effects on processes related to cardiovascular disorders such as improved endothelial function compared to human insulin.

The insulin derivatives of the present disclosure provide lower incidence of adverse cardiovascular events in patients with diabetes.

The disclosure provides insulin derivatives with improved stability in formulation.

The disclosure may also solve further problems that will be apparent from the disclosure of the exemplary embodiments.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows representative receptor binding curves for human insulin (●) and compound of example 15 (▲) from a competition binding assay with solubilised IR-A (CPM = counts per minute);

Fig. 2 shows representative dose-response curves for human insulin (●) and compound of example 15 (▲) from an IRpY1158 phosphorylation assay in CHO-hIR cells overexpressing the IR-A;

Fig. 3 shows representative dose-response curves for human insulin (●) and compound of example 15 (▲) from an AKT phosphorylation assay (phosphorylation of serine residue number 473) in CHO-hIR cells overexpressing the IR-A;

Fig. 4 shows representative dose-response curves for human insulin (●) and compound of example 15 (▲) from an ERK phosphorylation assay in CHO-hIR cells overexpressing the IR-A;

Fig. 5 shows representative dose-response curves for human insulin (●) and compound of example 15 (▲) from a lipogenesis assay in primary rat adipocytes (DPM = disintegrations per minute);

Fig. 6 shows representative dose-response curves for human insulin (●) and compound of example 15 (▲) from a glycogen synthesis assay in primary rat hepatocytes;

Fig. 7 shows representative dose-response curves for human insulin (●) and compound of example 15 (▲) from a quantitative real-time polymerase chain reaction (RT-PCR) assay for *fasn* performed on cDNA isolated from primary rat hepatocytes;

Fig. 8 shows representative dose-response curves for human insulin (●) and compound of example 15 (▲) from a quantitative real-time polymerase chain reaction (RT-PCR) assay for *g6pc* performed on cDNA isolated from primary rat hepatocytes;

Fig. 9 shows representative dose-response curves for human insulin (●) and compound of example 15 (▲) from a *de novo* lipogenesis assay in primary rat hepatocytes (CPM = counts per minute);

Fig. 10 shows representative curves for HbA1c levels from a sub-chronic *in vivo* study in diabetic STZ-DIO mice dosed subcutaneously twice daily for six weeks with vehicle, the PK Comparator (comparator no. 5) and compound of example 15.

CLAIMS

1. An insulin derivative, wherein said insulin derivative comprises B5Y or B5F and a substituent comprising an acyl group, or a pharmaceutically acceptable salt, amide or ester thereof.
2. The insulin derivative according to claim 1, wherein said insulin derivative further comprises B26G or B26A.
3. The insulin derivative according to any one of claims 1 or 2, wherein said substituent comprising an acyl group is attached to B28K, B26K or B29K.
4. The insulin derivative according to any one of claims 1-3, wherein said substituent has the following formula (I):

Acy-L1-L2-L3

wherein:

- Acy is an acyl group and is represented by lithocholic acid or comprises at least one functional group of formulae:

Chem. 1:-CO-$(CH_2)_x$-COOH; or

Chem. 2: -CO-$(CH_2)_x$-tetrazolyl;

wherein x represents an integer in the range of from 12 to 20; and the tetrazolyl group is 1*H*-tetrazol-5-yl.

or is a fatty acid of formula:

Chem. 3: -CO-$(CH_2)_x$-CH_3

wherein x represents an integer in the range from 8 to 16,

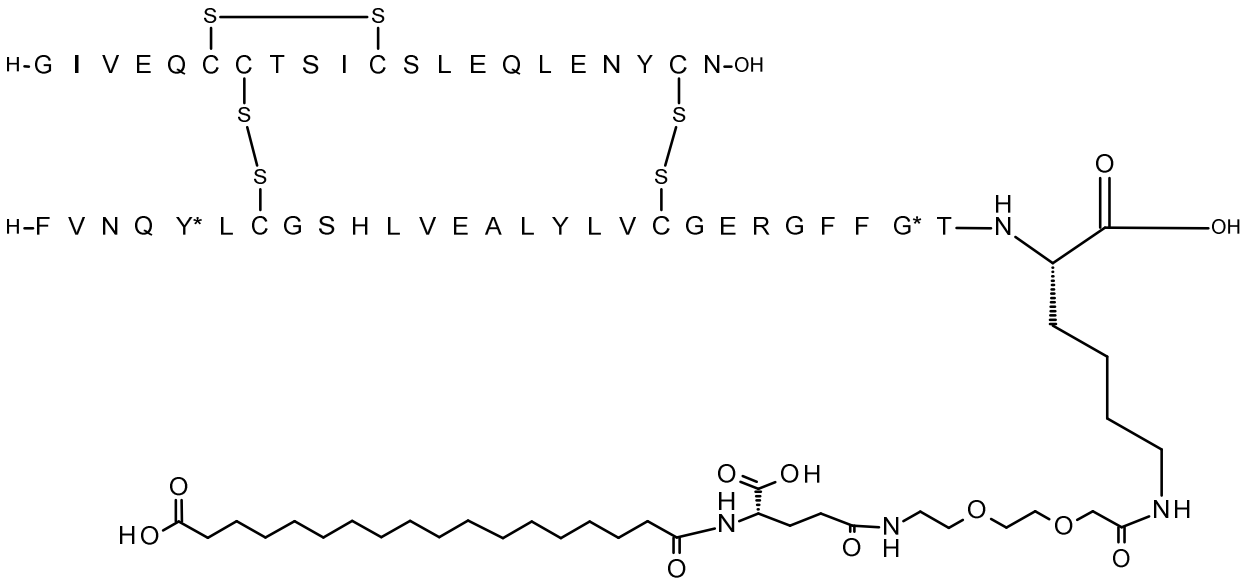
- L1 is absent and represents a covalent bond or represents OEG, gGlu, DgGlu or sulfonimide C-4
- L2 is absent and represents a covalent bond or represents OEG, gGlu, DgGlu or sulfonimide C-4
- L3 is absent and represents a covalent bond or represents OEG, gGlu, DgGlu or sulfonimide C-4

wherein gGlu represents a gamma glutamic acid residue and OEG represents [2-(2-aminoethoxy)ethoxy]acetyl.

5. The insulin derivative according to any one of the preceding claims, wherein said insulin derivative further comprises A14E and/or desB30 and/or desB29-30 and/or desB27-30.
6. The insulin derivative according to any of the previous claims, wherein said substitutions are selected from the group consisting of:
   i. A14E, B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 3 and 4)
   ii. A14E, B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 3 and 5)

iii. B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 1 and 7)
iv. B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 1 and 8)
v. B5Y, B28K, desB29-30 (SEQ ID NO: 1 and 9)
vi. A14E, B5F, B26G, B28K, desB29-30 (SEQ ID NO: 3 and 10)
vii. B5F, B28K, desB29-30 (SEQ ID NO: 1 and 11)
viii. B5Y, B26K, desB27-desB30 (SEQ ID 1 and 12)
ix. B5Y, desB30 (SEQ ID 1 and 13)
x. B5Y, B26G, desB30 (SEQ ID 1 and 14)
xi. B5Y, B26A, desB30 (SEQ ID 1 and 15)
xii. B5F, B26A, B28K, desB29-30 (SEQ ID 1 and 6)
xiii. B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 1 and 4)
xiv. B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 1 and 5)
xv. B5F, B26G, B28K, desB29-30 (SEQ ID NO: 1 and 10)
xvi. A14E, B5F, B26A, B28K, desB29-30 (SEQ ID 3 and 6)
xvii. A14E, B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 3 and 7)
xviii. A14E, B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 3 and 8)
xix. A14E, B5Y, B28K, desB29-30 (SEQ ID NO: 3 and 9)
xx. A14E, B5F, B28K, desB29-30 (SEQ ID NO: 3 and 11)
xxi. A14E, B5Y, B26K, desB27-desB30 (SEQ ID 3 and 12)
xxii. A14E, B5Y, desB30 (SEQ ID 3 and 13)
xxiii. A14E, B5Y, B26G, desB30 (SEQ ID 3 and 14)
xxiv. A14E, B5Y, B26A, desB30 (SEQ ID 3 and 15)

7. The insulin derivative according to claim 6, wherein Acy is selected from the group consisting of: lithocholic acid, 1,16-hexadecanedioic acid, 1,18-octadecanedioic acid, 1,20-eicosanedioic acid, tetrazole-C16, tetrazole-C17, tetrazole C18 and tetradecanoic acid.
8. The insulin derivative according to claim 6, wherein -L1-L2-L3 represents a divalent linking group selected from DgGlu, gGlu, gGlu–gGlu, gGlu-OEG, gGlu-OEG-OEG, OEG, sulfonimide-C4, and sulfonimide-C4-sulfonimide-C4.
9. The insulin derivative according to any of the previous claims, wherein said insulin derivative is compound of Example 15: N{Epsilon-B28}-[2-[2-[2-[[(4S)-4-carboxy-4-(17-carboxyheptadecanoylamino)butanoyl]amino]ethoxy]ethoxy]acetyl]-[GluA14,TyrB5,GlyB26,LysB28],des-(B29-B30)-Insulin



10. An intermediate product comprising a backbone selected from the group consisting of:

i. A14E, B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 3 and 4)
ii. A14E, B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 3 and 5)
iii. B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 1 and 7)
iv. B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 1 and 8)
v. B5Y, B28K, desB29-30 (SEQ ID NO: 1 and 9)
vi. A14E, B5F, B26G, B28K, desB29-30 (SEQ ID NO: 3 and 10)
vii. B5F, B28K, desB29-30 (SEQ ID NO: 1 and 11)
viii. B5Y, B26K, desB27-desB30 (SEQ ID 1 and 12)
ix. B5Y, desB30 (SEQ ID 1 and 13)
x. B5Y, B26G, desB30 (SEQ ID 1 and 14)
xi. B5Y, B26A, desB30 (SEQ ID 1 and 15)
xii. B5F, B26A, B28K, desB29-30 (SEQ ID 1 and 6)
xiii. B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 1 and 4)
xiv. B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 1 and 5)
xv. B5F, B26G, B28K, desB29-30 (SEQ ID NO: 1 and 10)
xvi. A14E, B5F, B26A, B28K, desB29-30 (SEQ ID 3 and 6)
xvii. A14E, B5Y, B26A, B28K, desB29-30 (SEQ ID NO: 3 and 7)
xviii. A14E, B5Y, B26G, B28K, desB29-30 (SEQ ID NO: 3 and 8)
xix. A14E, B5Y, B28K, desB29-30 (SEQ ID NO: 3 and 9)
xx. A14E, B5F, B28K, desB29-30 (SEQ ID NO: 3 and 11)
xxi. A14E, B5Y, B26K, desB27-desB30 (SEQ ID 3 and 12)

xxii. A14E, B5Y, desB30 (SEQ ID 3 and 13)

xxiii. A14E, B5Y, B26G, desB30 (SEQ ID 3 and 14)

xxiv. A14E, B5Y, B26A, desB30 (SEQ ID 3 and 15)

or a pharmaceutically acceptable salt, amide or ester thereof.

11. An insulin derivative according to any one of claims 1-9, for use as a medicament.

12. Use of an insulin derivative according to any one of claims 1-9, in the manufacture of a medicament for the prevention or treatment of diabetes, cardiovascular disease, atherosclerosis, and/or endothelial dysfunction and/or for preventing or reducing liver triglyceride content and/or for preventing or reducing body weight gain.

13. Use of an insulin derivative according to any one of claims 1-9, in the manufacture of a medicament for the treatment or prevention of diabetes, diabetes of Type 1, diabetes of Type 2, impaired glucose tolerance, hyperglycemia, dyslipidemia, obesity, metabolic syndrome (metabolic syndrome X, insulin resistance syndrome), hypertension, cognitive disorders, atherosclerosis, myocardial infarction, stroke, cardiovascular disorders, coronary heart disease, stroke, inflammatory bowel syndrome, dyspepsia, hypotension or gastric ulcers.

14. A method for determining selectivity of an insulin compound comprising the following steps:

- measuring the maximal AKT phosphorylation induced by said insulin compound relative to human insulin
- measuring the maximal ERK activation induced by said insulin compound relative to human insulin,

wherein the ERK/AKT ratio is less than 1.