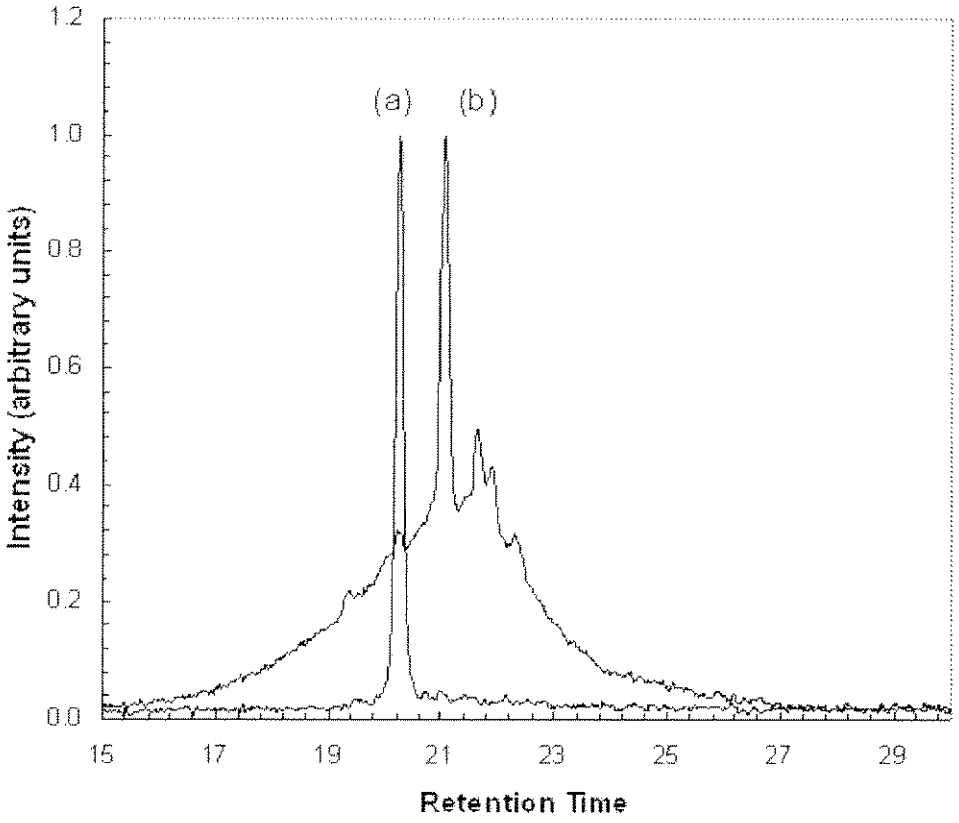

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Intensity (arbitrary units)
(a)
(b)
15
17
19
21
23
25
27
29
Retention Time

Figure 1

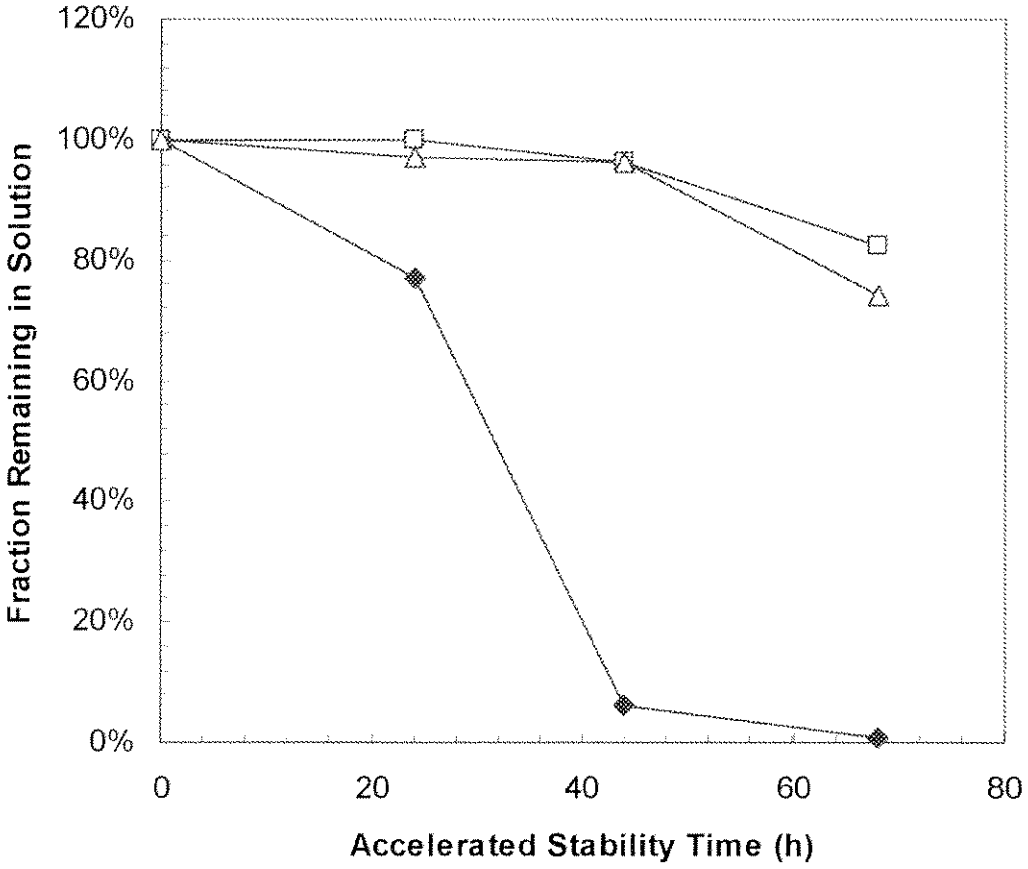

120%
100%
80%
60%
40%
20%
0%
Fraction Remaining in Solution
0
20
40
60
80
Accelerated Stability Time (h)

Figure 2

(a) IPC Peak Retention Times, %Free Insulin, and %Desamido Insulin

Sample	Peak 1 RT[1] (min)	Peak 2 RT (min)	Peak 3 RT (min)	% Purity IPC	% Free Insulin	% Desamido Insulin
HS-1-60-1 (fresh)	20.62	-	-	>99	<0.1	<0.1
HS-1-60-1 (AST, PBS)	20.61	19.19	-	85	<0.1	<0.1
HS-1-60-1 (AST, HEPES)	20.65	-	-	>99	<0.1	<0.1
TL-13-85 (fresh)	20.46	-	-	>99	<0.1	<0.1
TL-13-85 (AST, PBS)	20.40	19.84	18.91	30	<0.1	<0.1
TL-13-85 (AST, HEPES)	20.46	-	-	>99	<0.1	<0.1

[1]C8 reverse phase column, water:acetonitrile (0.1% TFA) elution gradient, RT = Retention Time

(b) Mass Spectroscopy Data on IPC's and IPC Breakdown Products

Sample	Peak 1 MW (Da)	Peak 2 MW (Da)	Peak 3 MW (Da)
HS-1-60-1 (fresh)	6730 [2]	-	-
HS-1-60-1 (AST, PBS)	6730	5947	-
HS-1-60-1 (AST, HEPES)	6730	-	-
TL-13-85 (fresh)	6829 [2]	-	-
TL-13-85 (AST, PBS)	6829	5944	7928
TL-13-85 (AST, HEPES)	6829	-	-

[2]The two IPC's shown here have different structures and, therefore, different MW's

Figure 3

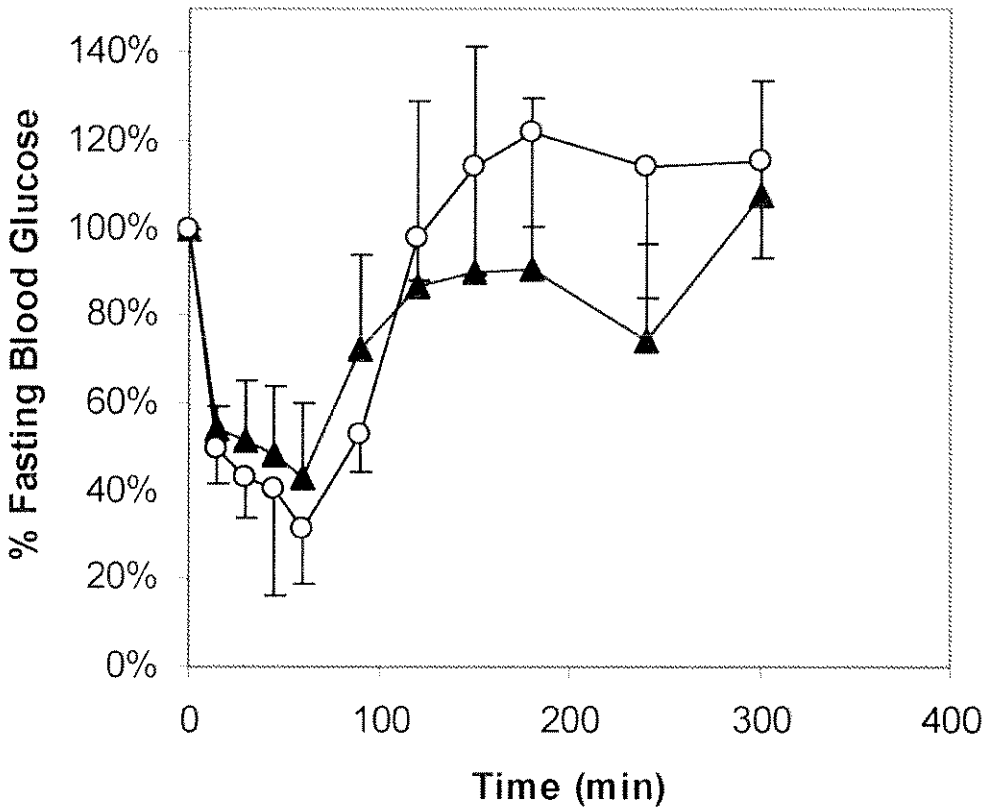

% Fasting Blood Glucose
140%
120%
100%
80%
60%
40%
20%
0%
0
100
200
300
400
Time (min)

Figure 4

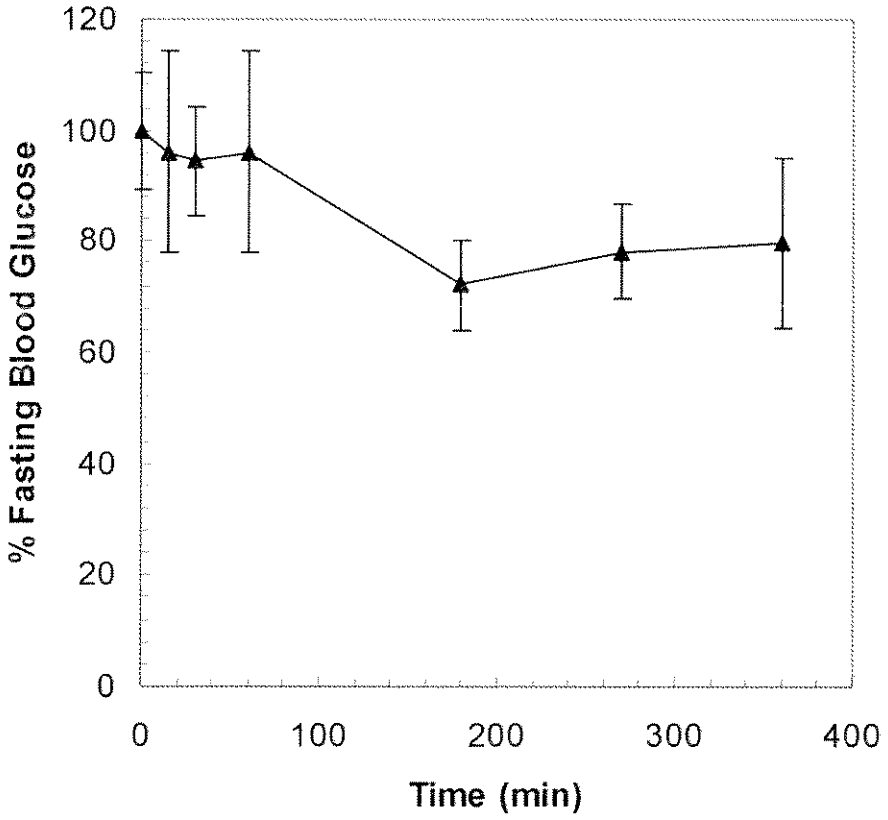

120
100
80
60
40
20
0
% Fasting Blood Glucose
0
100
200
300
400
Time (min)

Figure 5

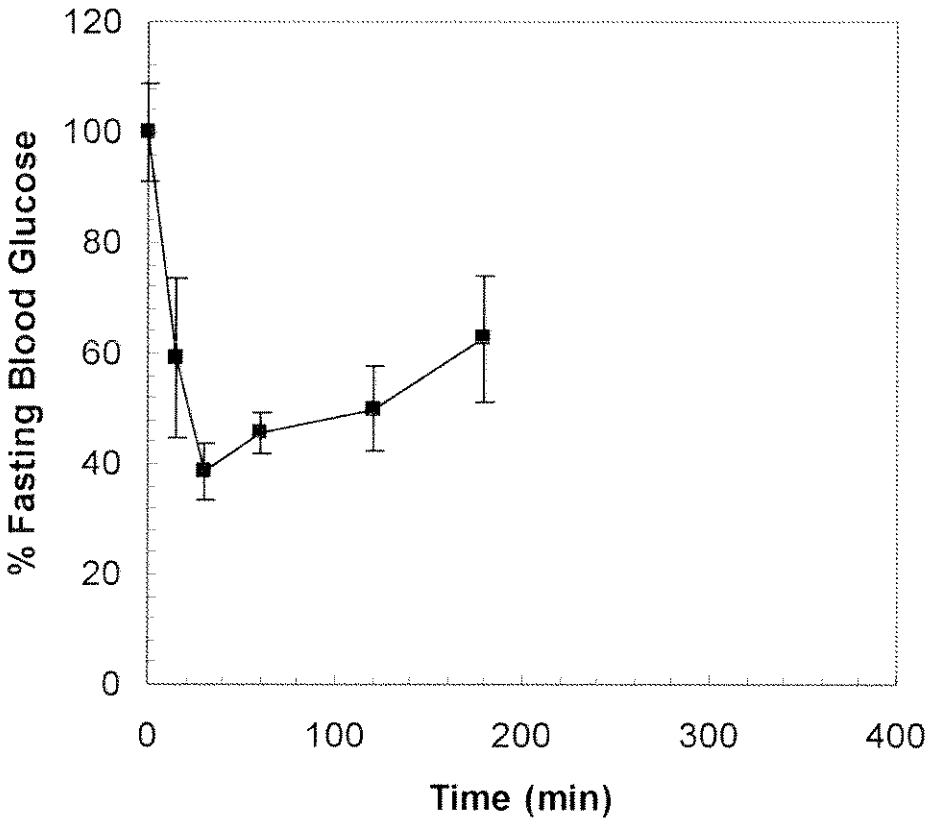

120
100
80
60
40
20
0
% Fasting Blood Glucose
0
100
200
300
400
Time (min)

Figure 6

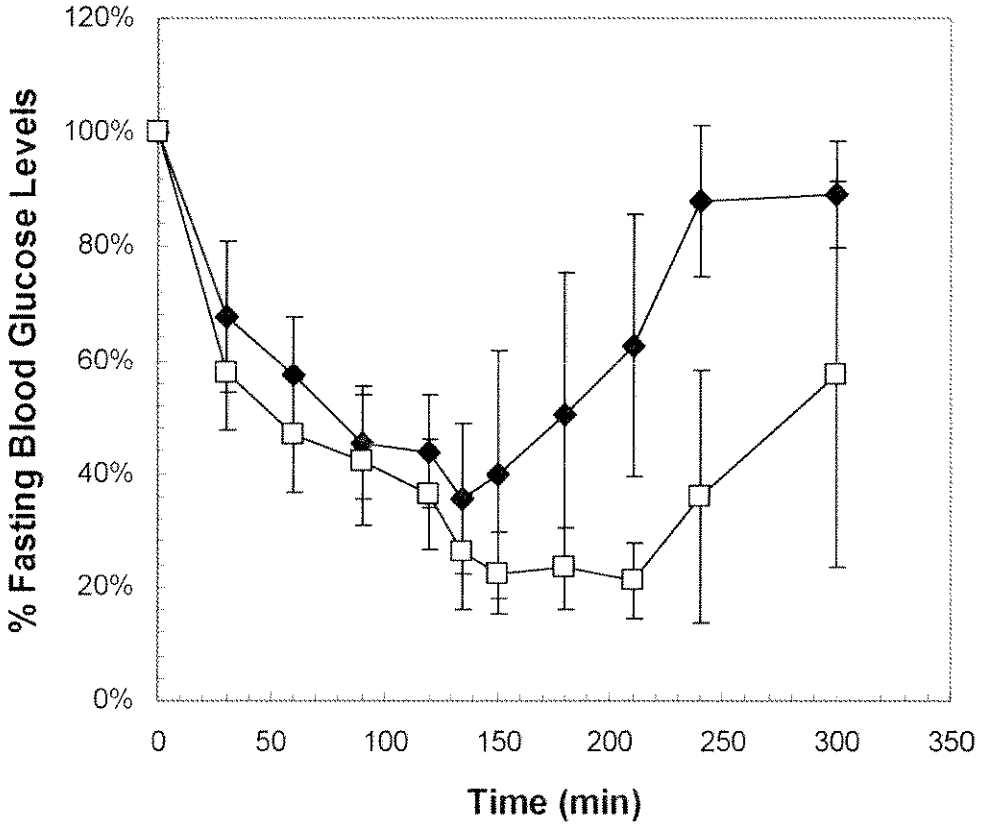

120%
100%
80%
60%
40%
20%
0%
% Fasting Blood Glucose Levels
0
50
100
150
200
250
300
350
Time (min)

Figure 7

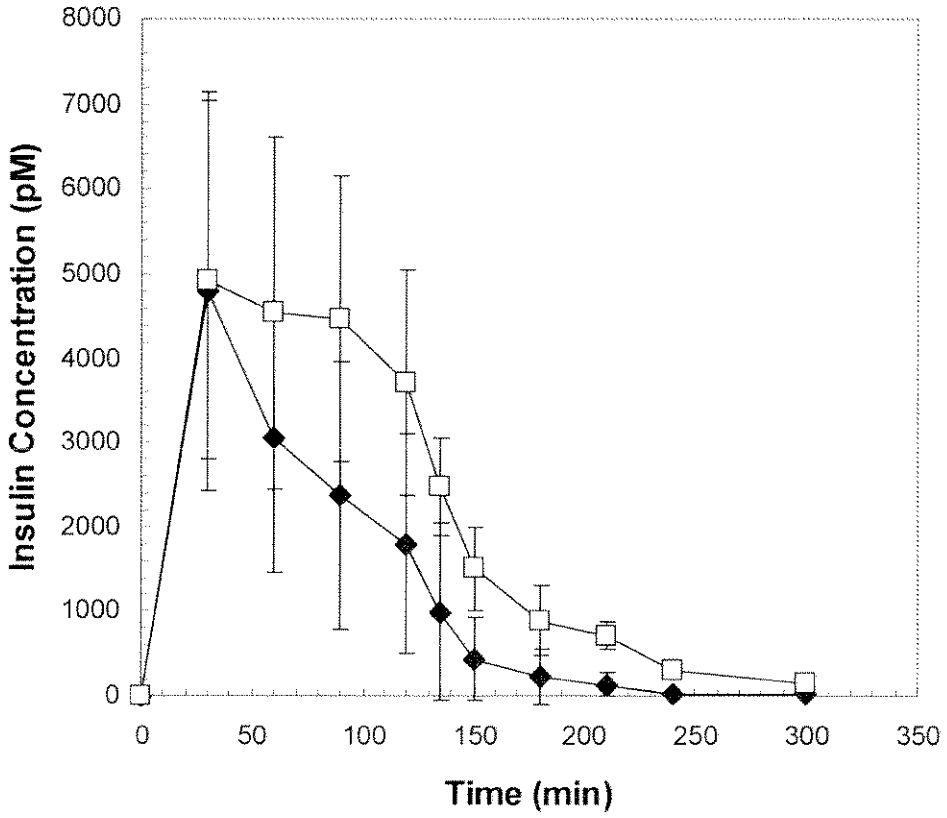

8000
7000
6000
5000
4000
3000
2000
1000
0
Insulin Concentration (pM)
0
50
100
150
200
250
300
350
Time (min)

Figure 8

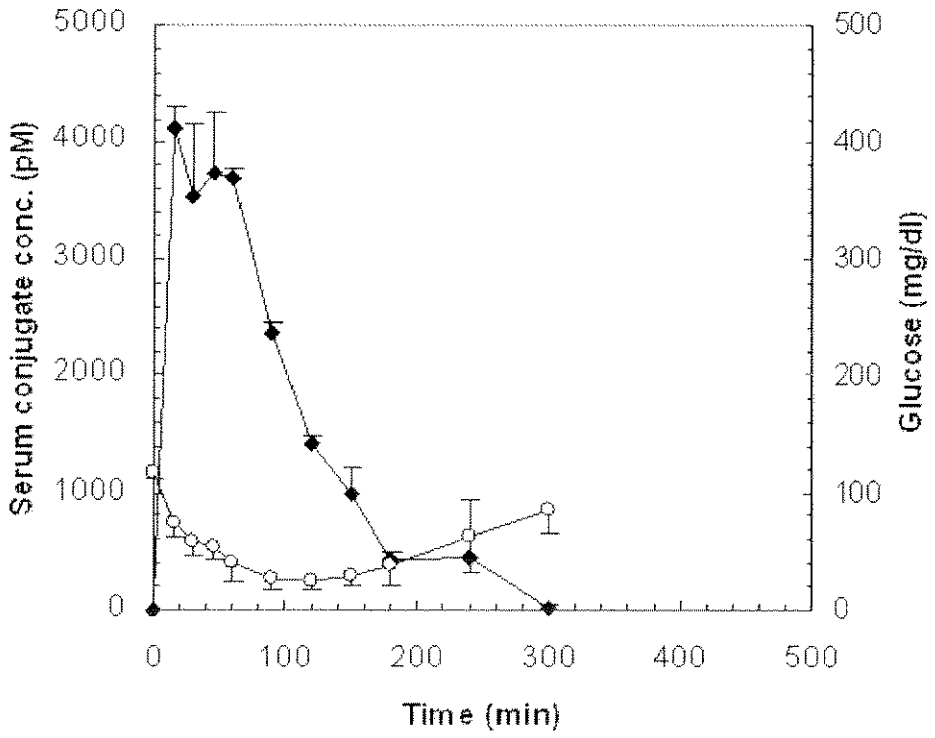

5000
4000
3000
2000
1000
0
Serum conjugate conc. (pM)
500
400
300
200
100
0
Glucose (mg/dl)
0
100
200
300
400
500
Time (min)

Figure 9

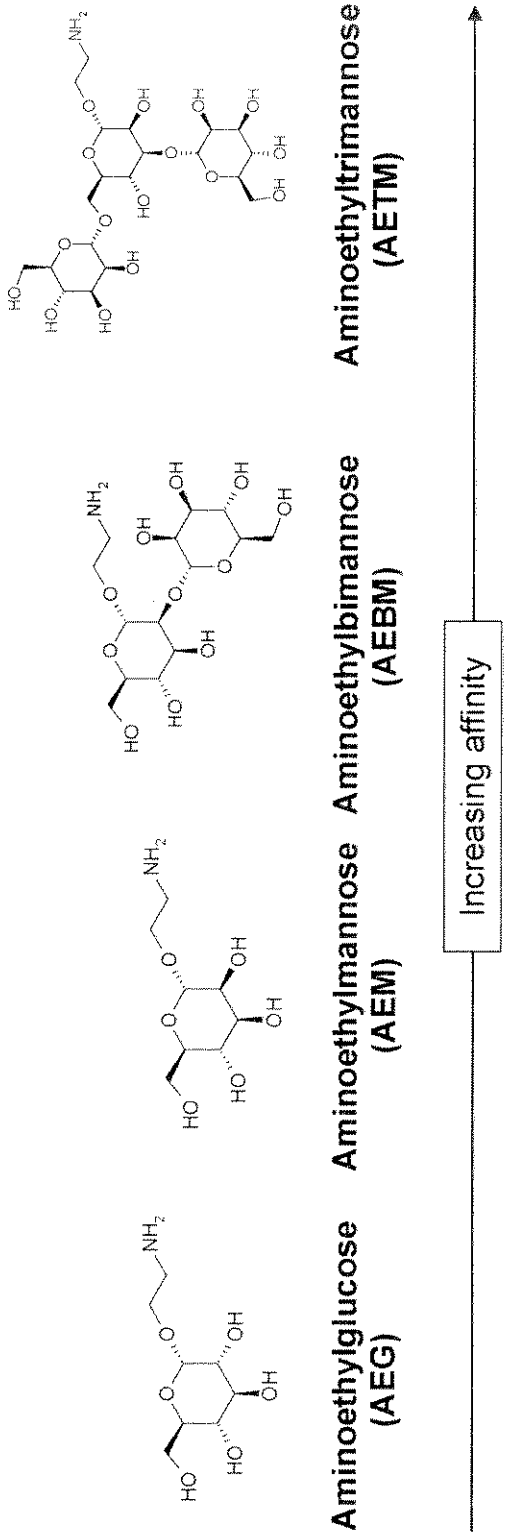

Aminoethylglucose (AEG)
Aminoethylmannose (AEM)
Aminoethylbimannose (AEBM)
Aminoethyltrimannose (AETM)
Increasing affinity

Figure 10

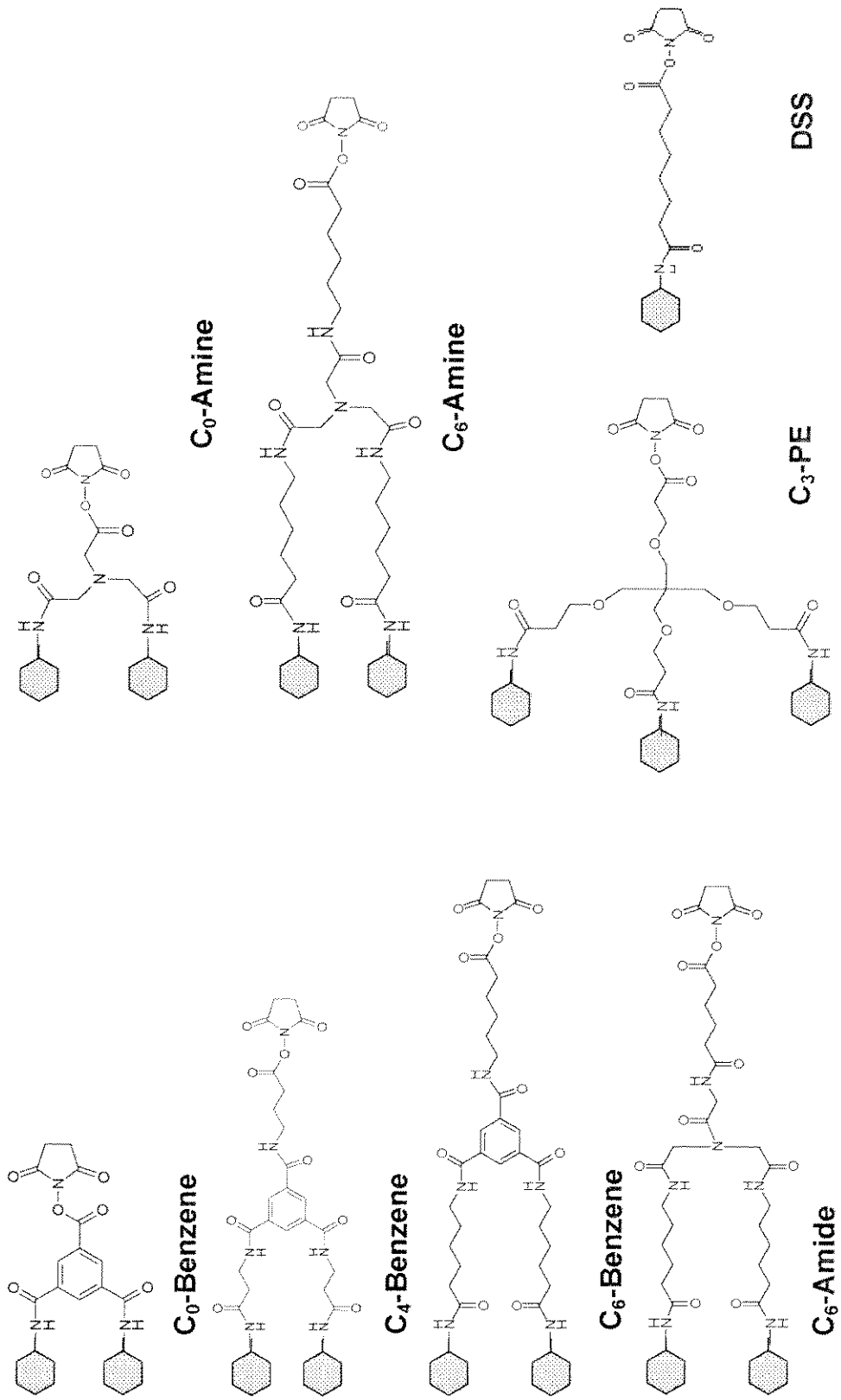

C0-Amine
C6-Amine
DSS
C3-PE
C0-Benzene
C4-Benzene
C6-Benzene
C6-Amide
= AEG, AEM, AEBM, AETM, AEGA, AEF

Figure 11

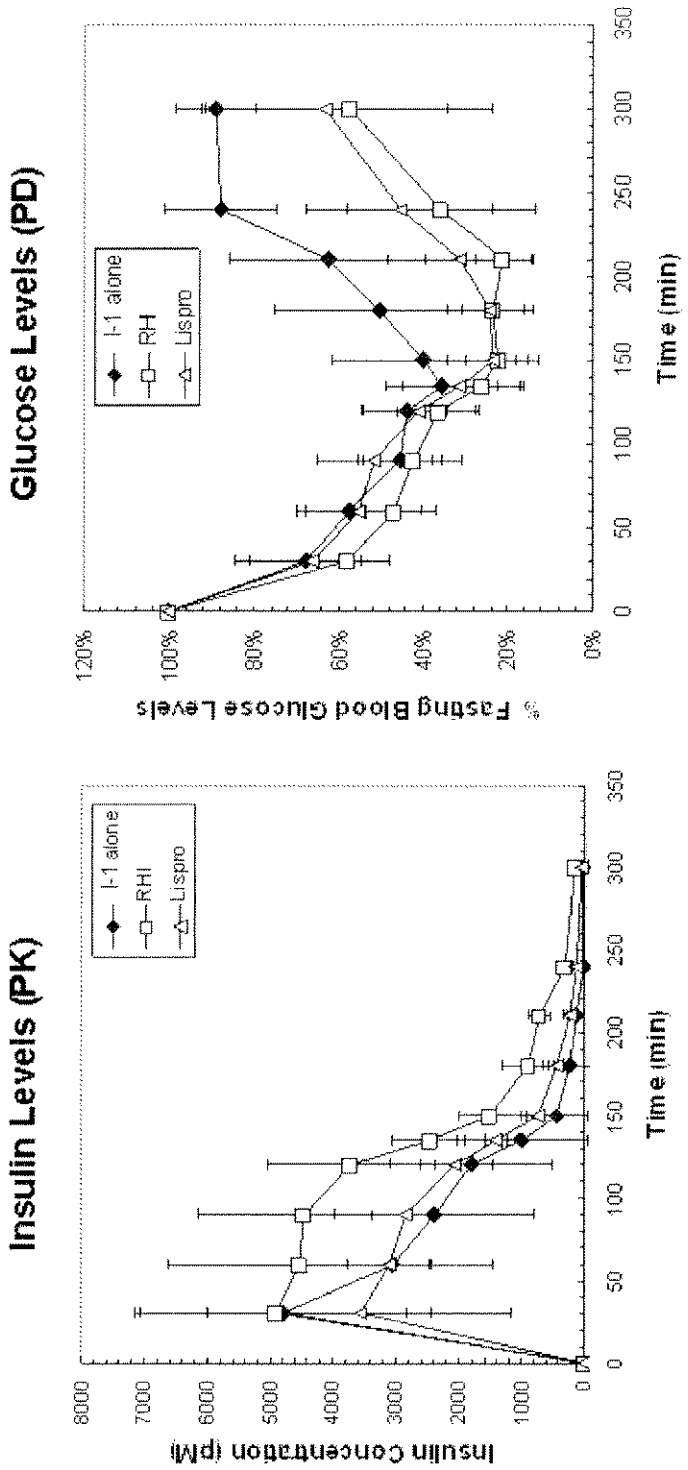

Glucose Levels (PD)
% Fasting Blood Glucose Levels
120%
100%
80%
60%
40%
20%
0%
0
50
100
150
200
250
300
350
Time (min)
I-1 alone
RHI
Lispro
Insulin Levels (PK)
Insulin Concentration (pM)
8000
7000
6000
5000
4000
3000
2000
1000
0
Time (min)
I-1 alone
RHI
Lispro

Figure 12

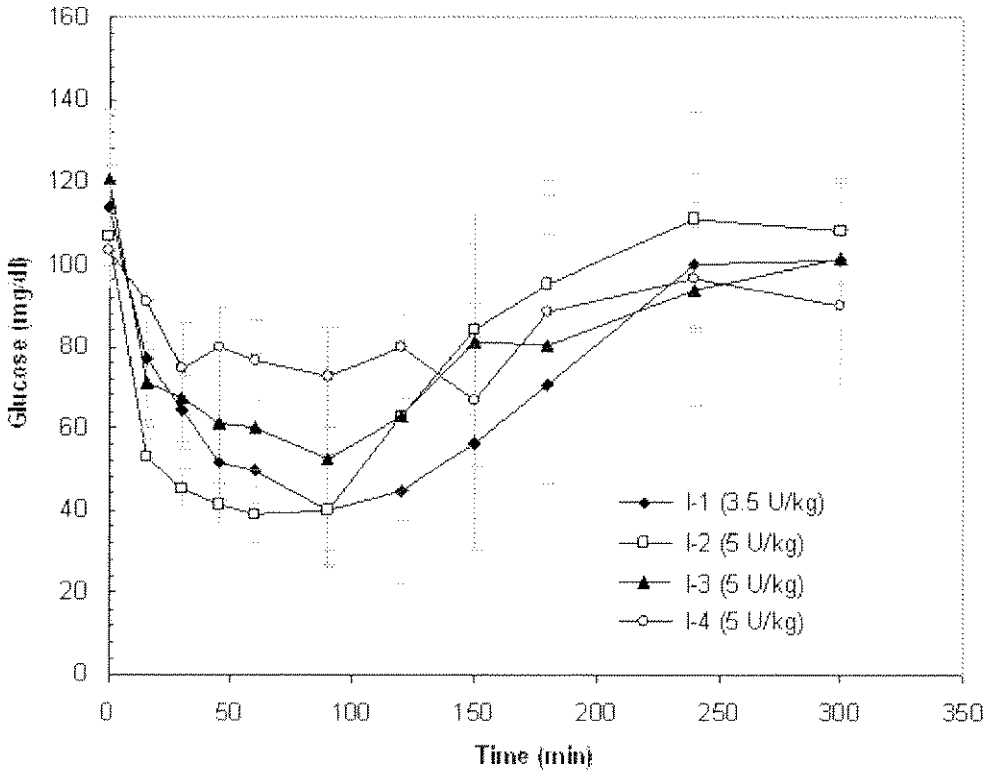

160
140
120
100
80
60
40
20
0
Glucose (mg/dl)
0
50
100
150
200
250
300
350
Time (min)
I-1 (3.5 U/kg)
I-2 (5 U/kg)
I-3 (5 U/kg)
I-4 (5 U/kg)

Figure 13

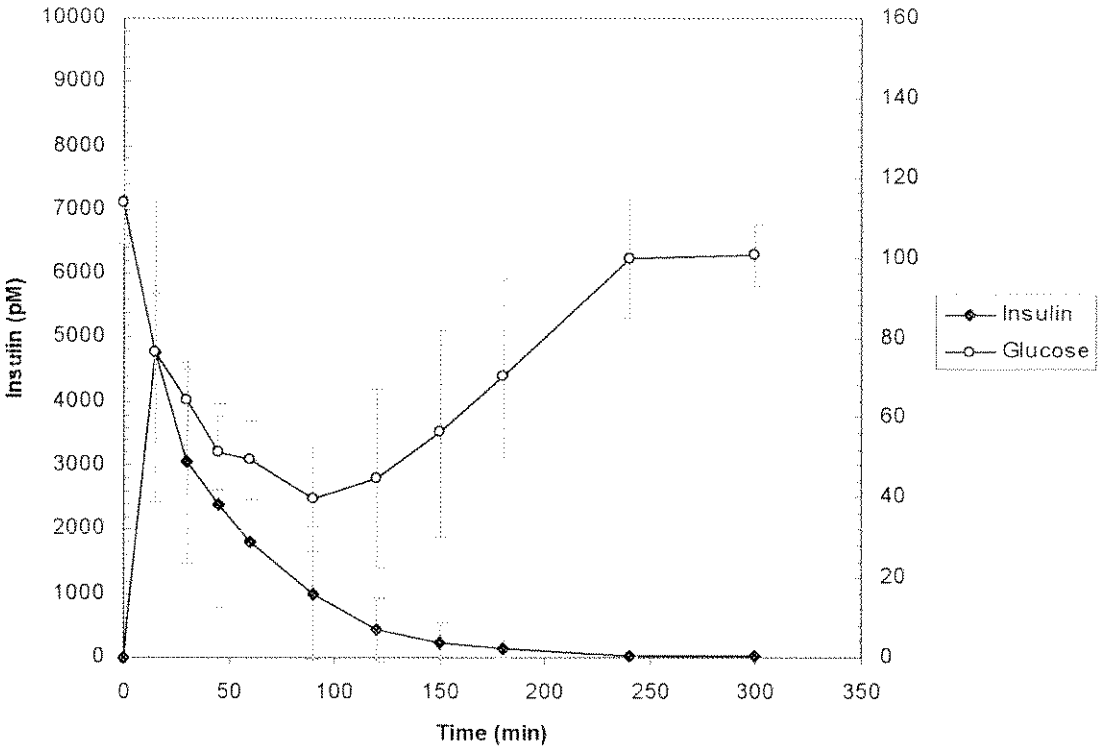

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Insulin (pM)
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
Time (min)
Insulin
Glucose

Figure 14

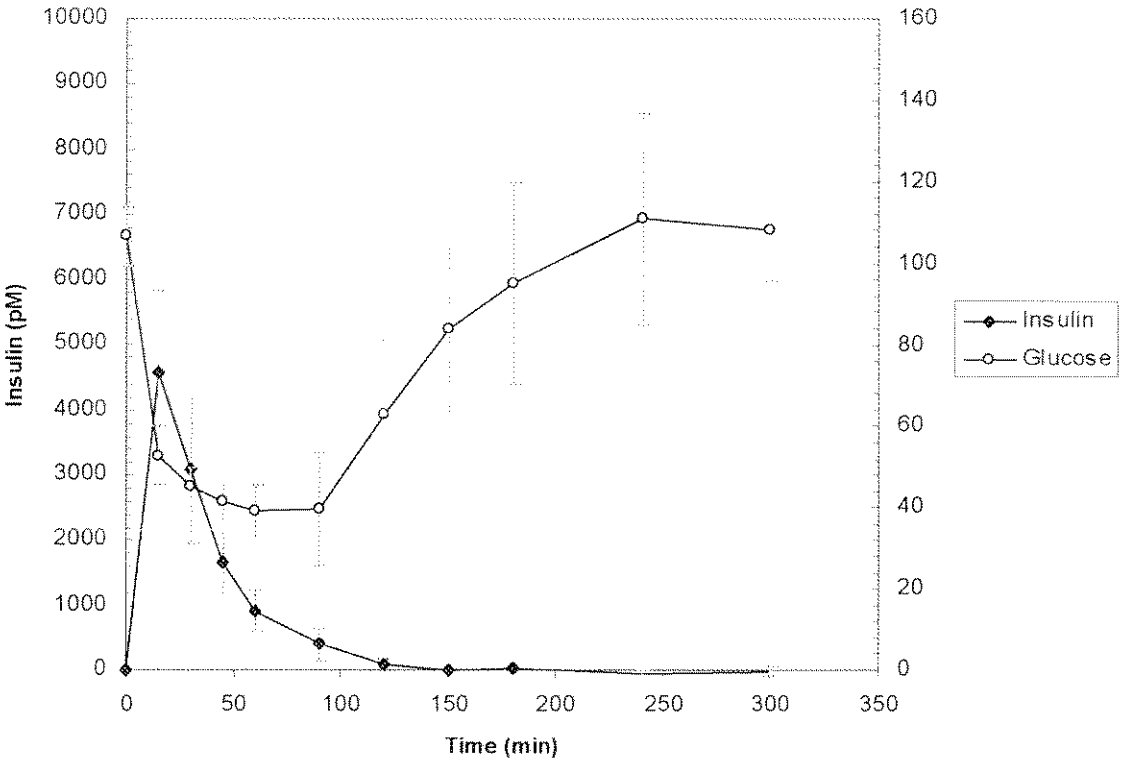

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Insulin (pM)
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
Time (min)
Insulin
Glucose

Figure 15

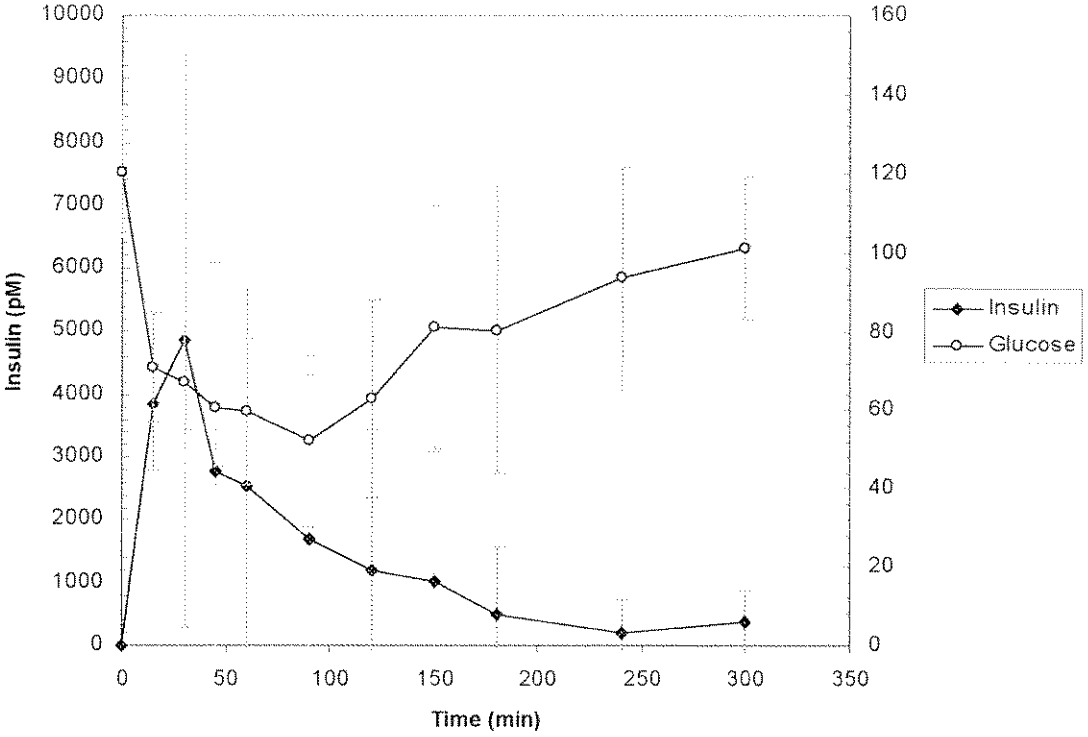

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Insulin (pM)
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
Time (min)
Insulin
Glucose

Figure 16

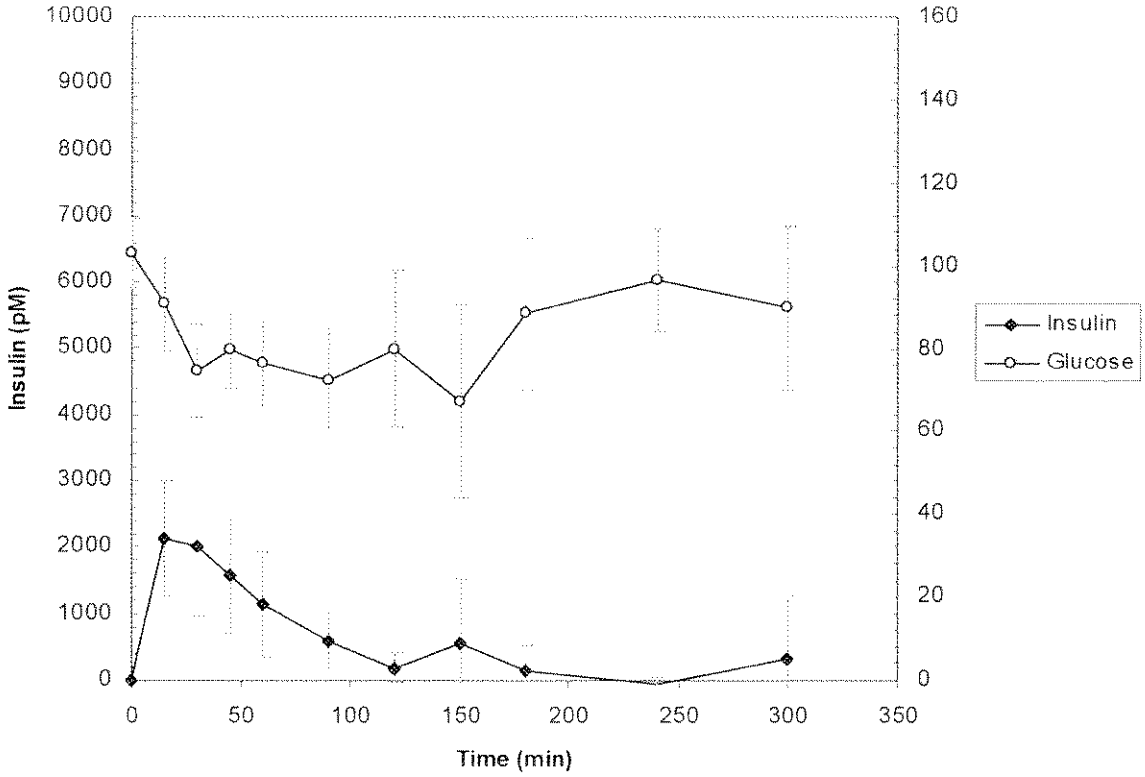

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Insulin (pM)
160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
Time (min)
Insulin
Glucose

Figure 17

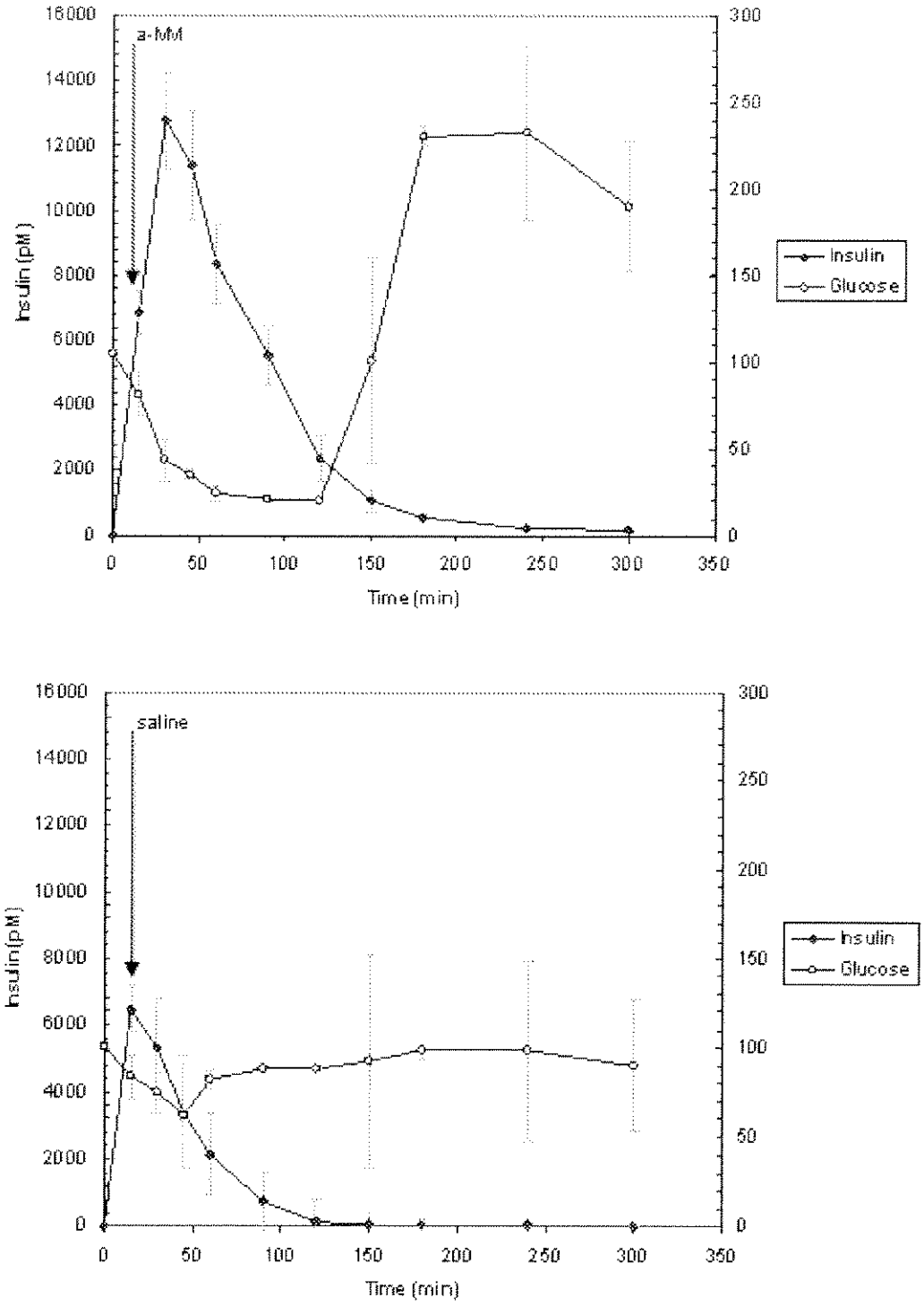

a-MM
Insulin (pM)
Time (min)
Insulin
Glucose
saline
Insulin (pM)
Time (min)
Insulin
Glucose

Figure 18

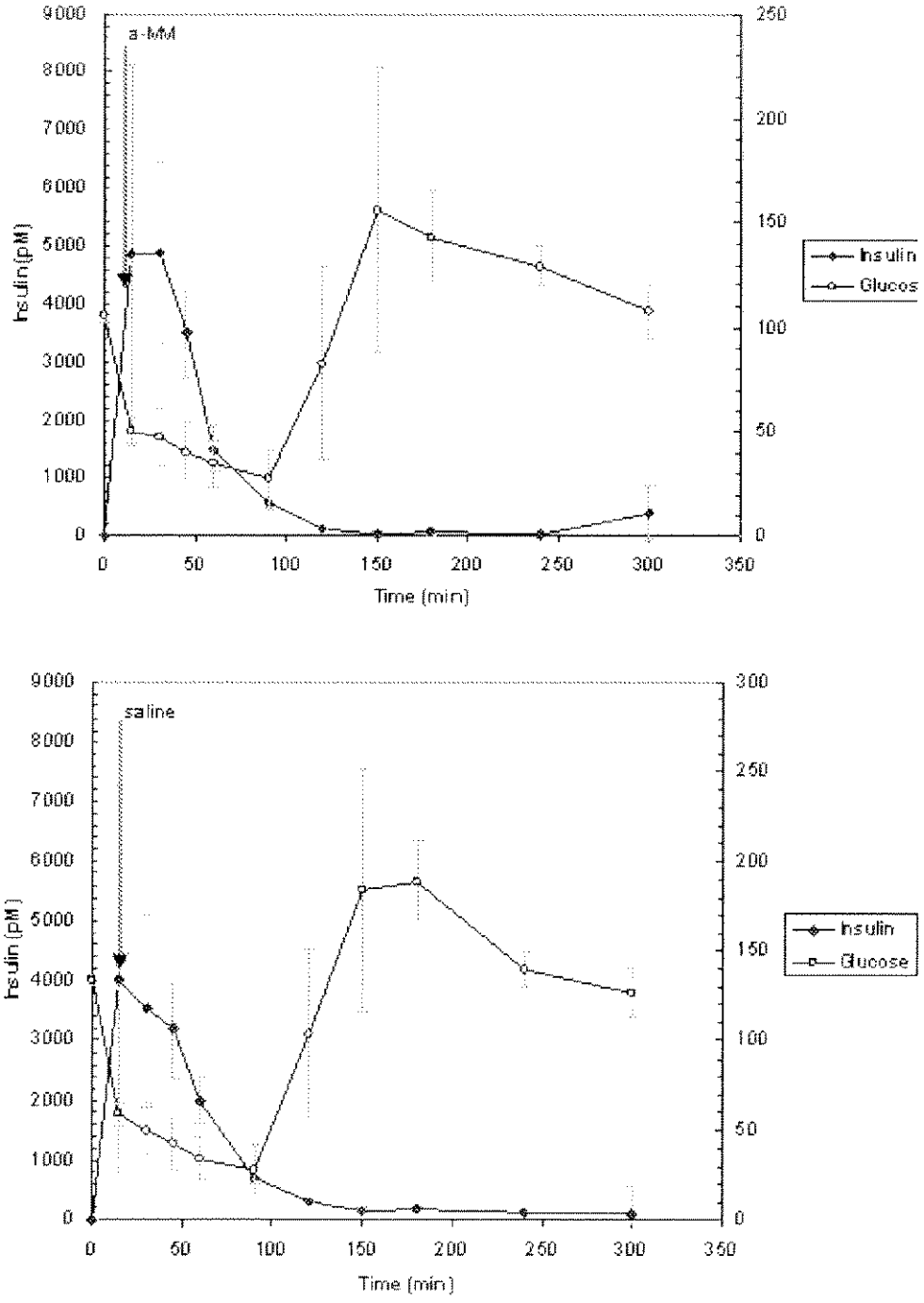

a-MM
Insulin (pM)
Time (min)
Insulin
Glucos
saline
Insulin (pM)
Time (min)
Insulin
Glucose

Figure 19

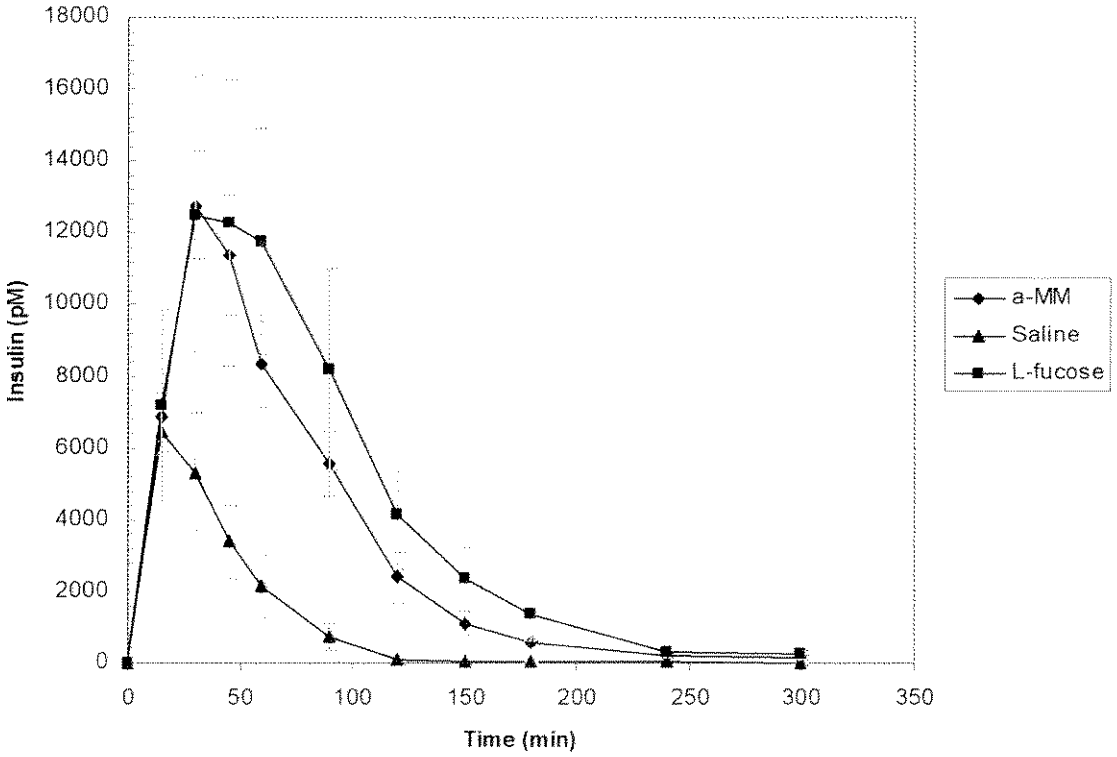

18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Insulin (pM)
0
50
100
150
200
250
300
350
Time (min)
a-MM
Saline
L-fucose

Figure 20

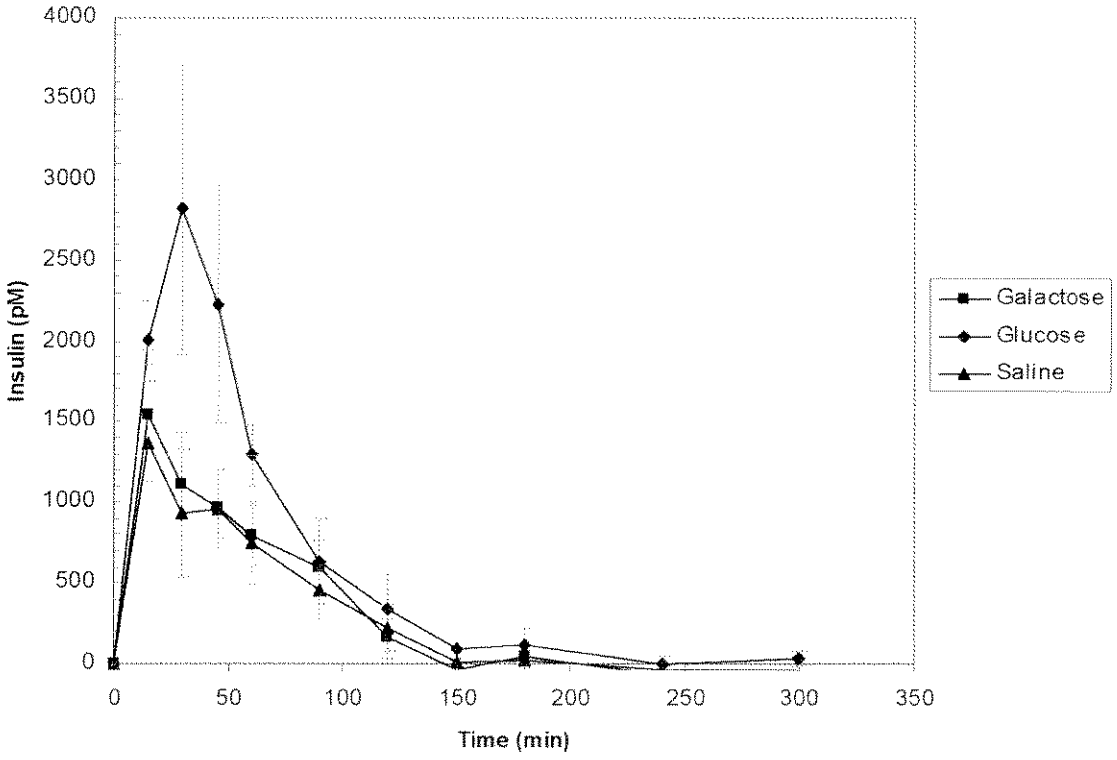

4000
3500
3000
2500
2000
1500
1000
500
0
Insulin (pM)
0
50
100
150
200
250
300
350
Time (min)
Galactose
Glucose
Saline

Figure 21

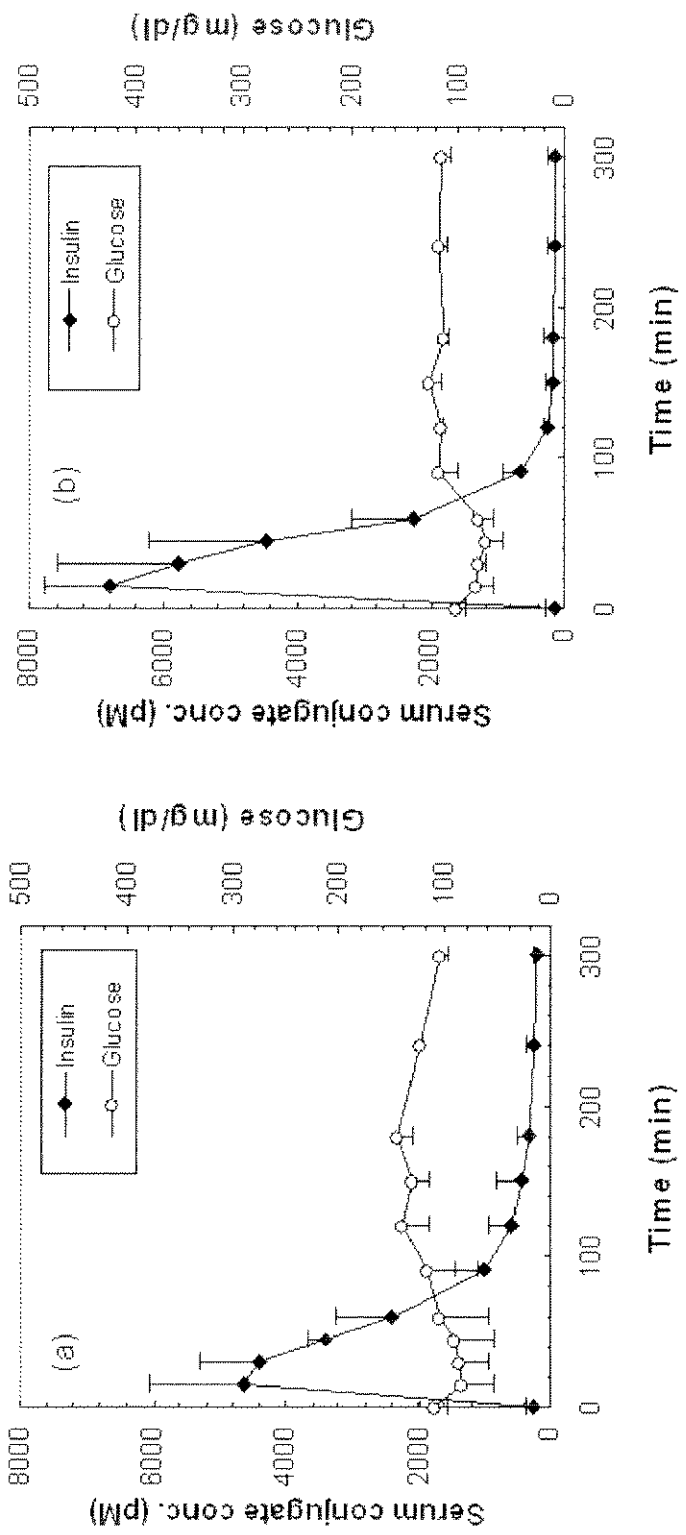

(a)
(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
2000
4000
6000
8000
100
200
300
400
500

Figure 22

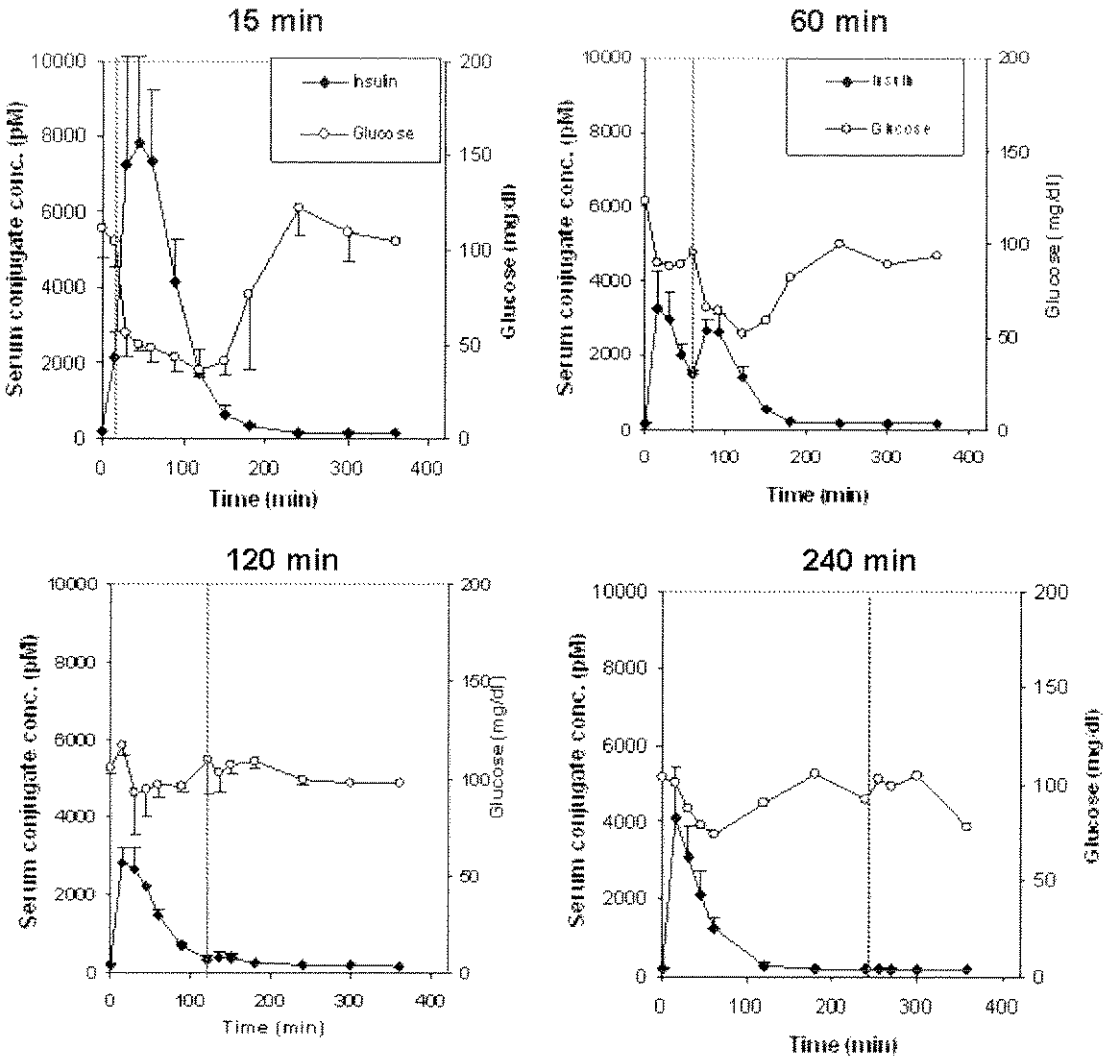

15 min
60 min
120 min
240 min
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
2000
4000
6000
8000
10000
50
100
150
200
300
400

Figure 23

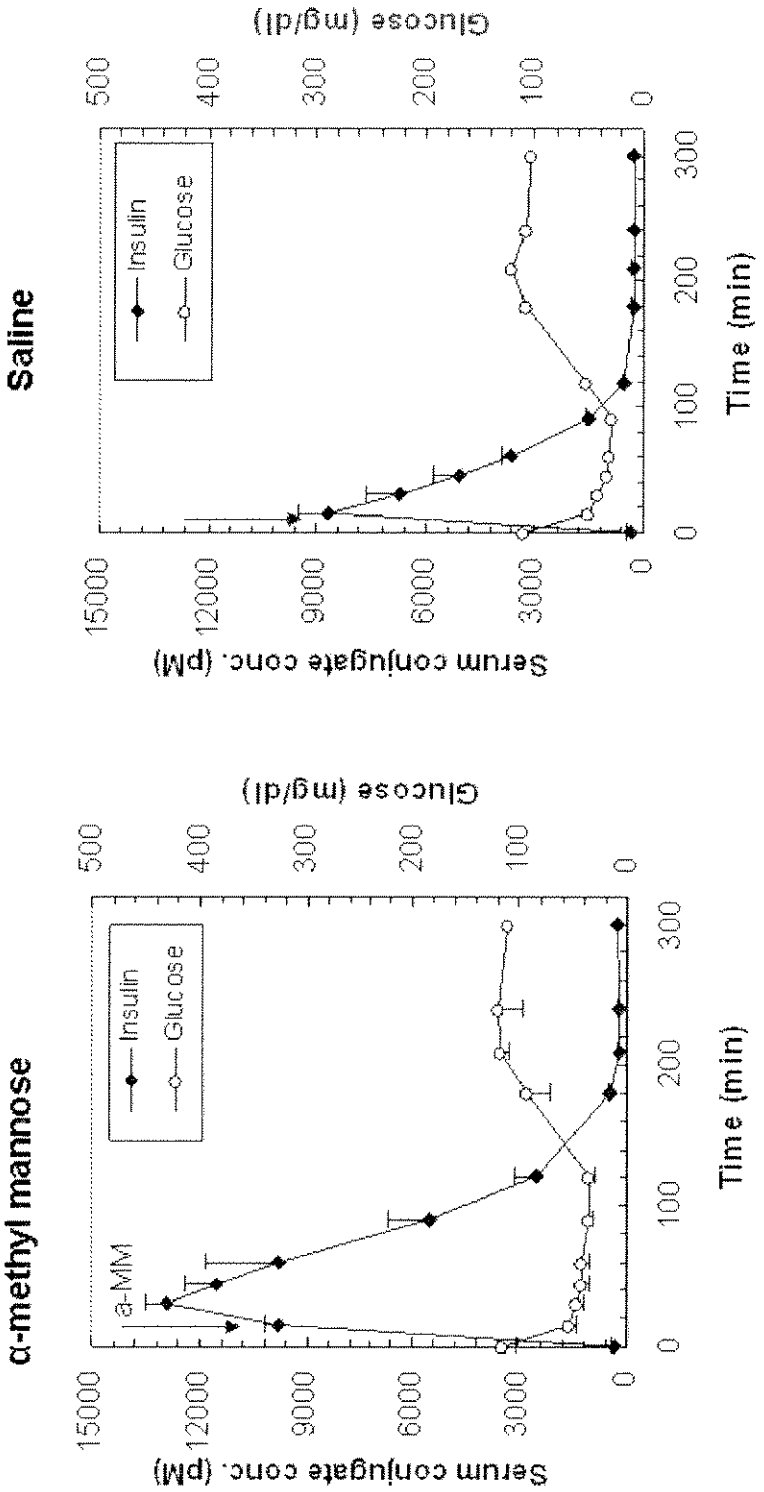

α-methyl mannose
Saline
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
Insulin
Glucose
a-MM
0
3000
6000
9000
12000
15000
100
200
300
400
500

Figure 24

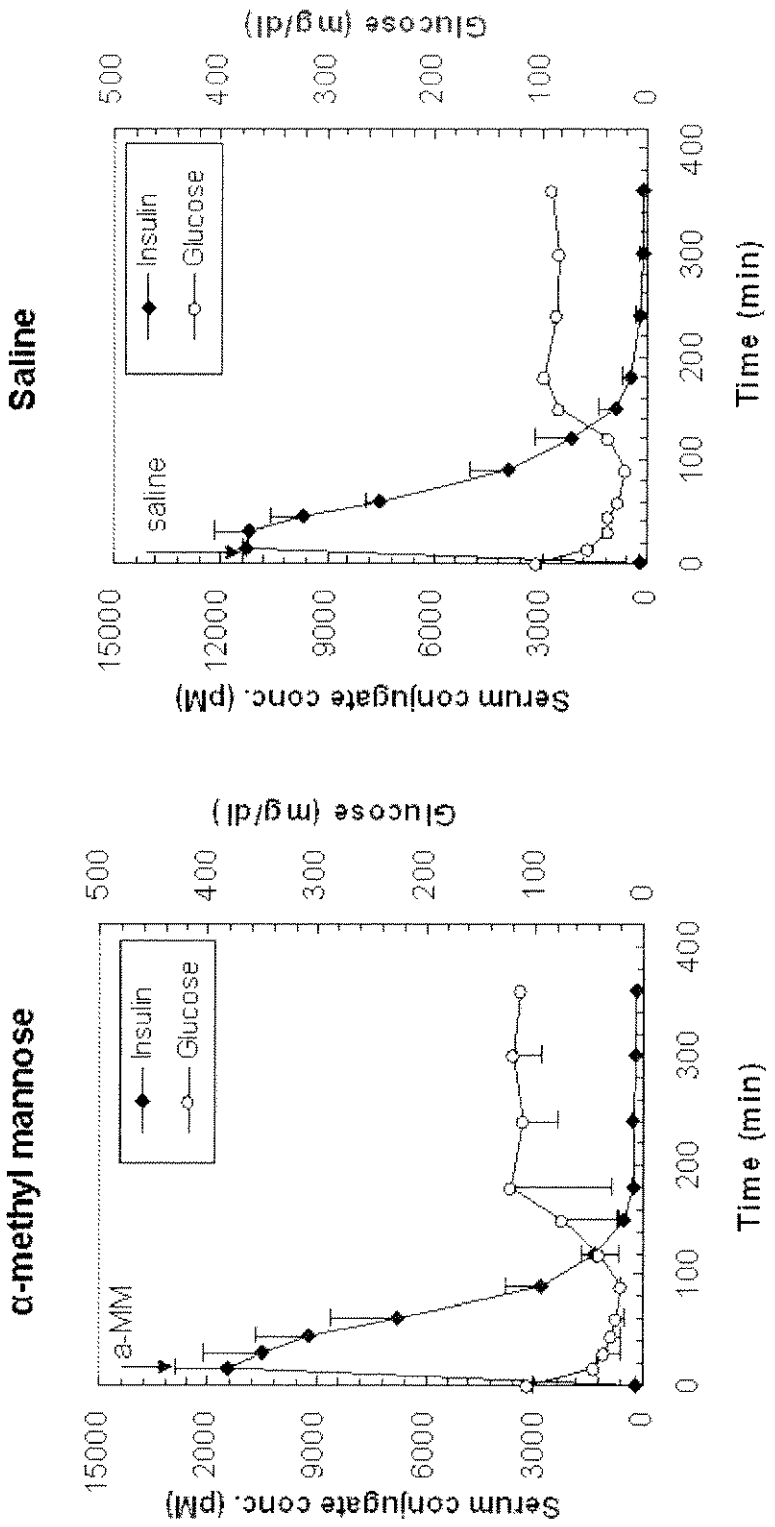

α-methyl mannose
Saline
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
Insulin
Glucose
α-MM
saline
0
3000
6000
9000
12000
15000
100
200
300
400
500

Figure 25

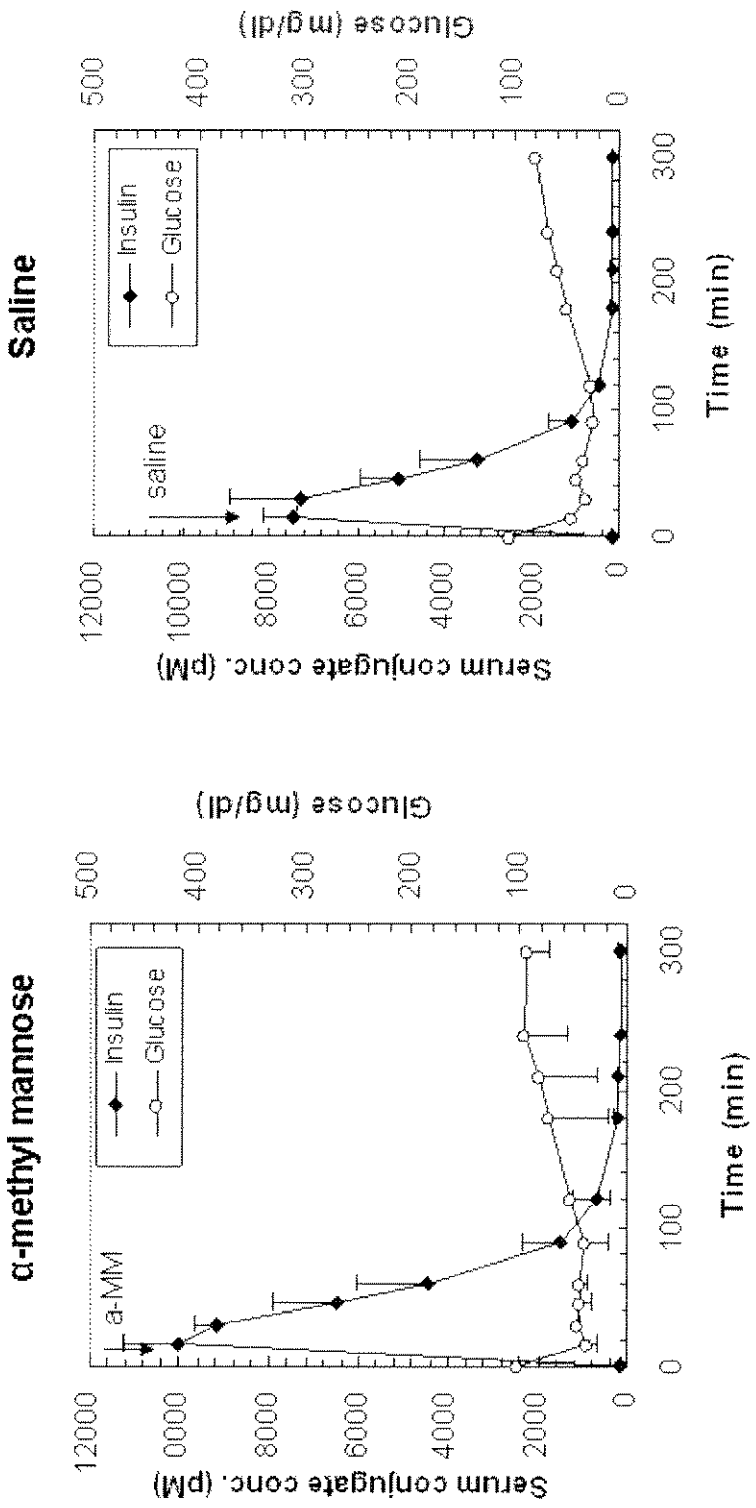

α-methyl mannose
Saline
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
Insulin
Glucose
a-MM
saline
0
2000
4000
6000
8000
10000
12000
100
200
300
400
500

Figure 26

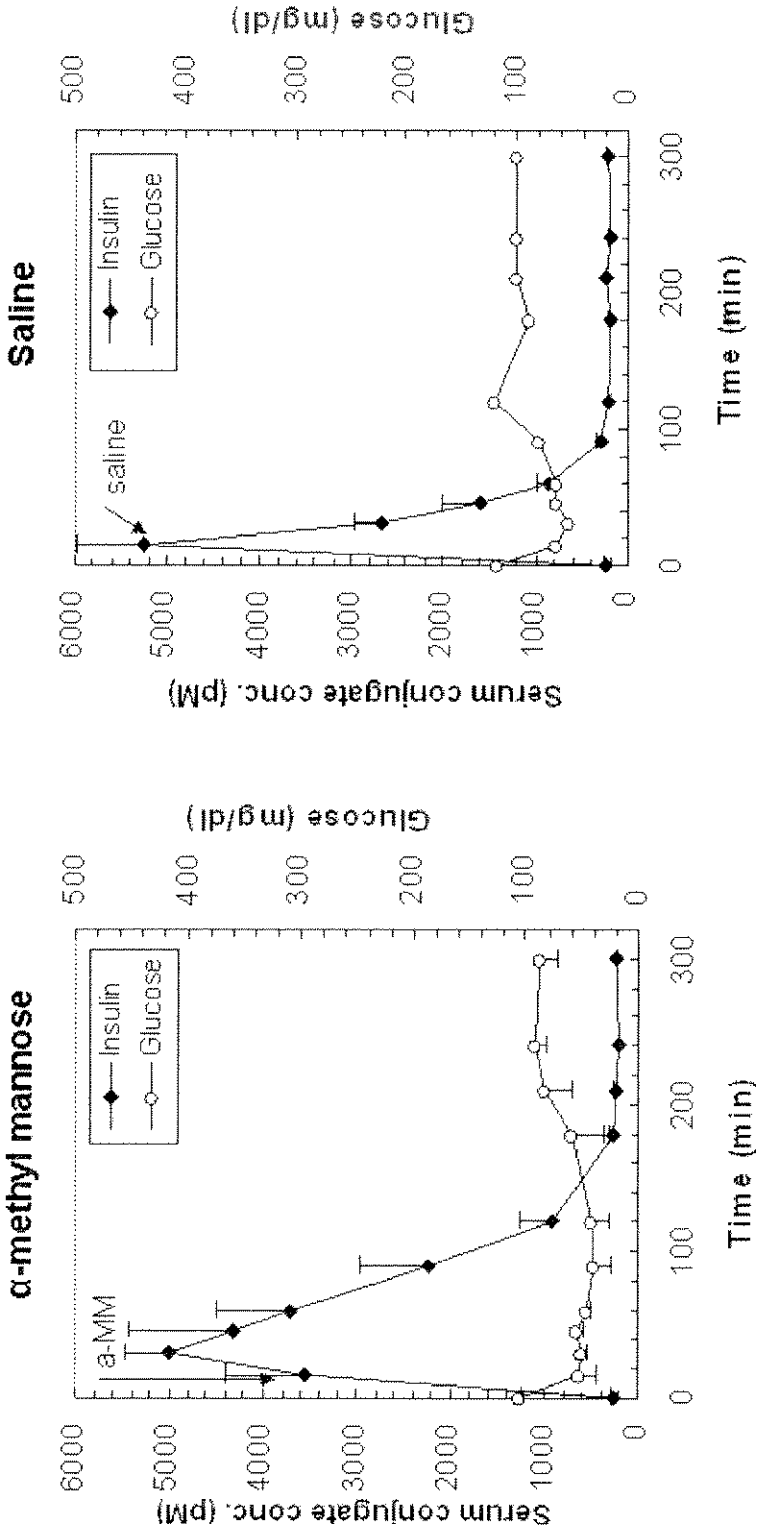

α-methyl mannose
Saline
Insulin
Glucose
a-MM
saline
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
6000
100
200
300
400
500

Figure 27

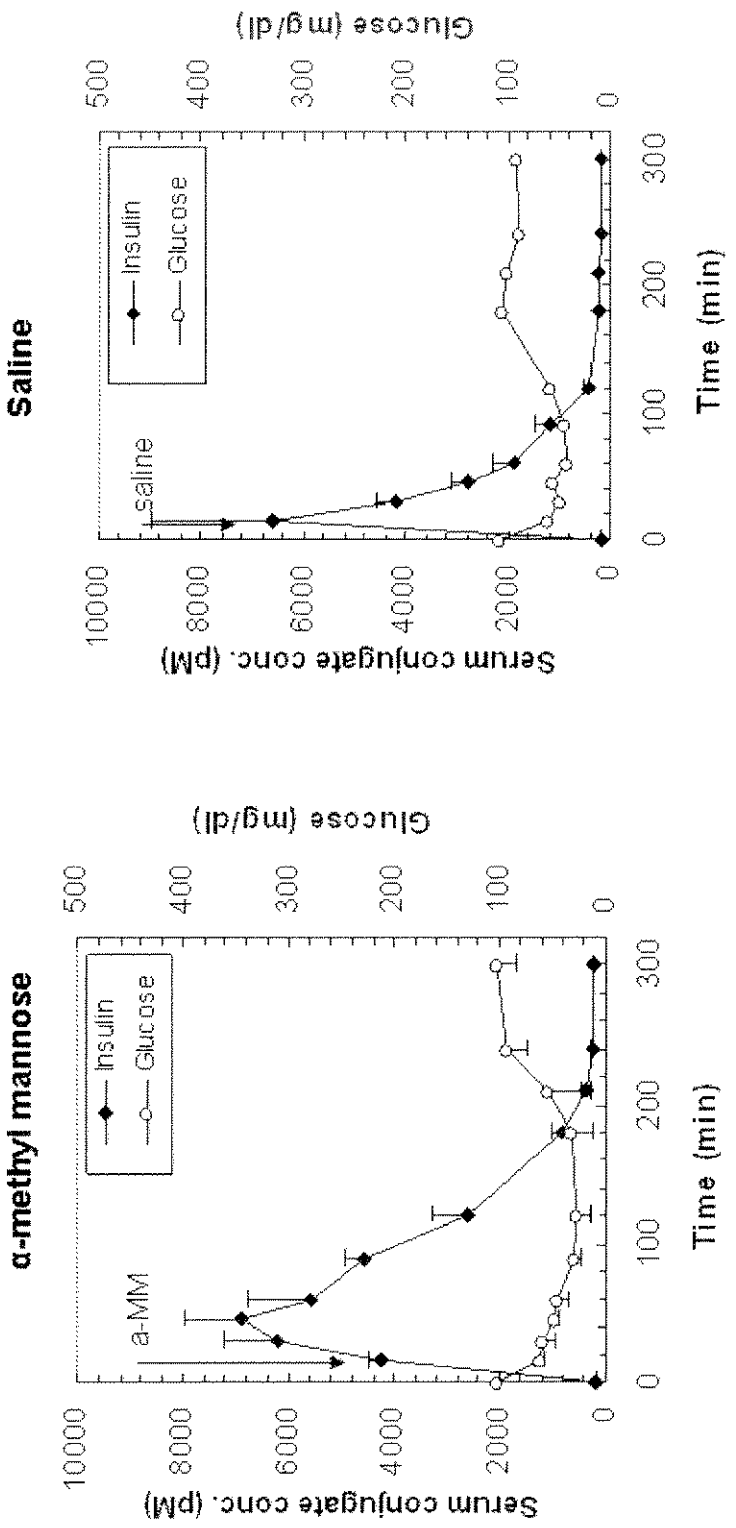

Saline
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
Insulin
Glucose
saline
α-methyl mannose
a-MM

Figure 28

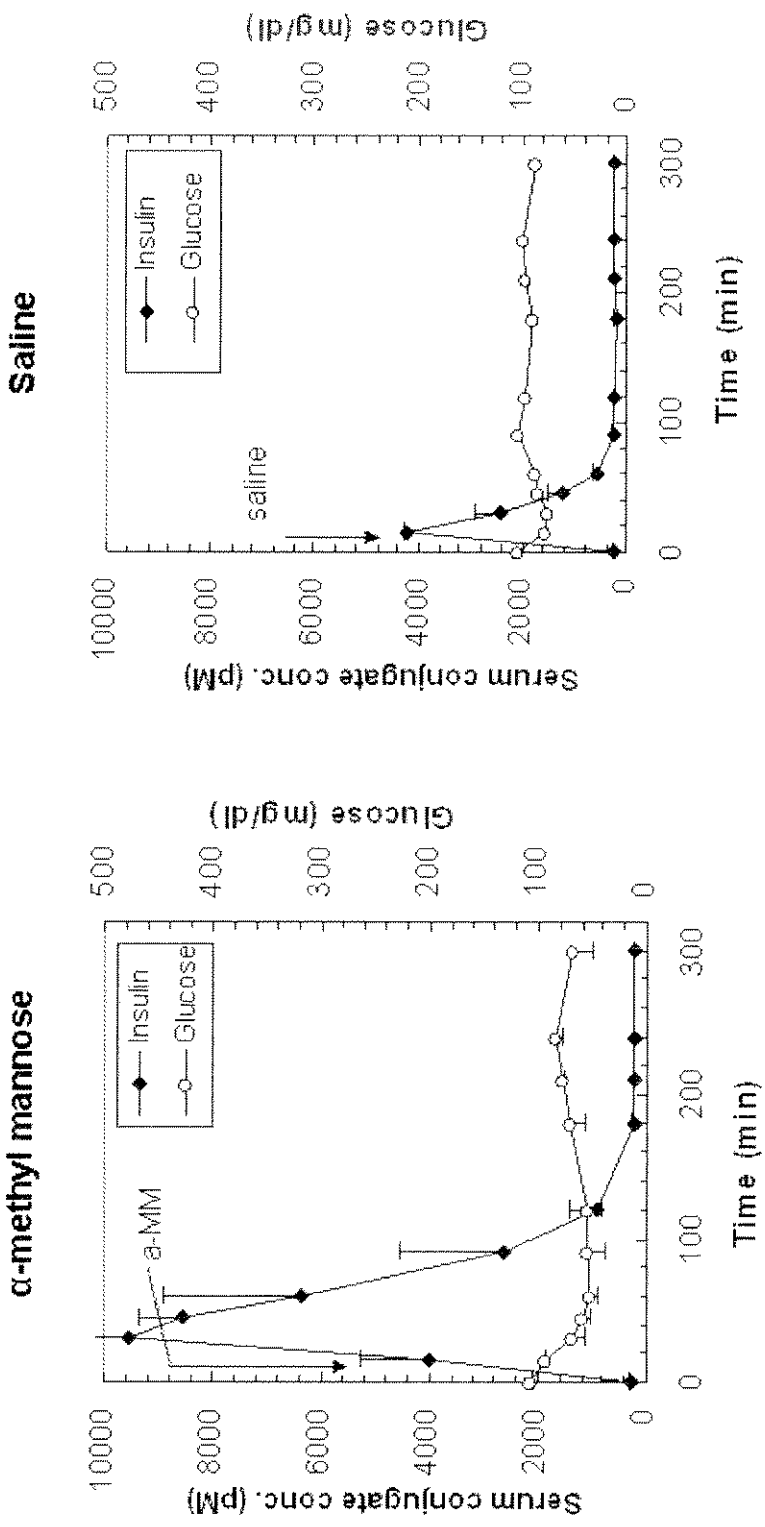

α-methyl mannose
Saline
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
Insulin
Glucose
a-MM
saline
0
2000
4000
6000
8000
10000
100
200
300
400
500

Figure 29

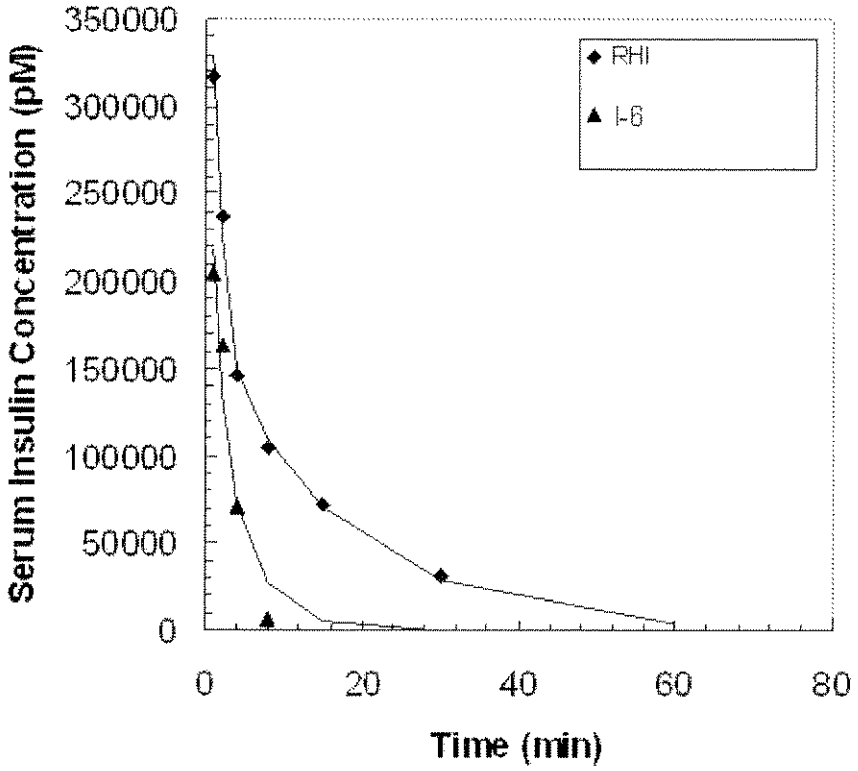
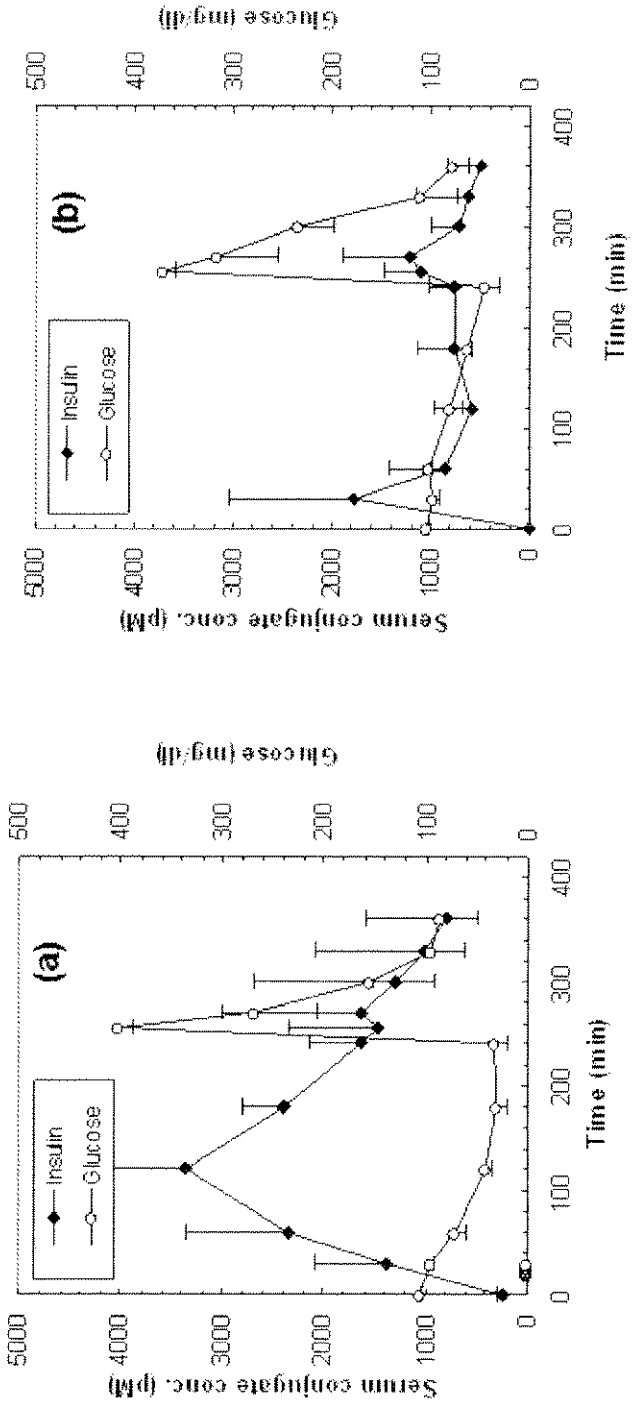

350000
300000
250000
200000
150000
100000
50000
0
Serum Insulin Concentration (pM)
0
20
40
60
80
Time (min)
RHI
I-6

Figure 30


(a)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
100
200
300
400
500

(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
100
200
300
400
500

Figure 31

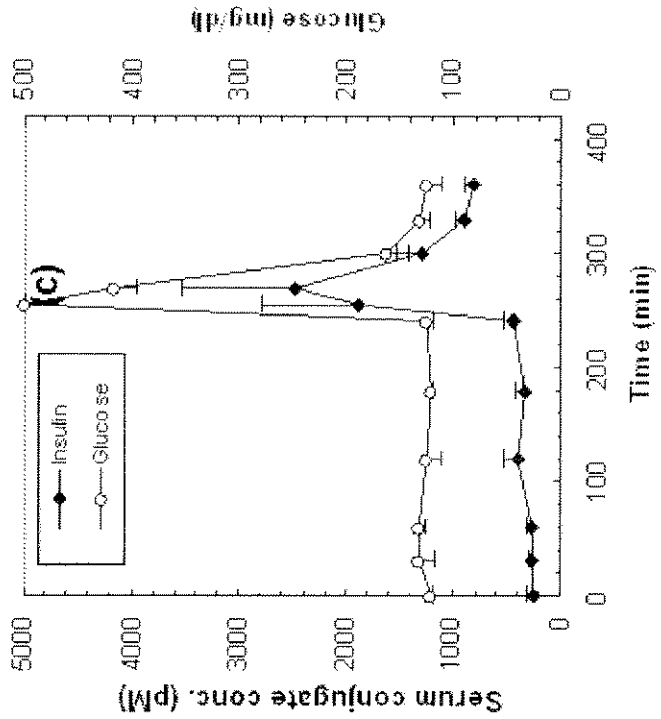


Figure 31 (continued)

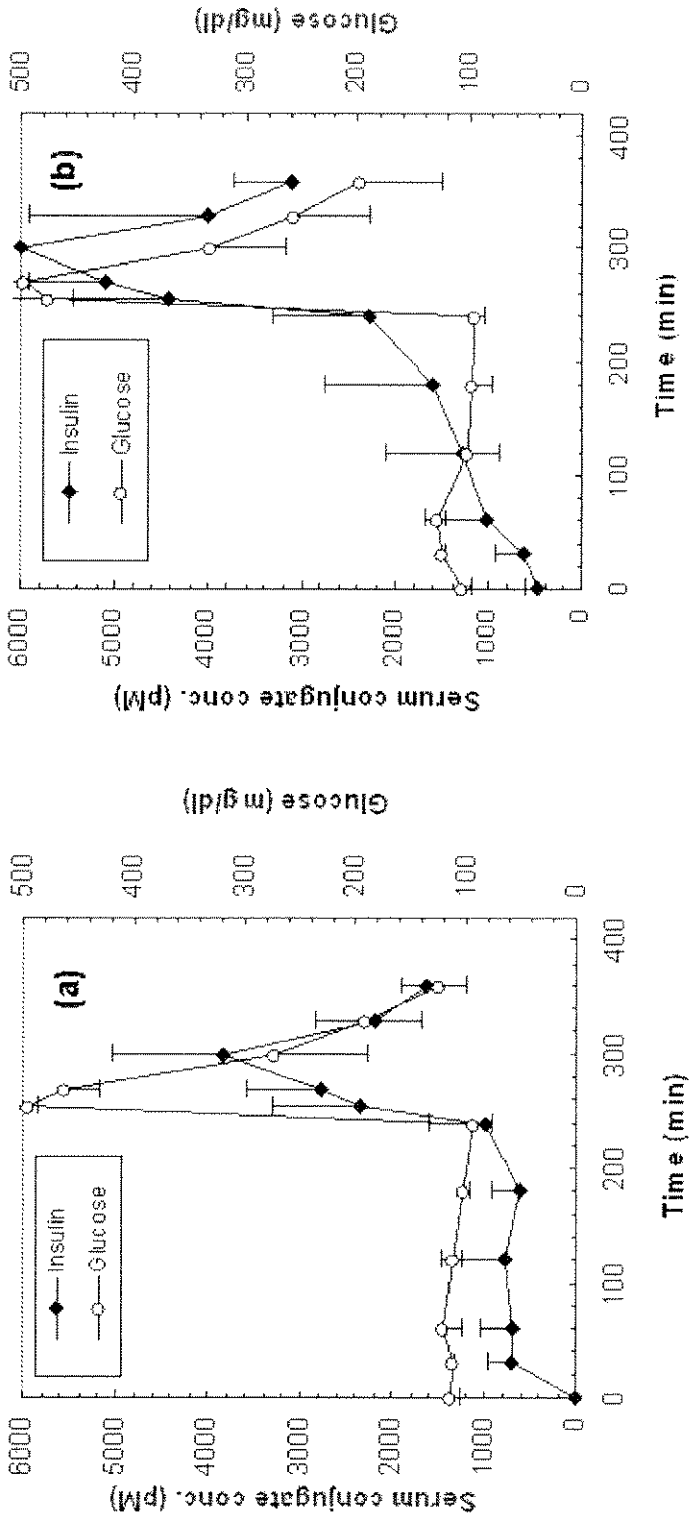

(a)
(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
6000
100
200
300
400
500

Figure 32

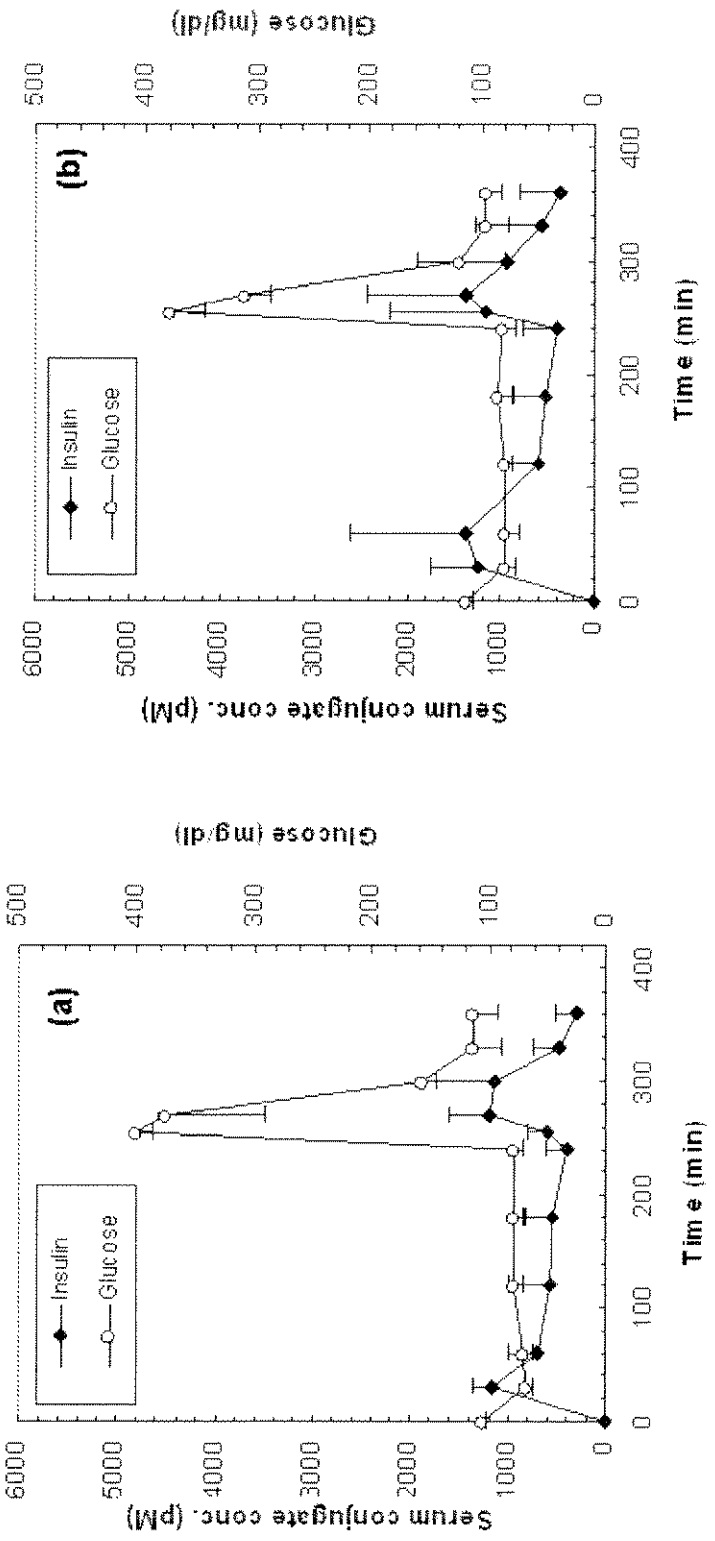

(a)
(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
6000
100
200
300
400
500

Figure 33

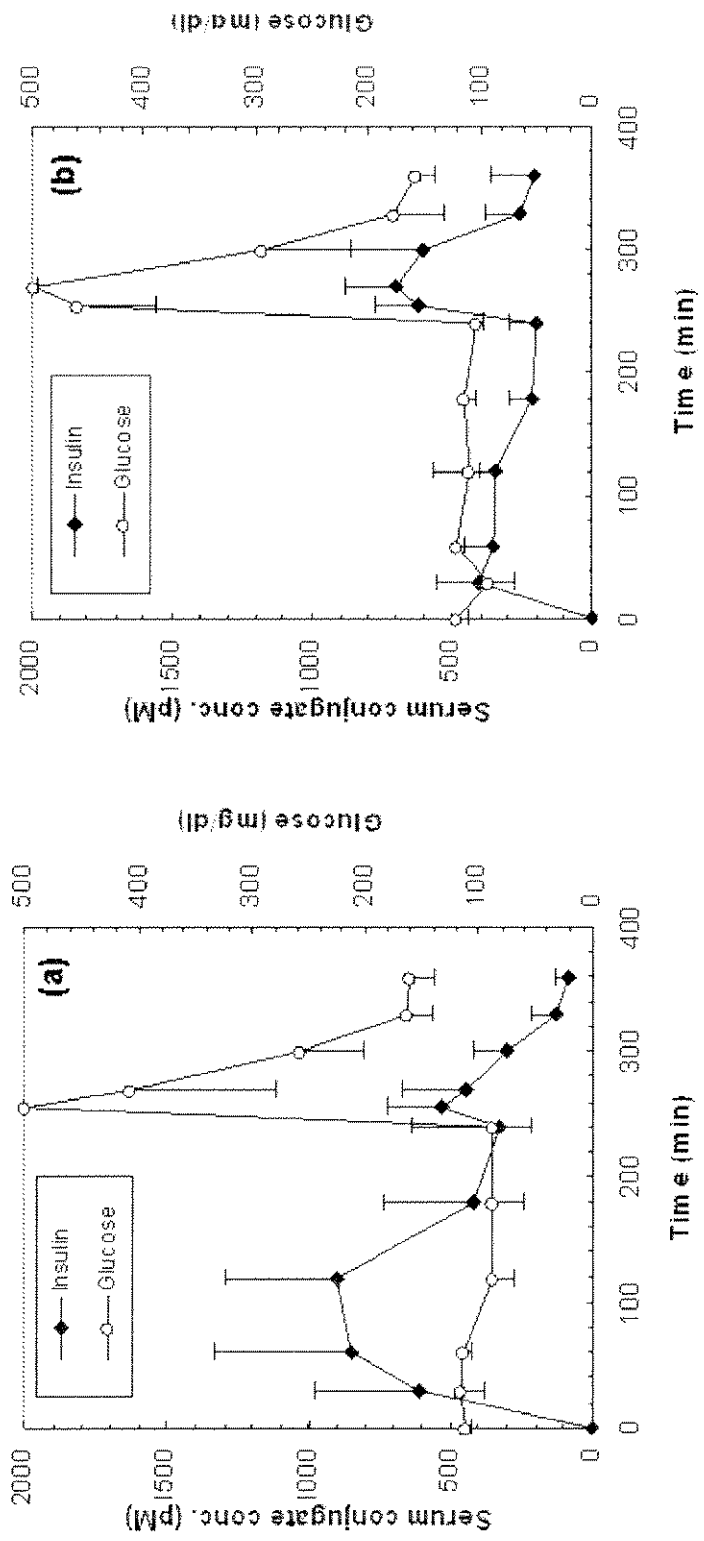

(a)
(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
100
200
300
400
500
1000
1500
2000

Figure 34

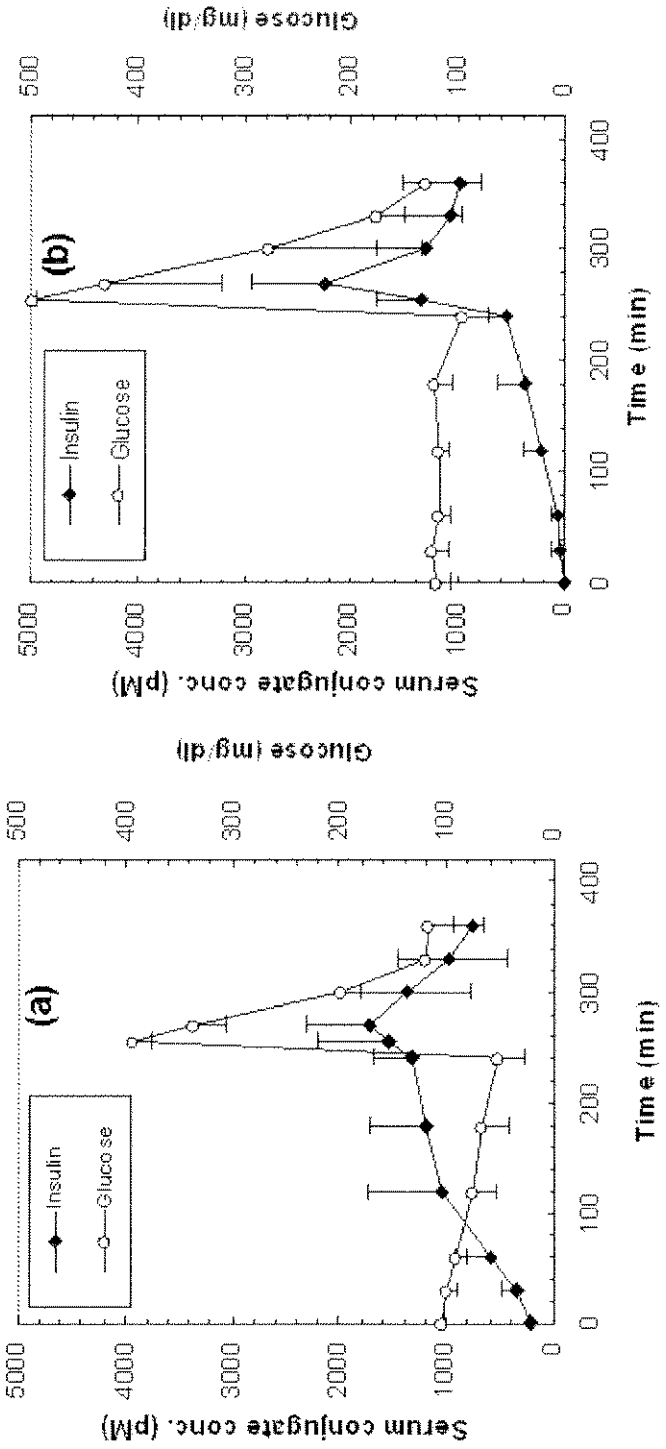

(a)
(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
100
200
300
400
500

Figure 35

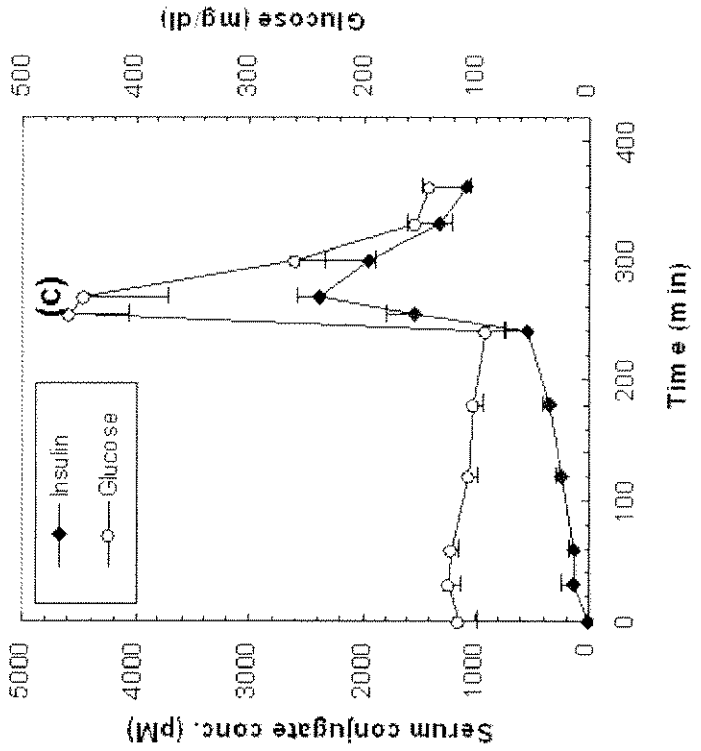


Figure 35 (continued)

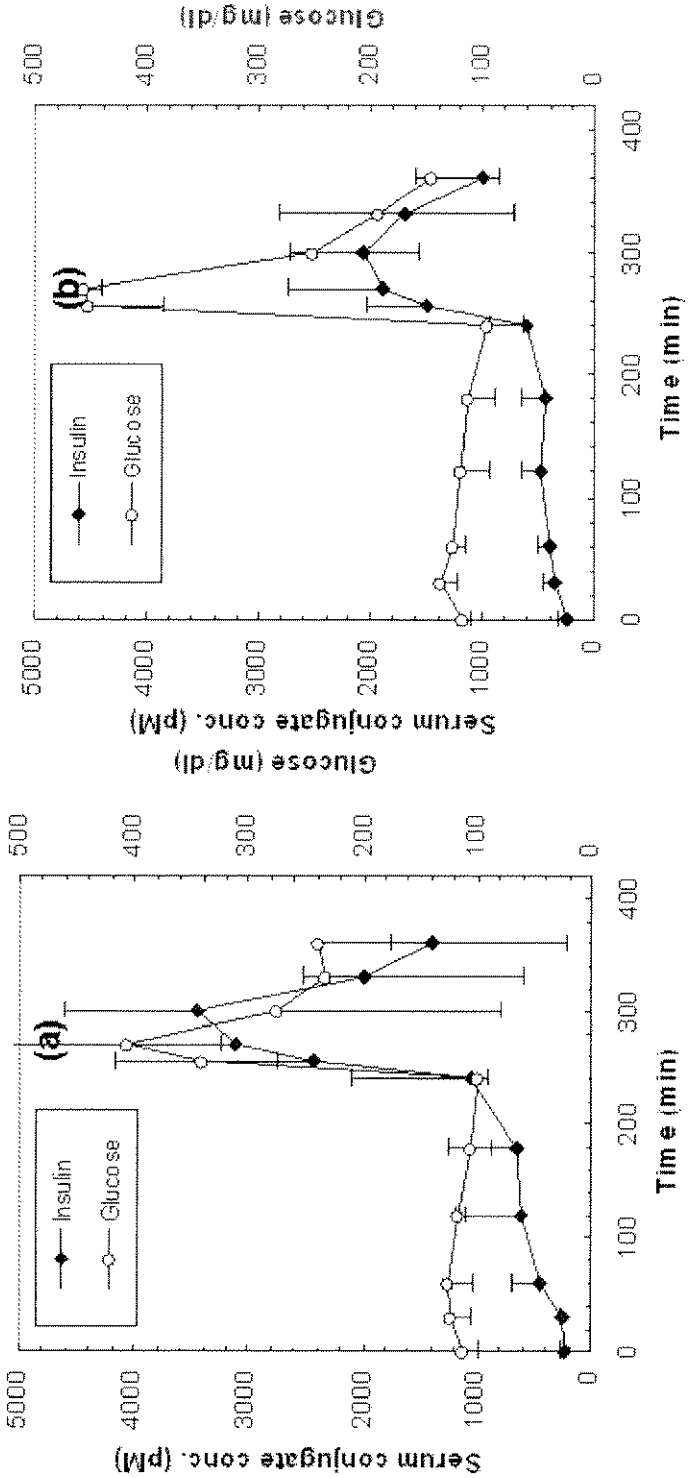

(a)
(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
100
200
300
400
500

Figure 36

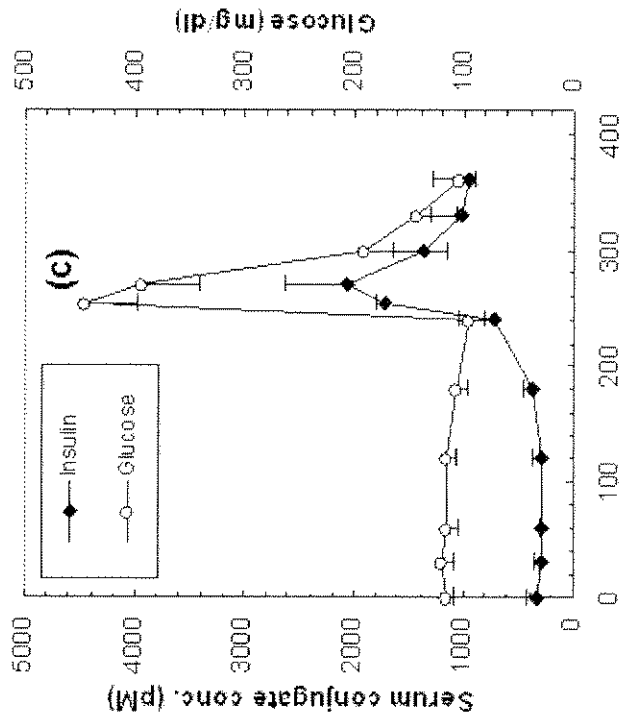


Figure 36 (continued)

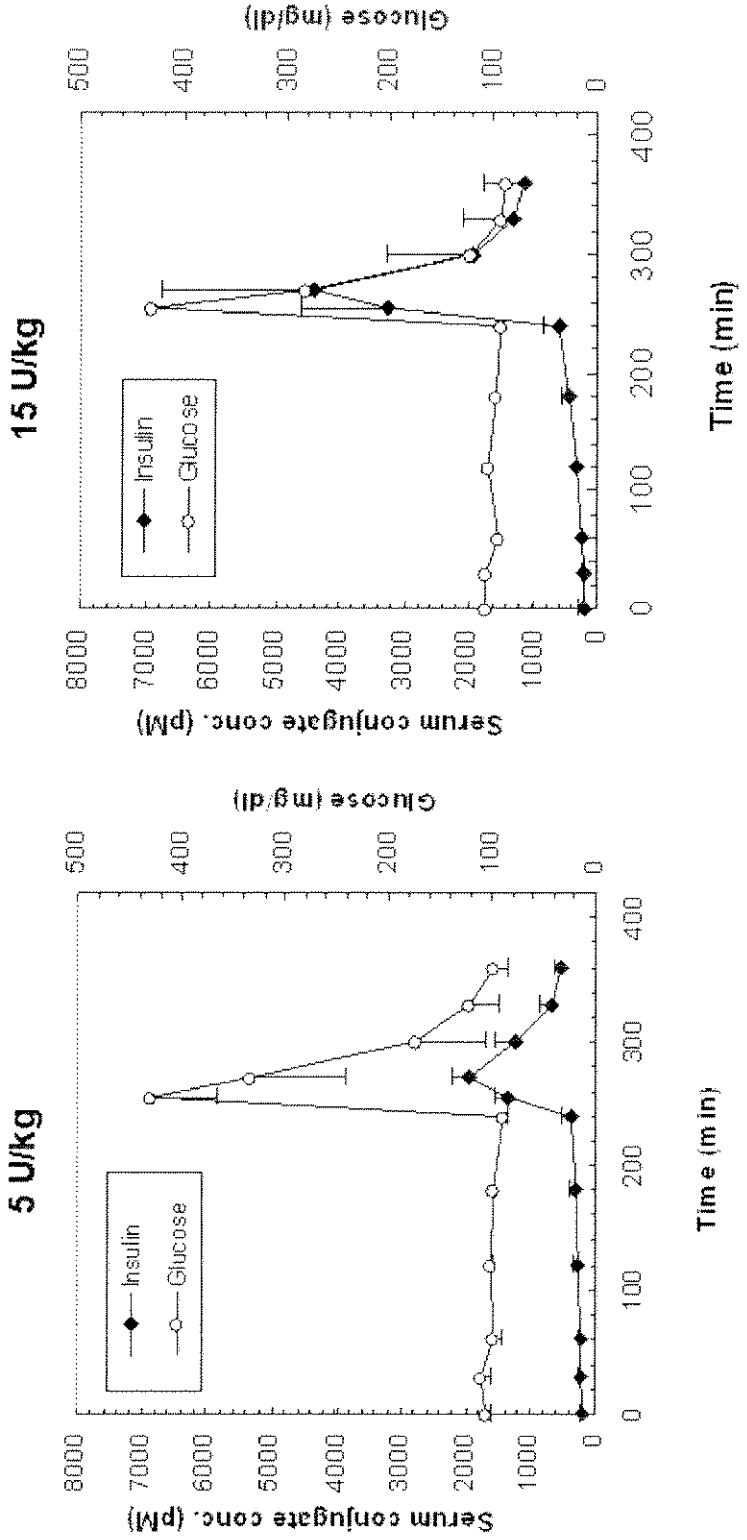

5 U/kg
15 U/kg
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
6000
7000
8000
100
200
300
400
500

Figure 37

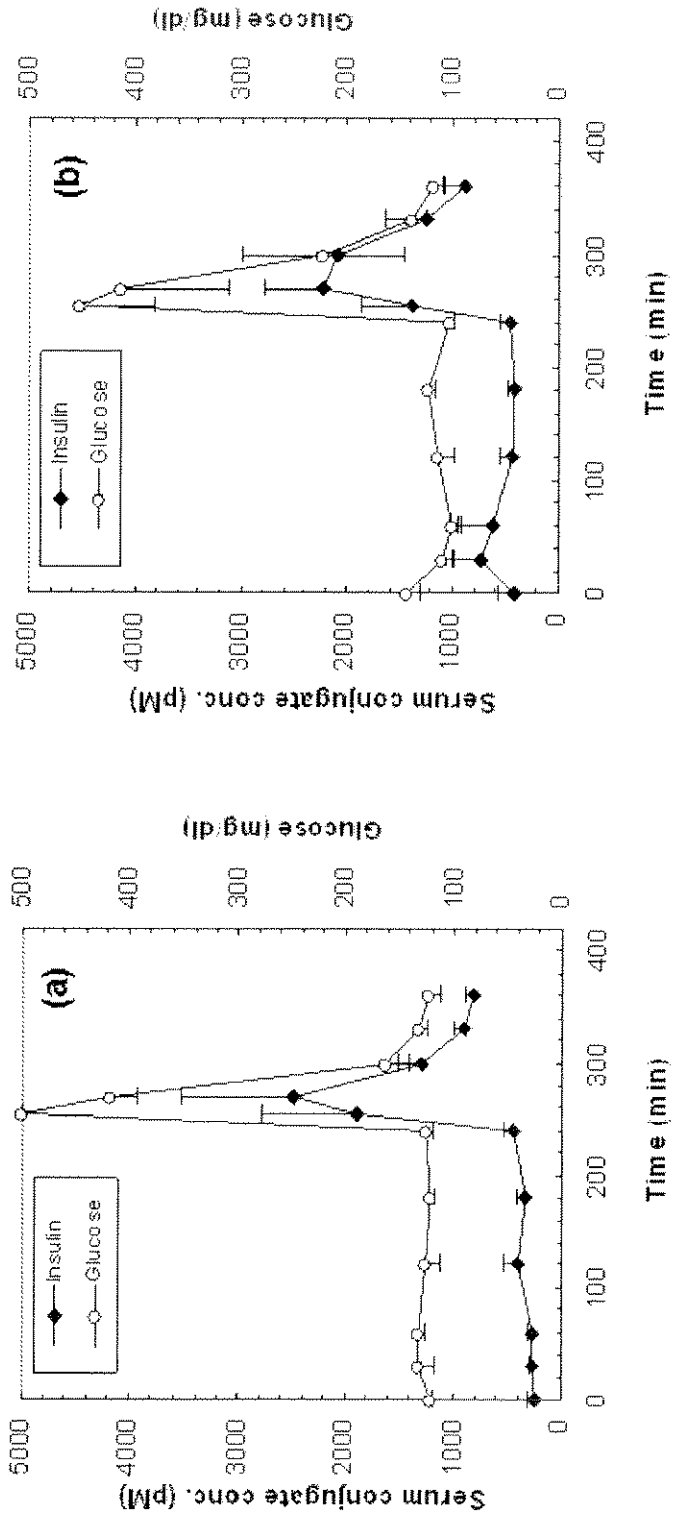

(a)
(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
100
200
300
400
500

Figure 38

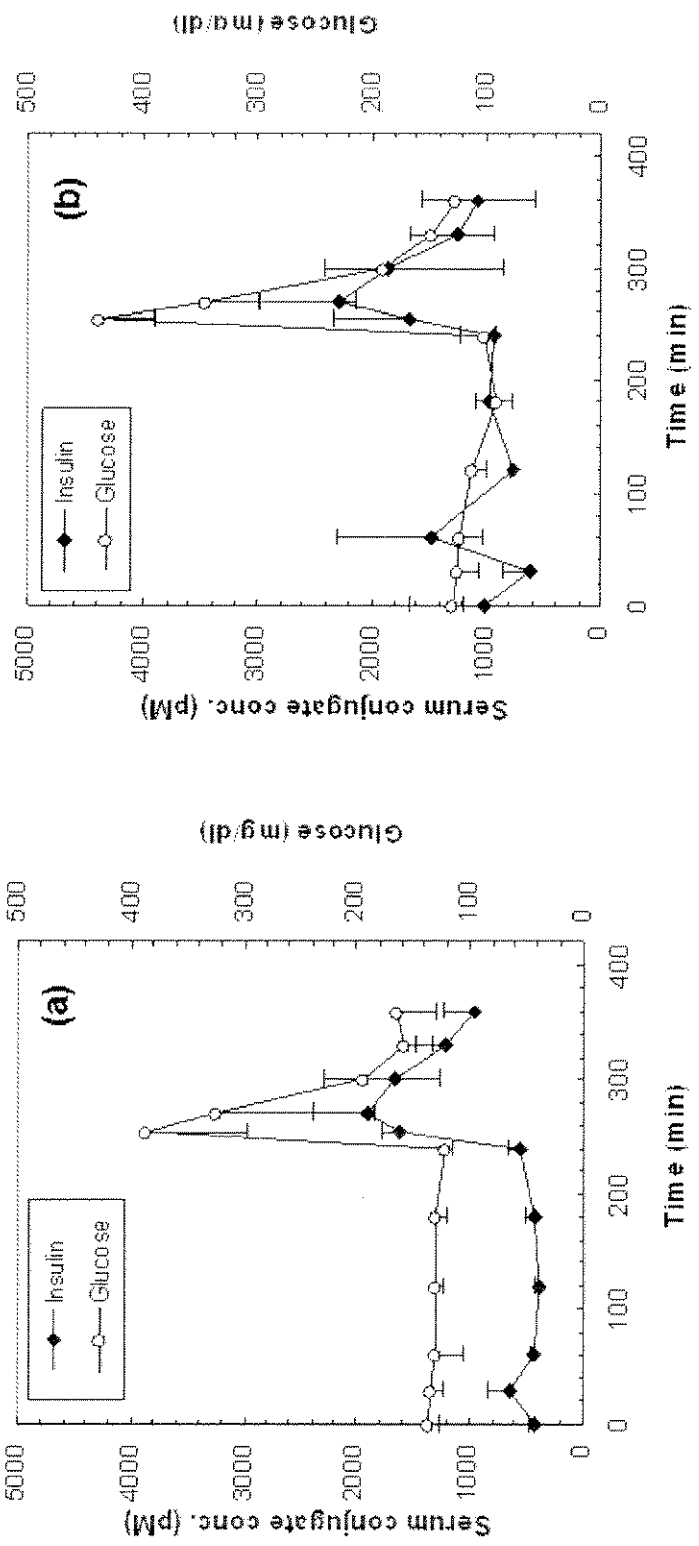
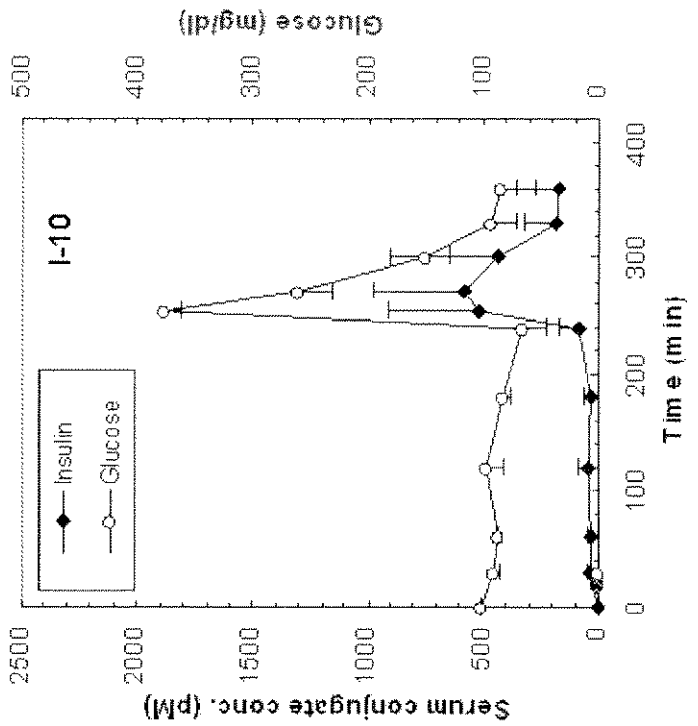

(a)
(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
100
200
300
400
500

Figure 39


I-10
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
500
1000
1500
2000
2500
100
200
300
400

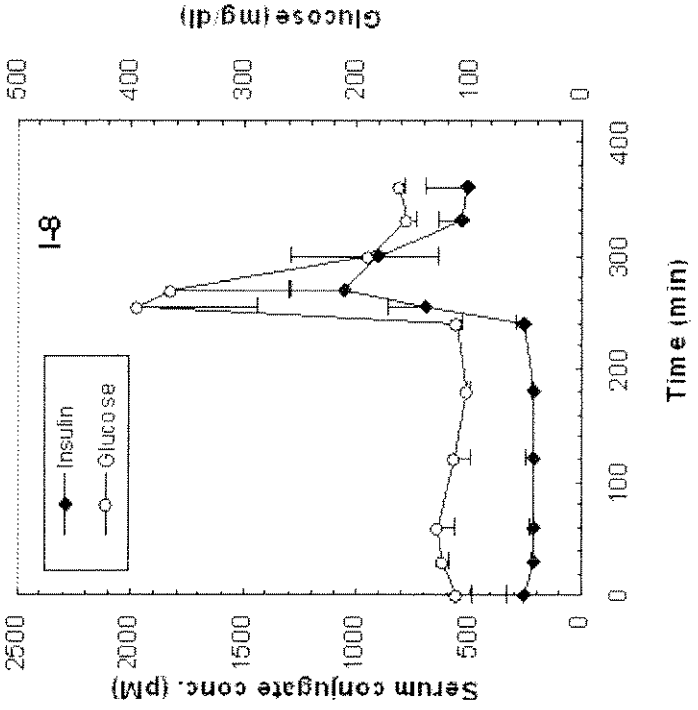

I-8
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
500
1000
1500
2000
2500
100
200
300
400

Figure 40

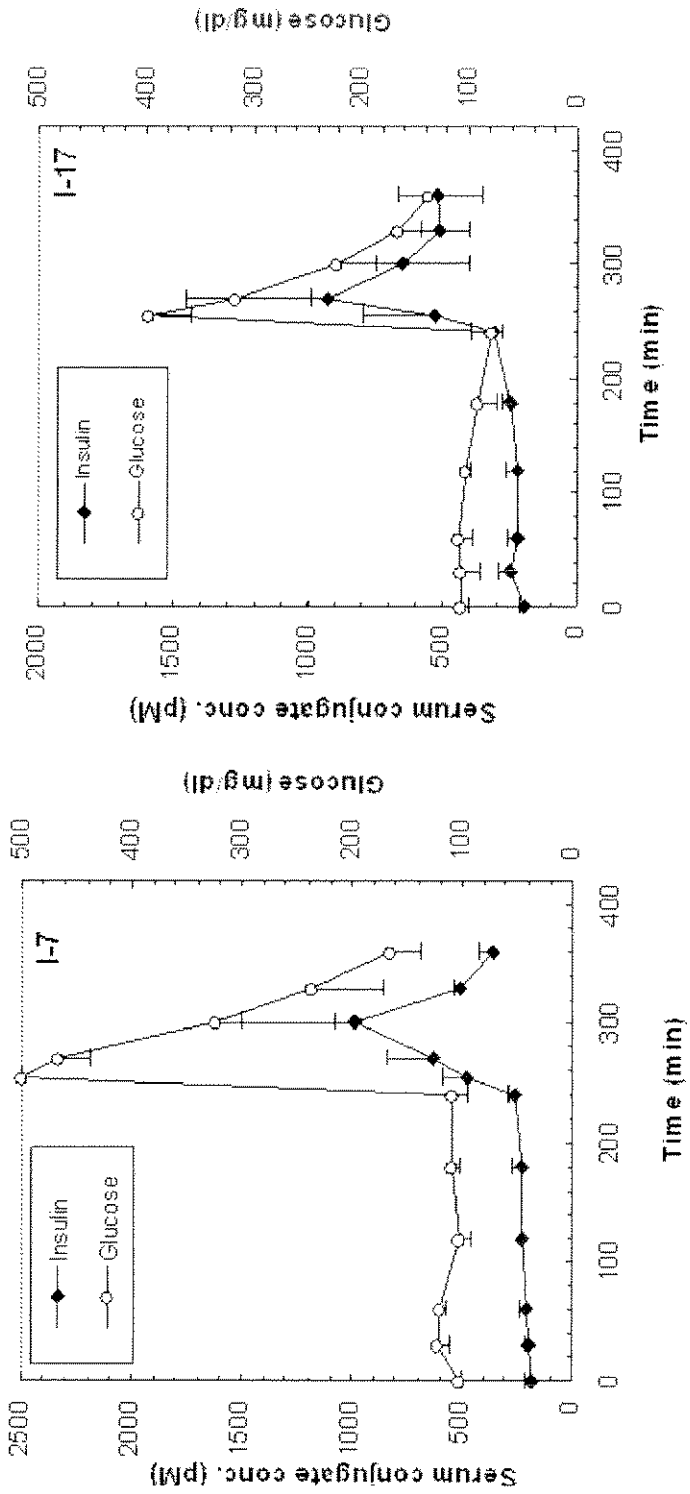


Figure 40 (continued)

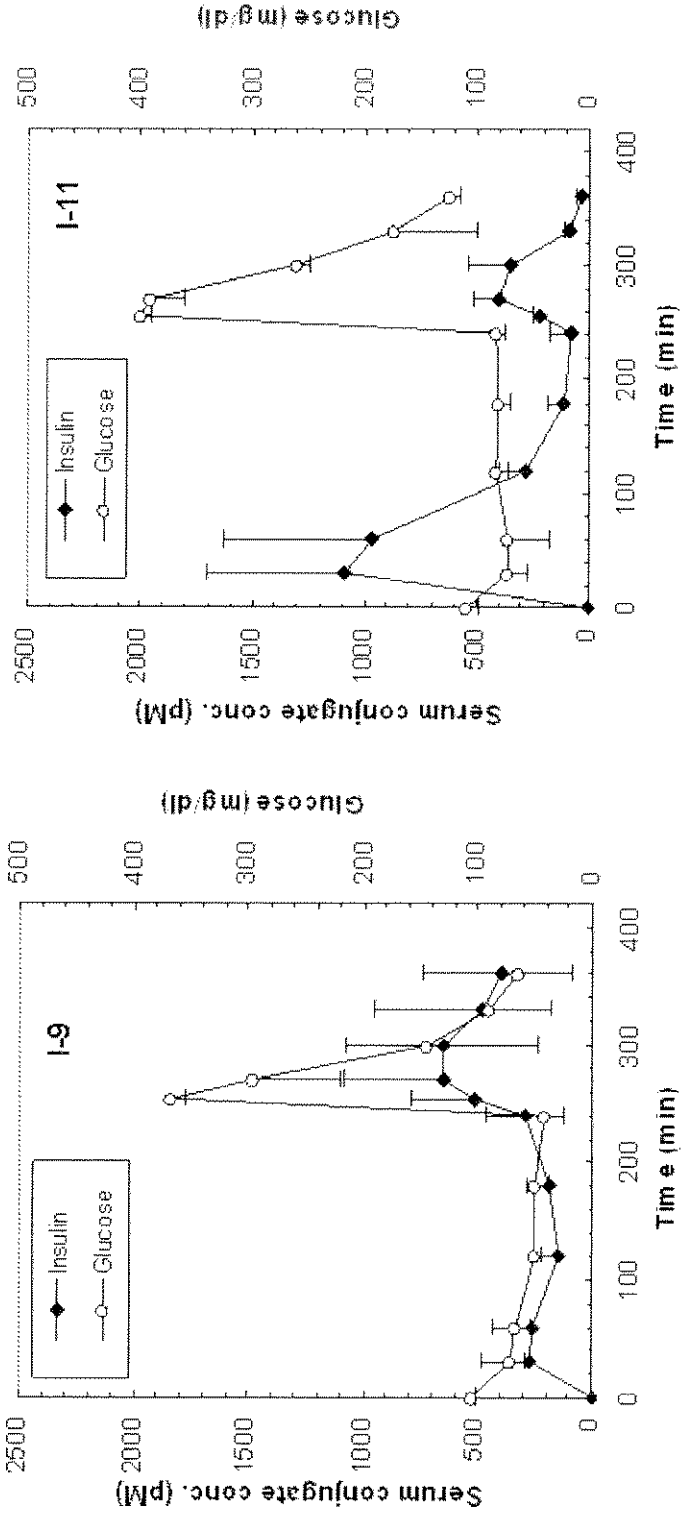


Figure 40 (continued)

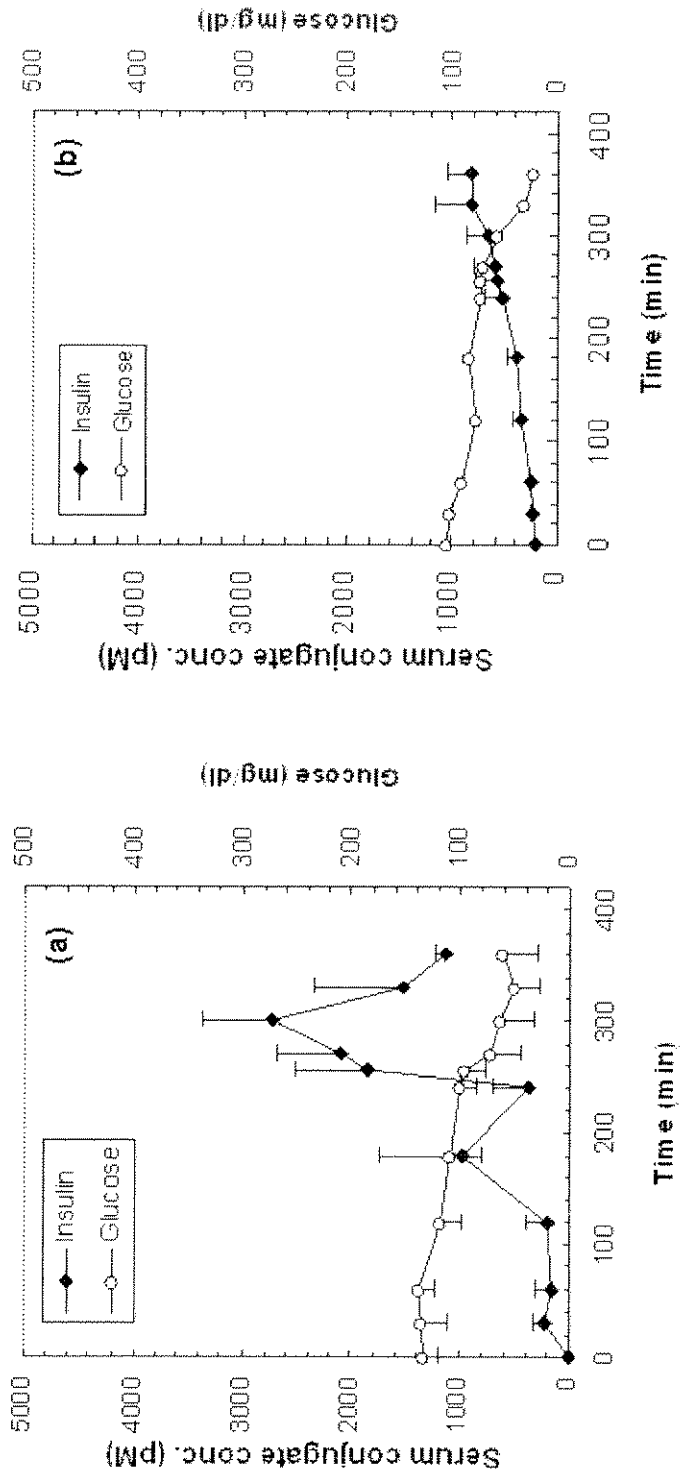
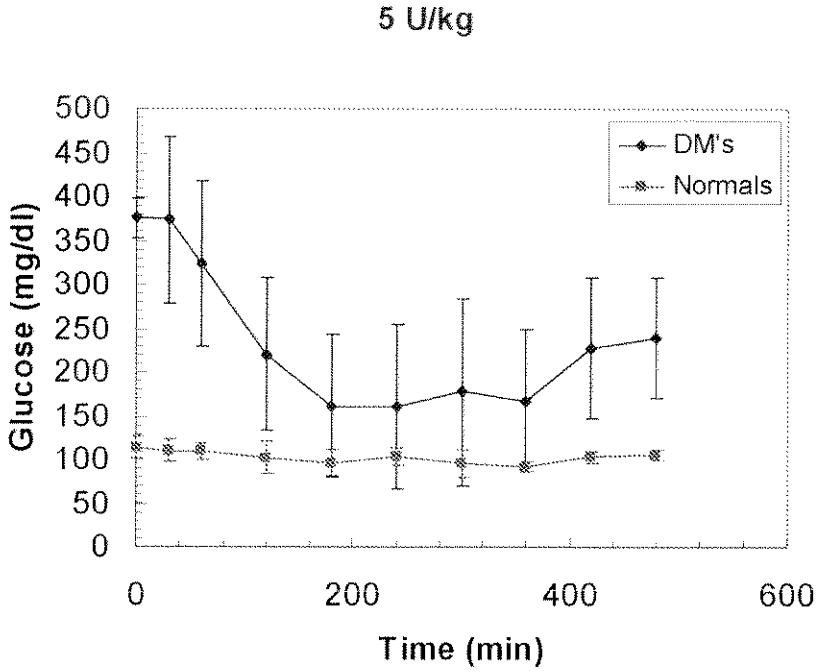

(a)
(b)
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
100
200
300
400
500

Figure 41


5 U/kg
Glucose (mg/dl)
500
450
400
350
300
250
200
150
100
50
0
0
200
400
600
Time (min)
DM's
Normals

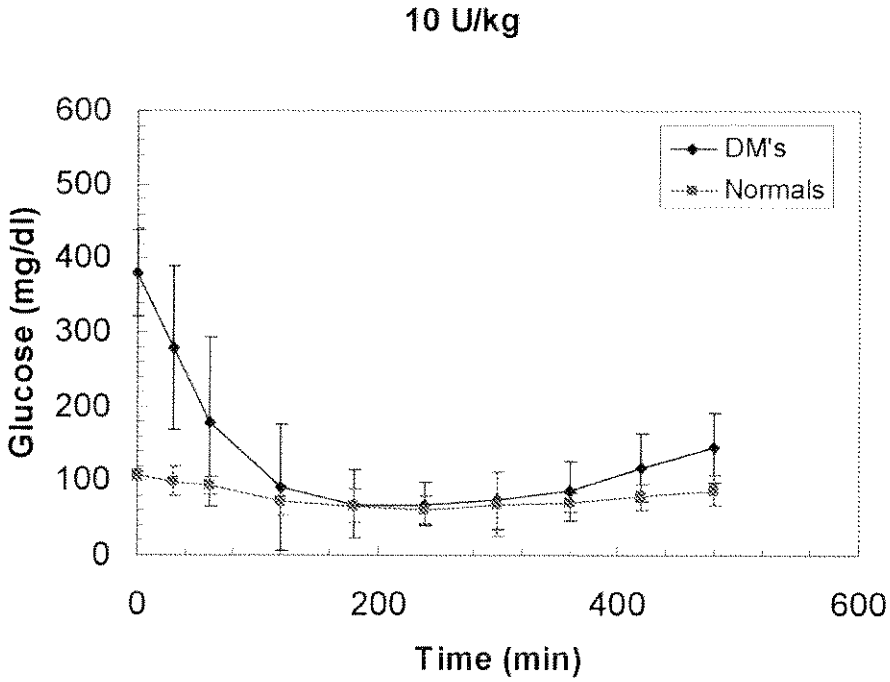

10 U/kg
Glucose (mg/dl)
600
500
400
300
200
100
0
0
200
400
600
Time (min)
DM's
Normals

Figure 42

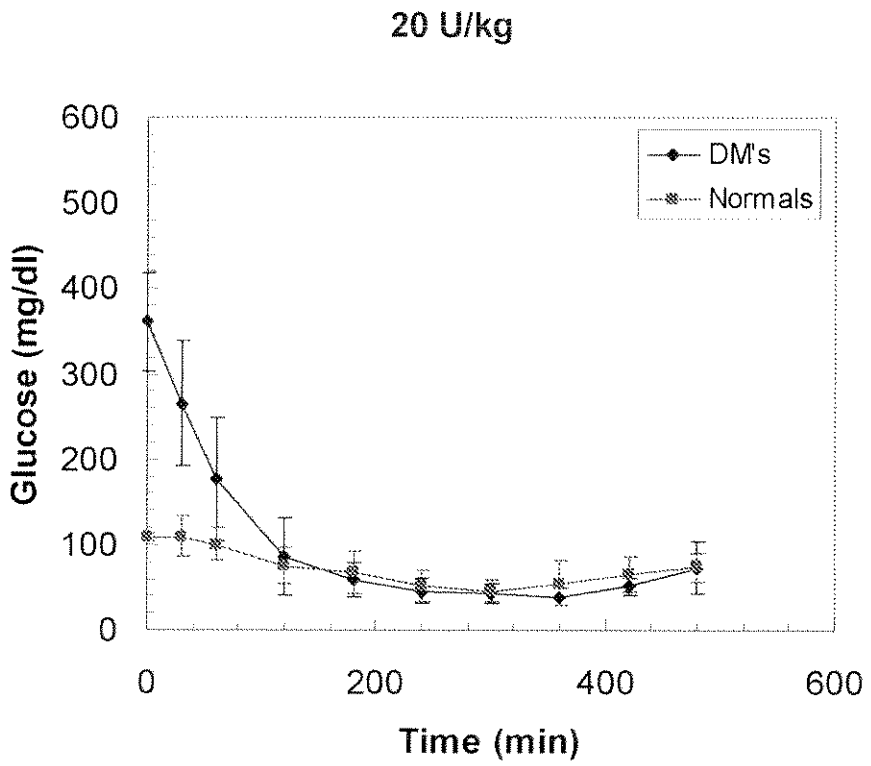


Figure 42 (continued)

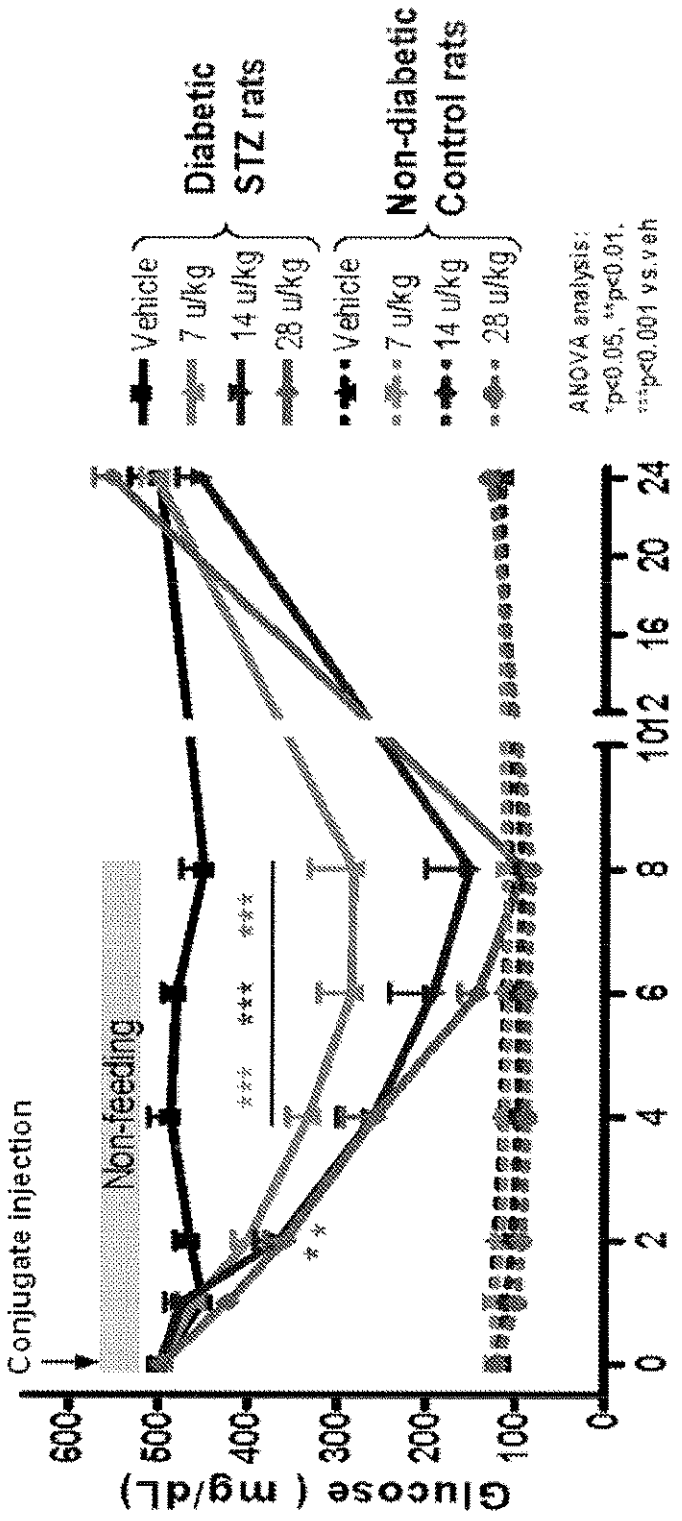

Conjugate injection
Non-feeding
Glucose (mg/dL)
Time (hour)
Vehicle
7 u/kg
14 u/kg
28 u/kg
Diabetic STZ rats
Vehicle
7 u/kg
14 u/kg
28 u/kg
Non-diabetic Control rats
ANOVA analysis:
*p<0.05, **p<0.01,
***p<0.001 vs.veh

Figure 43

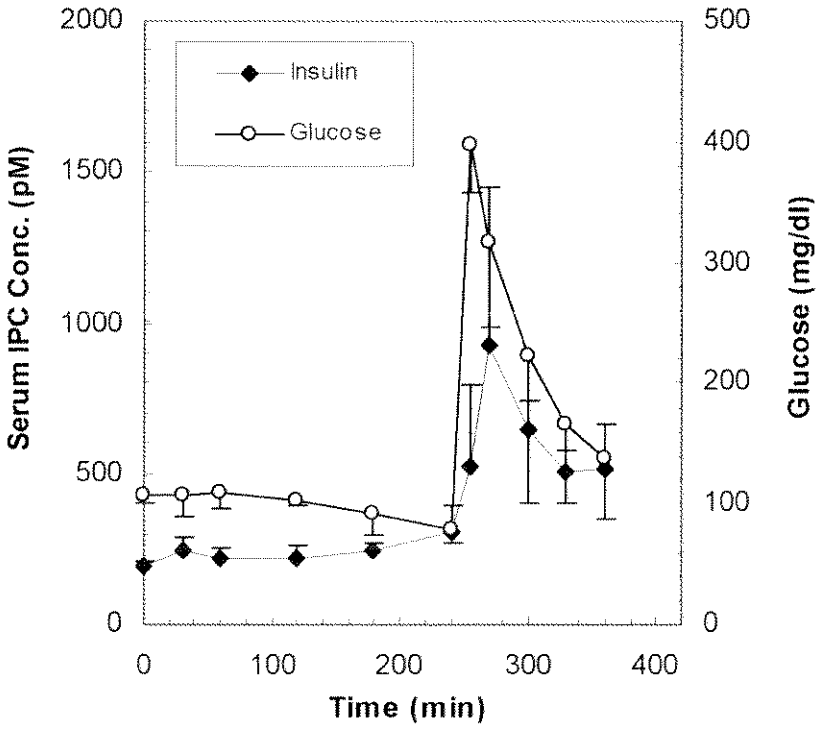
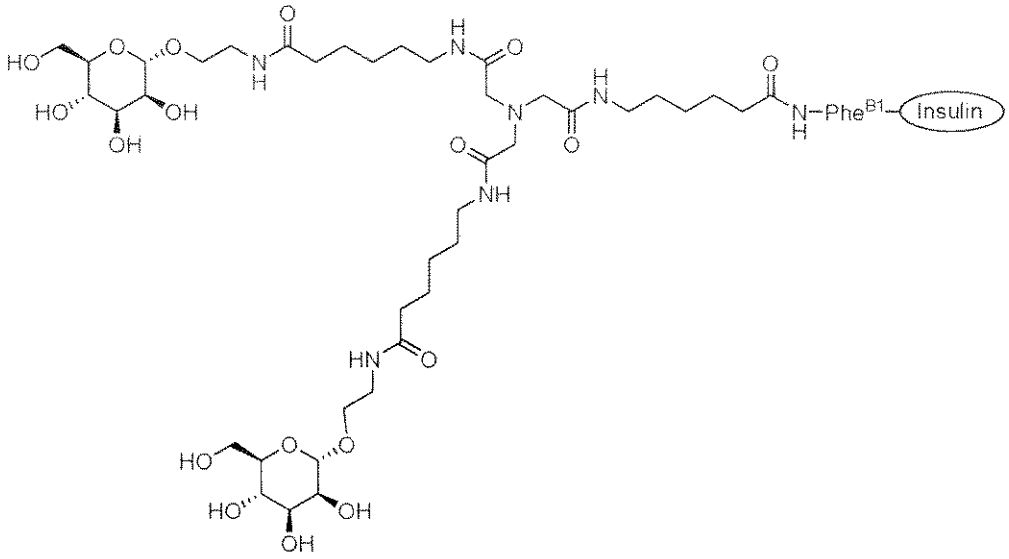
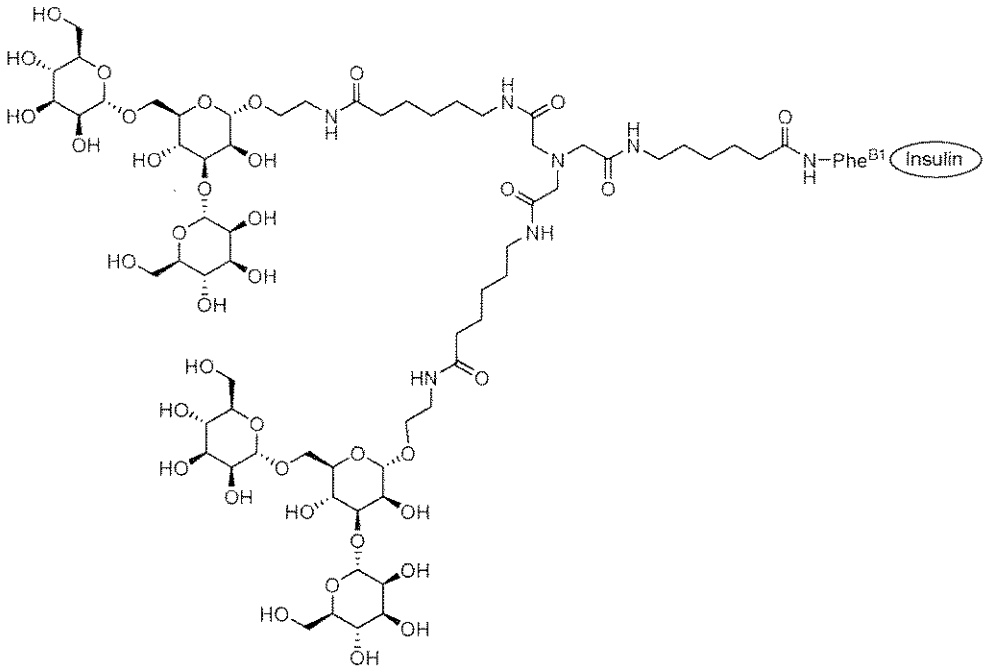

Insulin
Glucose
Serum IPC Conc. (pM)
Glucose (mg/dl)
Time (min)
2000
1500
1000
500
0
500
400
300
200
100
0
0
100
200
300
400

Figure 44

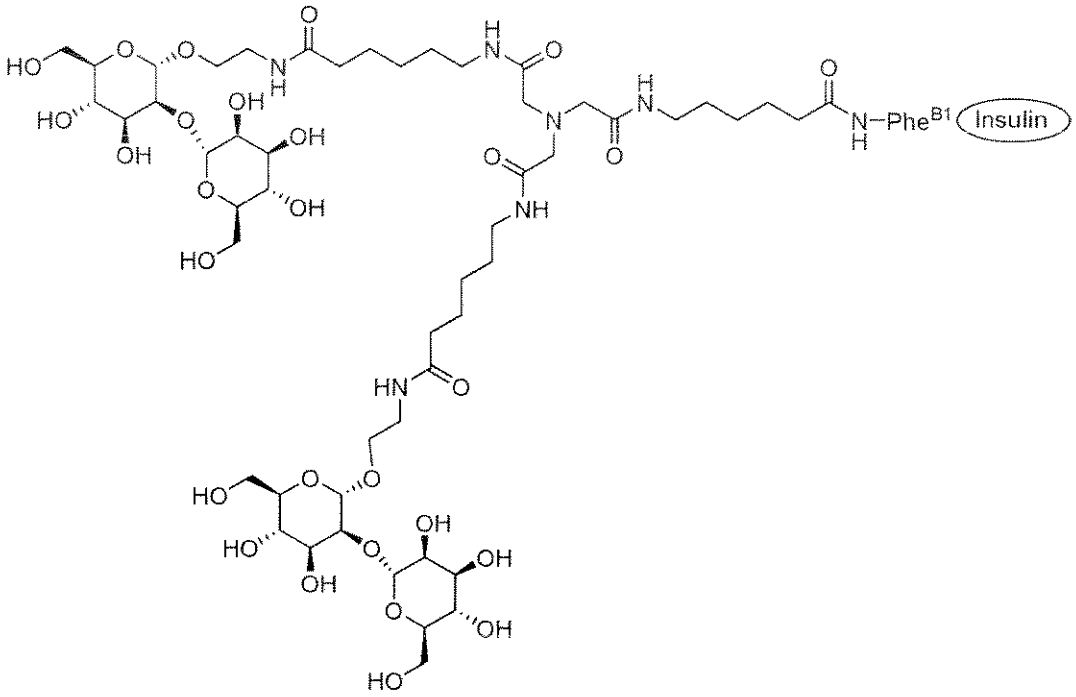


Conjugate I-1
TSAT-C6-AEM-2 (B1)

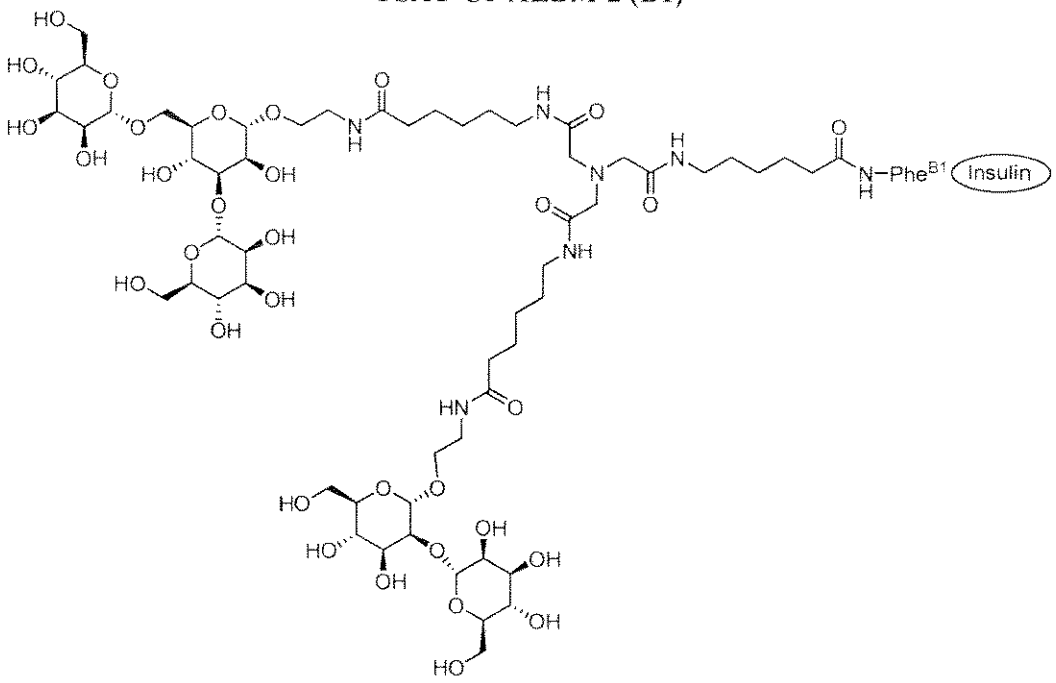


Conjugate I-2
TSAT-C6-AETM-2 (B1)

Figure 45

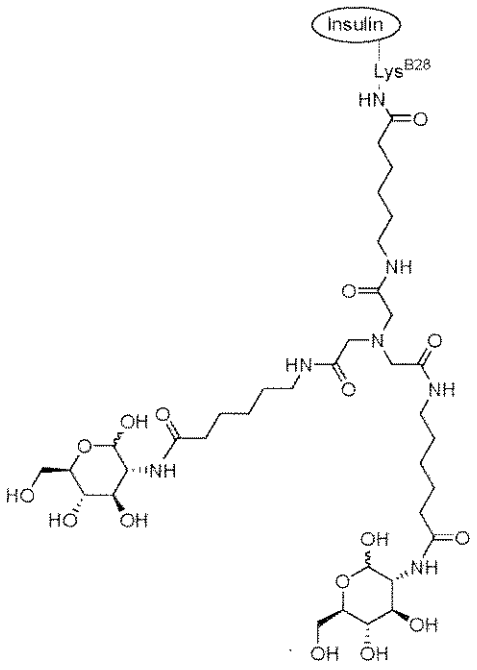


Conjugate I-3
TSAT-C6-AEBM-2 (B1)



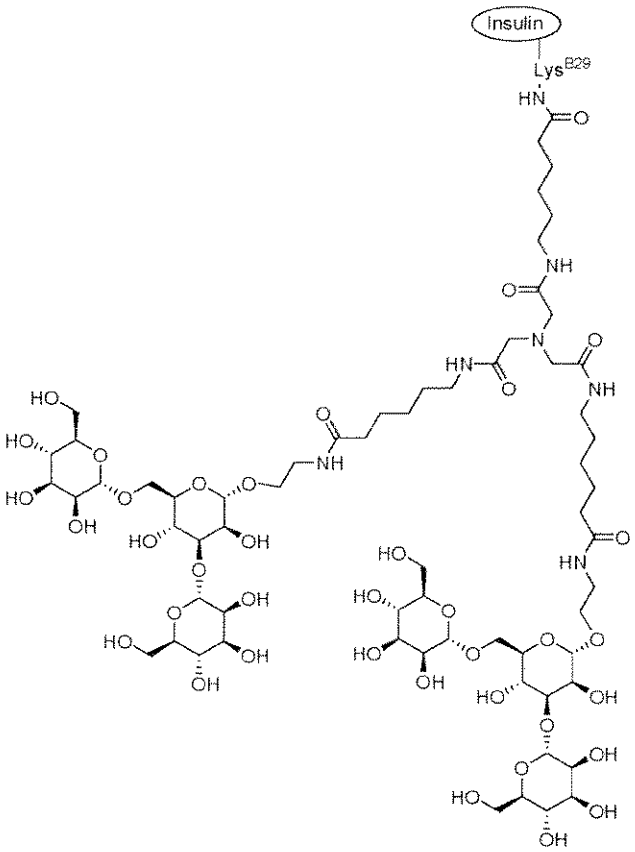
Conjugate I-4
TSAT-C6-AEBM-1-AETM-1 (B1)

Figure 45 (continued)



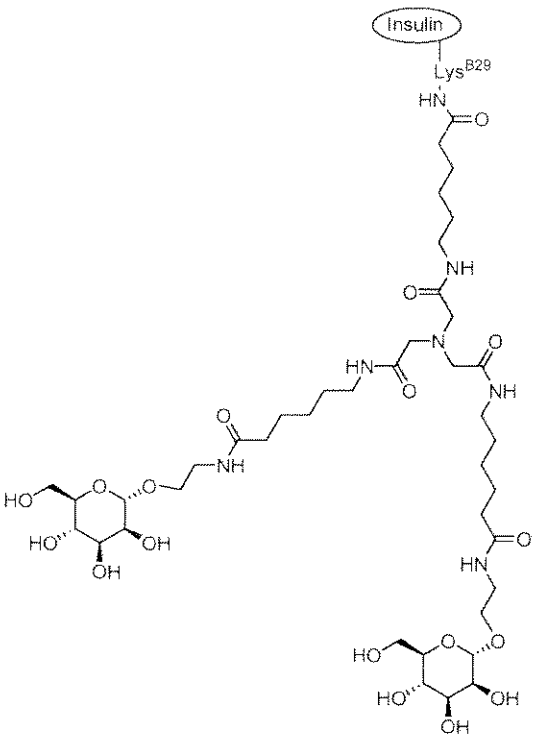
Conjugate I-5
TSAT-C6-GA-2 (B29)

Figure 45 (continued)

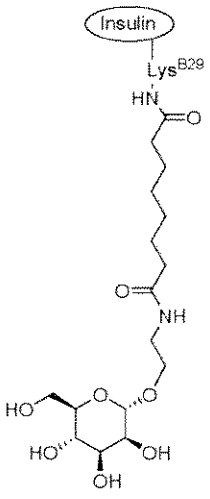


Conjugate I-6
TSAT-C6-AETM-2 (B29)

Figure 45 (continued)

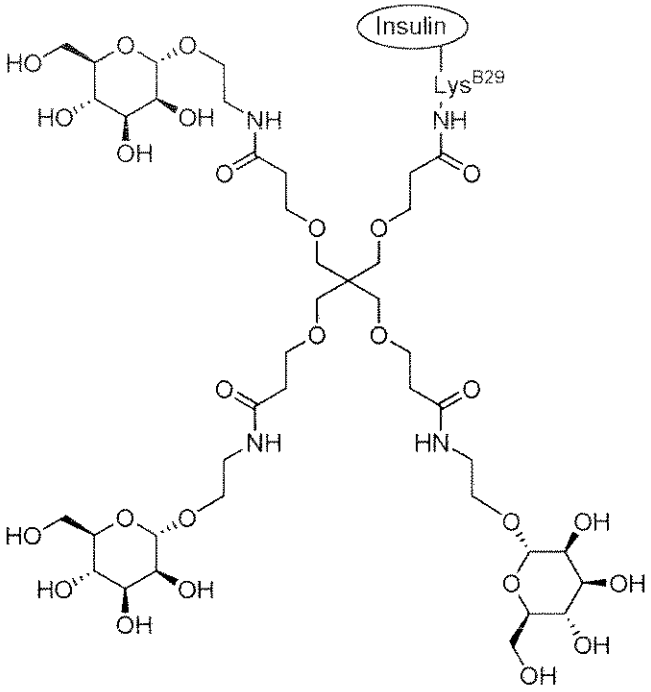


Conjugate I-7
TSAT-C6-AEM-2 (B29)

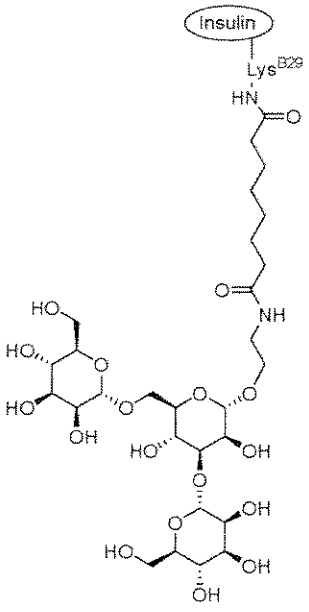


Conjugate I-8
DSS-AEM-1 (B29)

Figure 45 (continued)

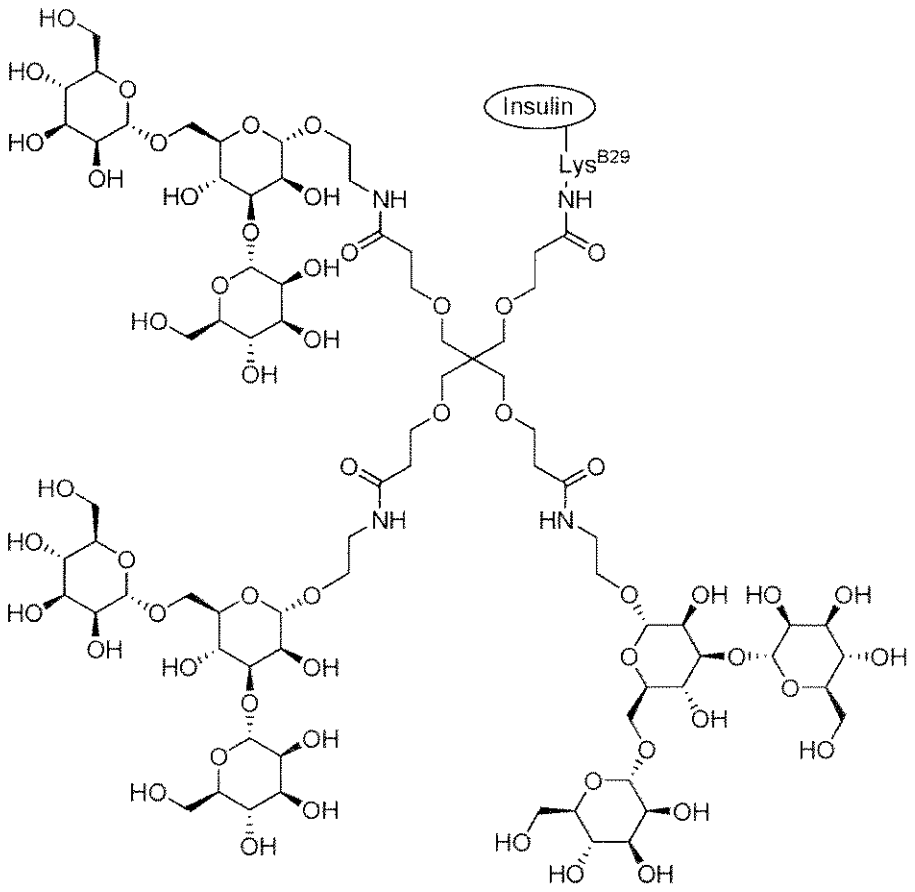


Conjugate I-9
TSPE-AEM-3 (B29)



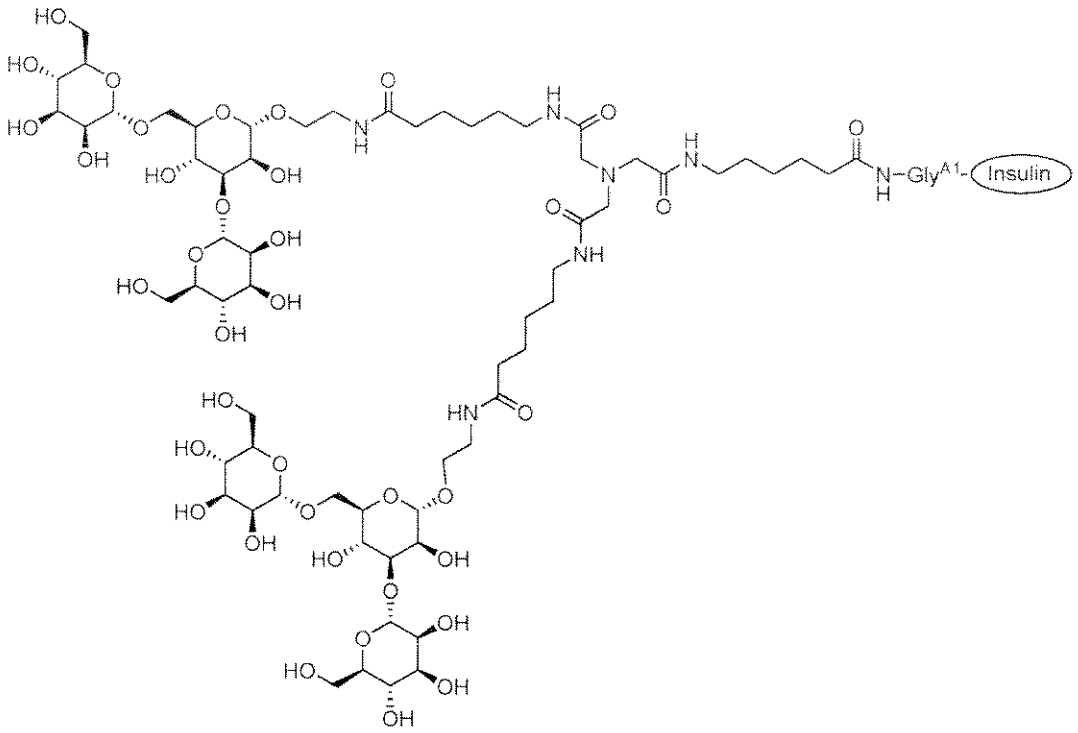
Conjugate I-10
DSS-AETM-1 (B29)

Figure 45 (continued)

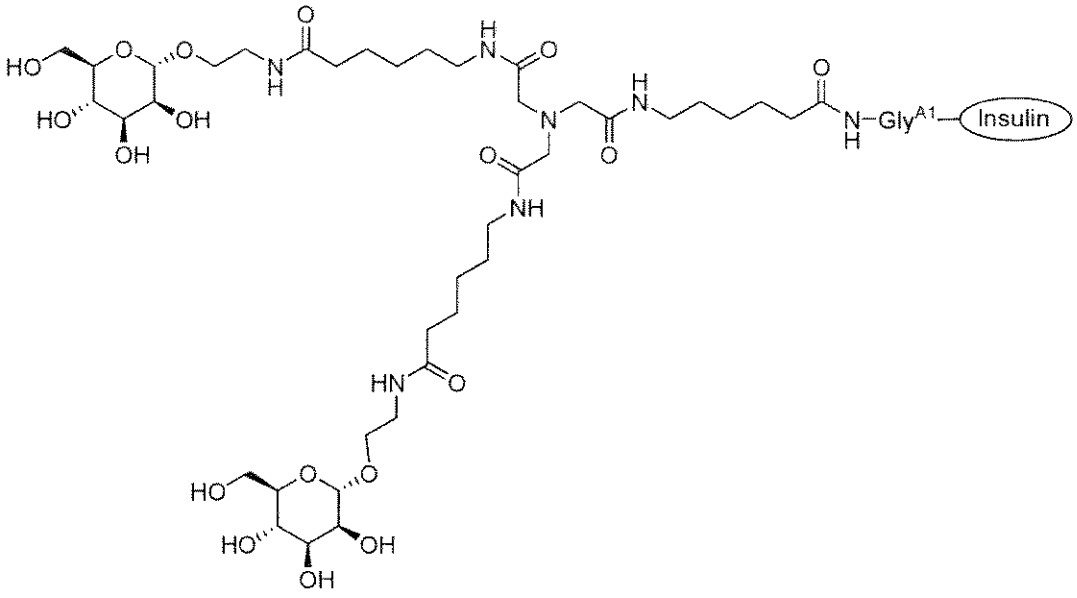


Conjugate I-11
TSPE-AETM-3 (B29)

Figure 45 (continued)

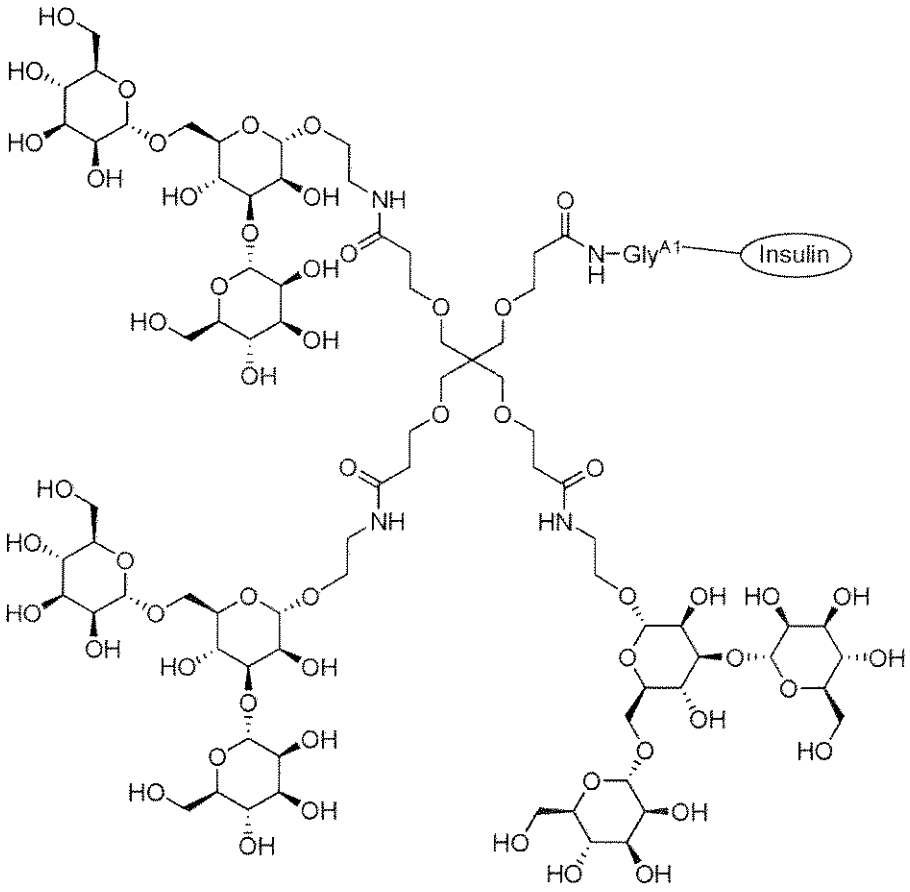


Conjugate I-12
TSAT-C6-AETM-2 (A1)



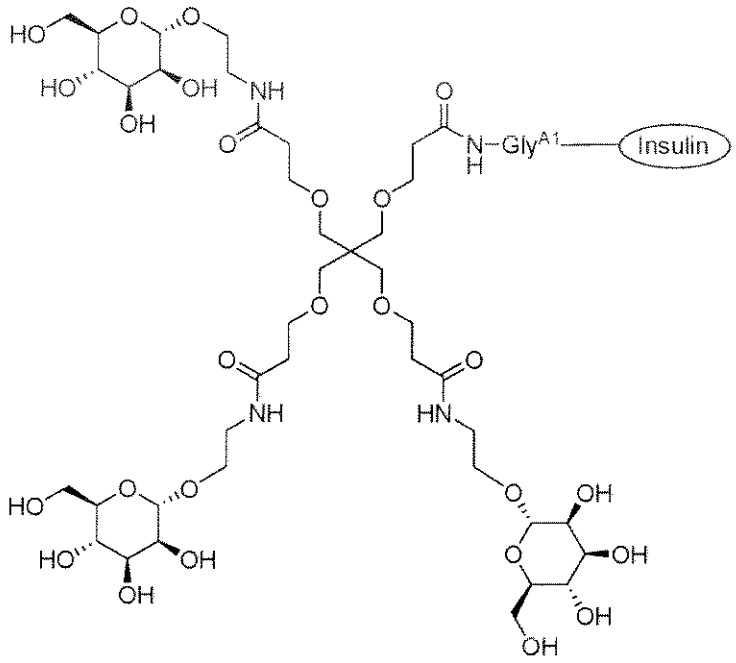
Conjugate I-13
TSAT-C6-AEM-2 (A1)

Figure 45 (continued)

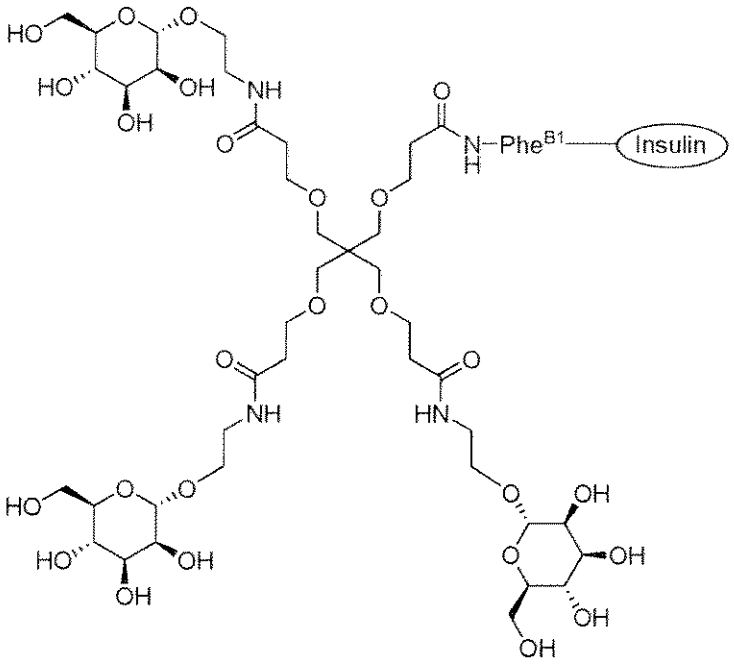


Conjugate I-14
TSPE-AETM-3 (A1)

Figure 45 (continued)



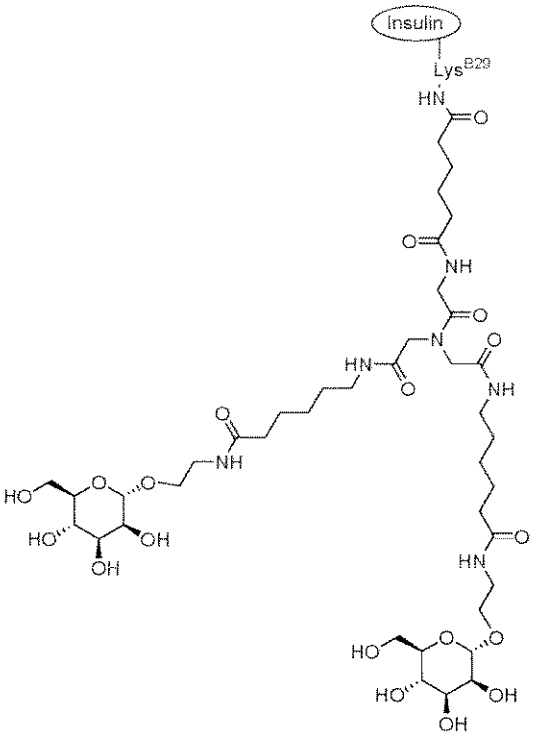
Conjugate I-15
TSPE-AEM-3 (A1)



Conjugate I-16

TSPE-AEM-3 (B1)

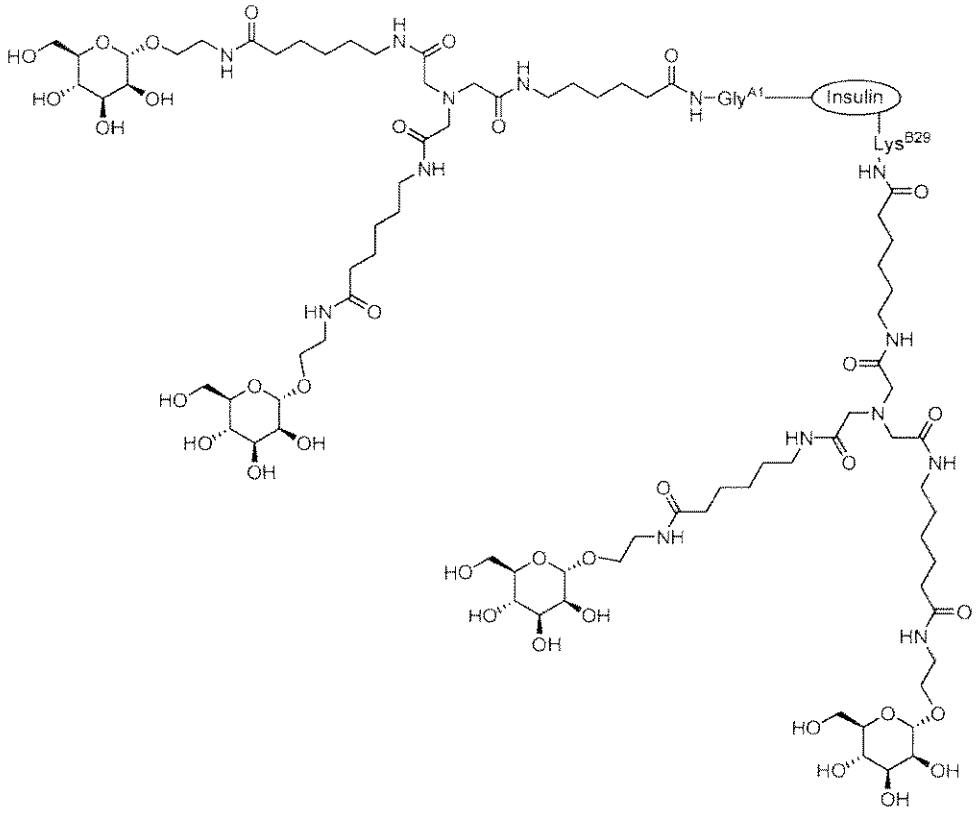
Figure 45 (continued)



Conjugate I-17

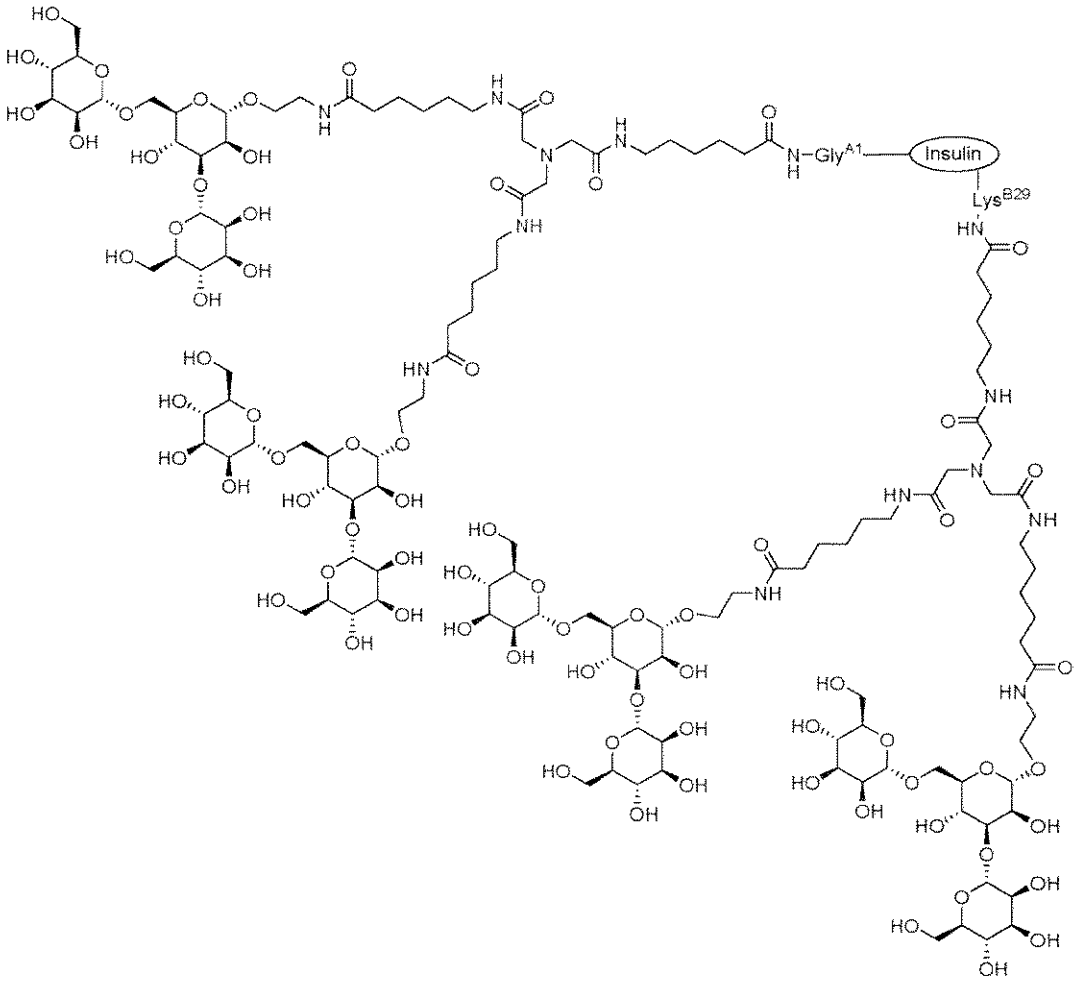
C6-Amide-AEM-2 (B29)

Figure 45 (continued)



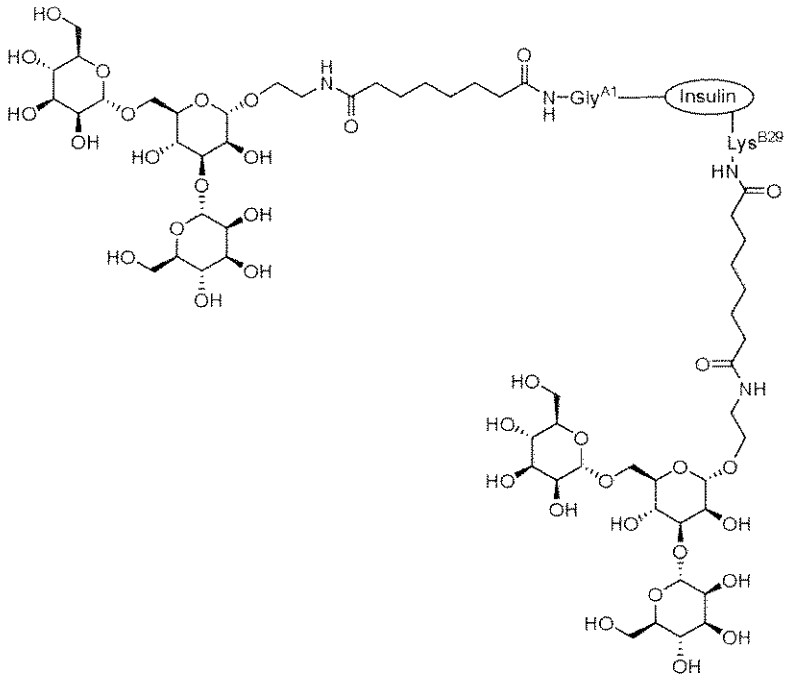
Conjugate II-1
TSAT-C6-Di-sub-AEM-2 (A1,B29)

Figure 45 (continued)

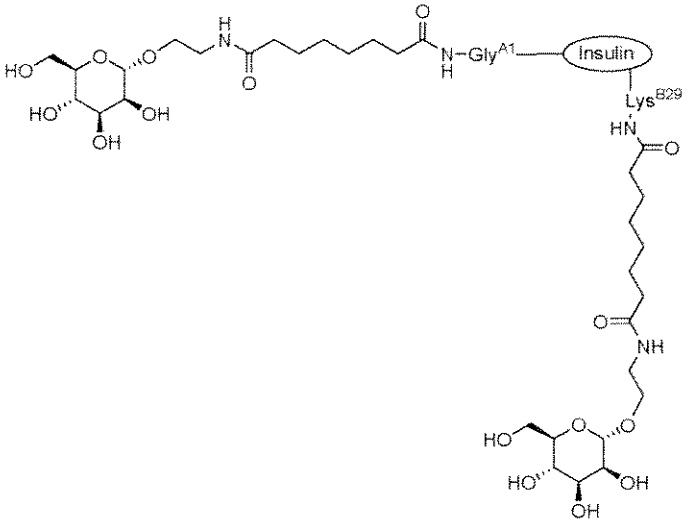


Conjugate II-2
TSAT-C6-Di-sub-AETM-2 (A1,B29)

Figure 45 (continued)

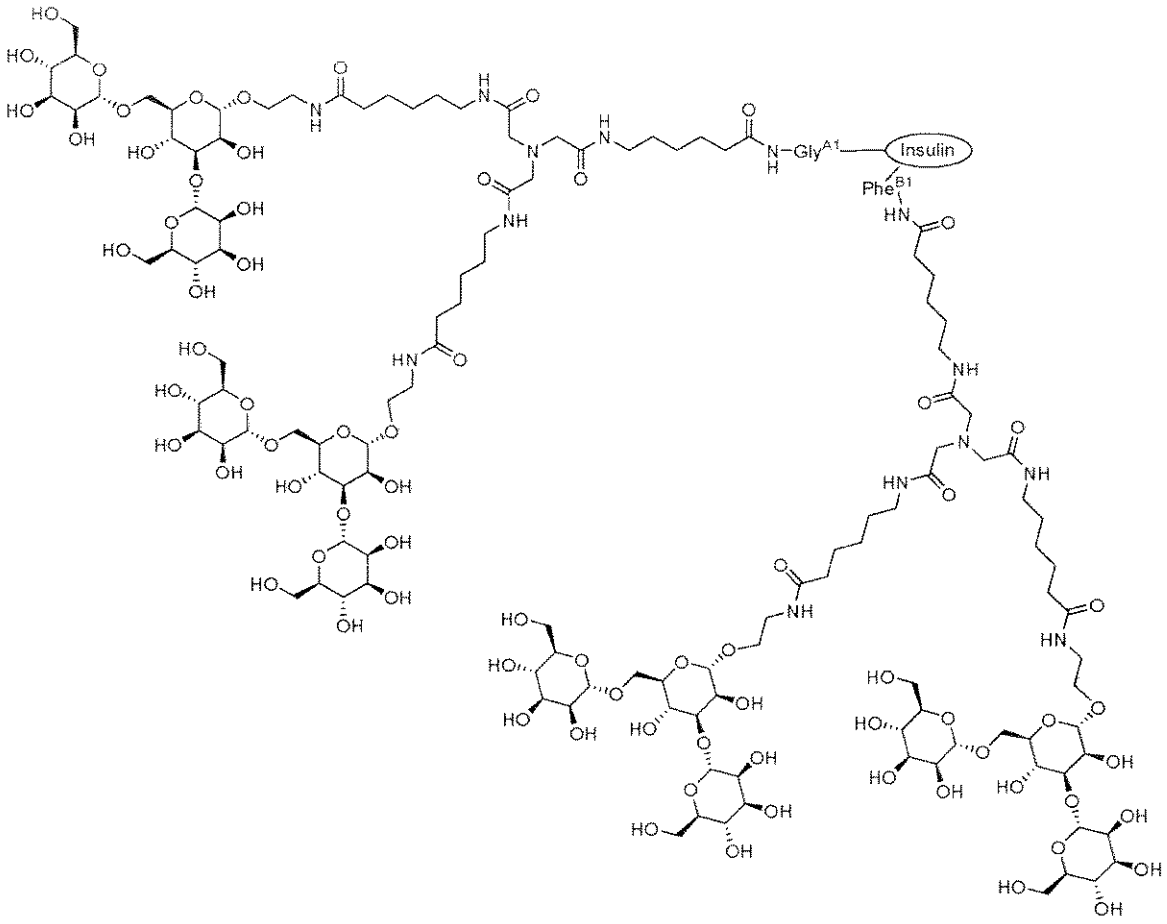


Conjugate II-3
DSS-Di-sub-AETM-1 (A1,B29)



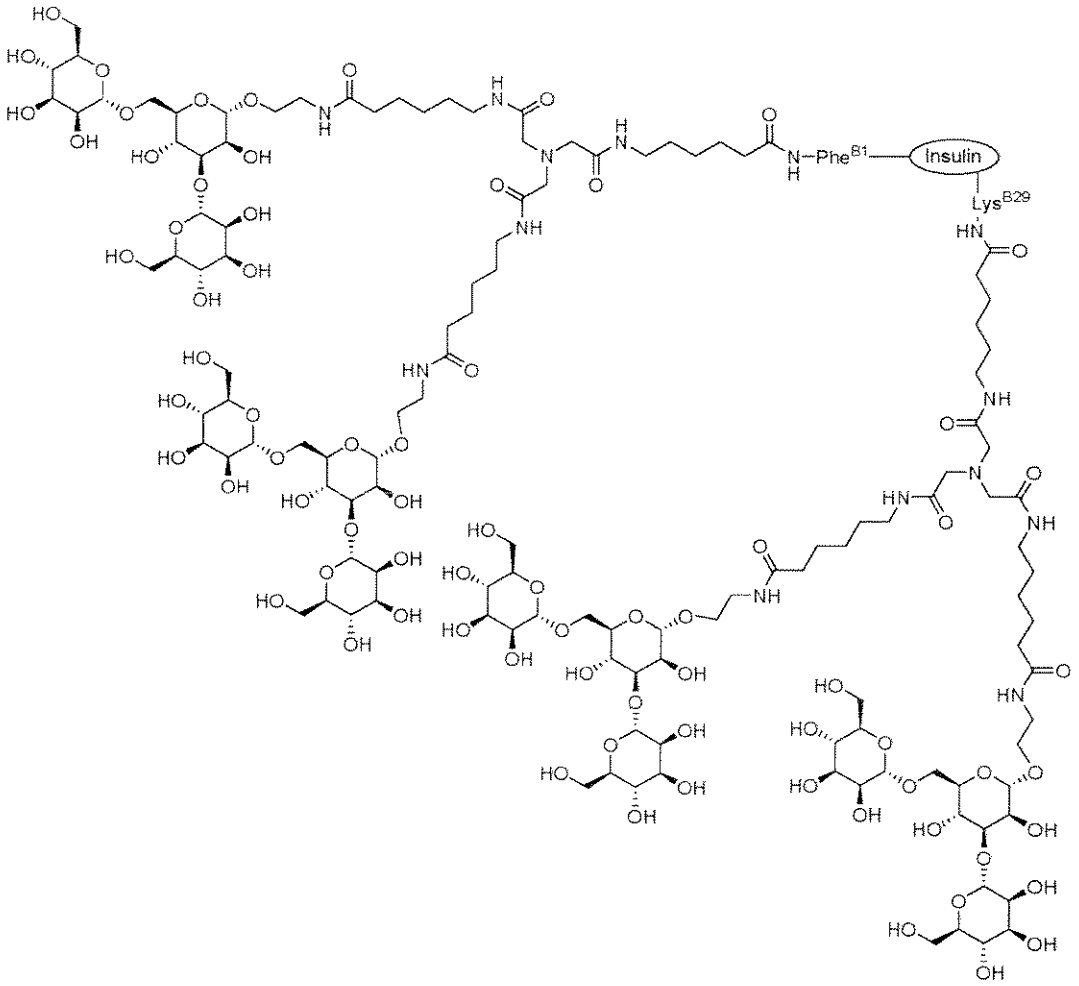
Conjugate II-4
DSS-Di-sub-AEM-1 (A1,B29)

Figure 45 (continued)



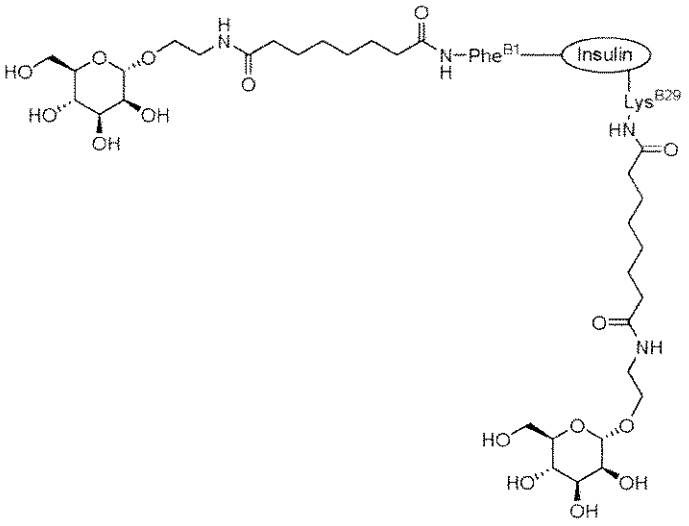
Conjugate II-5
TSAT-C6-Di-sub-AETM-2 (A1,B1)

Figure 45 (continued)



Conjugate II-6
TSAT-C6-Di-sub-AETM-2 (B1,B29)

Figure 45 (continued)



Conjugate II-7
DSS-Di-sub-AETM-2 (B1,B29)

Figure 45 (continued)

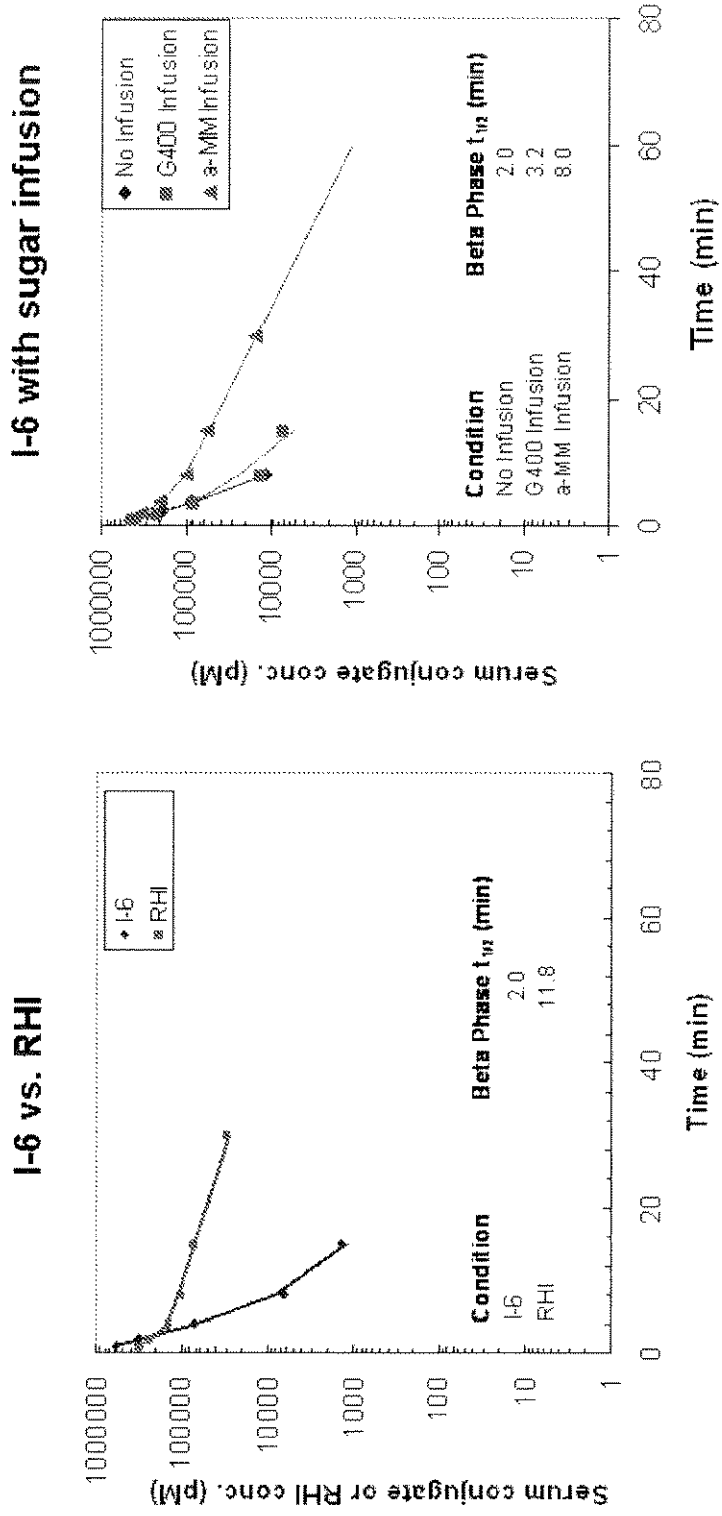

I-6 vs. RHI
Serum conjugate or RHI conc. (pM)
Time (min)
I-6
RHI
Condition
Beta Phase t1/2 (min)
I-6 2.0
RHI 11.8
I-6 with sugar infusion
Serum conjugate conc. (pM)
Time (min)
No Infusion
G400 Infusion
a-MM Infusion
Condition
Beta Phase t1/2 (min)
No Infusion 2.0
G400 Infusion 3.2
a-MM Infusion 8.0

Figure 46

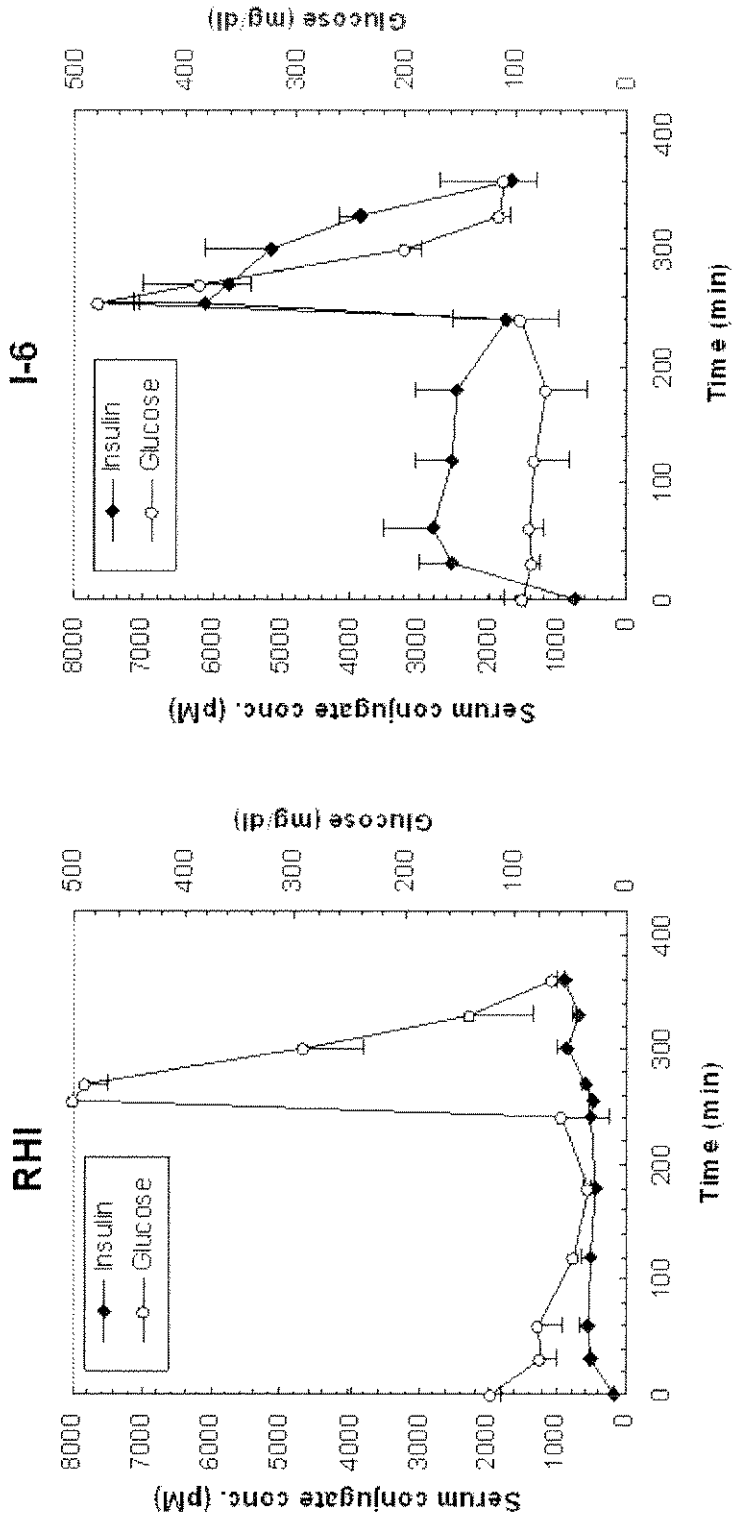

I-6
RHI
Insulin
Glucose
Serum conjugate conc. (pM)
Glucose (mg/dl)
Time (min)
0
1000
2000
3000
4000
5000
6000
7000
8000
100
200
300
400
500

Figure 47

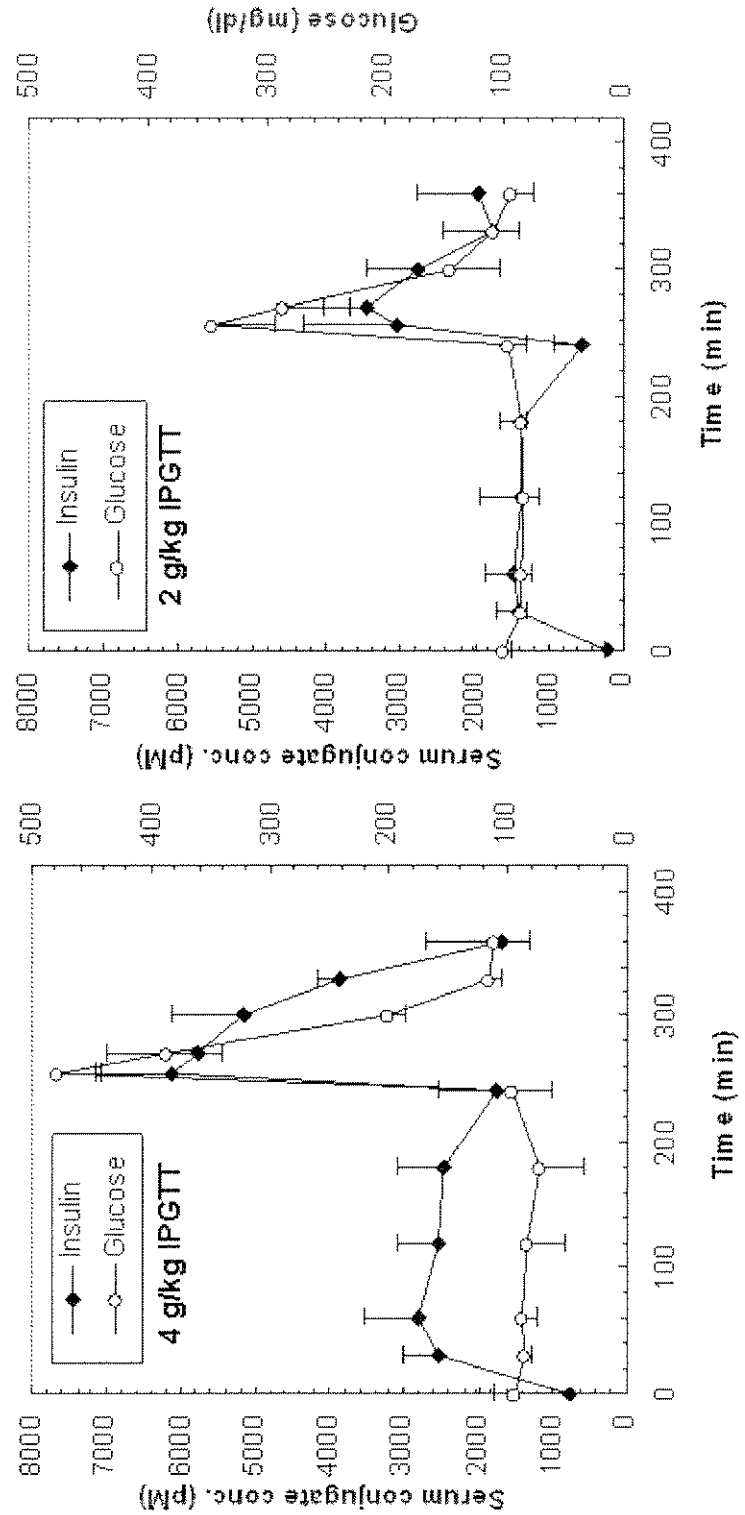


Figure 47 (continued)

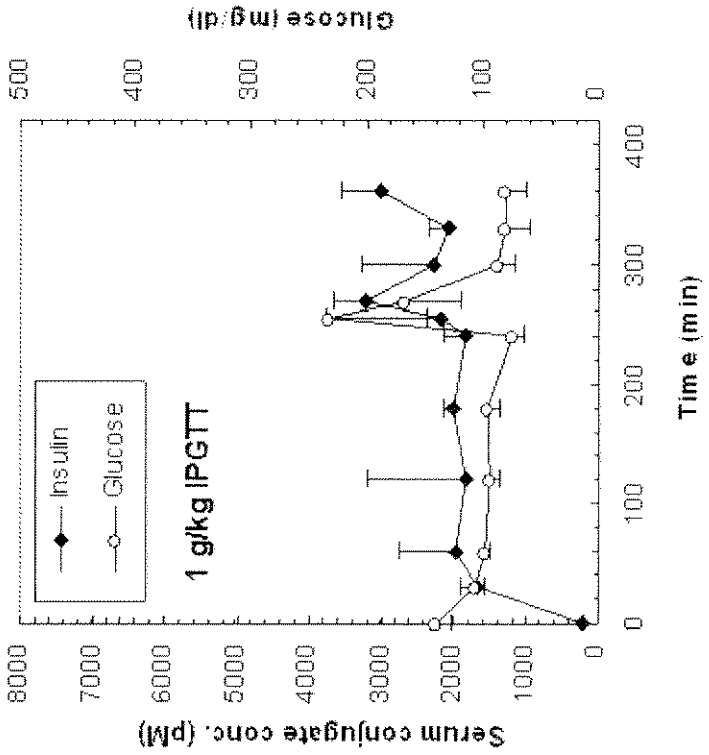
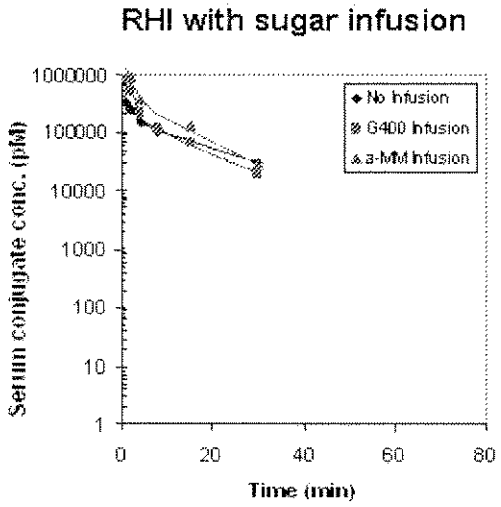
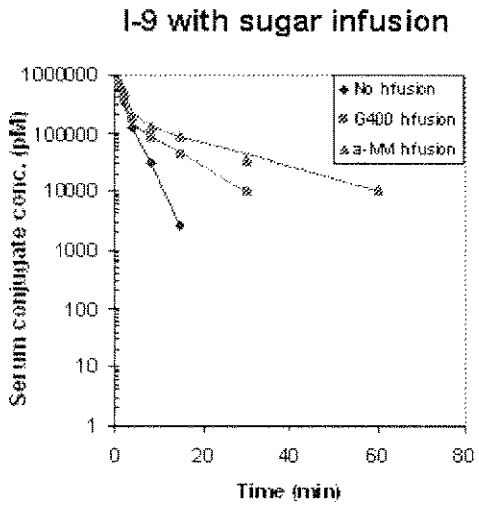


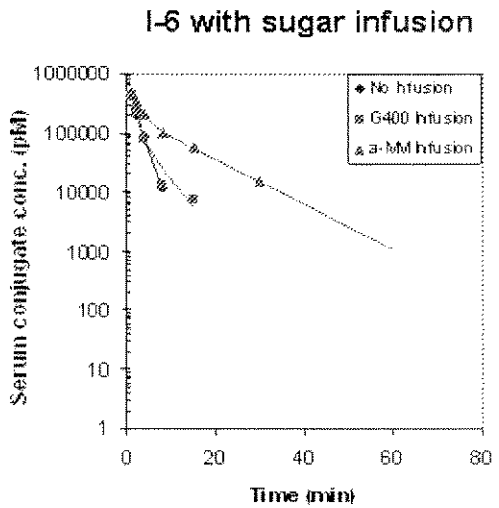
Figure 47 (continued)



Condition	Beta Phase $t_{1/2}$ (min)
No Infusion	11.8
G400 Infusion	8.6
a-MM Infusion	8.0

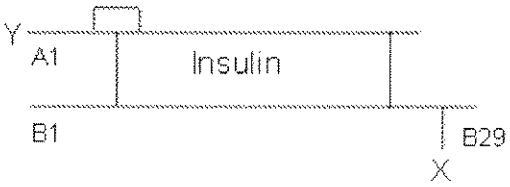


Condition	Beta Phase $t_{1/2}$ (min)
No Infusion	2.0
G400 Infusion	6.9
a-MM Infusion	14.4



Condition	Beta Phase $t_{1/2}$ (min)
No Infusion	2.0
G400 Infusion	3.2
a-MM Infusion	8.0

Figure 48



Formulation	Scaffold – X	Scaffold – Y	Sugar (n)
RHI	n/a	n/a	n/a
I-7: AEM-2	n/a	TSAT-C_6	AEM (2)
I-6: AETM-2	n/a	TSAT-C_6	AETM (2)
I-11: AETM-3	n/a	TSPE	AETM (3)
II-2: Di-sub-AETM-2	TSAT-C_6	TSAT-C_6	AETM (2x2)

Figure 49

	β-phase half-life (min) for each type of infusion		
Formulation	Saline	Glucose	α-methyl mannose
RHI	2.7	2.3	3.3
I-7: AEM-2	5.5	5.8	5.4
I-6: AETM-2	4.8	3.3	8.1
I-11: AETM-3	6.7	3.7	10.8
II-2: Di-sub-AETM-2	2.5	n/a	20.3

Figure 50

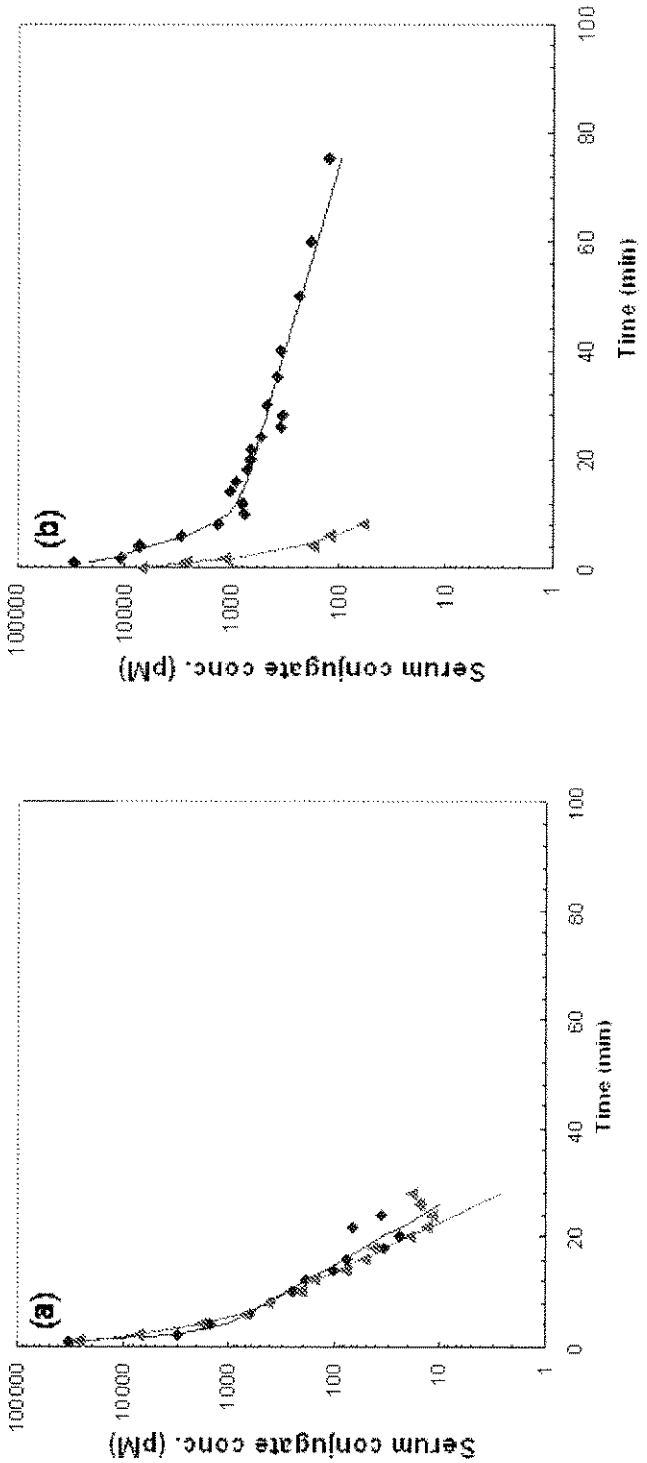

(a)
(b)
Serum conjugate conc. (pM)
Time (min)
100000
10000
1000
100
10
1
0
20
40
60
80
100

Figure 51

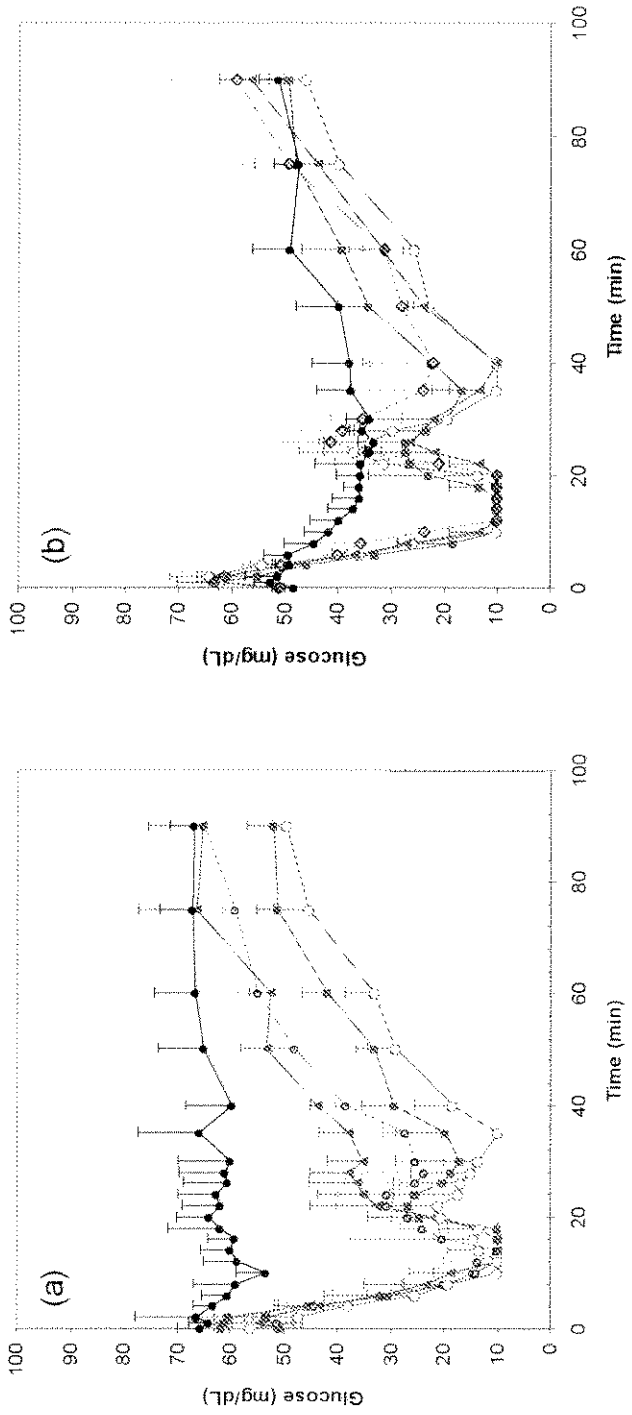

(a)
(b)
Glucose (mg/dL)
Time (min)
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100

Figure 52

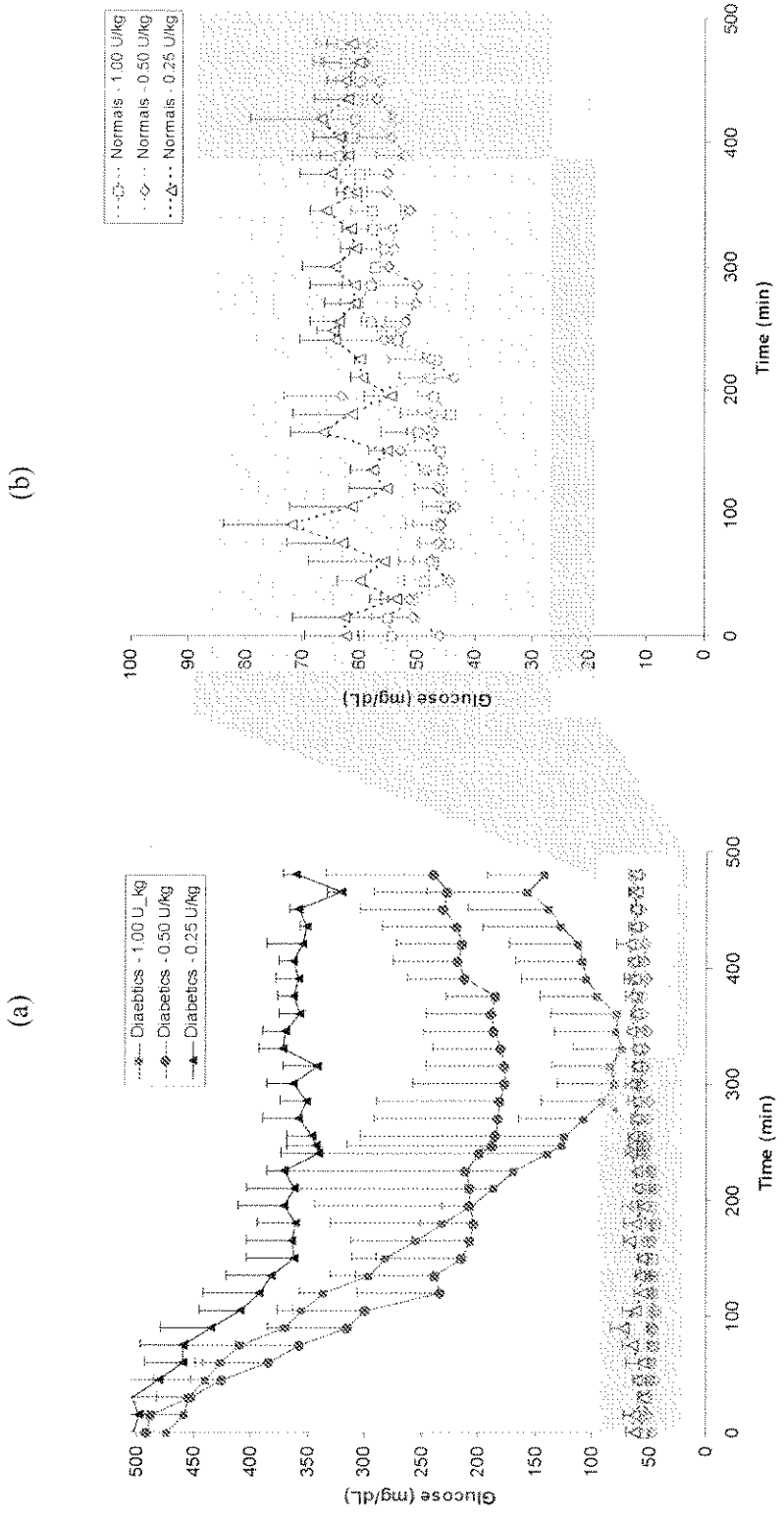

(a)
(b)
Diabetics - 1.00 U_Kg
Diabetics - 0.50 U/kg
Diabetics - 0.25 U/kg
Normals - 1.00 U/kg
Normals - 0.50 U/kg
Normals - 0.25 U/kg
Glucose (mg/dL)
Time (min)

Figure 53

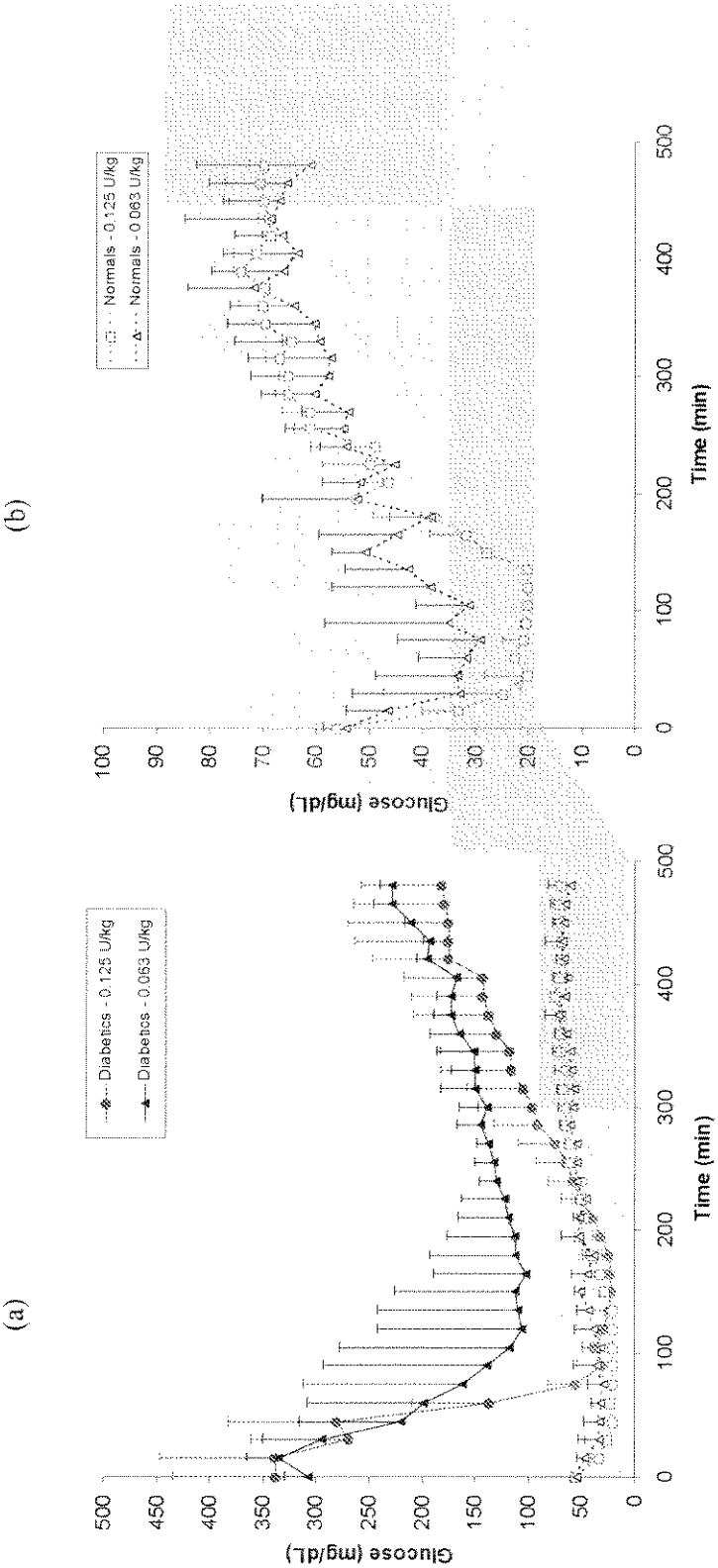
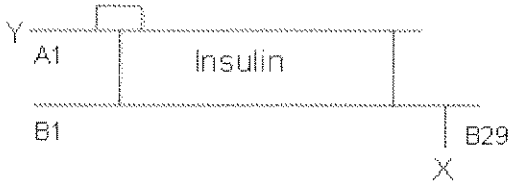
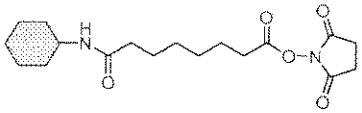

(a)
Diabetics - 0.125 U/kg
Diabetics - 0.063 U/kg
Glucose (mg/dL)
Time (min)
(b)
Normals - 0.125 U/kg
Normals - 0.063 U/kg
Glucose (mg/dL)
Time (min)

Figure 54





DSS

Formulation	Scaffold – X	Scaffold – Y	Sugar (n)
I-12: AETM-2	n/a	TSAT-C_6	AETM (2)
I-13: AEM-2	n/a	TSAT-C_6	AEM (2)
I-15: AEM-3	n/a	TSPE	AEM (3)
I-14: AETM-3	n/a	TSPE	AETM (3)
II-1: Di-sub-AEM-2	TSAT-C_6	TSAT-C_6	AEM (2x2)
II-3: Di-sub-AETM-1	DSS	DSS	AETM (2x1)
II-4: Di-sub-AEM-1	DSS	DSS	AEM (2x1)

Figure 55

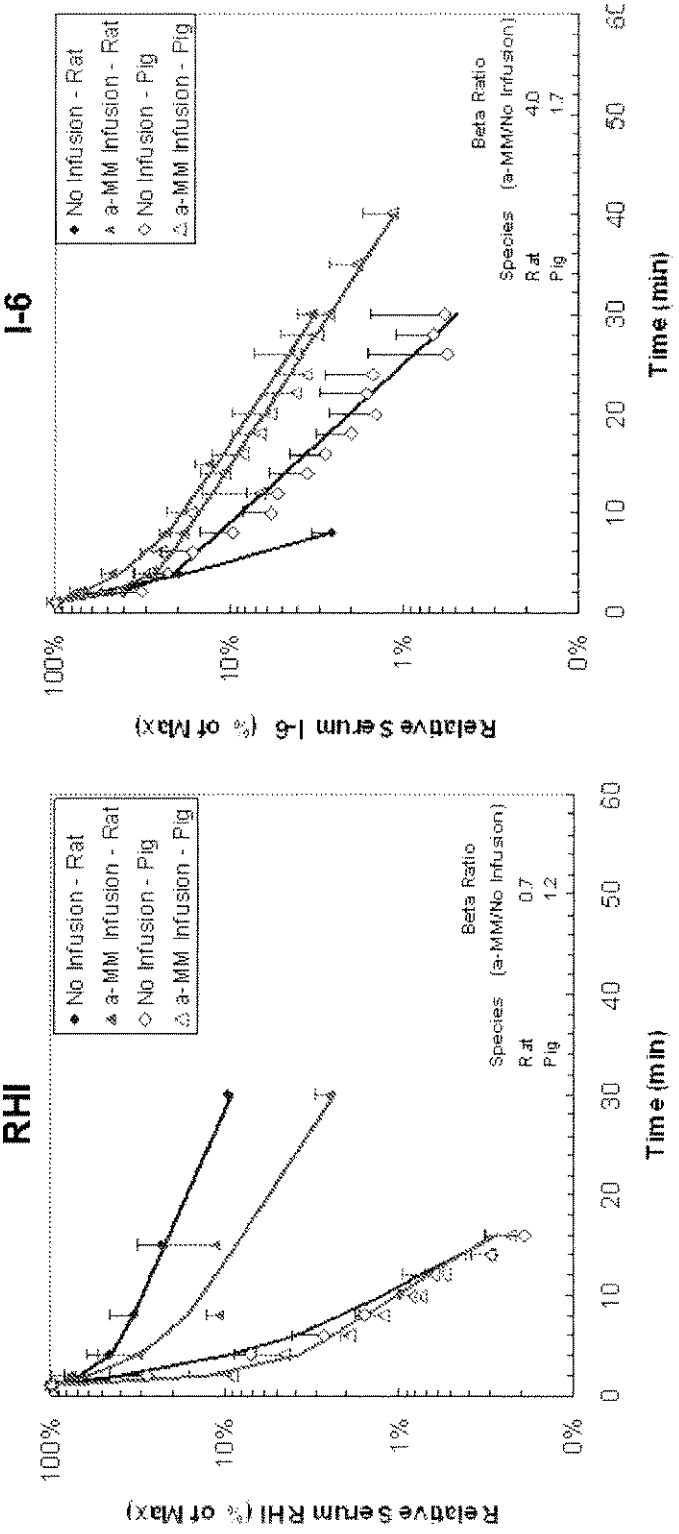

RHI
Relative Serum RHI (% of Max)
100%
10%
1%
0%
0
10
20
30
40
50
60
Time (min)
No Infusion - Rat
a-MM Infusion - Rat
No Infusion - Pig
a-MM Infusion - Pig
Beta Ratio
Species (a-MM/No Infusion)
Rat 0.7
Pig 1.2
I-6
Relative Serum I-6 (% of Max)
100%
10%
1%
0%
0
10
20
30
40
50
60
Time (min)
No Infusion - Rat
a-MM Infusion - Rat
No Infusion - Pig
a-MM Infusion - Pig
Beta Ratio
Species (a-MM/No Infusion)
Rat 4.0
Pig 1.7

Figure 56

Insulin-conjugate	Sugar Composition	$t_{1/2,\beta}$ (a-MM)/ $t_{1/2,\beta}$ (No Inf)
II-2	B29: AETMx2, A1: AETMx2	8.1
I-12	A1: AETMx2	2.3
I-6	B29: AETMx2	1.7
I-11	B29: AETMx3	1.6
I-9	B29: AEMx3	1.6
II-1	B29: AEMx2, A1: AEMx2	1.5
II-3	B29: AETMx1, A1: AETMx1	1.3
RHI	n/a	1.2
I-7	B29: AEMx2	1.0
II-4	B29: AEMx1, A1: AEMx1	0.9

Figure 57

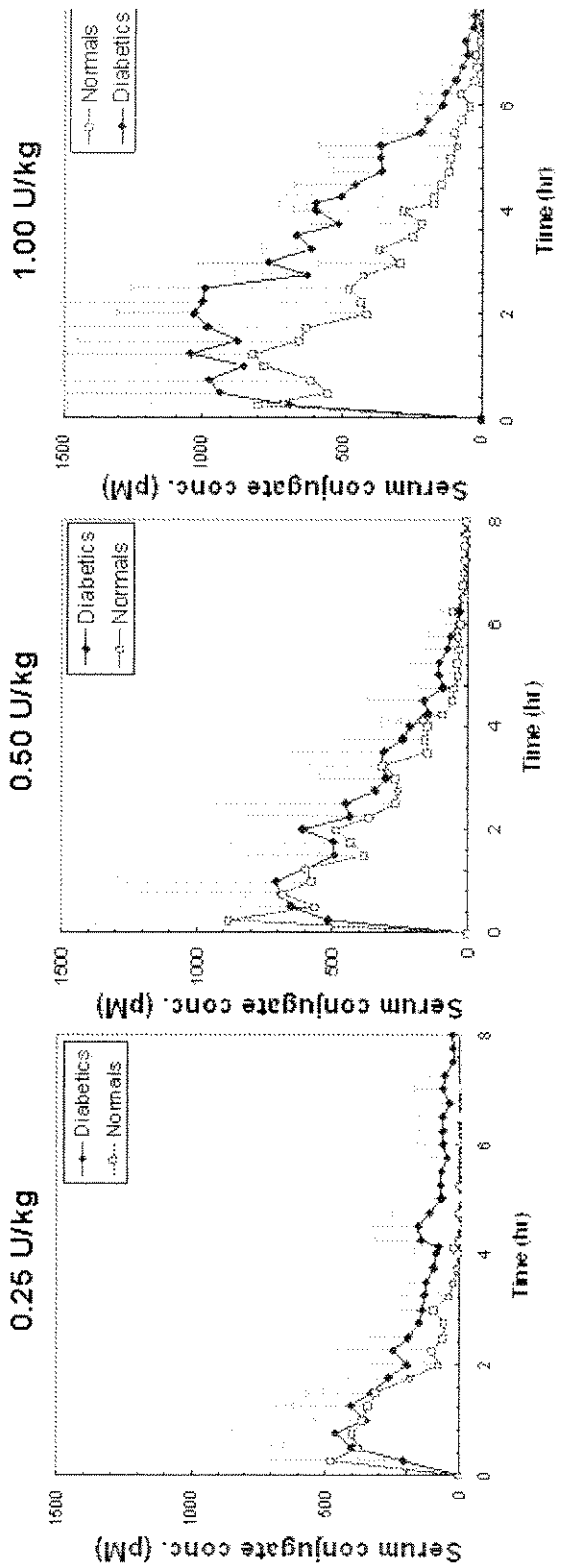
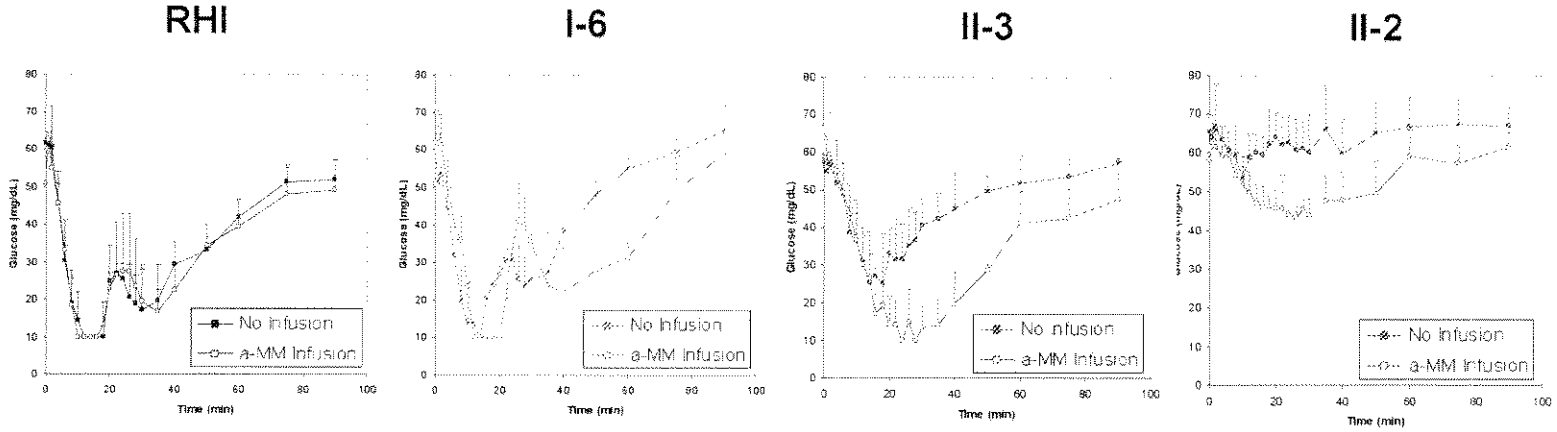

0.25 U/kg
0.50 U/kg
1.00 U/kg
Serum conjugate conc. (pM)
Time (hr)
Diabetics
Normals
0
500
1000
1500
0
2
4
6
8

Figure 58



Formulation	AOC Ratio (a-MM/no infusion)	$t_{1/2,\beta}$ (a-MM)/ $t_{1/2,\beta}$ (no infusion)
RHI	0.99	1.2
I-6	1.36	1.6
II-3	2.34 ←	1.3 ←
II-2	2.58 ←	8.1 ←

Lack of correlation between sugar-dependent half-life and bioactivity

Figure 59

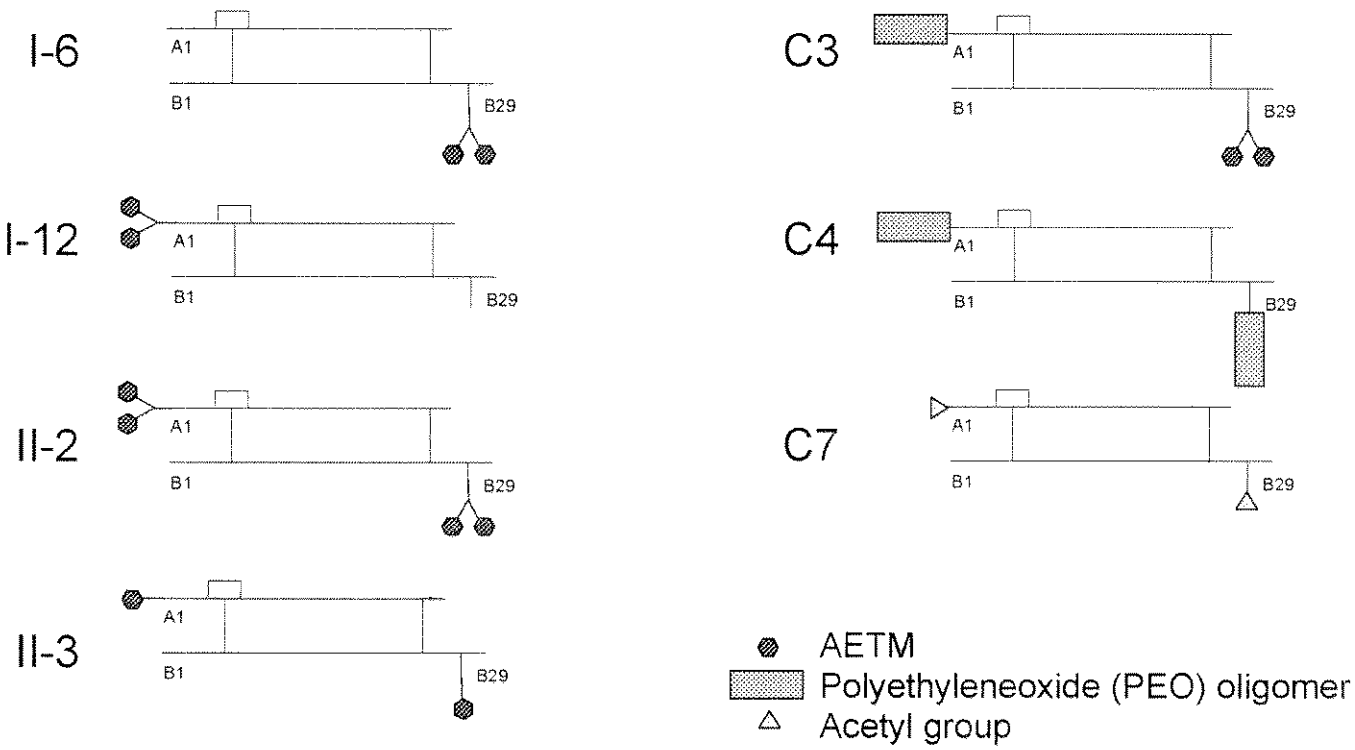

I-6
A1
B1
B29
I-12
A1
B1
B29
II-2
A1
B1
B29
II-3
A1
B1
B29
C3
A1
B1
B29
C4
A1
B1
B29
C7
A1
B1
B29
AETM
Polyethyleneoxide (PEO) oligomer
Acetyl group

Figure 60

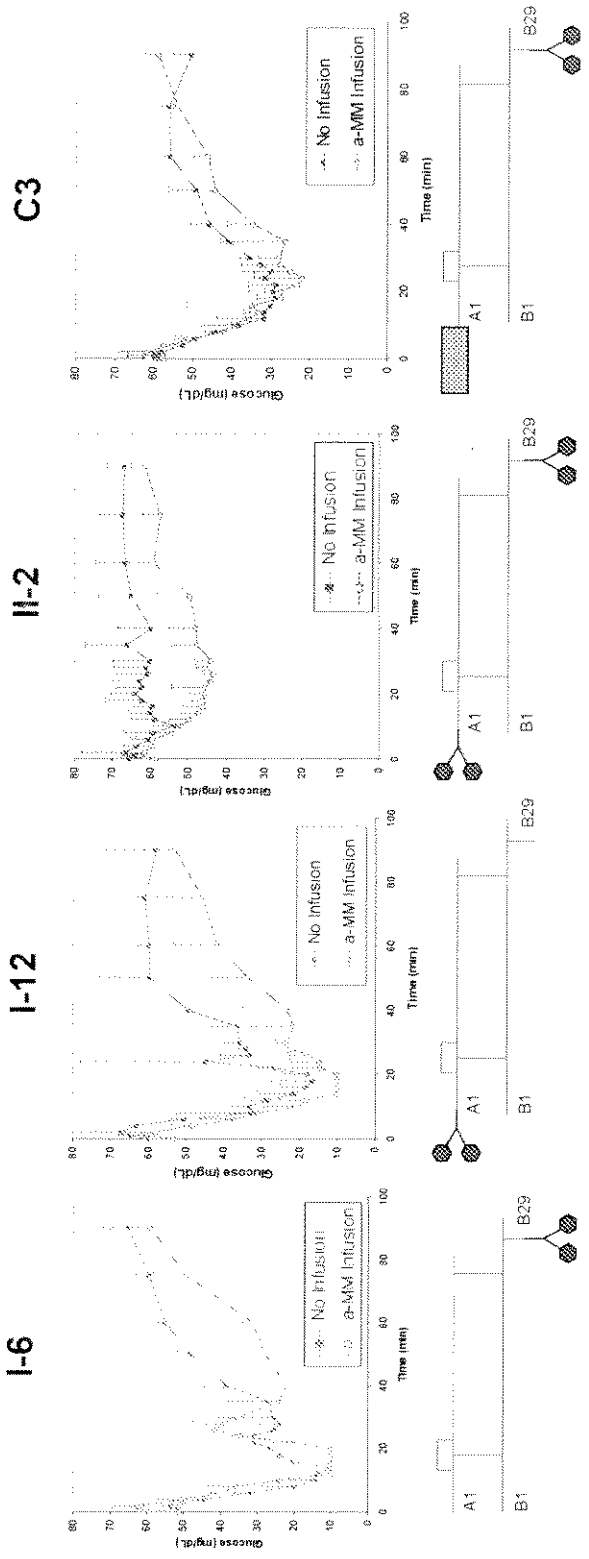

I-6
I-12
II-2
C3
Glucose (mg/dL)
Time (min)
No Infusion
a-MM Infusion
A1
B1
B29

Figure 61

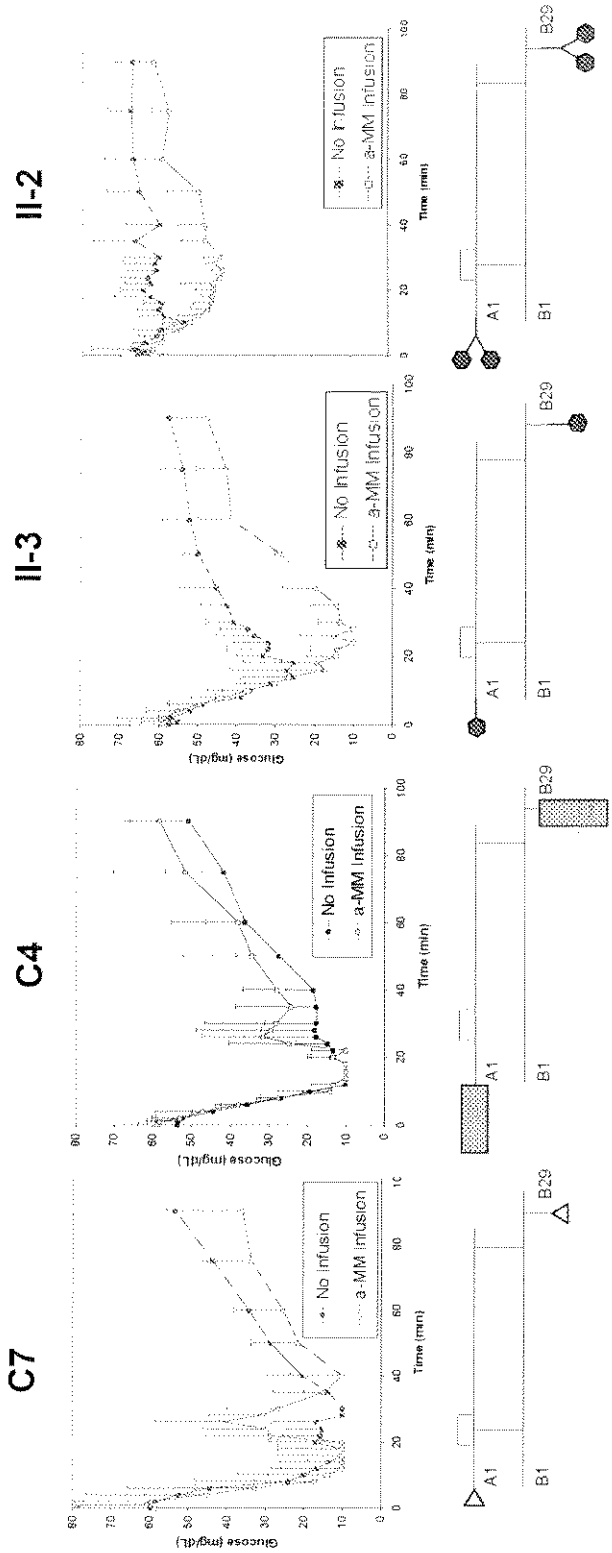

II-2
II-3
C4
C7
No Infusion
a-MM Infusion
Glucose (mg/dL)
Time (min)
A1
B1
B29

Figure 62

A-Chain
Gly-Ile-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
S
S
S
S
S
B-Chain
Phe-Val-Asn-Gln-His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Figure 63