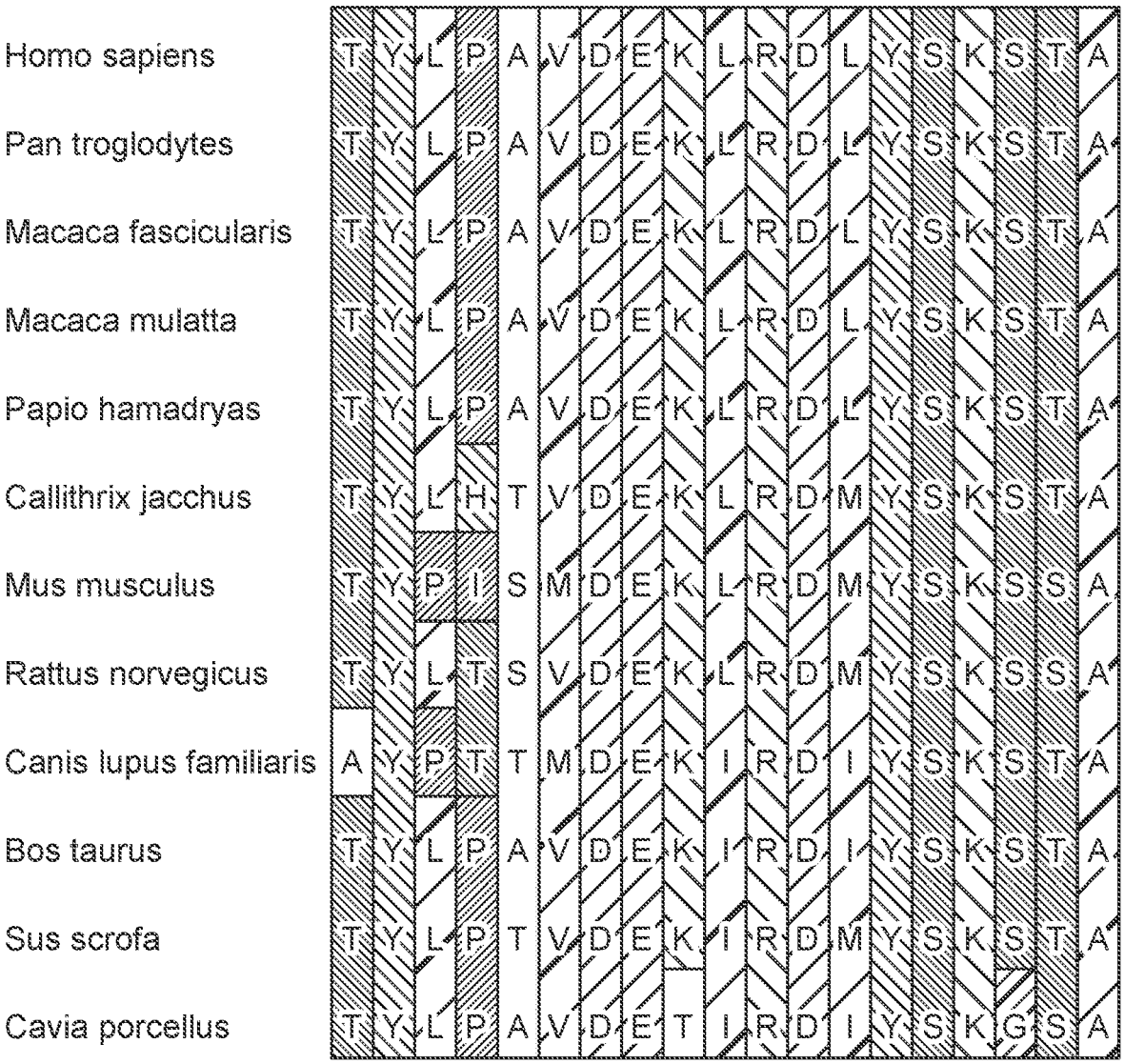
Homo sapiens T Y L P A V D E K L R D L Y S K S T A
Pan troglodytes T Y L P A V D E K L R D L Y S K S T A
Macaca fascicularis T Y L P A V D E K L R D L Y S K S T A
Macaca mulatta T Y L P A V D E K L R D L Y S K S T A
Papio hamadryas T Y L P A V D E K L R D L Y S K S T A
Callithrix jacchus T Y L H T V D E K L R D M Y S K S T A
Mus musculus T Y P I S M D E K L R D M Y S K S S A
Rattus norvegicus T Y L T S V D E K L R D M Y S K S S A
Canis lupus familiaris A Y P T T M D E K I R D I Y S K S T A
Bos taurus T Y L P A V D E K I R D I Y S K S T A
Sus scrofa T Y L P T V D E K I R D M Y S K S T A
Cavia porcellus T Y L P A V D E T I R D I Y S K G S A

FIG. 1

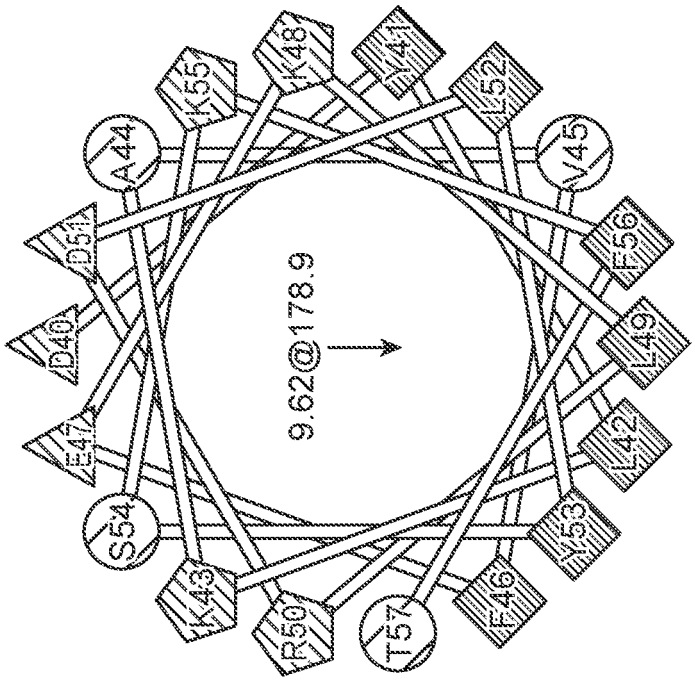
Delta4': DYLKAVFEKLRDLYSKFT
9.62@178.9
T57
R50
K43
S54
E47
D46
D51
A44
K55
K48
V41
L52
V45
F56
L49
L42
V53
F46

FIG. 2B

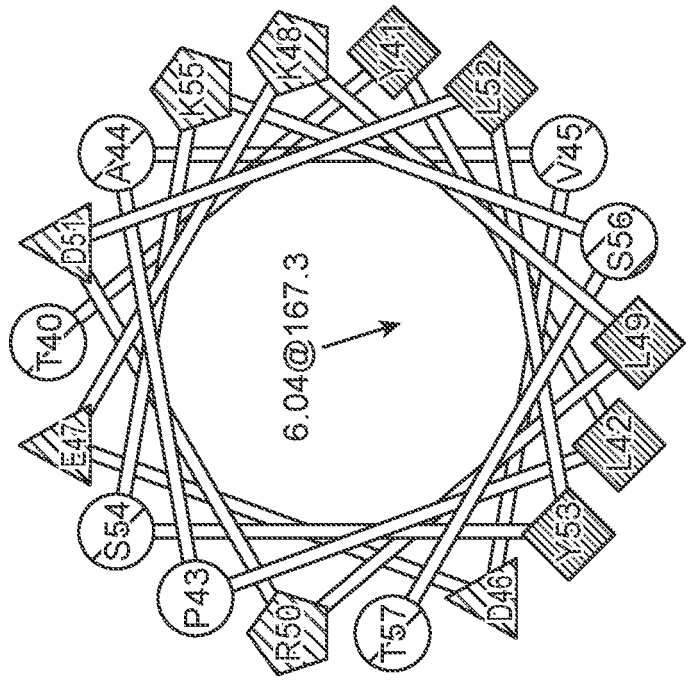
Native apoC-II
(amino acid residues 40-57):
TYLPAVDEKLRDLYSKST
6.04@167.3
T57
R50
P43
S54
E47
T40
D51
A44
K55
K48
V41
L52
V45
S56
L49
L42
V53
D46

FIG. 2A

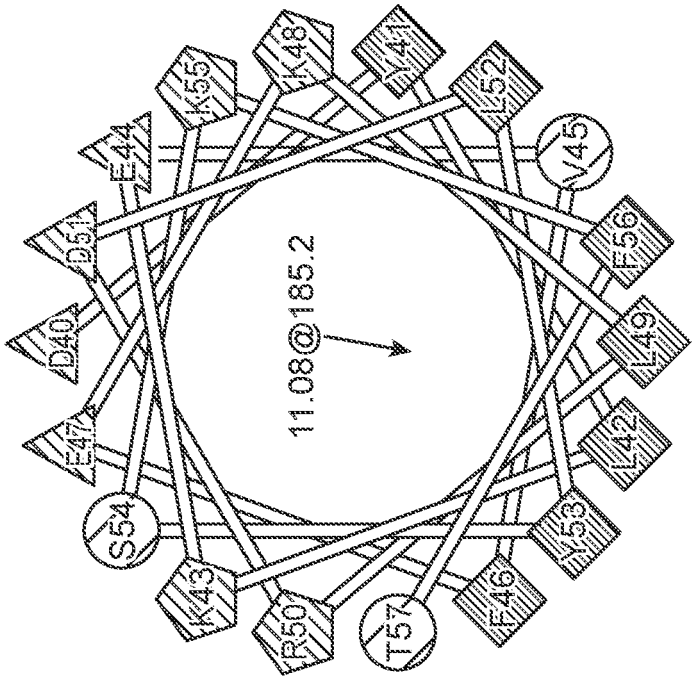
Delta5': DYLKEVFEKLRDLYSKFT
11.08@185.2
D40
D51
E44
K55
K48
L41
L52
V45
E56
L49
L42
Y53
F46
T57
R50
K43
S54
E47

FIG. 3B

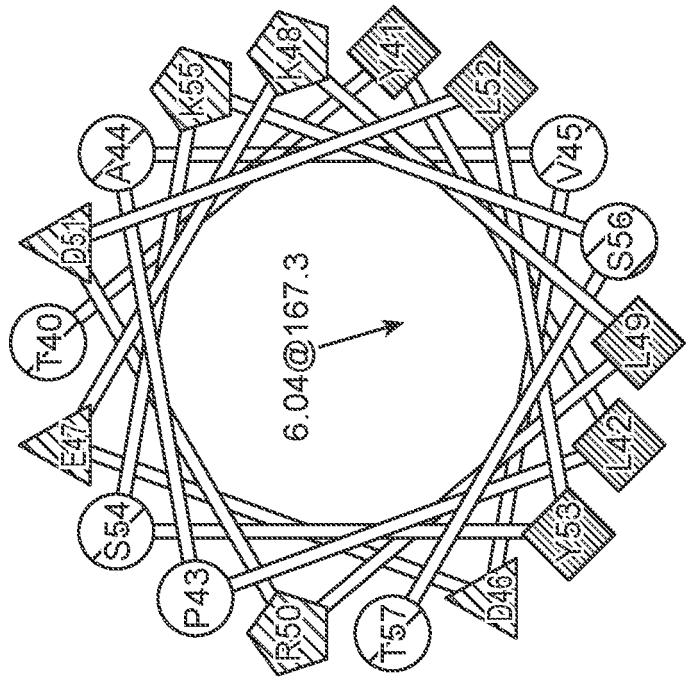
Native apoC-II
(amino acid residues 40-57):
TYLPAVDEKLRDLYSKST
6.04@167.3
T40
D51
A44
K55
K48
L41
L52
V45
S56
L49
L42
Y53
D46
T57
R50
P43
S54
E47

FIG. 3A

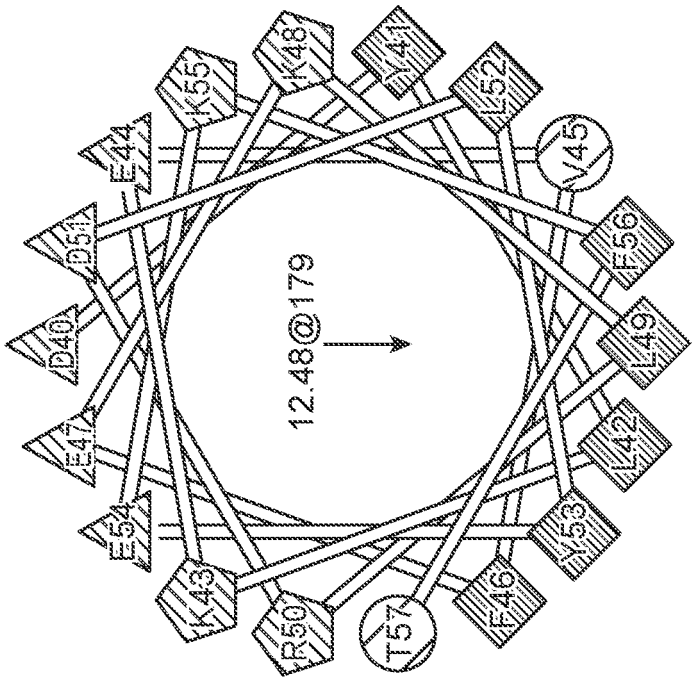
Delta6': DYLKEVFEKLRDLYEKFT
12.48@179
T57
R50
K43
E54
E47
D46
D51
E44
K55
K48
V41
L52
V45
F56
L49
L42
V53
F46

FIG. 4B

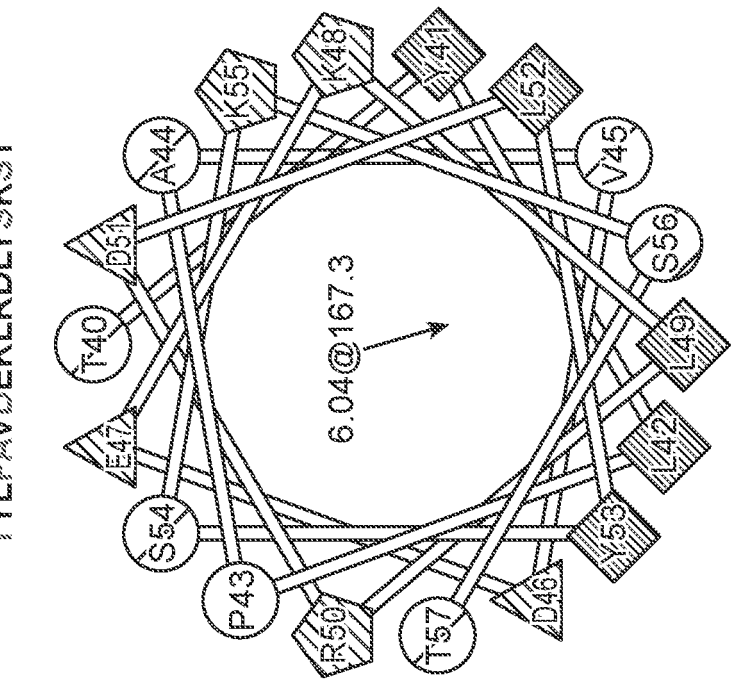
Native apoC-II
(amino acid residues 40-57):
TYLPAVDEKLRDLYSKST
6.04@167.3
T57
R50
P43
S54
E47
T40
D51
A44
K55
K48
V41
L52
V45
S56
L49
L42
V53
D46

FIG. 4A

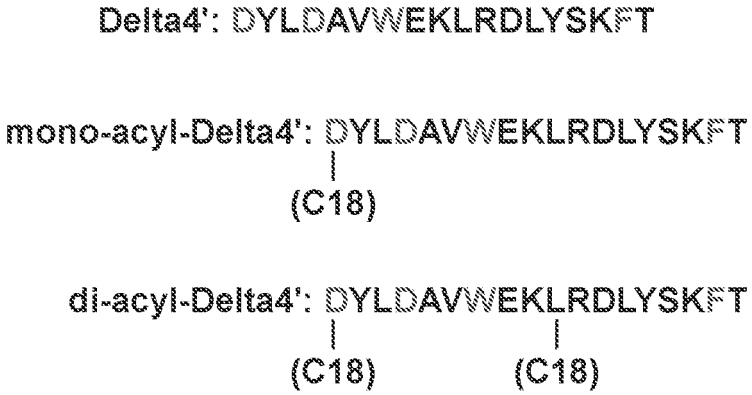
Delta4': DYLDAVWEKLRDLYSKFT
mono-acyl-Delta4': DYLDAVWEKLRDLYSKFT
(C18)
di-acyl-Delta4': DYLDAVWEKLRDLYSKFT
(C18)
(C18)

FIG. 5

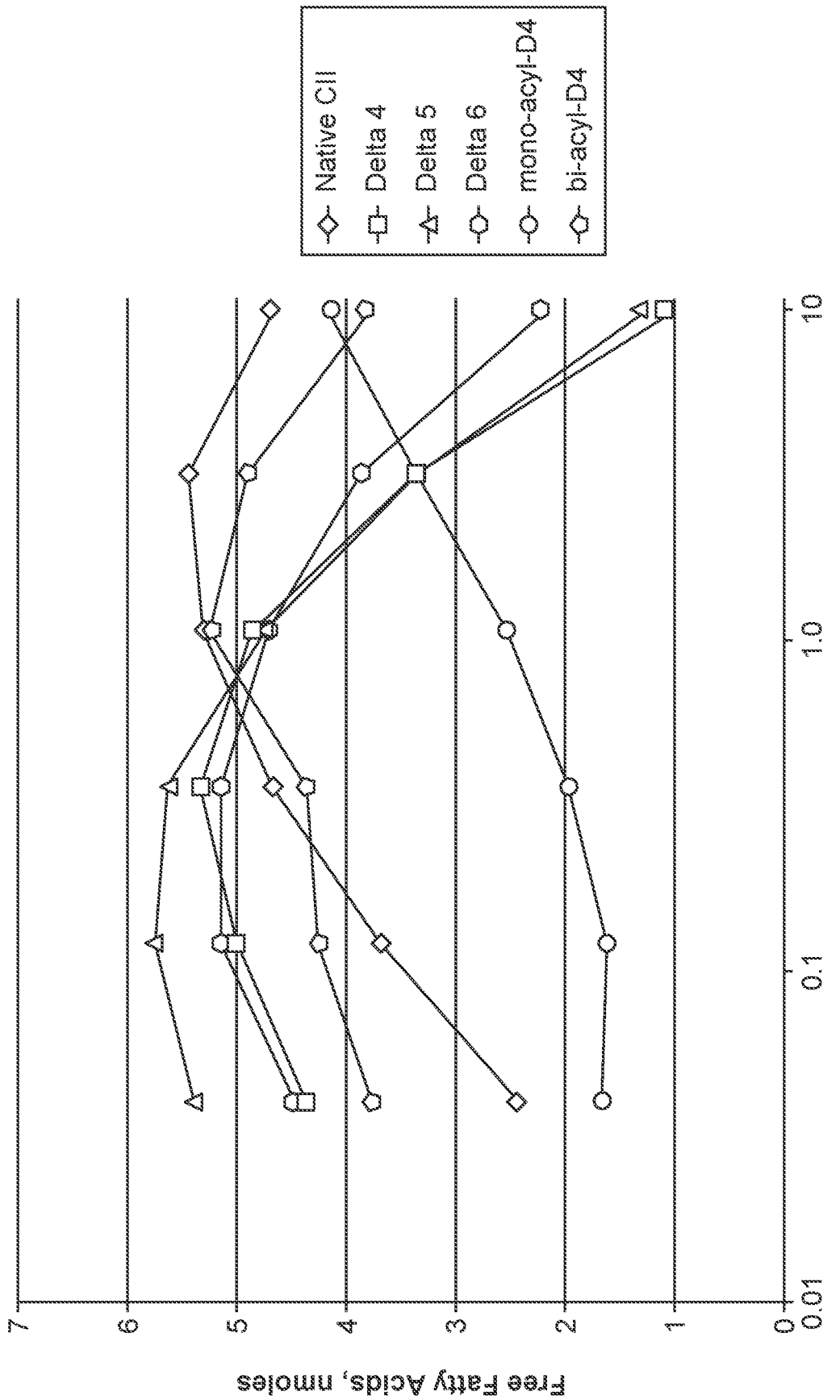
Native CII
Delta 4
Delta 5
Delta 6
mono-acyl-D4
bi-acyl-D4
Free Fatty Acids, nmoles
0
1
2
3
4
5
6
7
0.01
0.1
1.0
10
Peptide, uM

FIG. 6

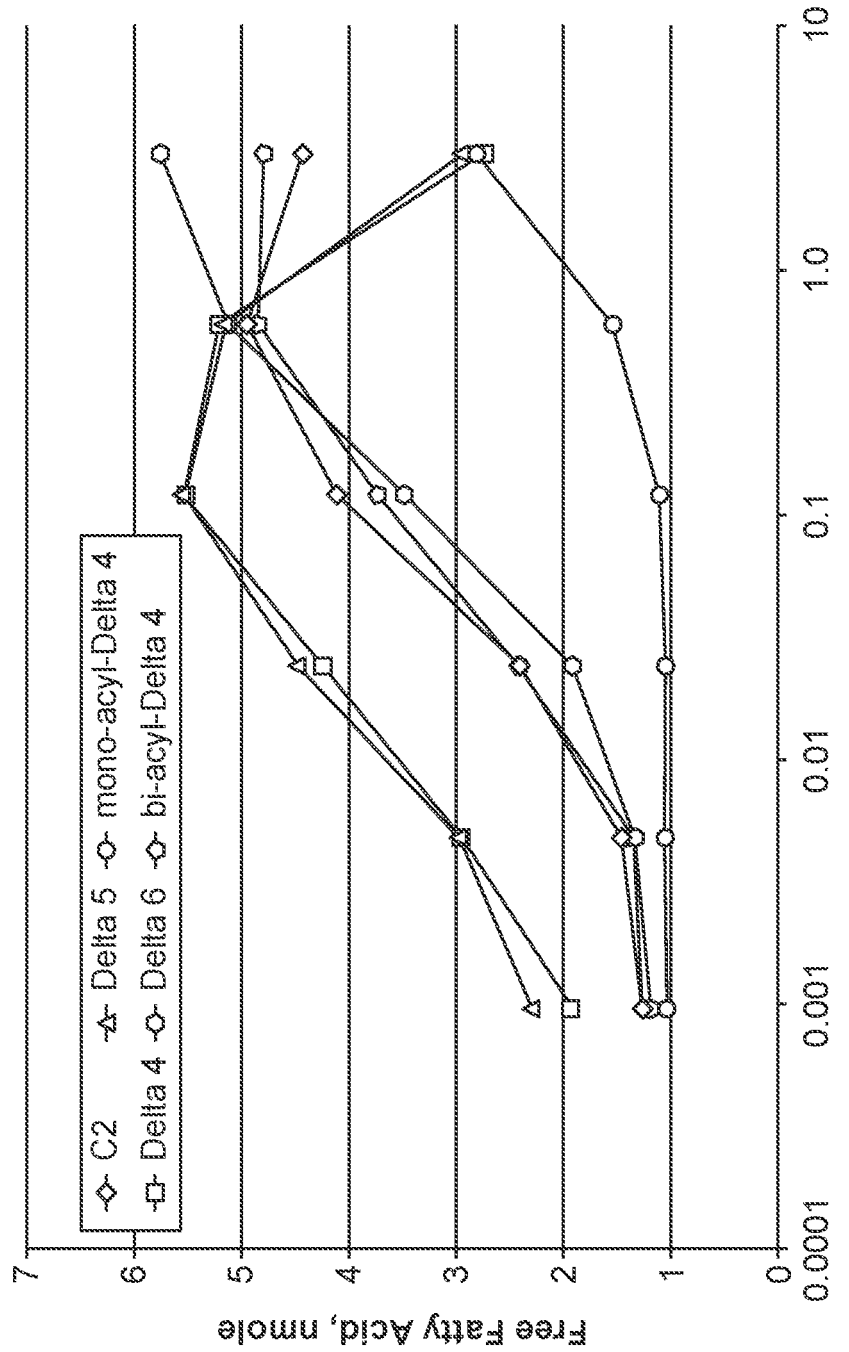
C2
Delta 5
mono-acyl-Delta 4
Delta 4
Delta 6
bi-acyl-Delta 4
Free Fatty Acid, nmole
0
1
2
3
4
5
6
7
0.0001
0.001
0.01
0.1
1.0
10
Peptide, uM

FIG. 7

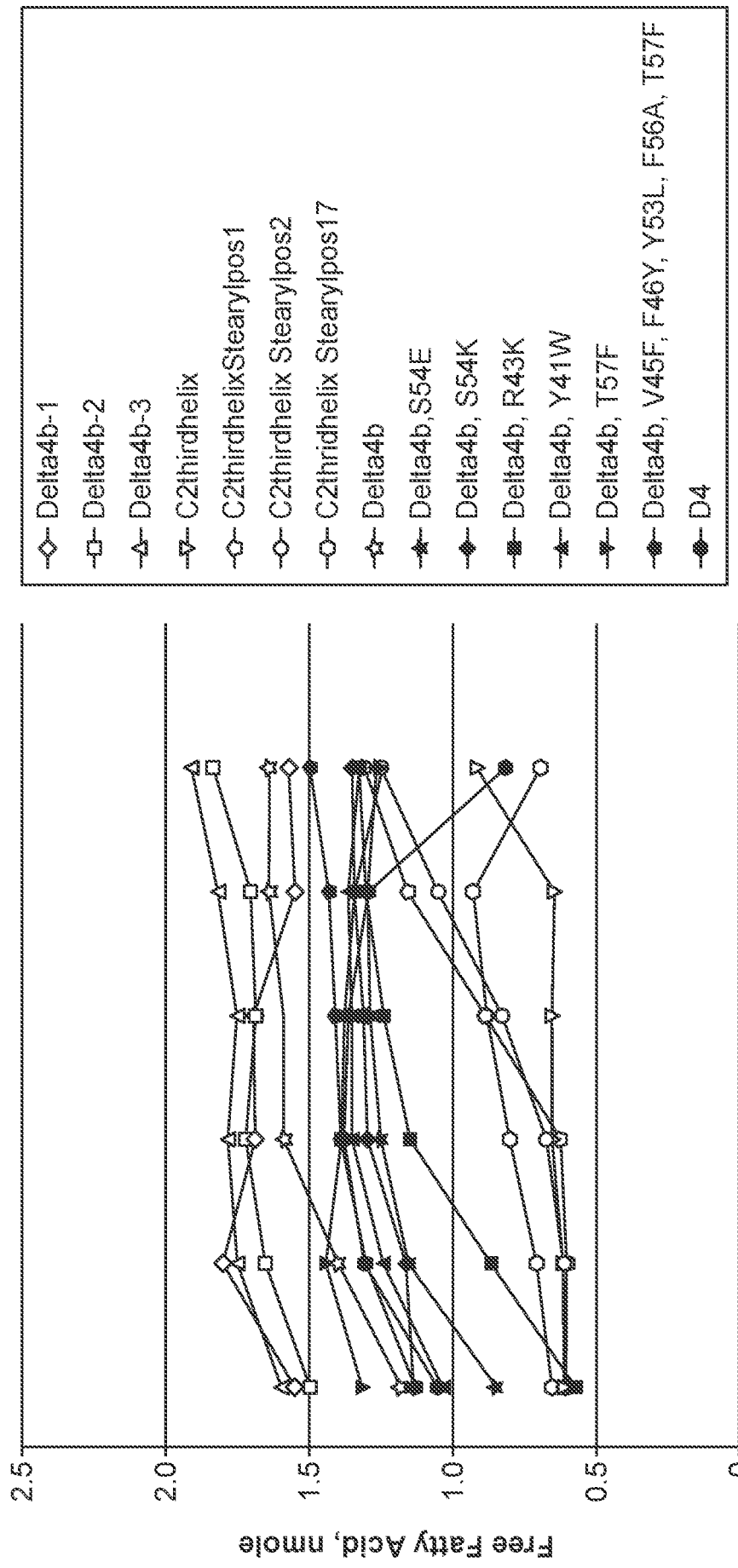
Delta4b-1
Delta4b-2
Delta4b-3
C2thirdhelix
C2thirdhelixStearylpos1
C2thirdhelix Stearylpos2
C2thridhelix Stearylpos17
Delta4b
Delta4b,S54E
Delta4b, S54K
Delta4b, R43K
Delta4b, Y41W
Delta4b, T57F
Delta4b, V45F, F46Y, Y53L, F56A, T57F
D4
Free Fatty Acid, nmole
2.5
2.0
1.5
1.0
0.5
0
0.01
0.1
1.0
10
100
Peptide, uM

FIG. 8

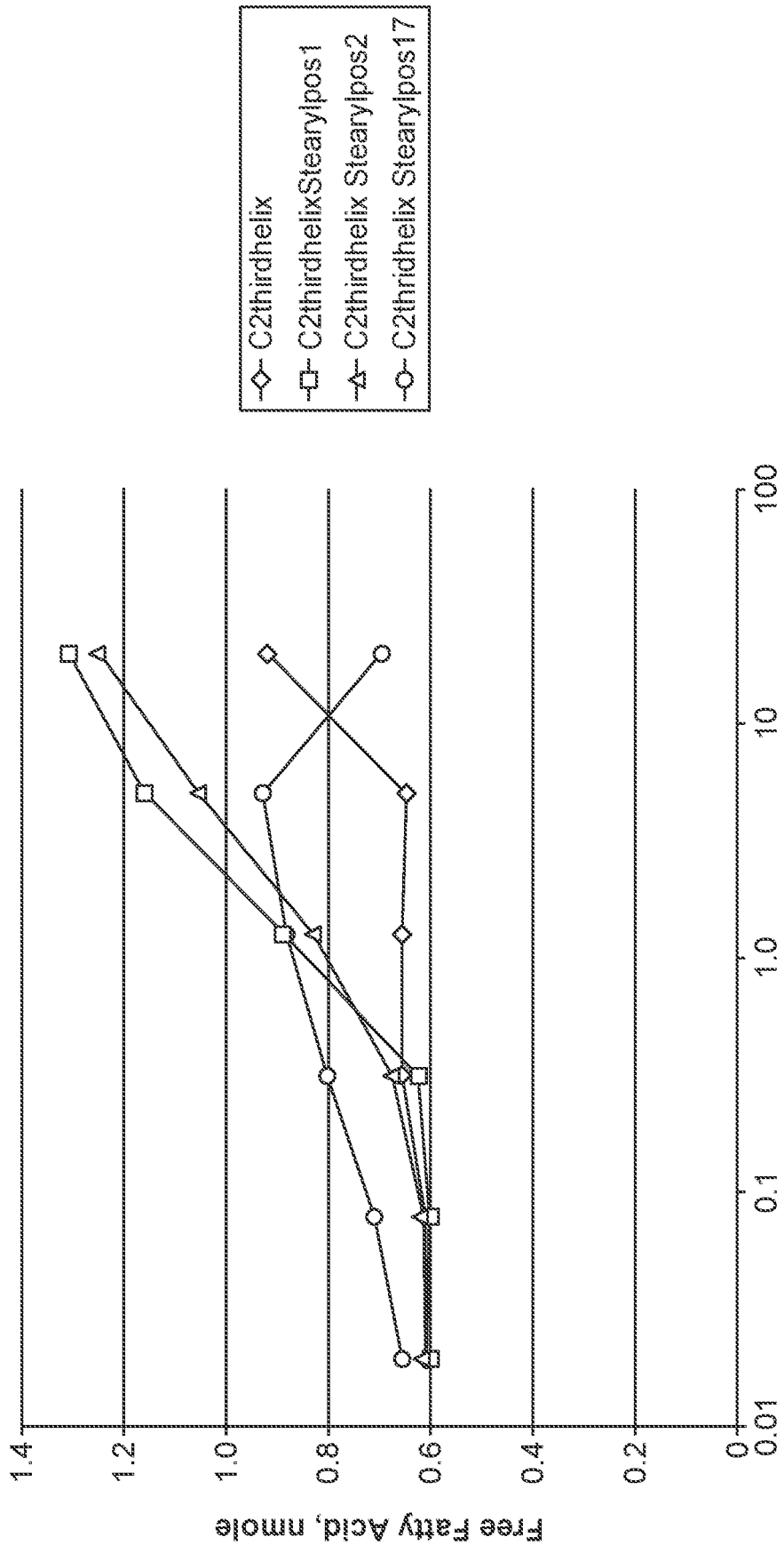
C2thirdhelix
C2thirdhelixStearylpos1
C2thirdhelix Stearylpos2
C2thridhelix Stearylpos17
Free Fatty Acid, nmole
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0.01
0.1
1.0
10
100
Peptide, uM

FIG. 9

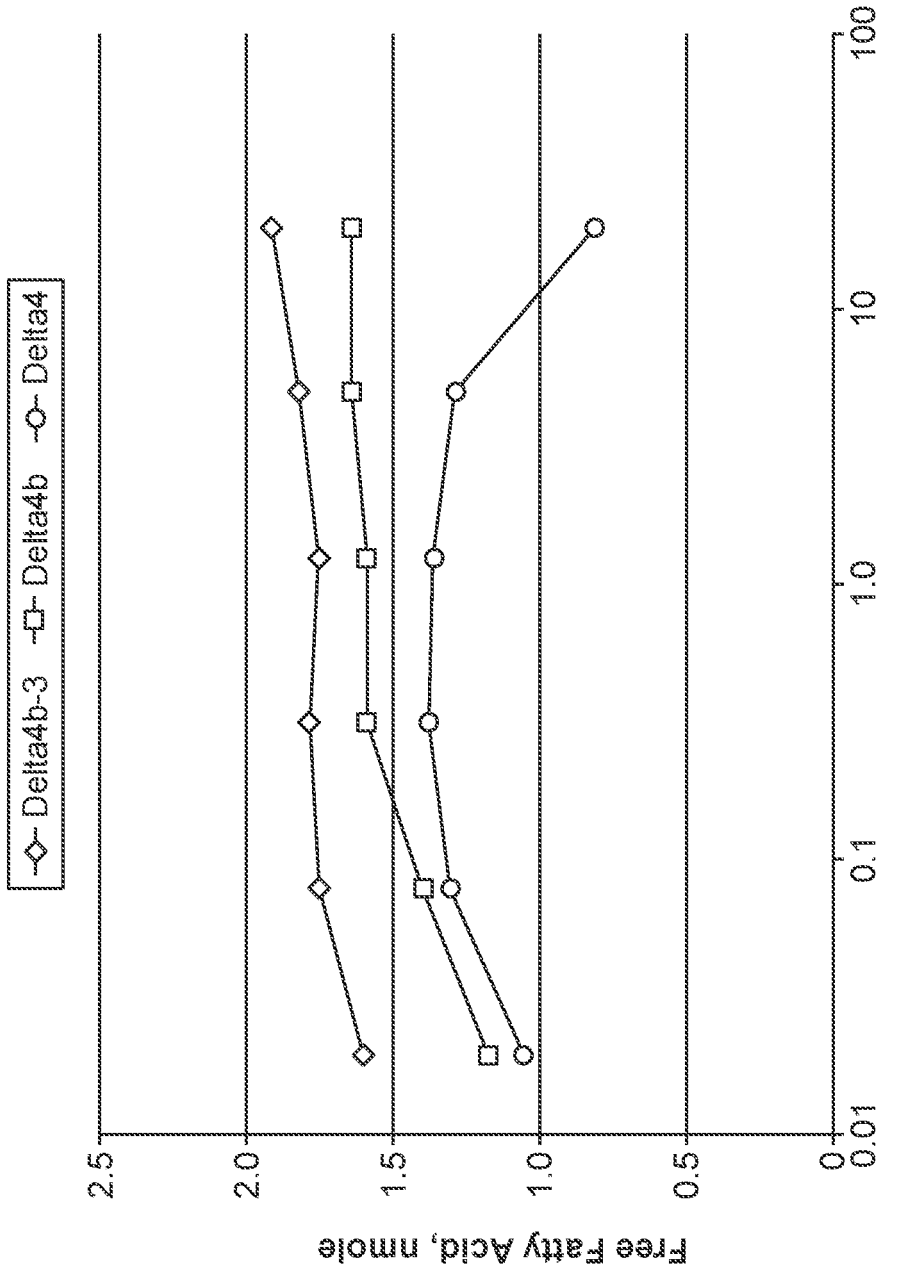
Delta4b-3
Delta4b
Delta4
Free Fatty Acid, nmole
2.5
2.0
1.5
1.0
0.5
0
0.01
0.1
1.0
10
100
Peptide, uM

FIG. 10

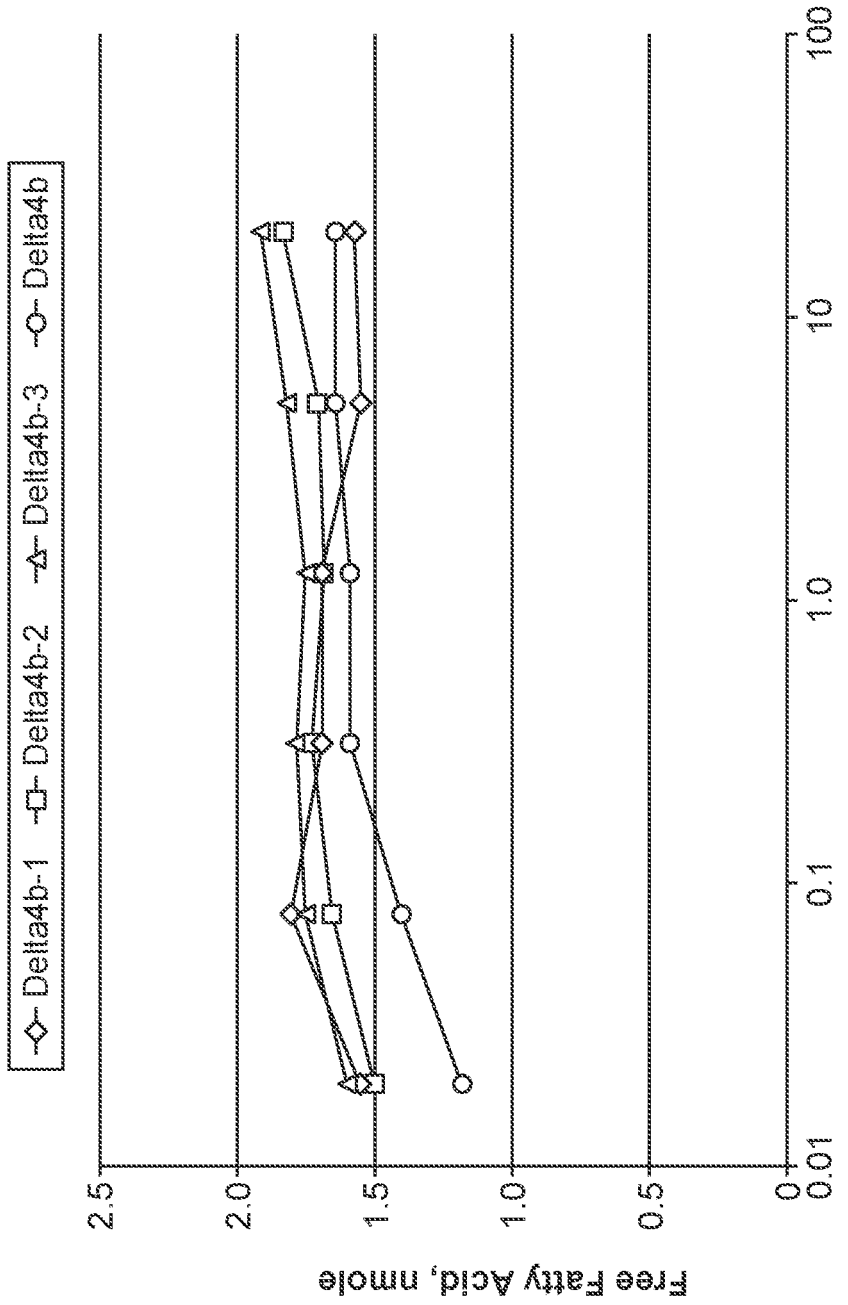
Delta4b-1
Delta4b-2
Delta4b-3
Delta4b
Free Fatty Acid, nmole
2.5
2.0
1.5
1.0
0.5
0
0.01
0.1
1.0
10
100
Peptide, uM

FIG. 11

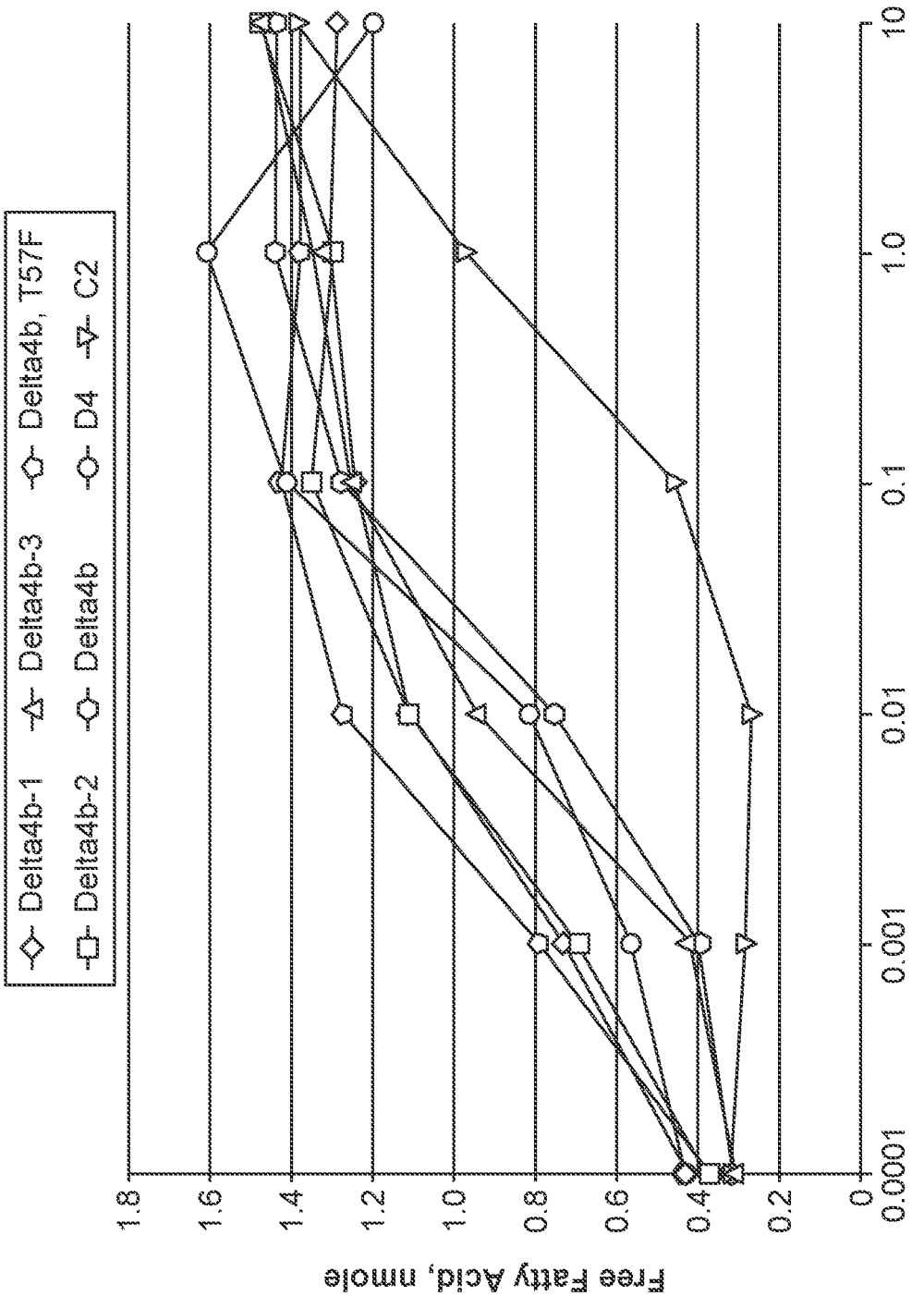
Delta4b-1
Delta4b-3
Delta4b, T57F
Delta4b-2
Delta4b
D4
C2
Free Fatty Acid, nmole
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0.0001
0.001
0.01
0.1
1.0
10
Peptide, uM

FIG. 12

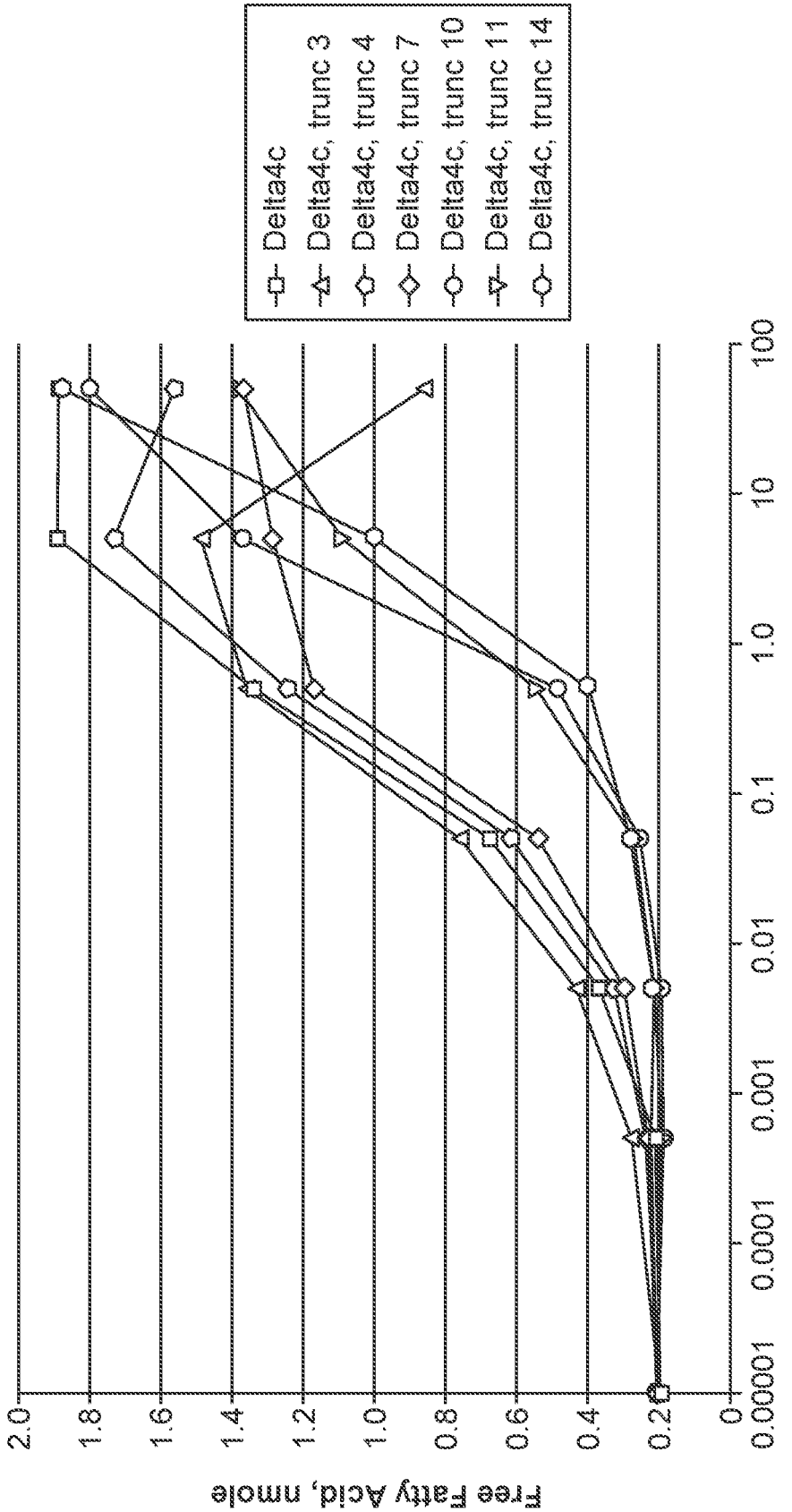
Delta4c
Delta4c, trunc 3
Delta4c, trunc 4
Delta4c, trunc 7
Delta4c, trunc 10
Delta4c, trunc 11
Delta4c, trunc 14
Free Fatty Acid, nmole
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0.00001
0.0001
0.001
0.01
0.1
1.0
10
100
Peptide, uM

FIG. 13

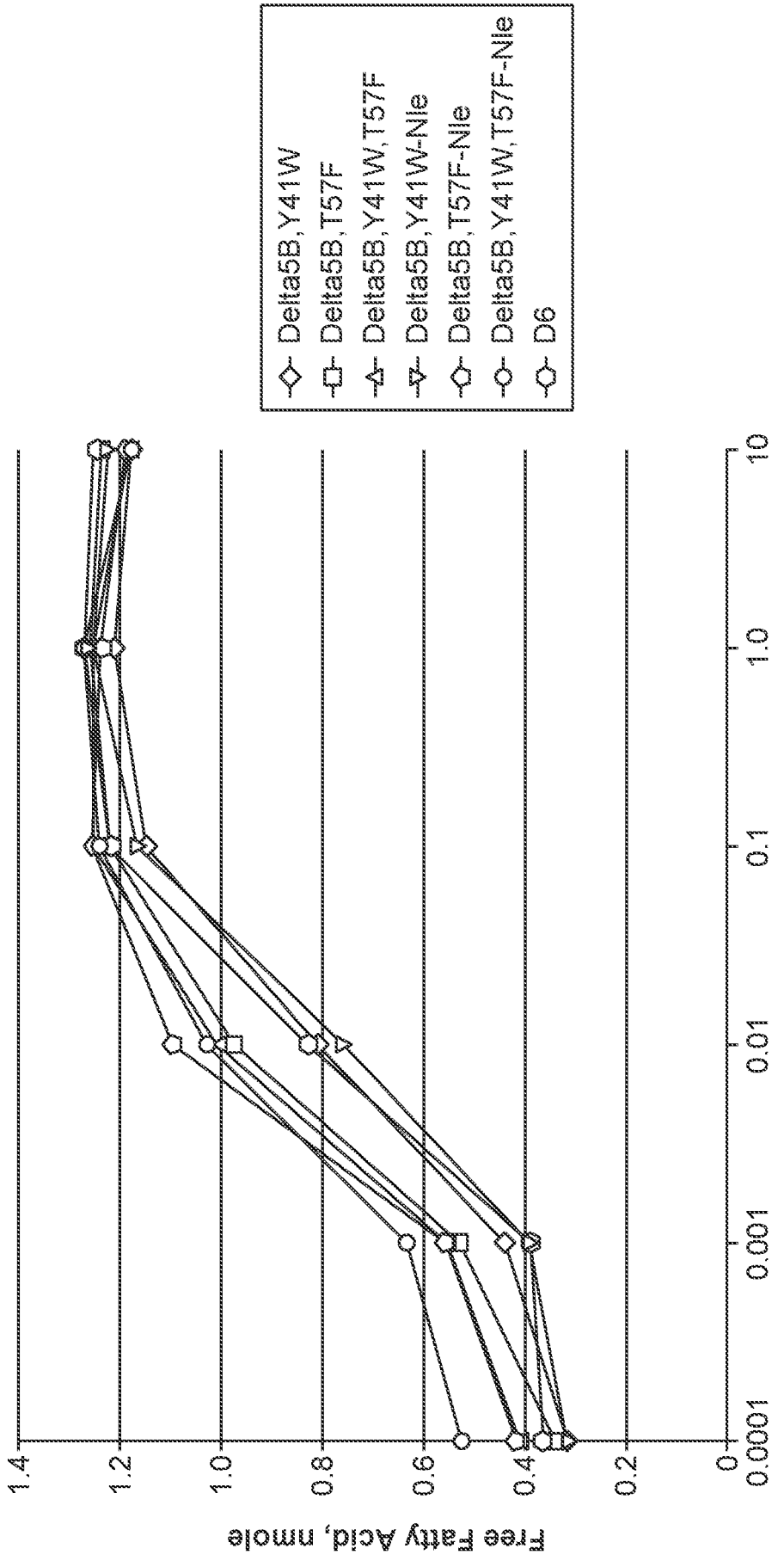
Delta5B,Y41W
Delta5B,T57F
Delta5B,Y41W,T57F
Delta5B,Y41W-Nle
Delta5B,T57F-Nle
Delta5B,Y41W,T57F-Nle
D6
Free Fatty Acid, nmole
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0.0001
0.001
0.01
0.1
1.0
10
Peptides, uM

FIG. 14

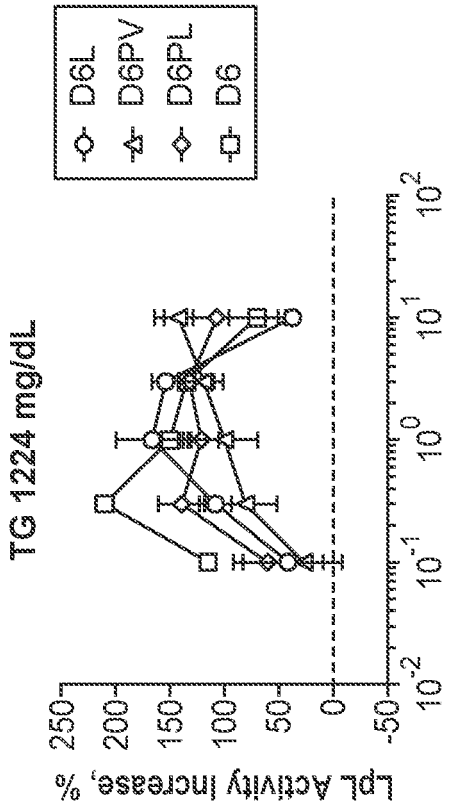

FIG. 15B

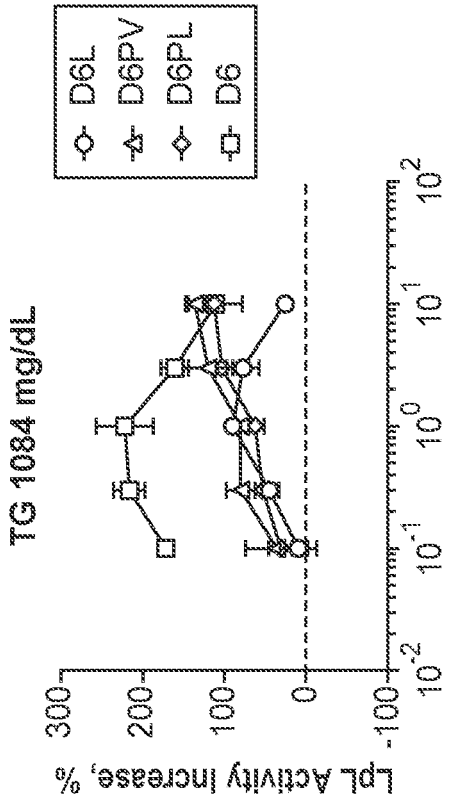

FIG. 15A

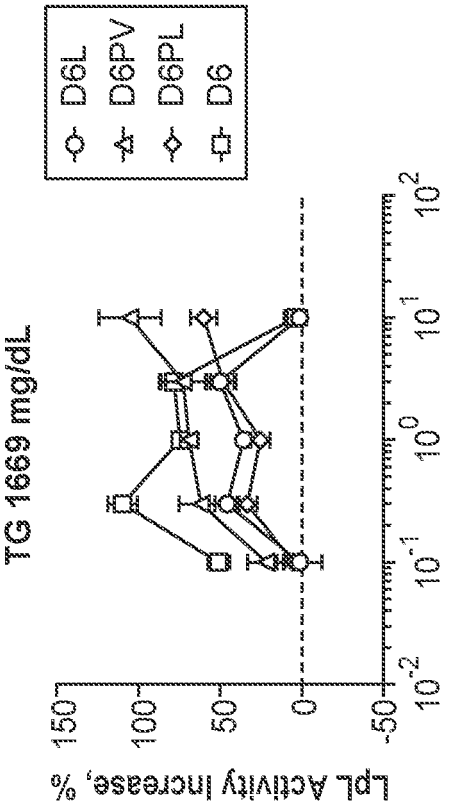

FIG. 15D

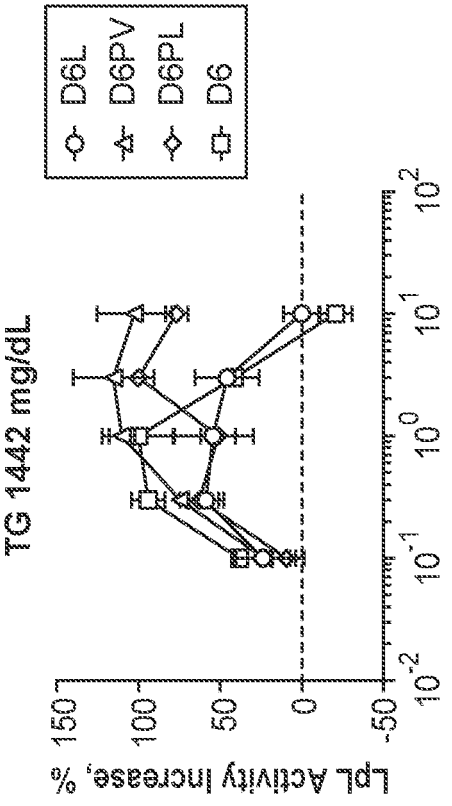

FIG. 15C

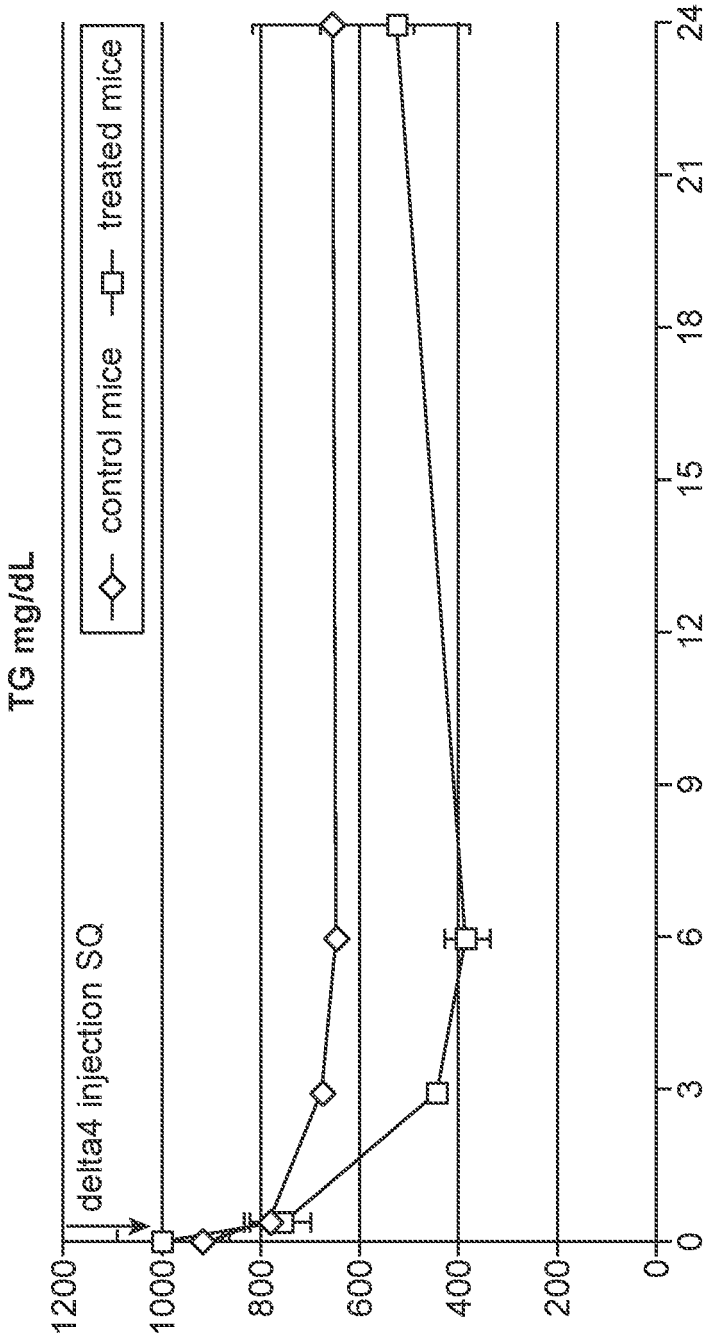
TG mg/dL
control mice
treated mice
delta4 injection SQ
1200
1000
800
600
400
200
0
0
3
6
9
12
15
18
21
24
hours

FIG. 16

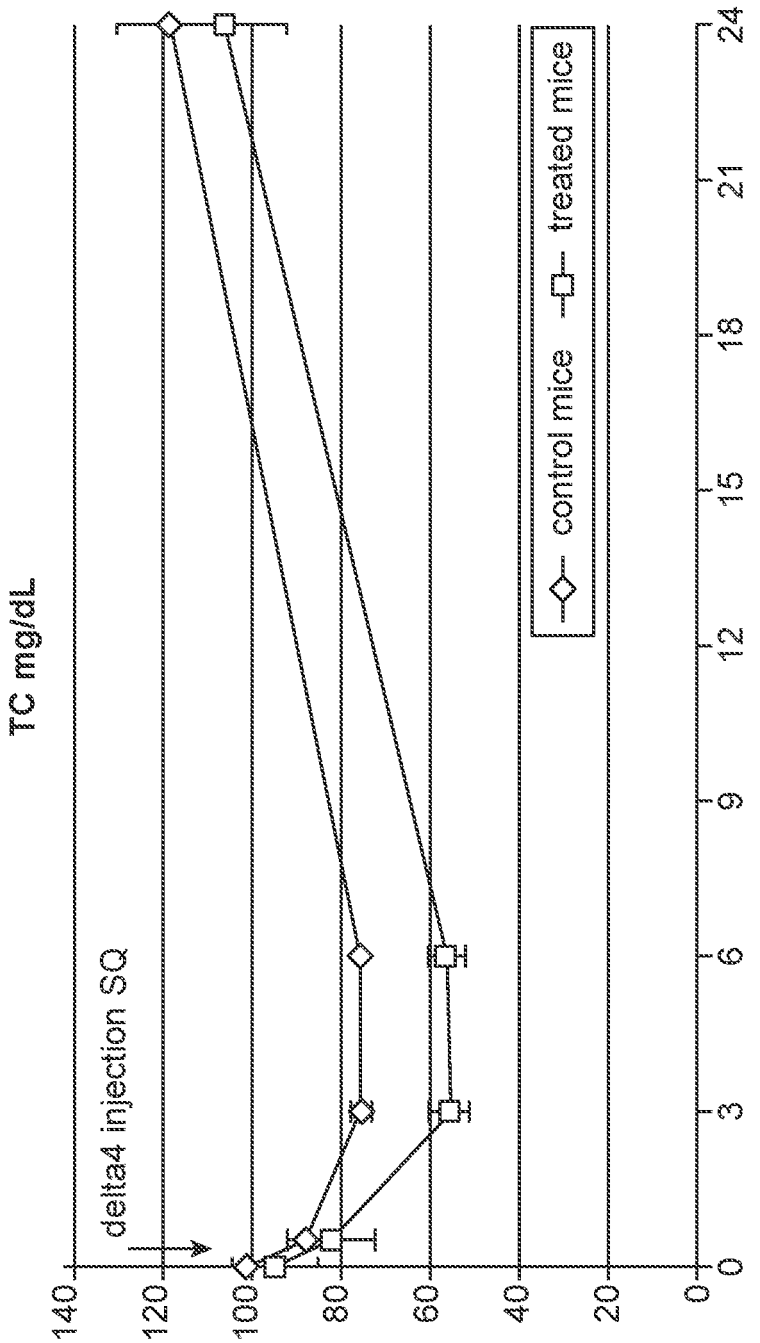
TC mg/dL
delta4 injection SQ
140
120
100
80
60
40
20
0
0
3
6
9
12
15
18
21
24
hours
control mice
treated mice

FIG. 17

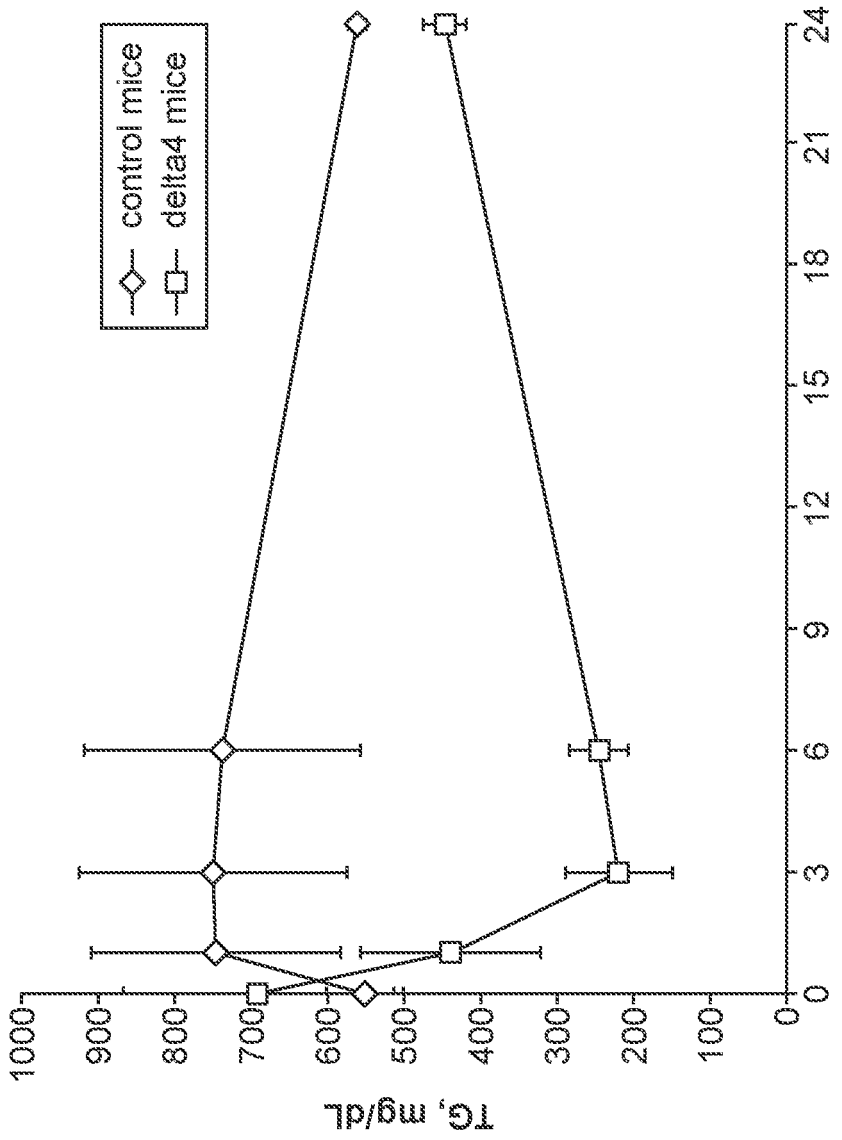
control mice
delta4 mice
TG, mg/dL
1000
900
800
700
600
500
400
300
200
100
0
0
3
6
9
12
15
18
21
24
Hour

FIG. 18

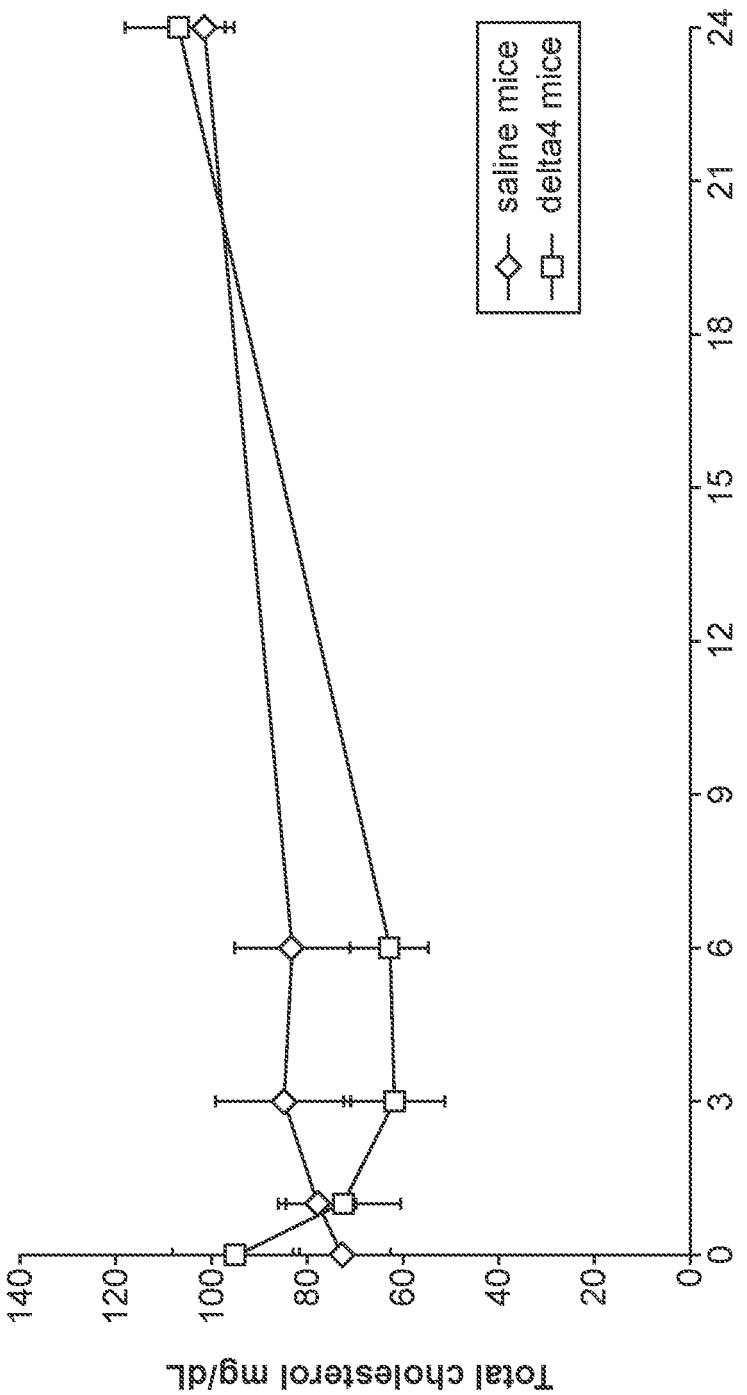
Total cholesterol mg/dL
140
120
100
80
60
40
20
0
0
3
6
9
12
15
18
21
24
Hours
saline mice
delta4 mice

FIG. 19

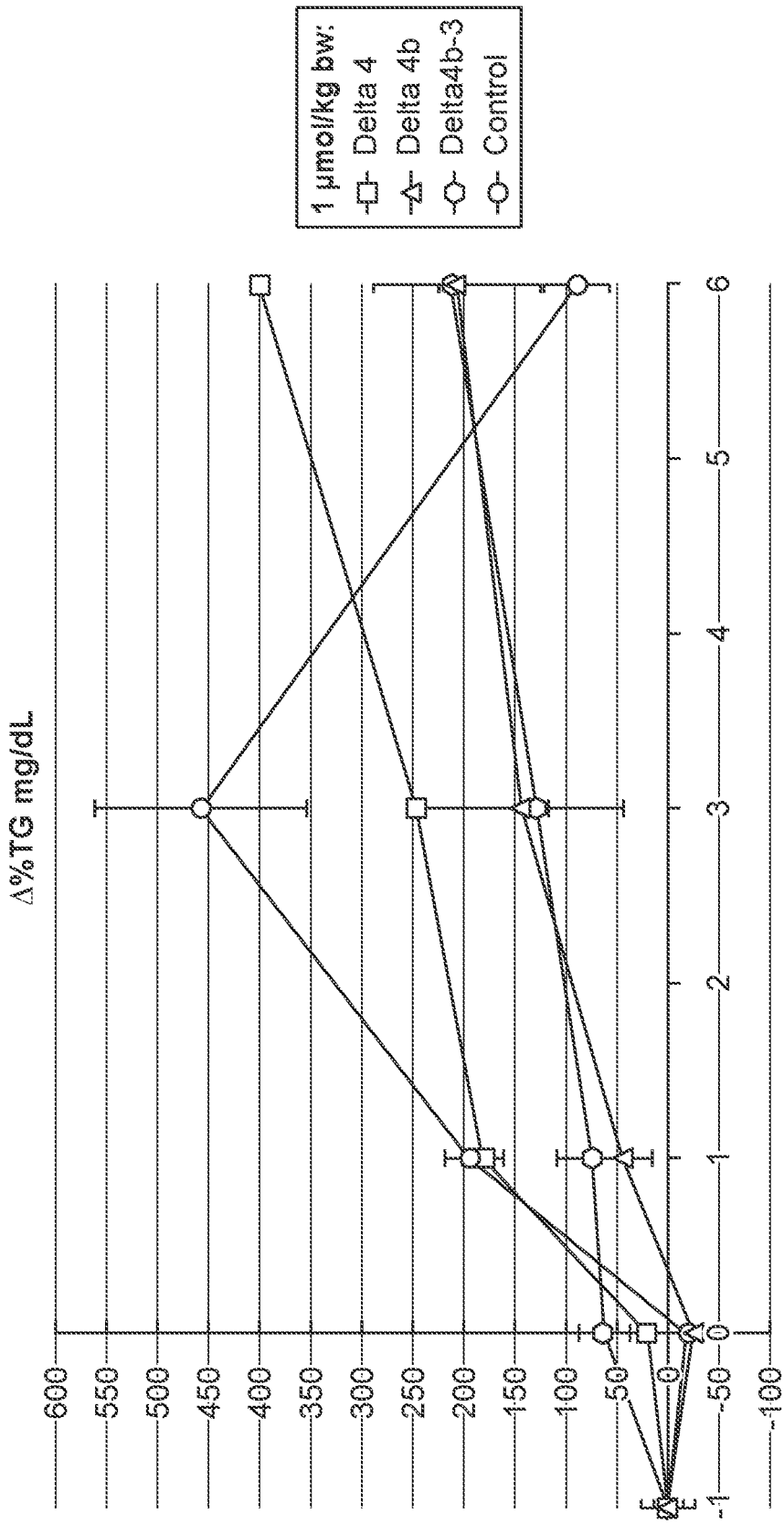
1 µmol/kg bw:
Delta 4
Delta 4b
Delta4b-3
Control
Δ%TG mg/dL
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
-100
-1
0
1
2
3
4
5
6
peptides IP
vegetable oil gavage

FIG. 20

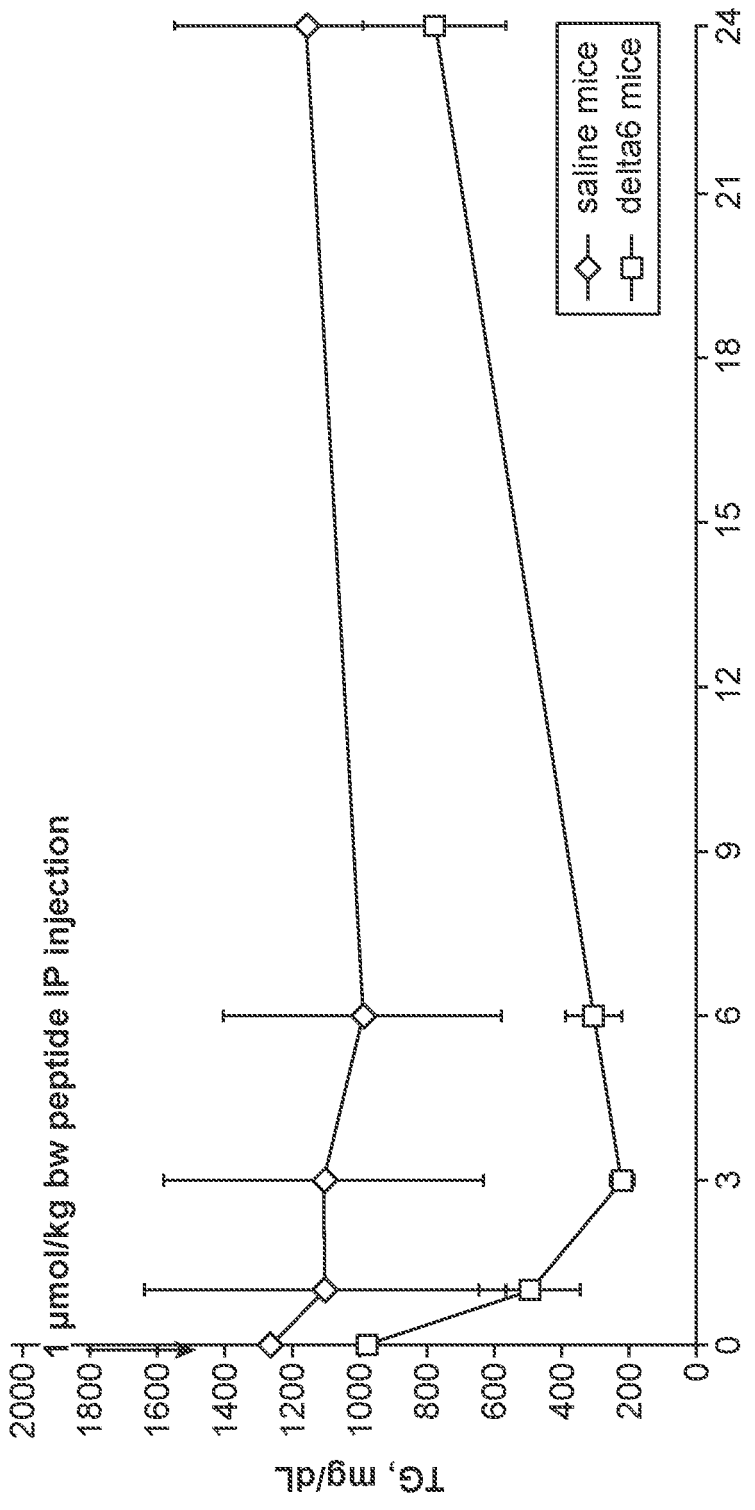
TG, mg/dL
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1 µmol/kg bw peptide IP injection
0
3
6
9
12
15
18
21
24
Hour
saline mice
delta6 mice

FIG. 21

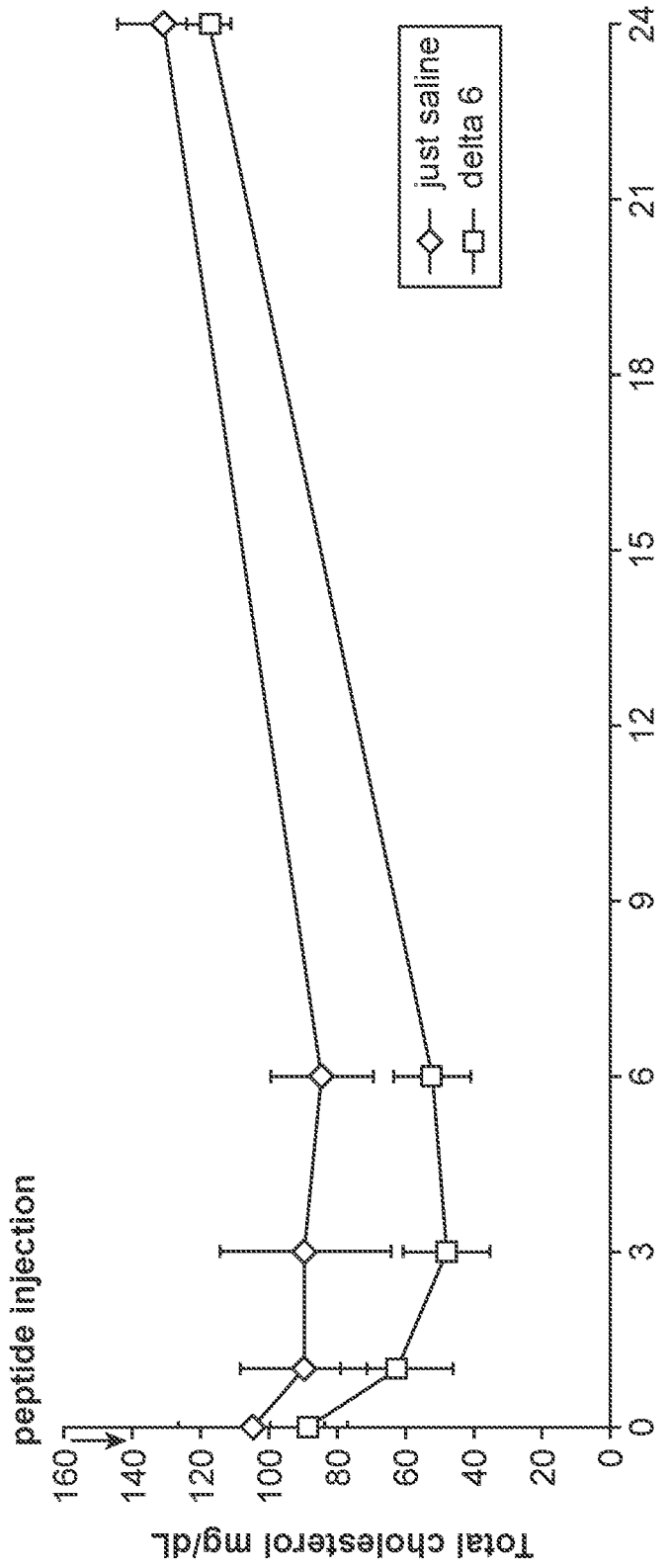
peptide injection
total cholesterol mg/dL
160
140
120
100
80
60
40
20
0
0
3
6
9
12
15
18
21
24
hours
just saline
delta 6

FIG. 22

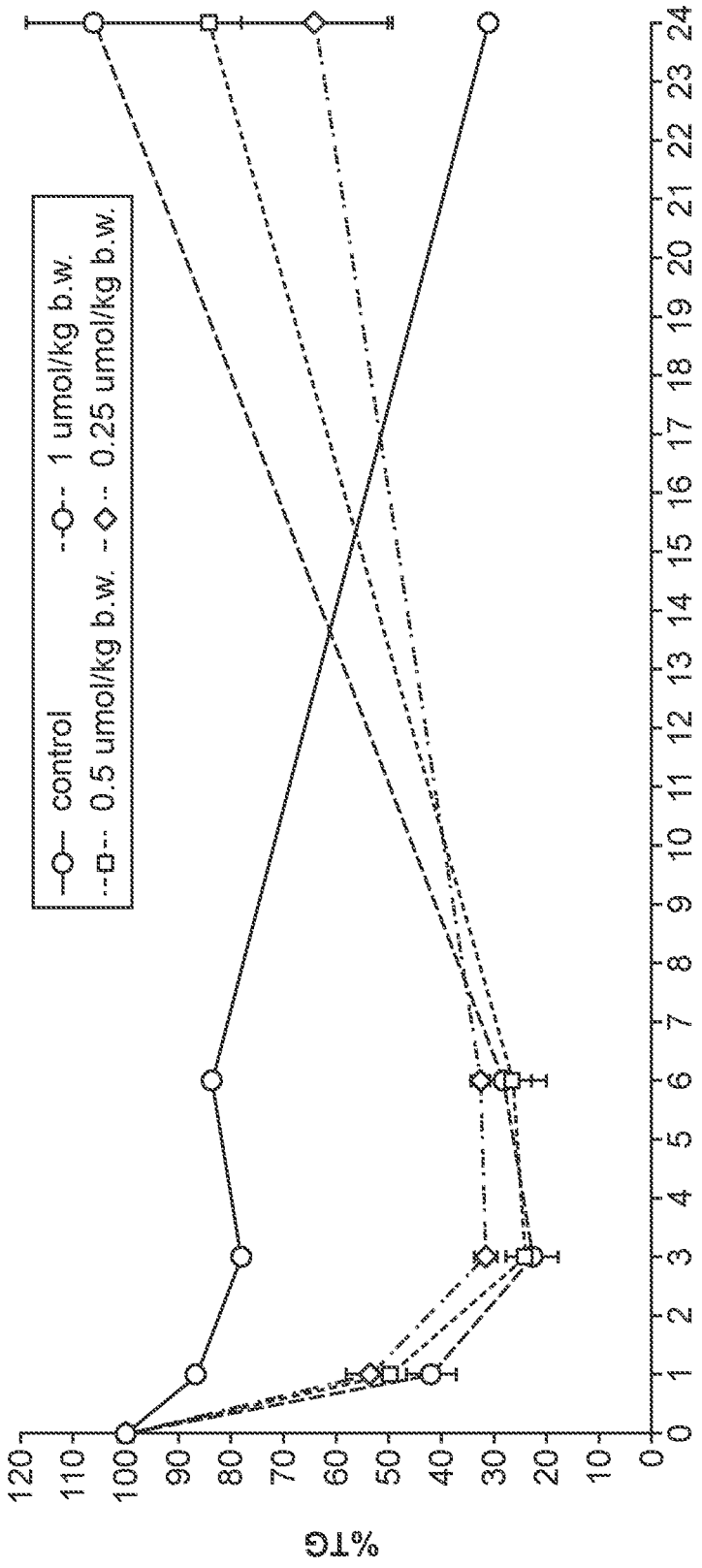
control
1 umol/kg b.w.
0.5 umol/kg b.w.
0.25 umol/kg b.w.
%TG
Hour
120
110
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

FIG. 23

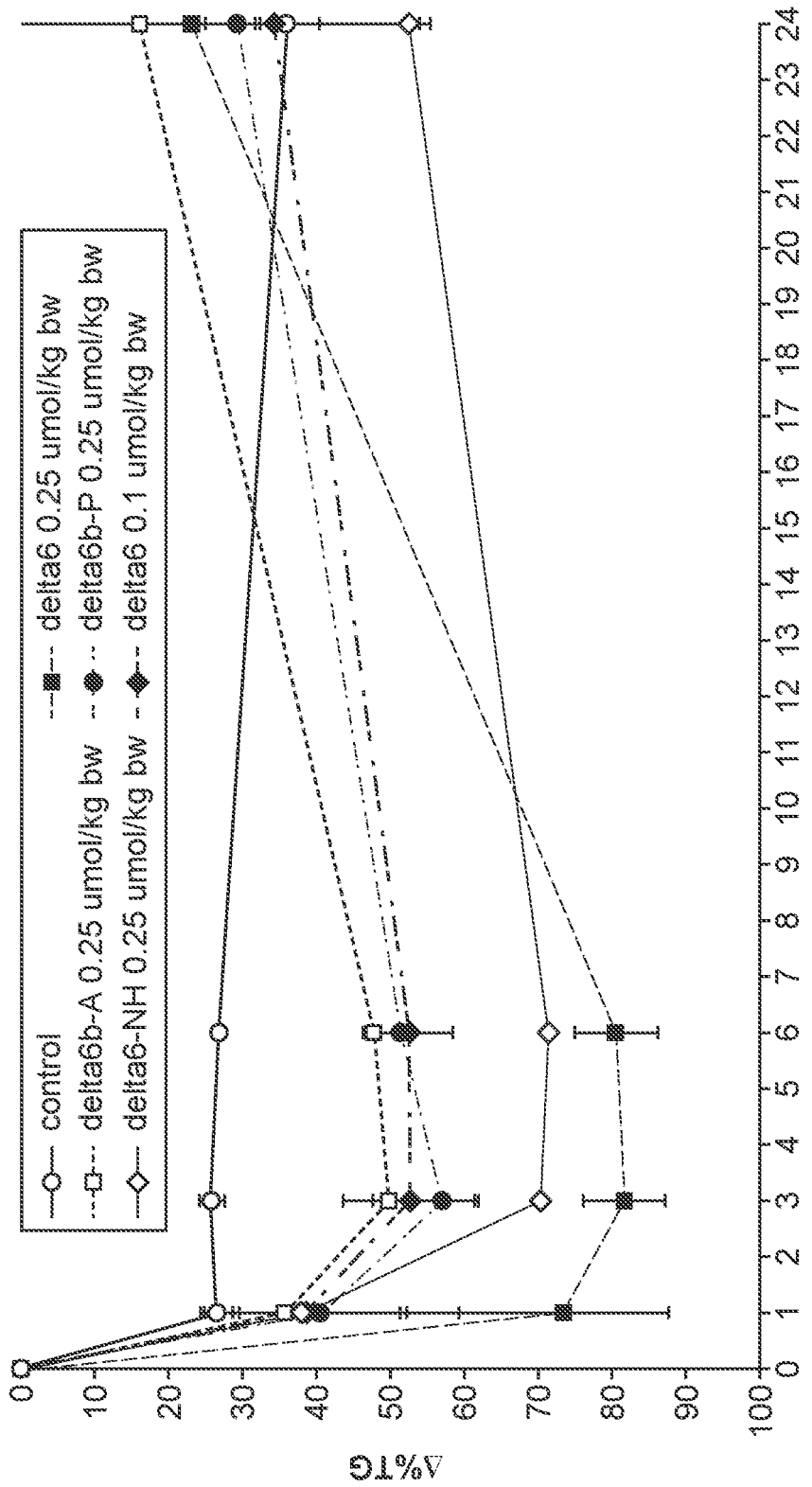
control
delta6b-A 0.25 umol/kg bw
delta6-NH 0.25 umol/kg bw
delta6 0.25 umol/kg bw
delta6b-P 0.25 umol/kg bw
delta6 0.1 umol/kg bw
Δ%TG
Hour
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

FIG. 24

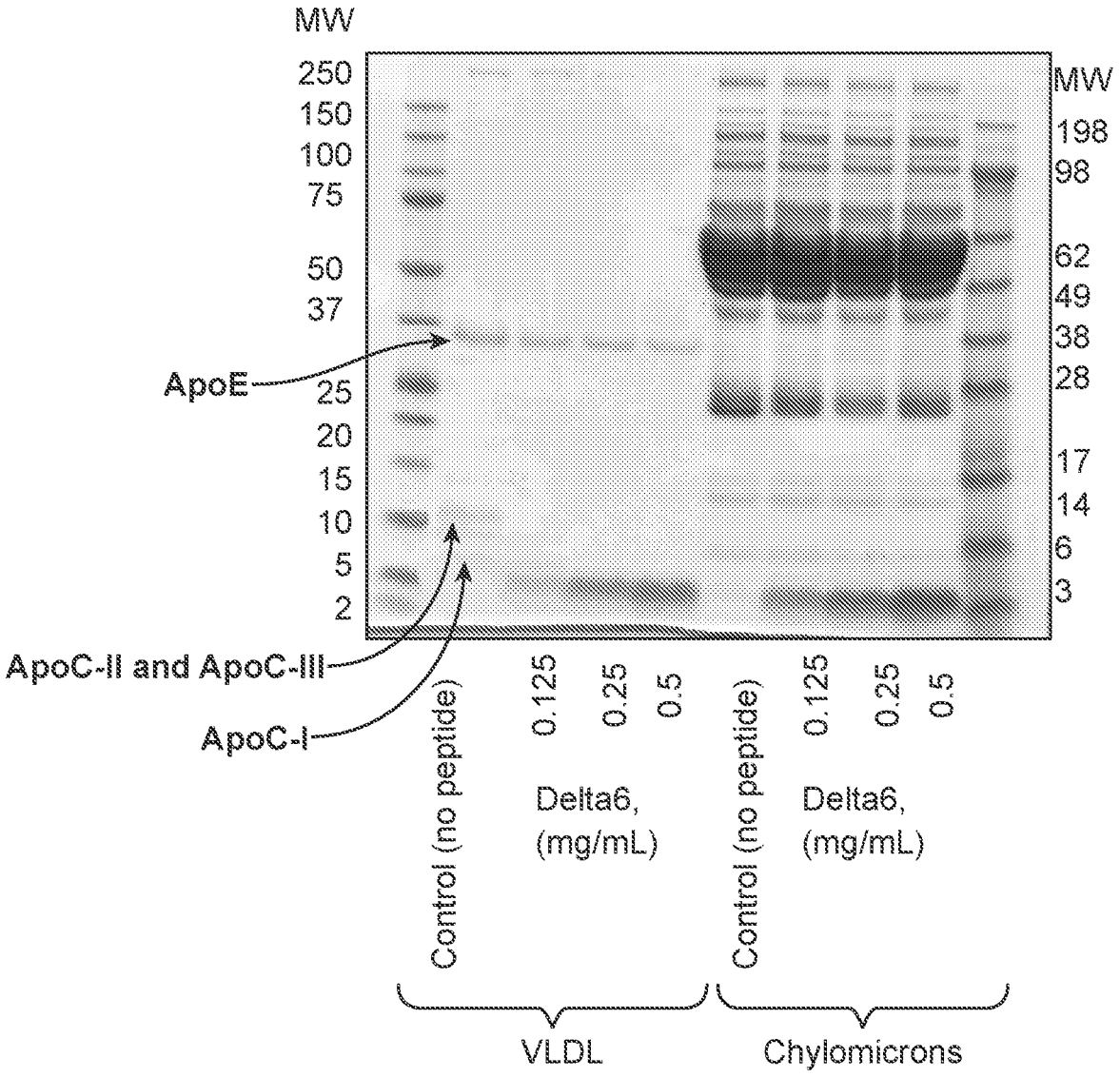
MW
250
150
100
75
50
37
25
20
15
10
5
2
MW
198
98
62
49
38
28
17
14
6
3
ApoE
ApoC-II and ApoC-III
ApoC-I
Control (no peptide)
0.125
0.25
0.5
Delta6, (mg/mL)
Control (no peptide)
0.125
0.25
0.5
Delta6, (mg/mL)
VLDL
Chylomicrons

FIG. 25

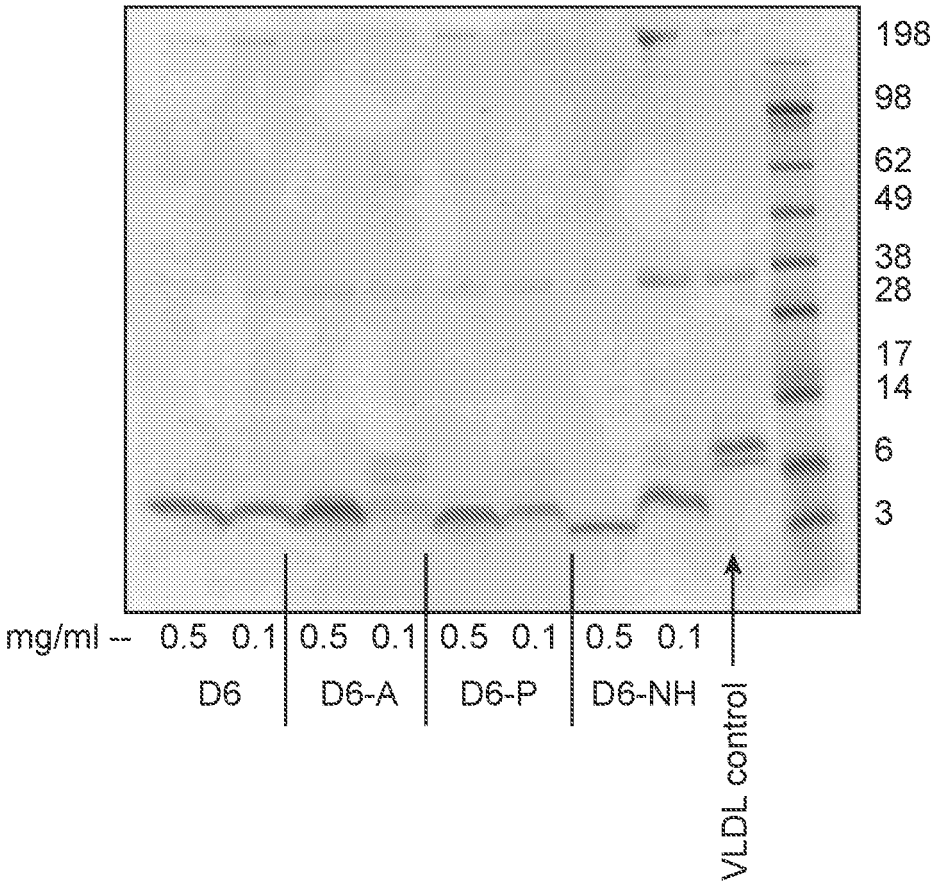
198
98
62
49
38
28
17
14
6
3
mg/ml --
0.5
0.1
0.5
0.1
0.5
0.1
0.5
0.1
D6
D6-A
D6-P
D6-NH
VLDL control

FIG. 26

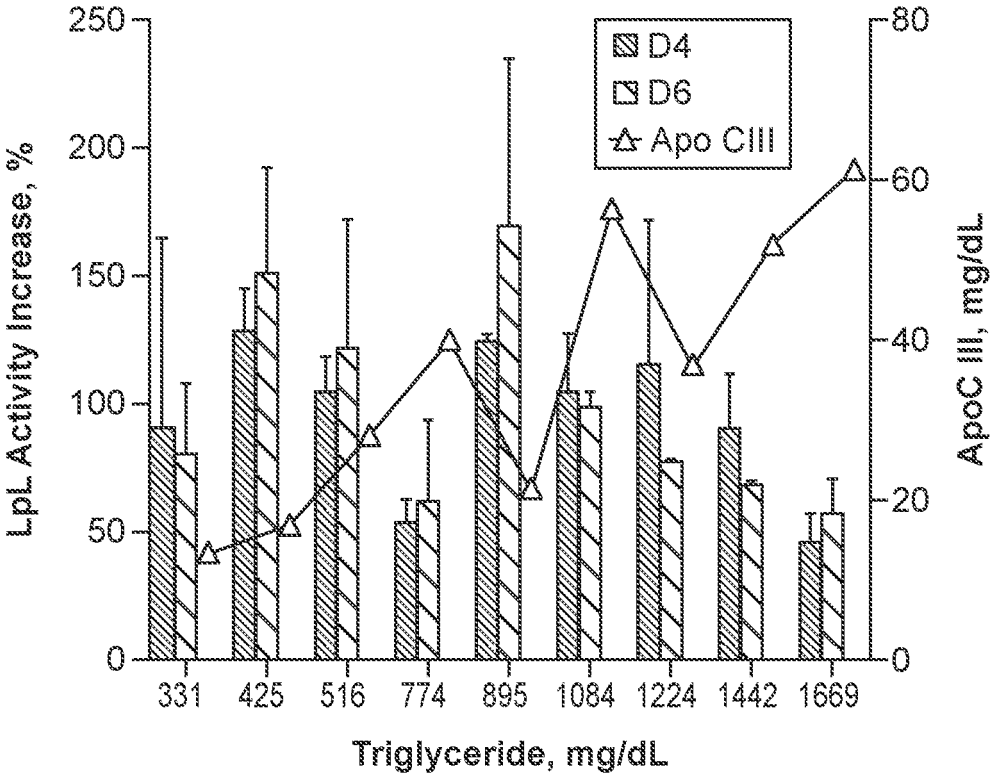
250
200
150
100
50
0
80
60
40
20
0
D4
D6
Apo CIII
LpL Activity Increase, %
ApoC III, mg/dL
331
425
516
774
895
1084
1224
1442
1669
Triglyceride, mg/dL

FIG. 27

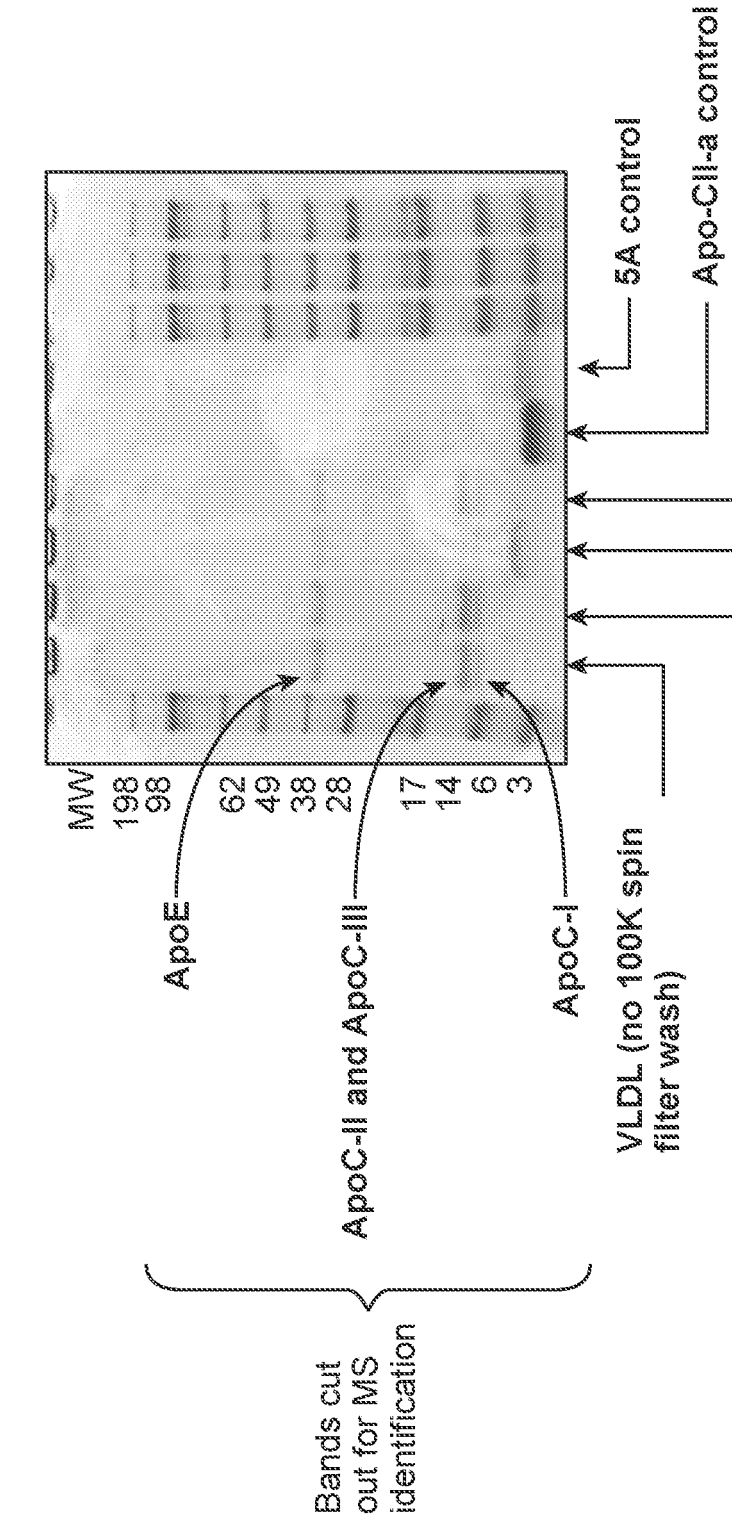
MW
198
98
62
49
38
28
17
14
6
3
ApoE
ApoC-II and ApoC-III
ApoC-I
Bands cut out for MS identification
VLDL (no 100K spin filter wash)
VLDL (3 x 100K spin filter wash)
VLDL + Apo-CII-a (3 x 100K spin filter wash)
VLDL + 5A peptide (3 x 100K spin filter wash)
Apo-CII-a control
5A control

FIG. 28

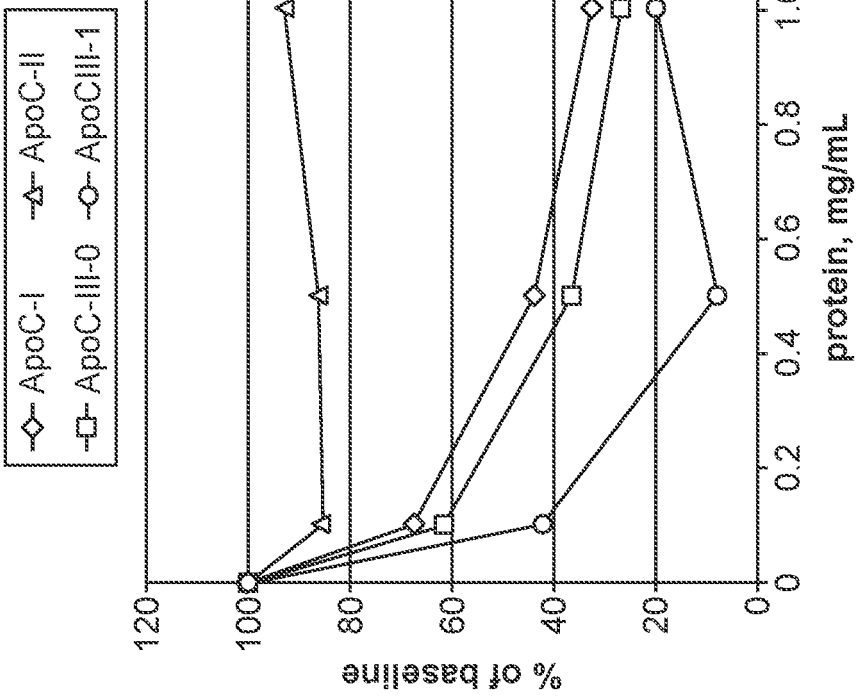

FIG. 29A

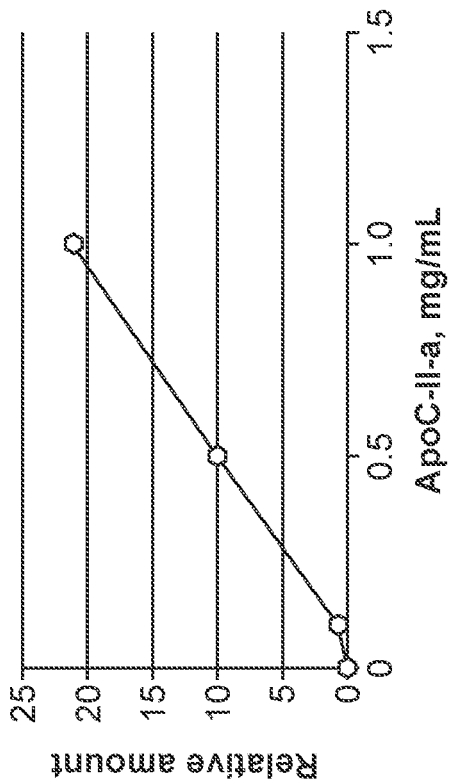

FIG. 29B

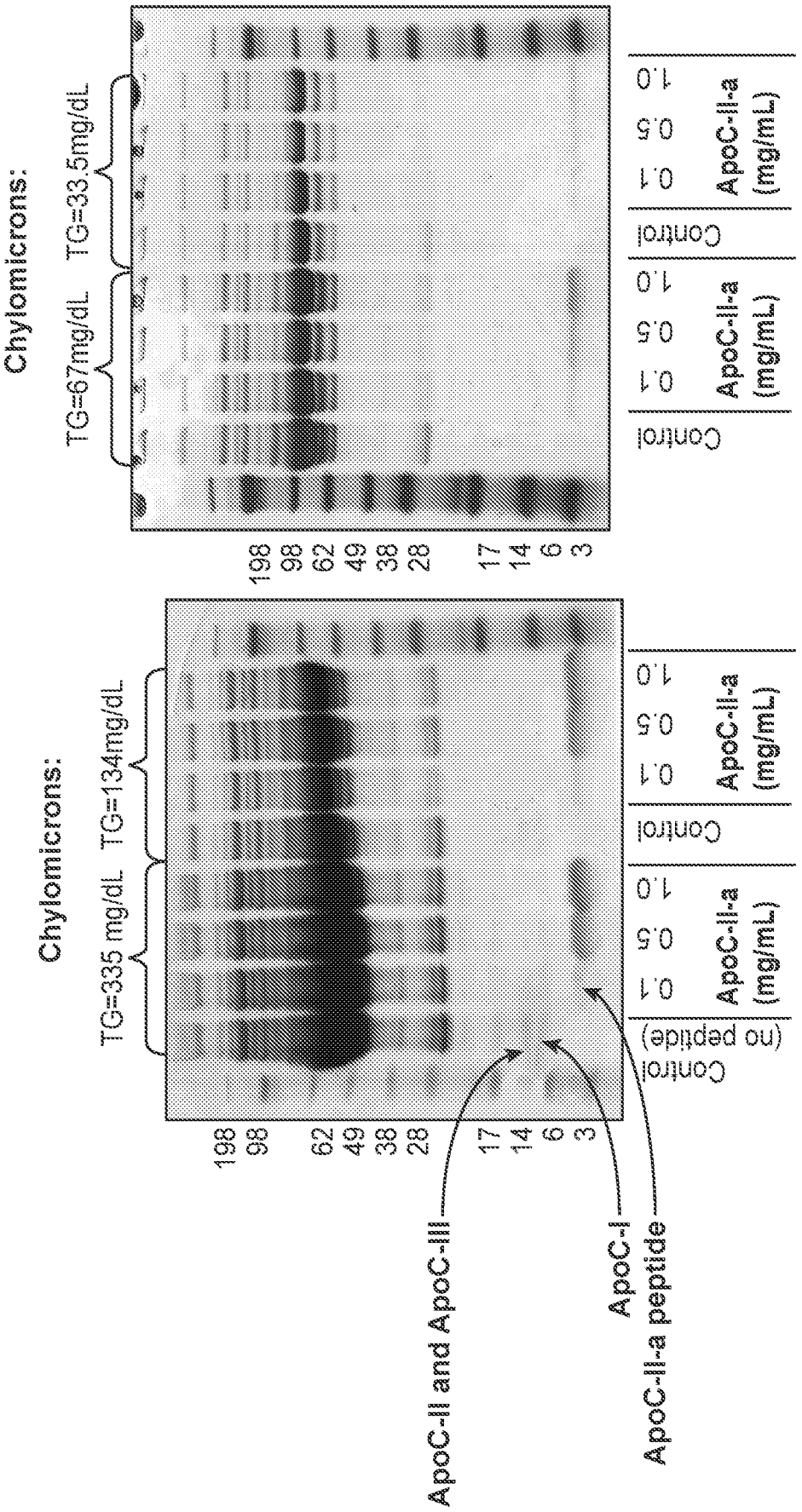
Chylomicrons:
TG=335 mg/dL
TG=134mg/dL
198
98
62
49
38
28
17
14
6
3
Control (no peptide)
Control
0.1
0.5
1.0
ApoC-II-a (mg/mL)
ApoC-II and ApoC-III
ApoC-I
ApoC-II-a peptide
Chylomicrons:
TG=67mg/dL
TG=33.5mg/dL

FIG. 30

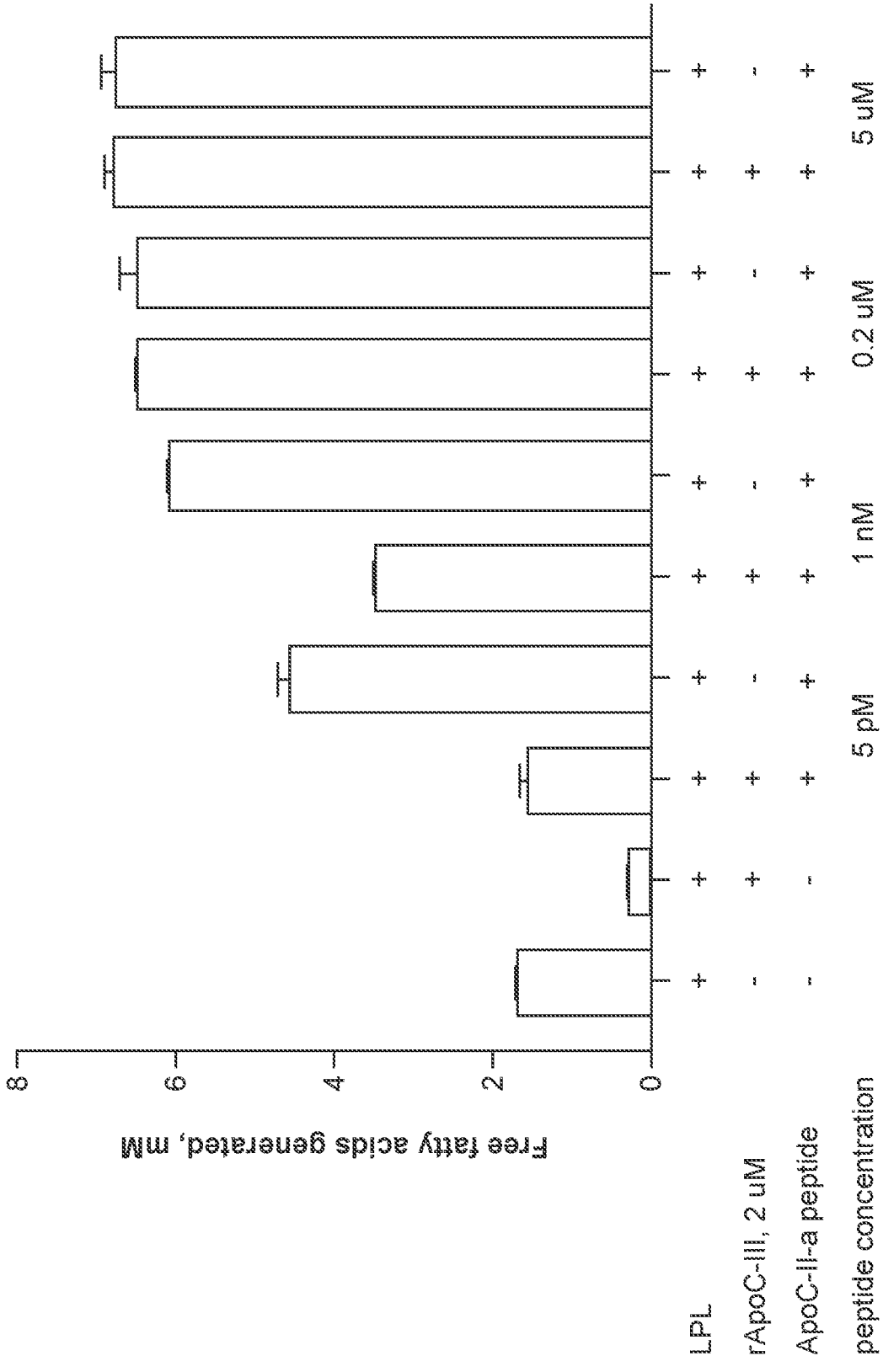
Free fatty acids generated, mM
0
2
4
6
8
LPL
rApoC-III, 2 uM
ApoC-II-a peptide
peptide concentration
5 pM
1 nM
0.2 uM
5 uM

FIG. 31

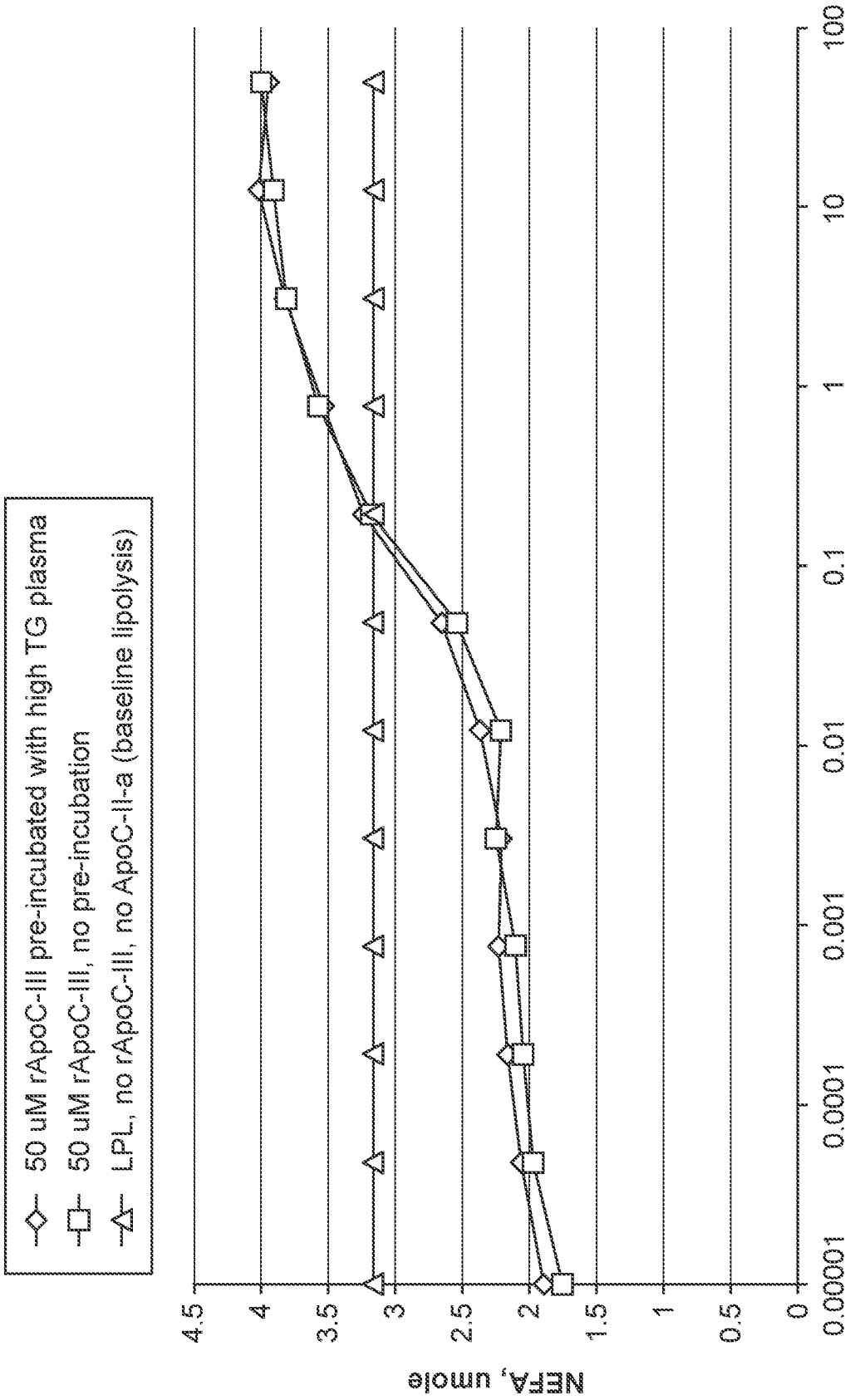
50 uM rApoC-III pre-incubated with high TG plasma
50 uM rApoC-III, no pre-incubation
LPL, no rApoC-III, no ApoC-II-a (baseline lipolysis)
NEFA, umole
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
ApoC-II-a concentration, uM

FIG. 32

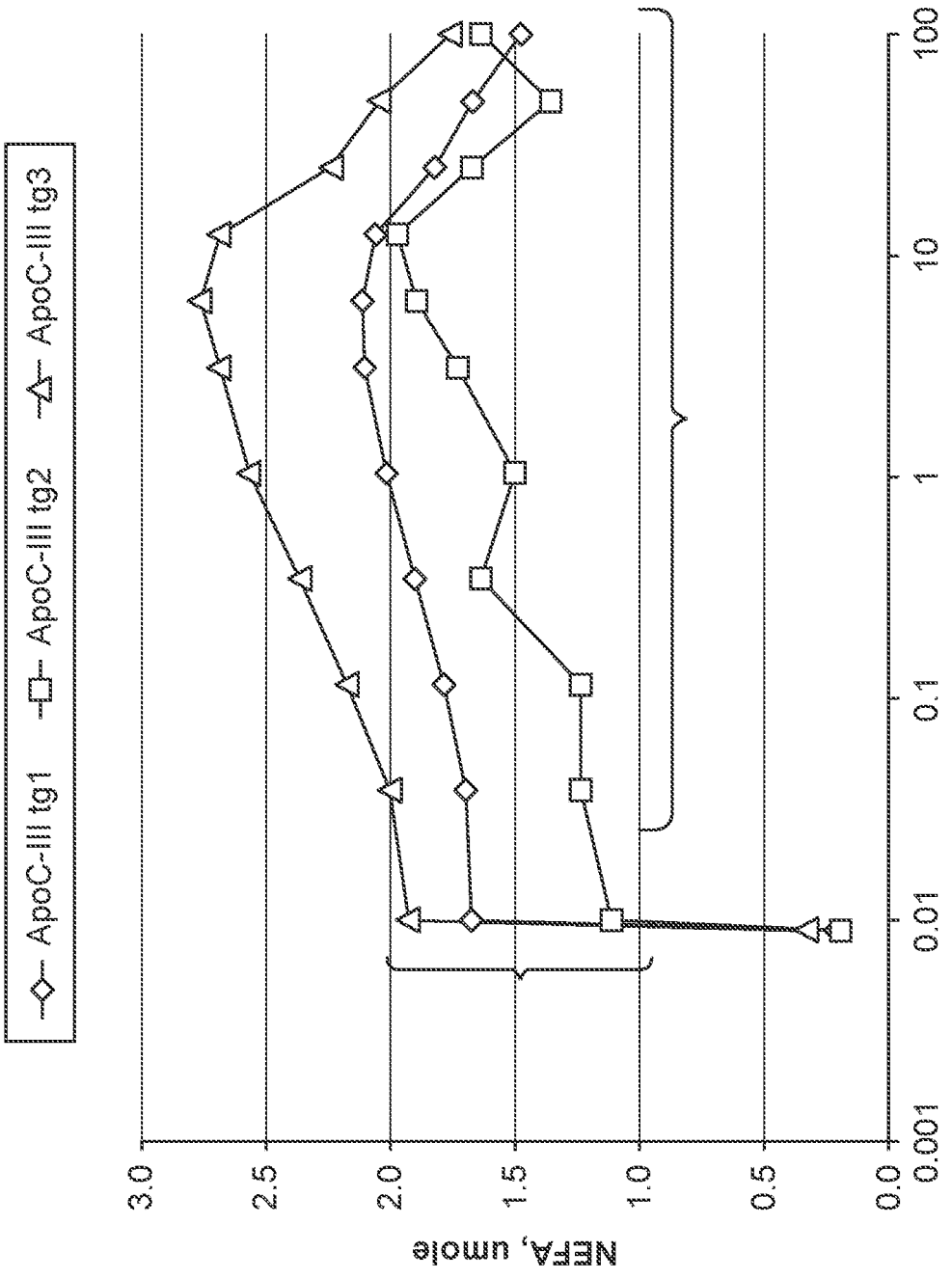
ApoC-III tg1
ApoC-III tg2
ApoC-III tg3
NEFA, umole
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
ApoC-II-a peptide, uM

FIG. 33

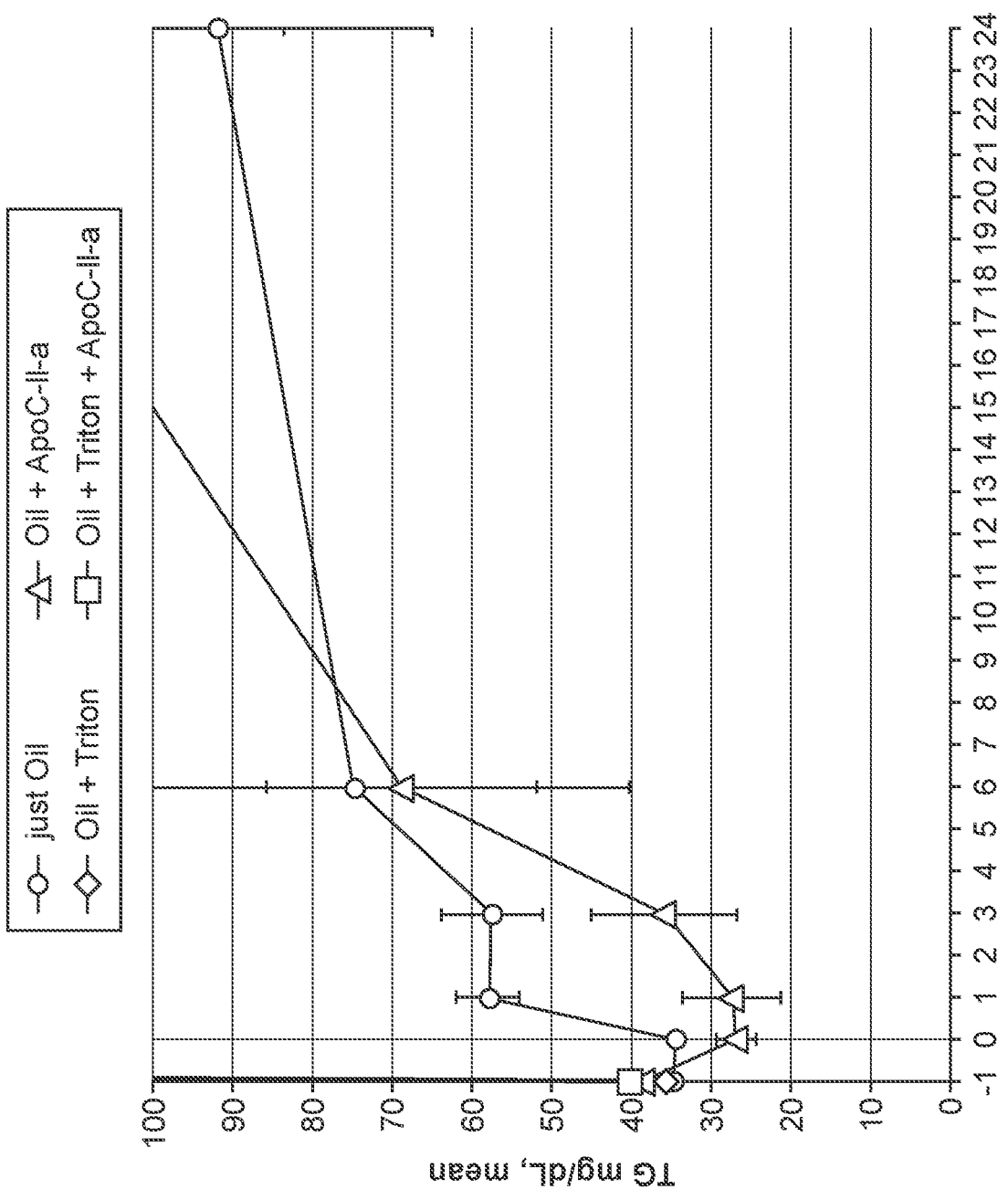
just Oil
Oil + ApoC-II-a
Oil + Triton
Oil + Triton + ApoC-II-a
TG mg/dL, mean
100
90
80
70
60
50
40
30
20
10
0
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
hour

FIG. 34A

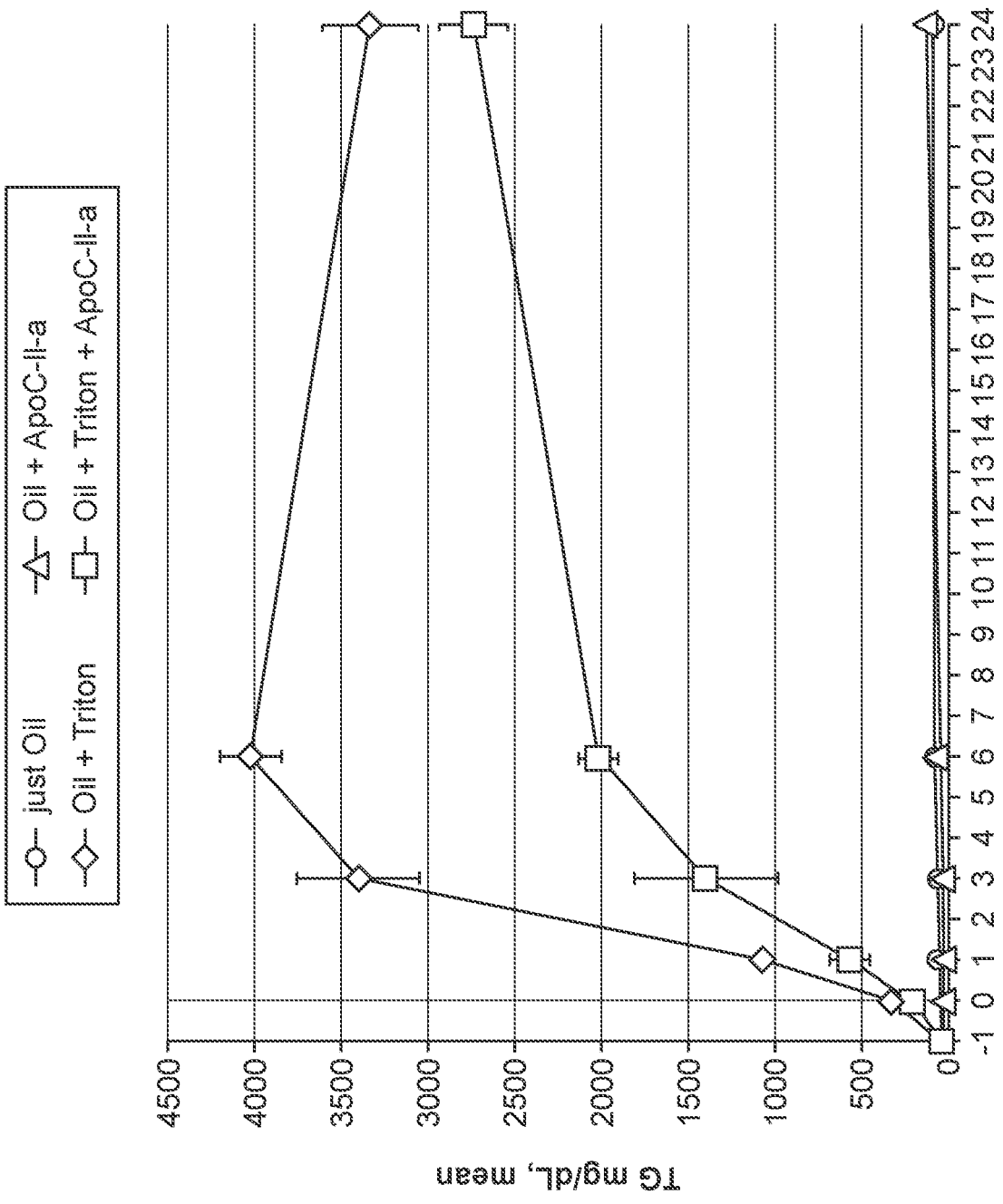
just Oil
Oil + ApoC-II-a
Oil + Triton
Oil + Triton + ApoC-II-a
TG mg/dL, mean
4500
4000
3500
3000
2500
2000
1500
1000
500
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
hour

FIG. 34B

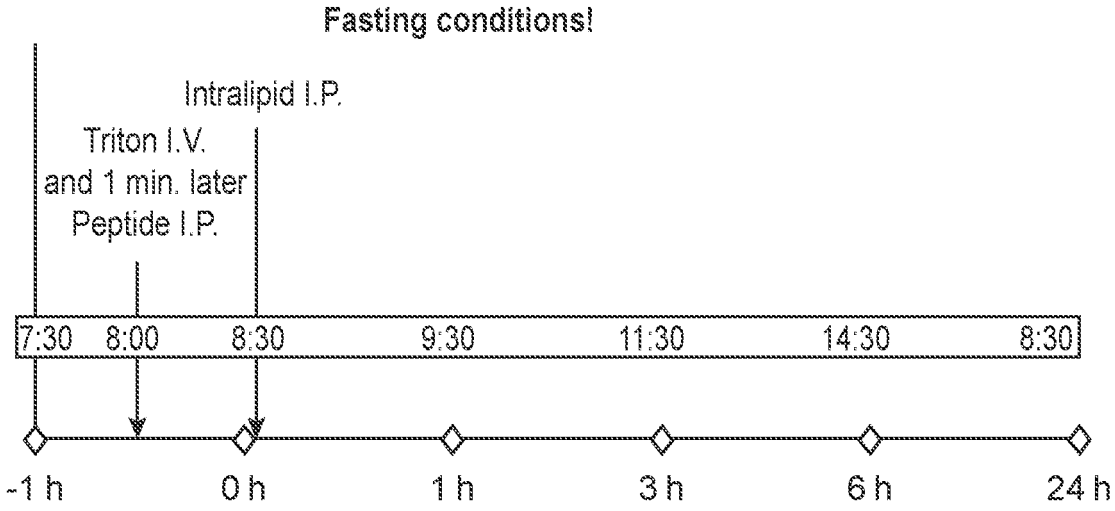

FIG. 35A

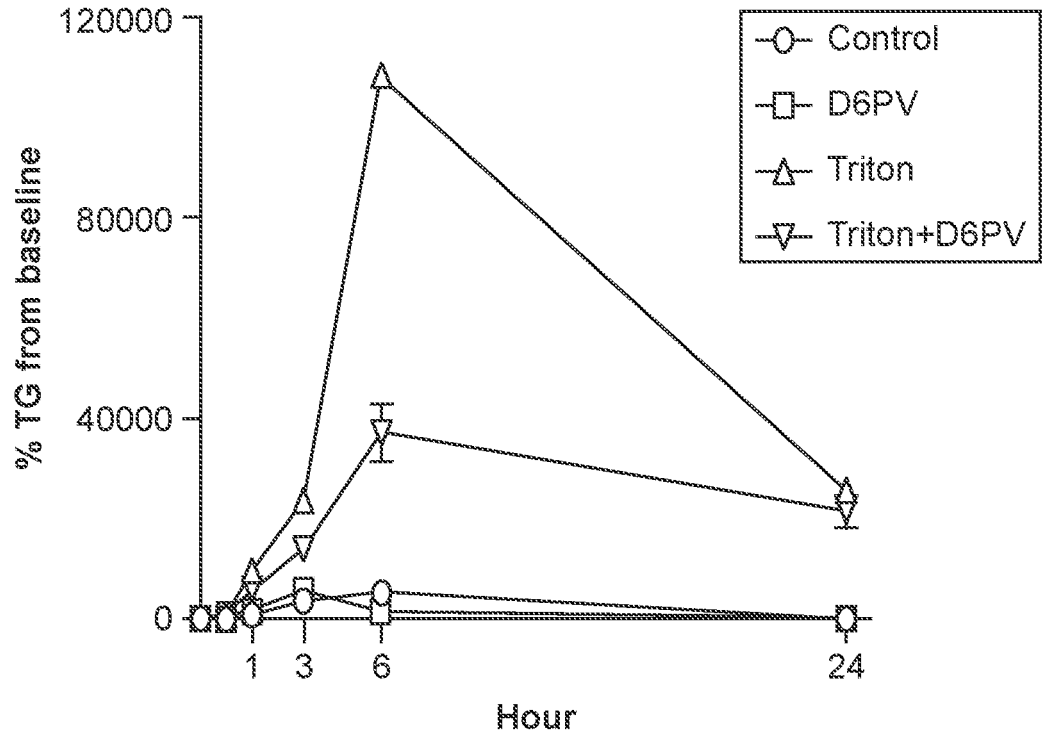

FIG. 35B

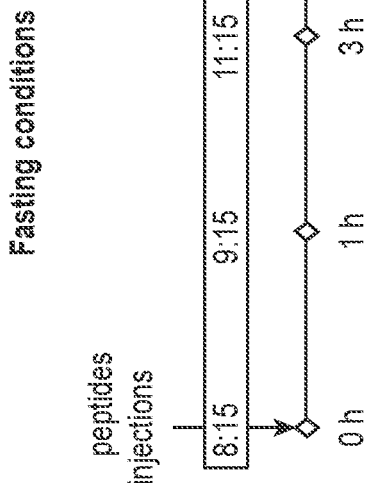

FIG. 36A

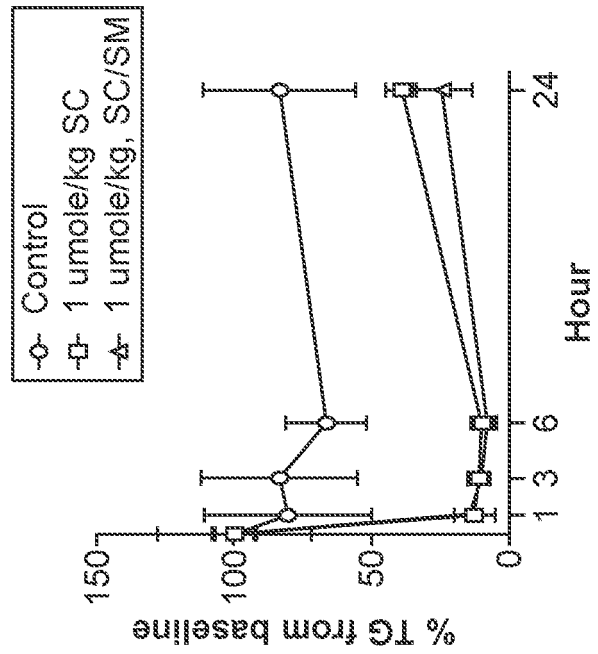

FIG. 36C

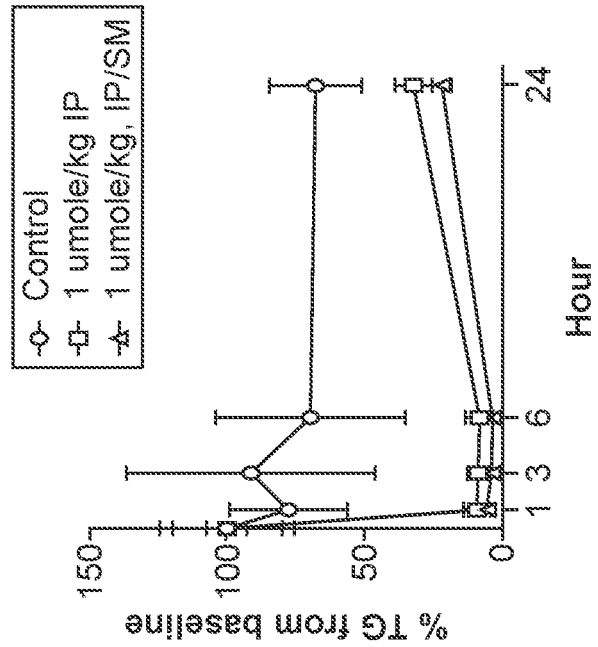

FIG. 36B

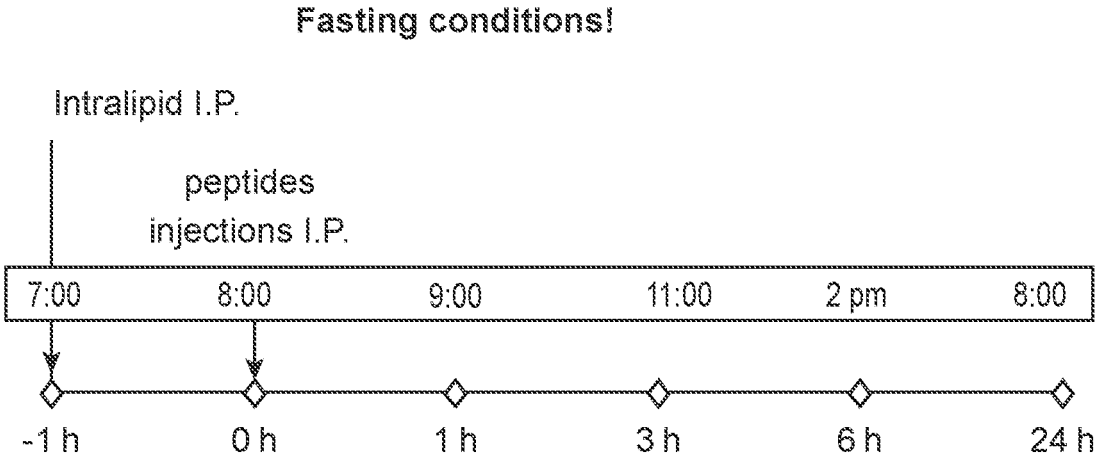

FIG. 37A

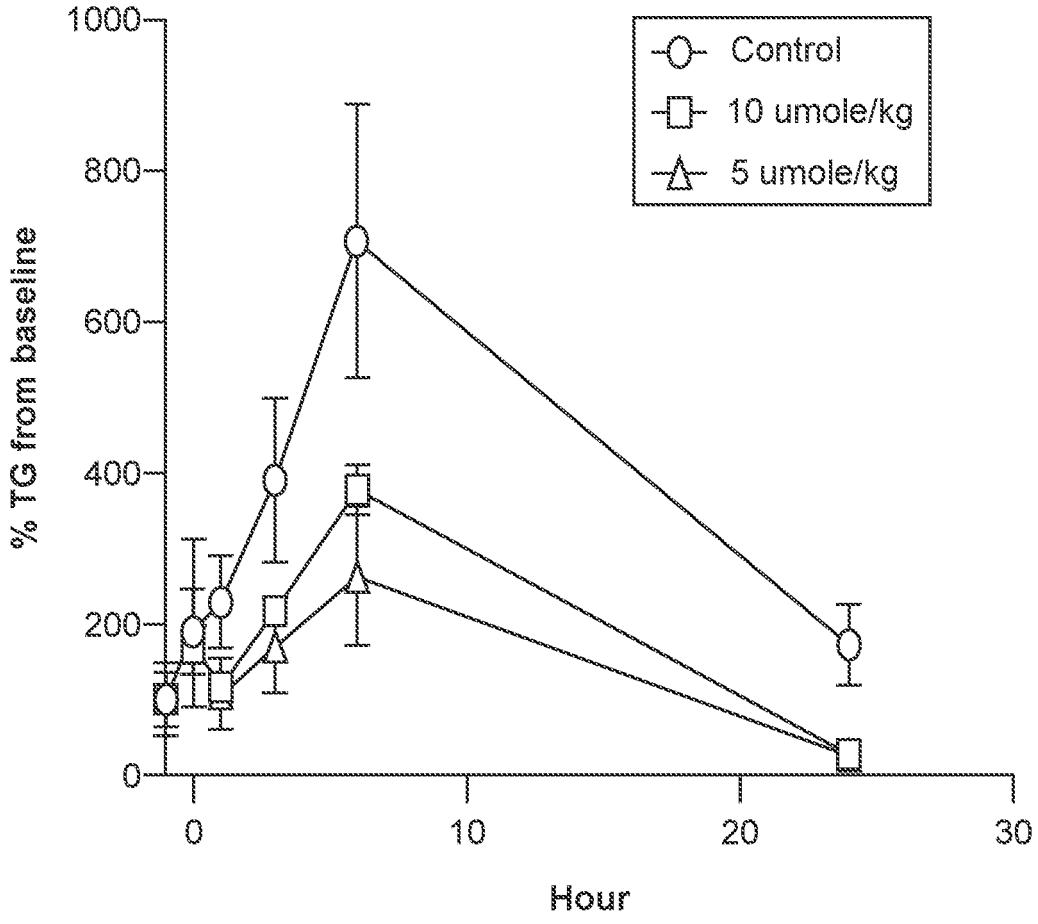

FIG. 37B

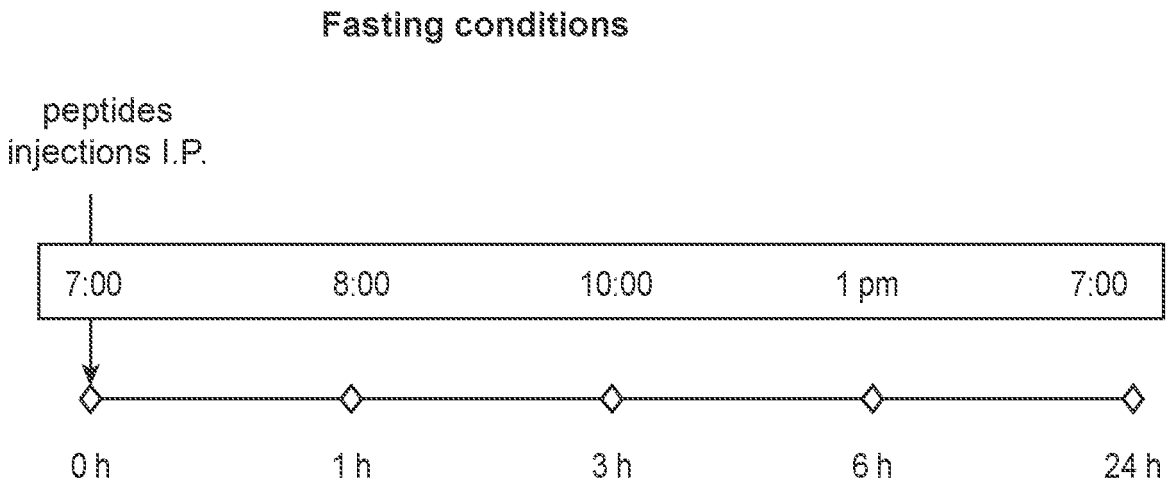

FIG. 38A

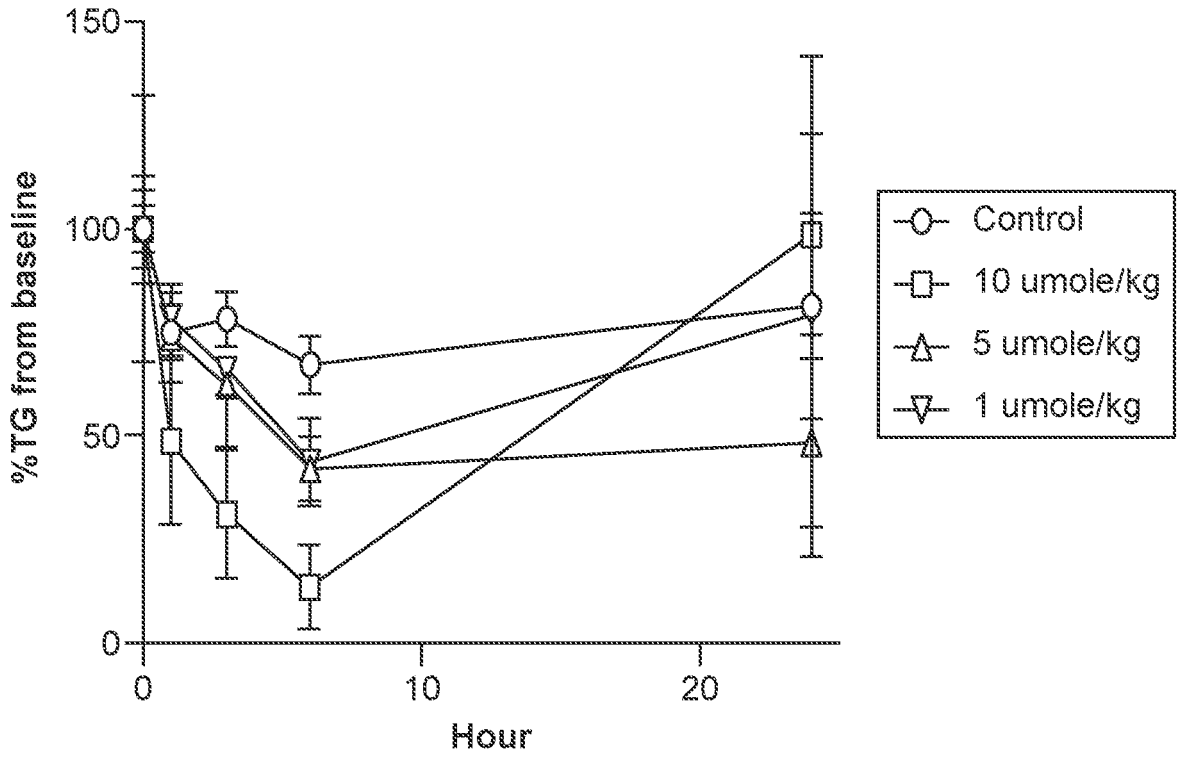

FIG. 38B

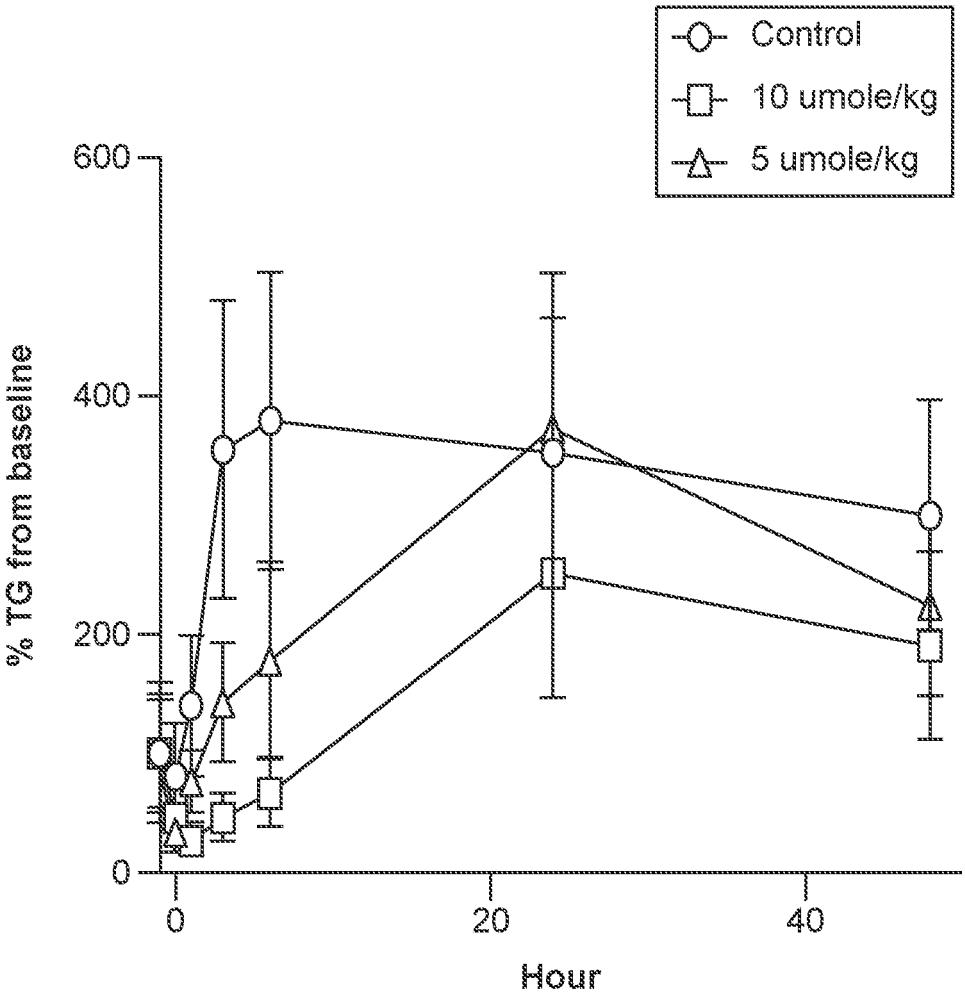
Control
10 umole/kg
5 umole/kg
600
400
200
0
% TG from baseline
0
20
40
Hour

FIG. 39

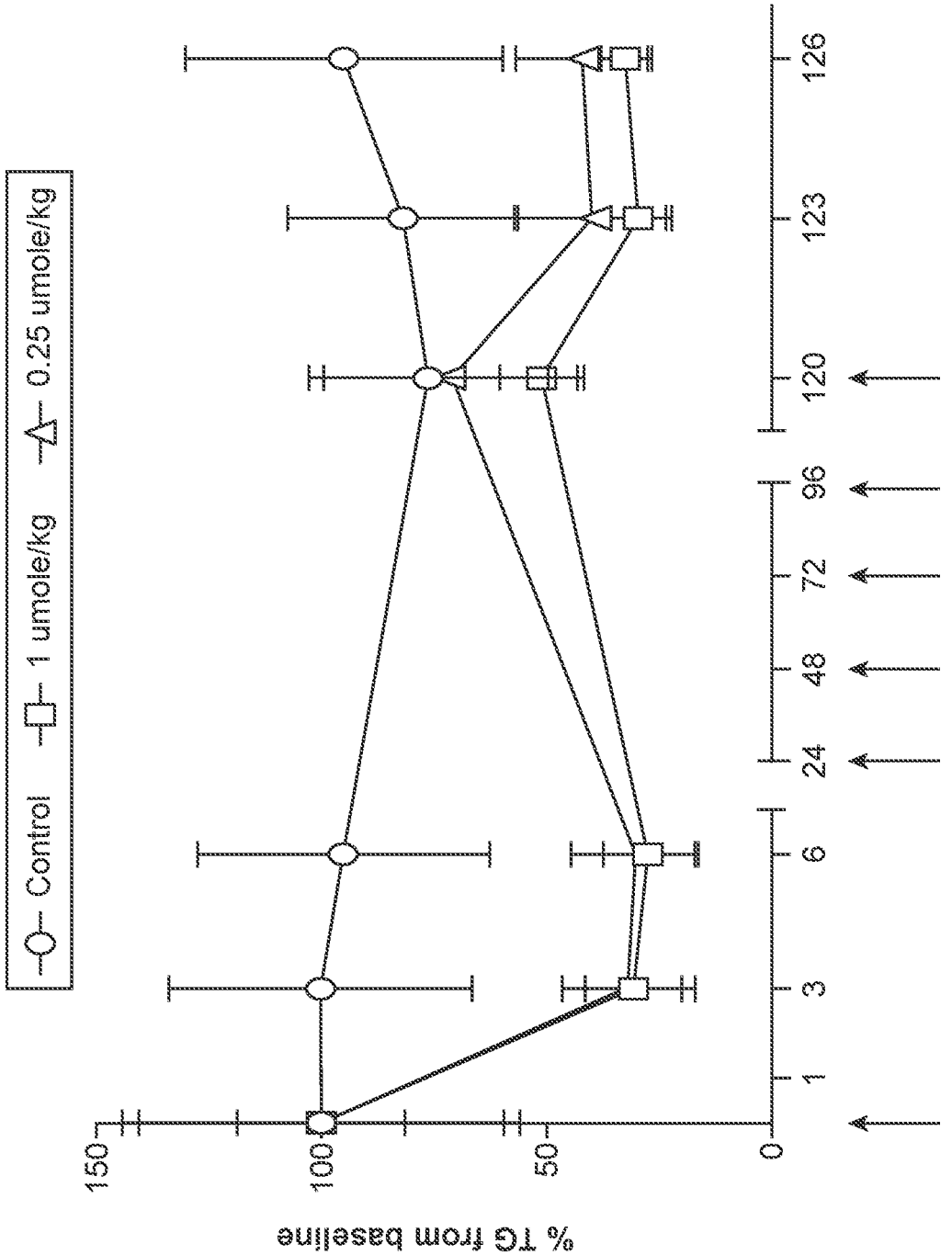
Control
1 umole/kg
0.25 umole/kg
% TG from baseline
150
100
50
0
1
3
6
24
48
72
96
120
123
126
Hour

FIG. 40

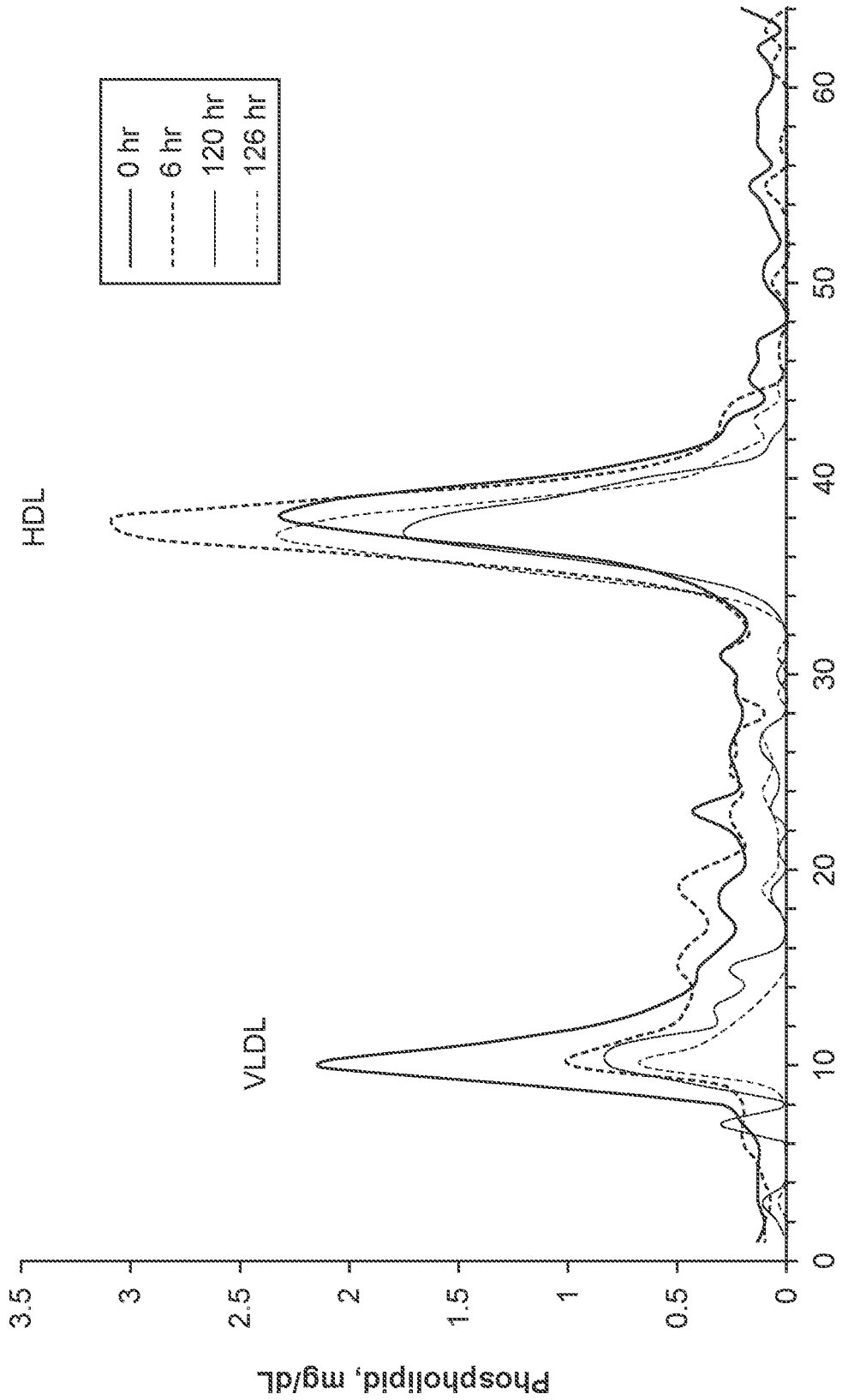
0 hr
6 hr
120 hr
126 hr
HDL
VLDL
Phospholipid, mg/dL
Fraction
0
0.5
1
1.5
2
2.5
3
3.5
10
20
30
40
50
60

FIG. 41

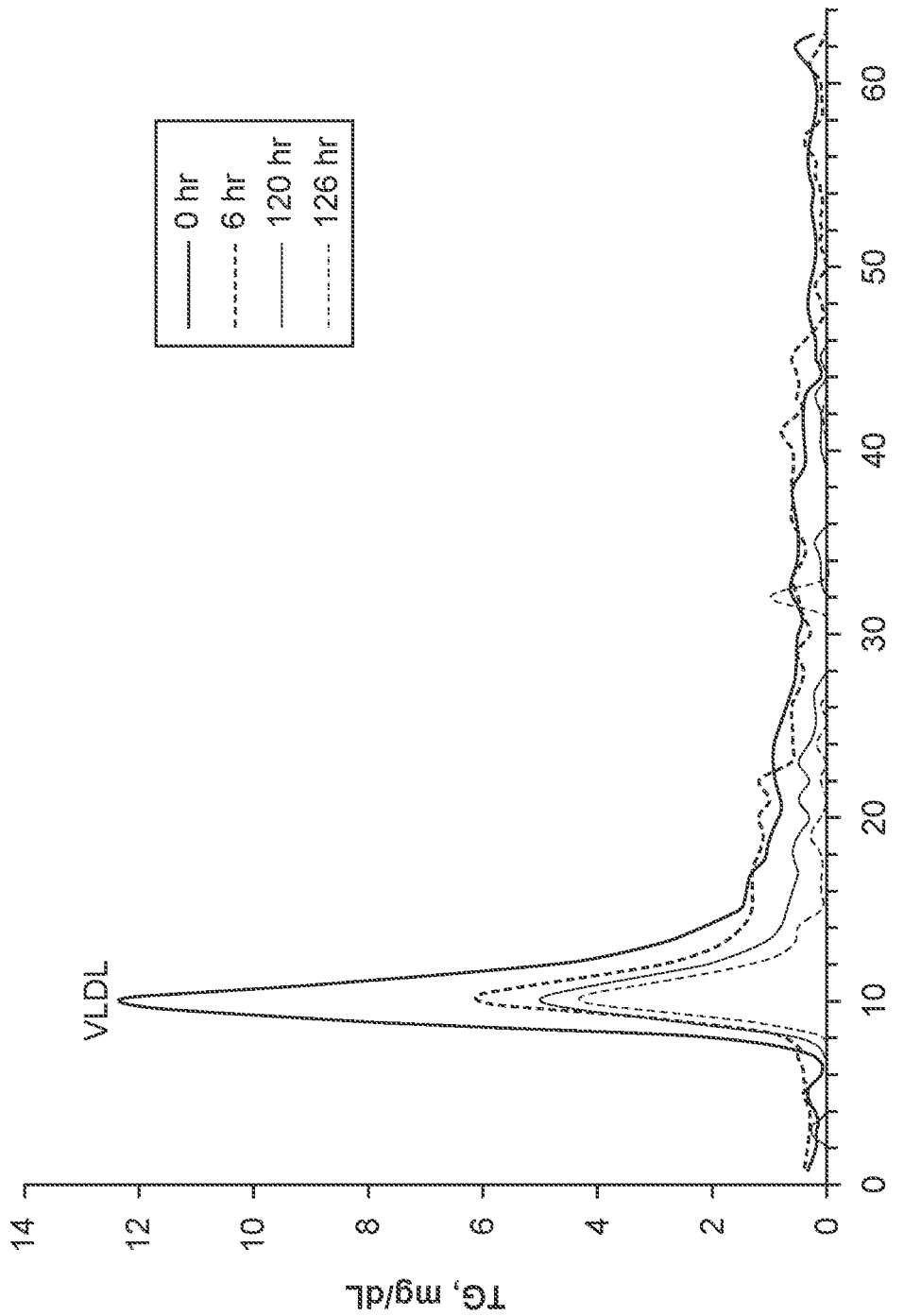
0 hr
6 hr
120 hr
126 hr
VLDL
TG, mg/dL
Fraction
0
2
4
6
8
10
12
14
0
10
20
30
40
50
60

FIG. 42

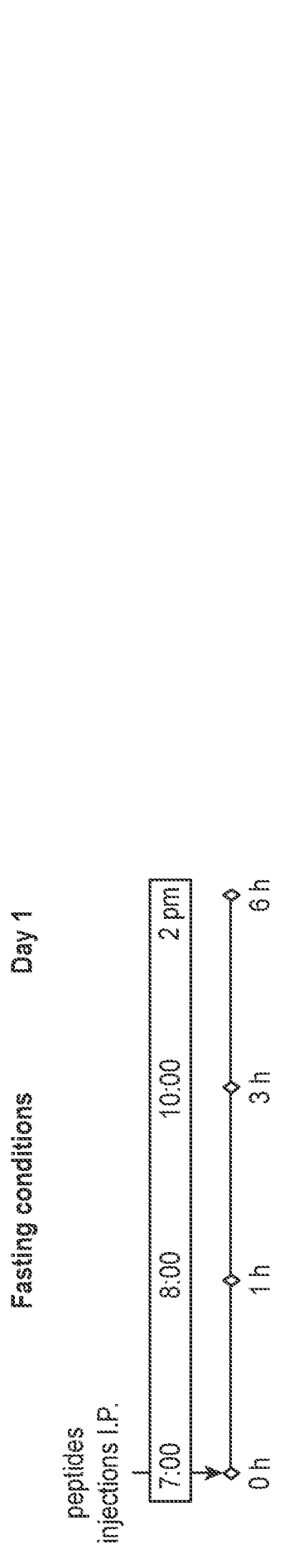

FIG. 43A

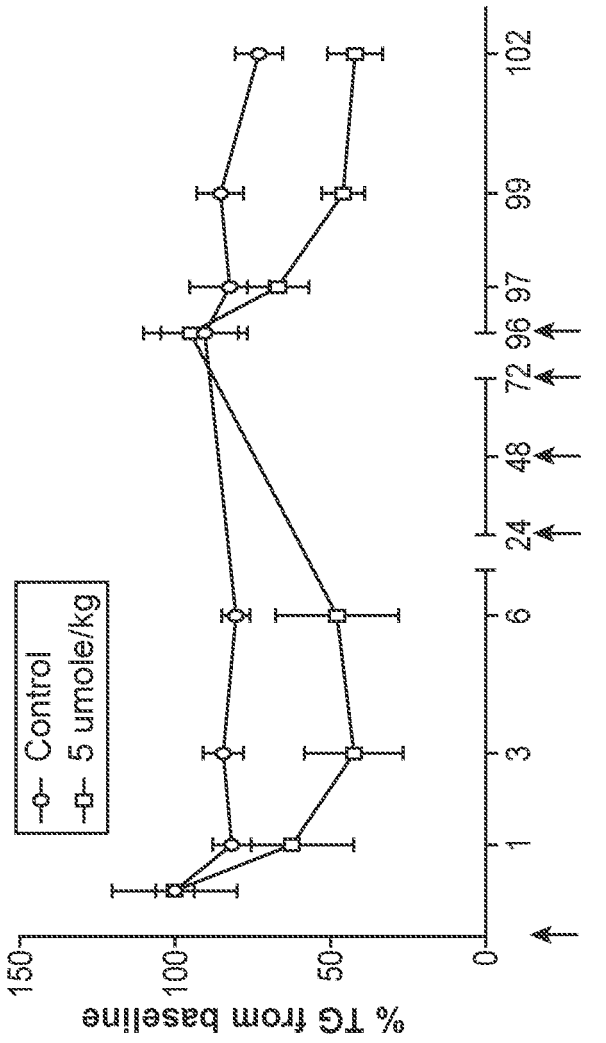

FIG. 43B

SUBSTITUTE SHEET (RULE 26)

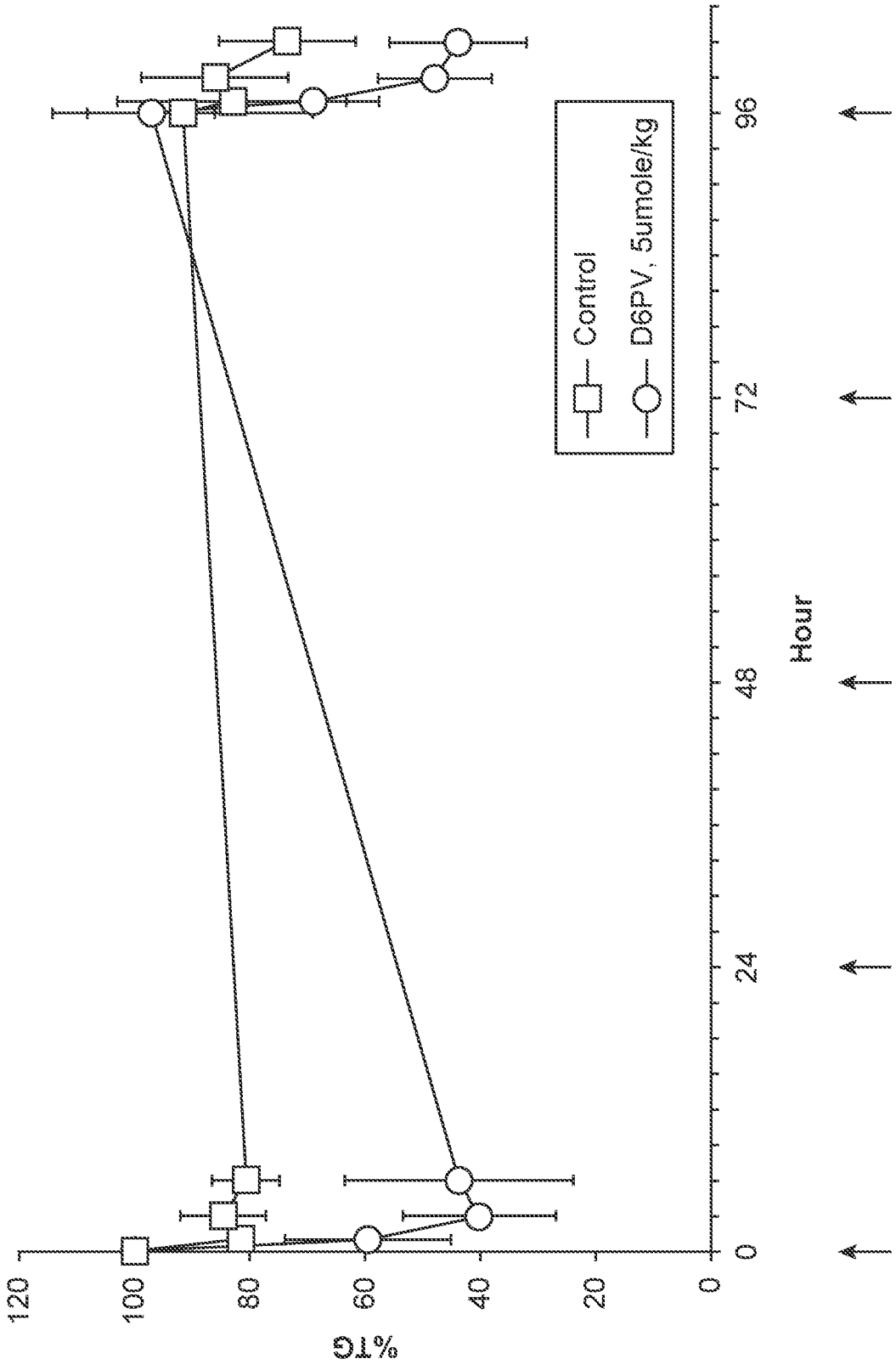

FIG. 44

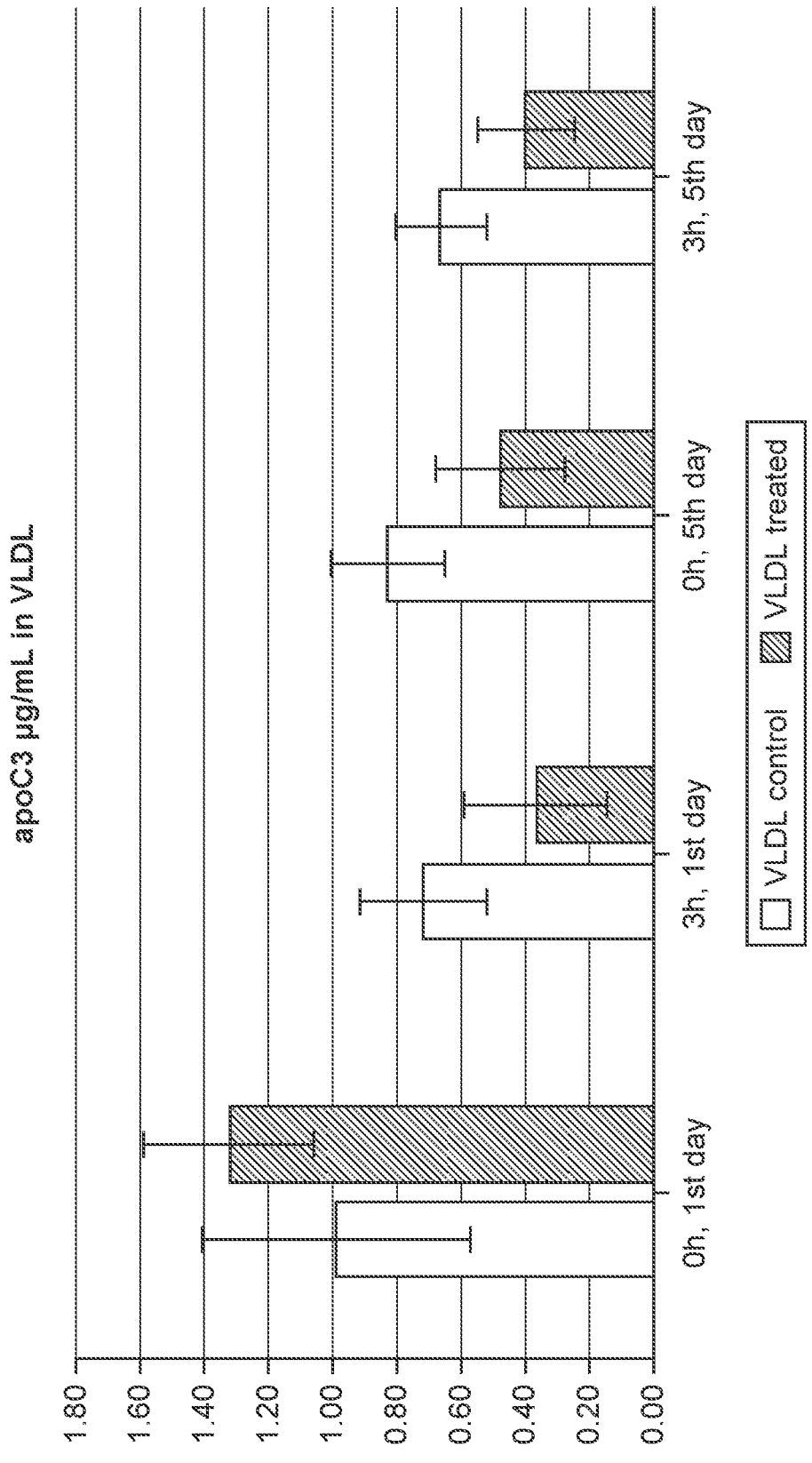
apoC3 µg/mL in VLDL
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0h, 1st day
3h, 1st day
0h, 5th day
3h, 5th day
VLDL control
VLDL treated

FIG. 45A

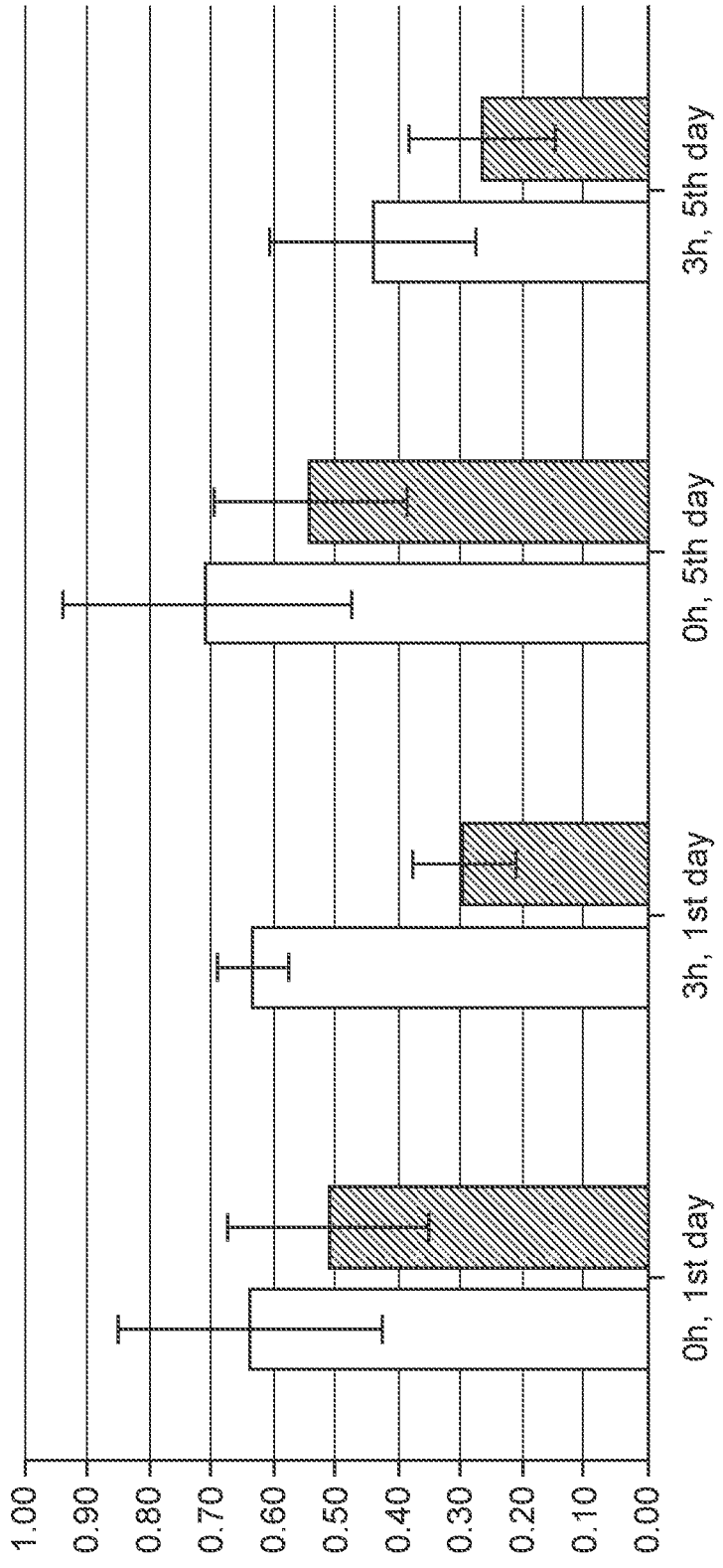
apoC3 µg/mL in HDL
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0h, 1st day
3h, 1st day
0h, 5th day
3h, 5th day
HDL control
HDL treated

FIG. 45B

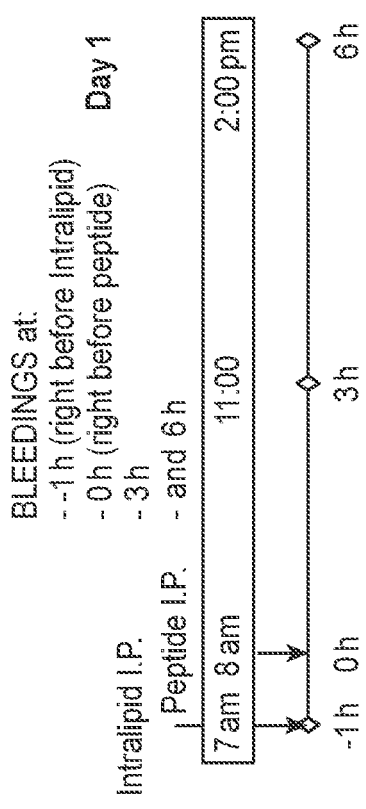

FIG. 46A

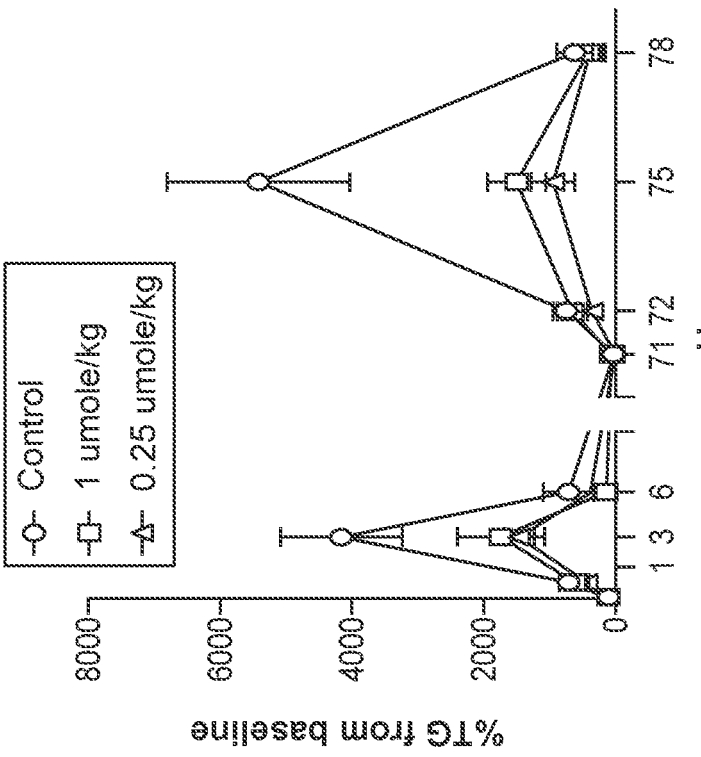

FIG. 46B

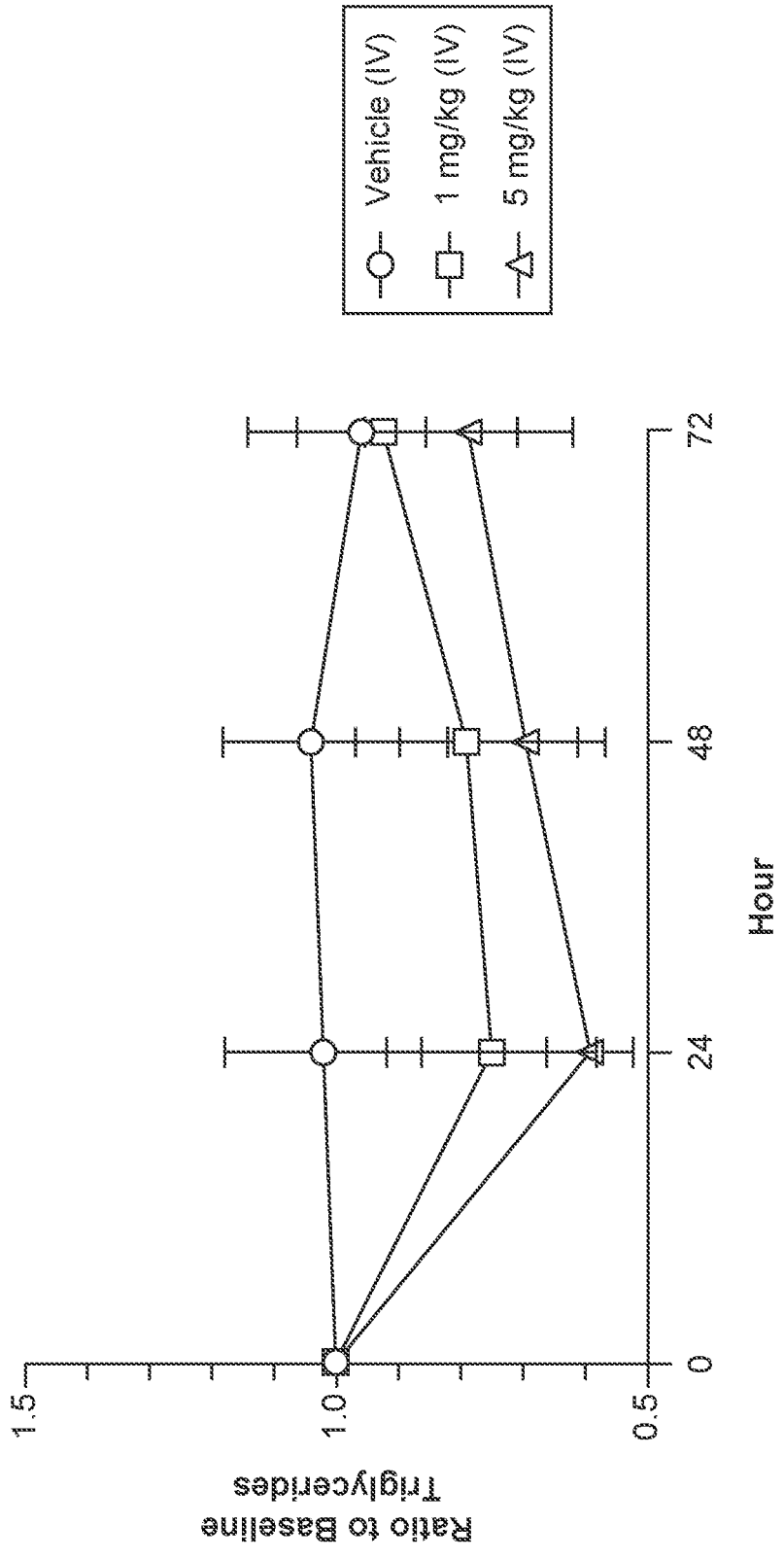
Vehicle (IV)
1 mg/kg (IV)
5 mg/kg (IV)
Ratio to Baseline
Triglycerides
1.5
1.0
0.5
0
24
48
72
Hour

FIG. 47

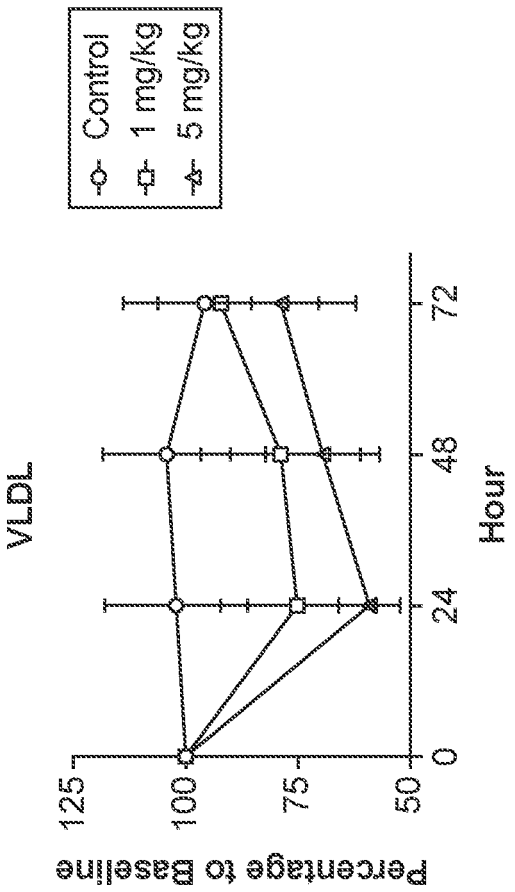

FIG. 48A

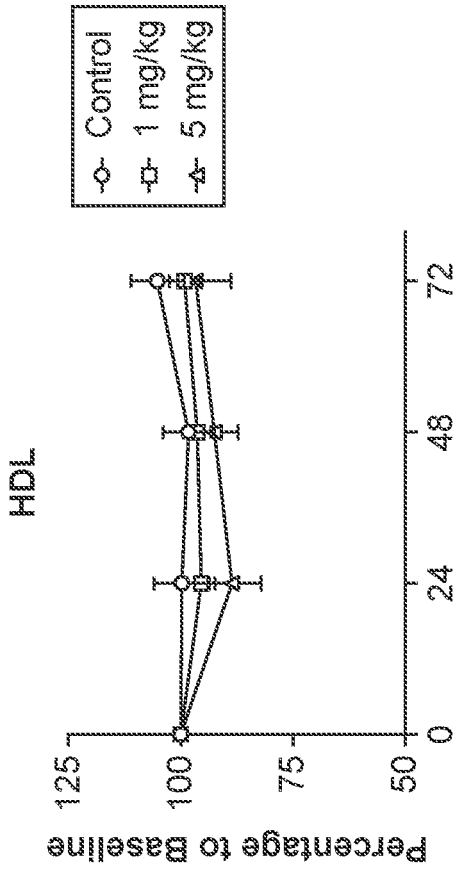

FIG. 48C

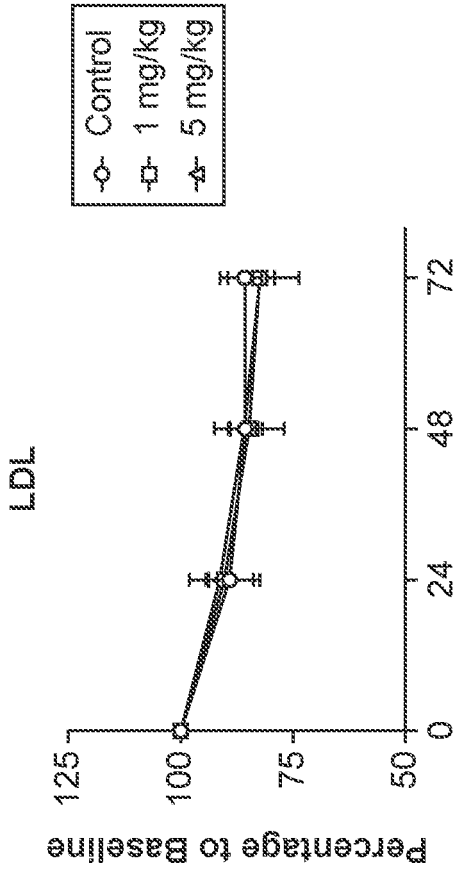

FIG. 48B

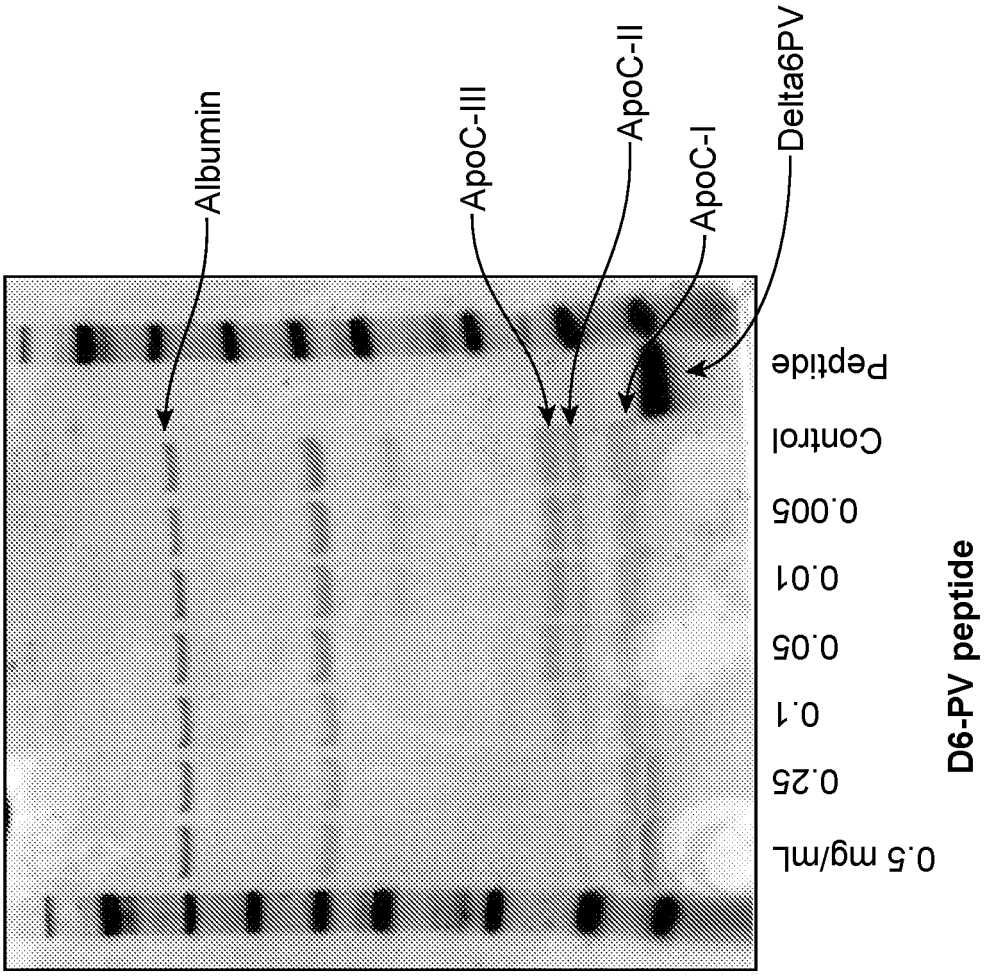
Albumin
ApoC-III
ApoC-II
ApoC-I
Delta6PV
0.5 mg/mL
0.25
0.1
0.05
0.01
0.005
Control
Peptide
D6-PV peptide

FIG. 49

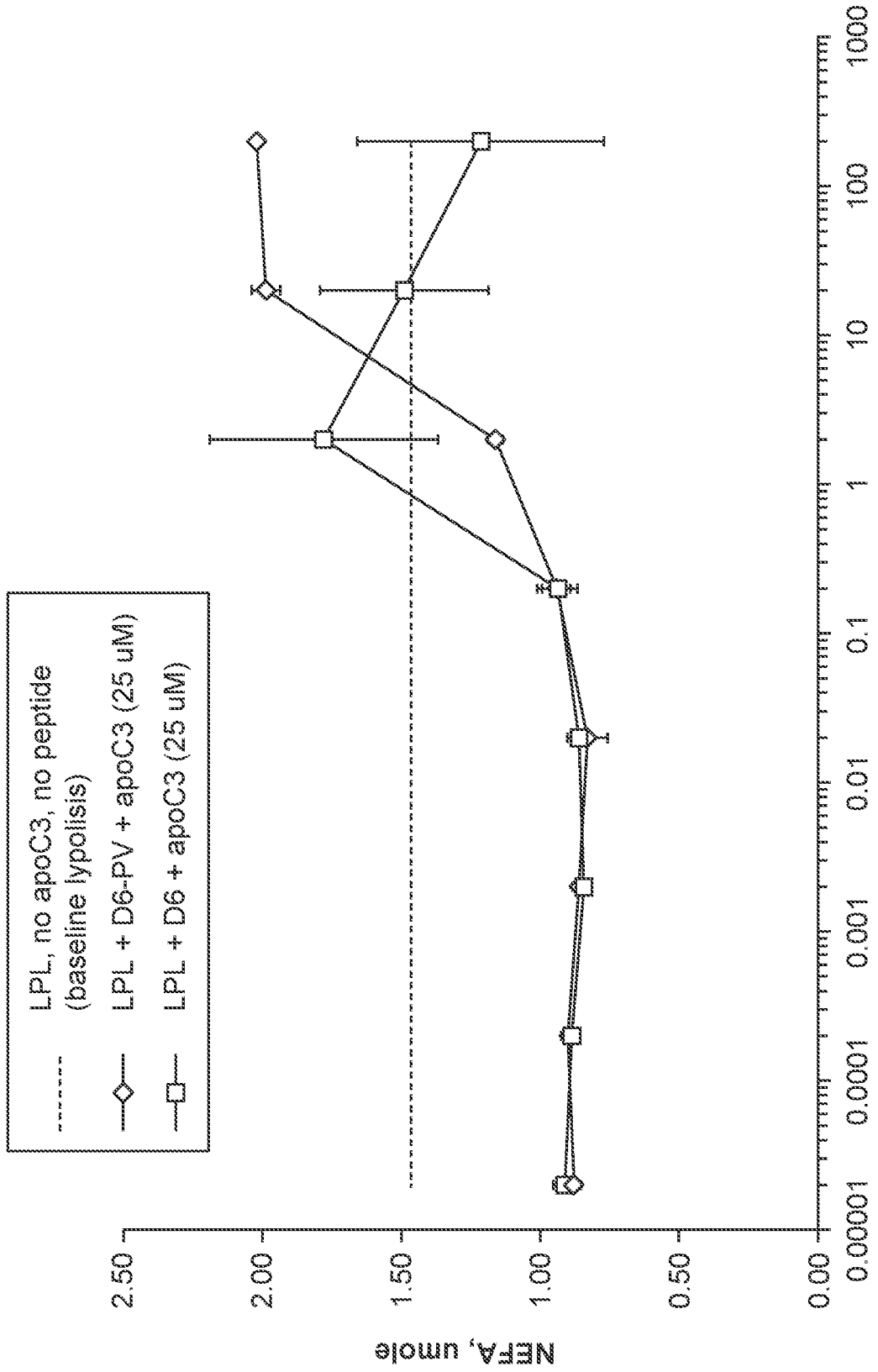
LPL, no apoC3, no peptide
(baseline lypolisis)
LPL + D6-PV + apoC3 (25 uM)
LPL + D6 + apoC3 (25 uM)
NEFA, umole
2.50
2.00
1.50
1.00
0.50
0.00
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
Peptide concentration, uM

FIG. 50

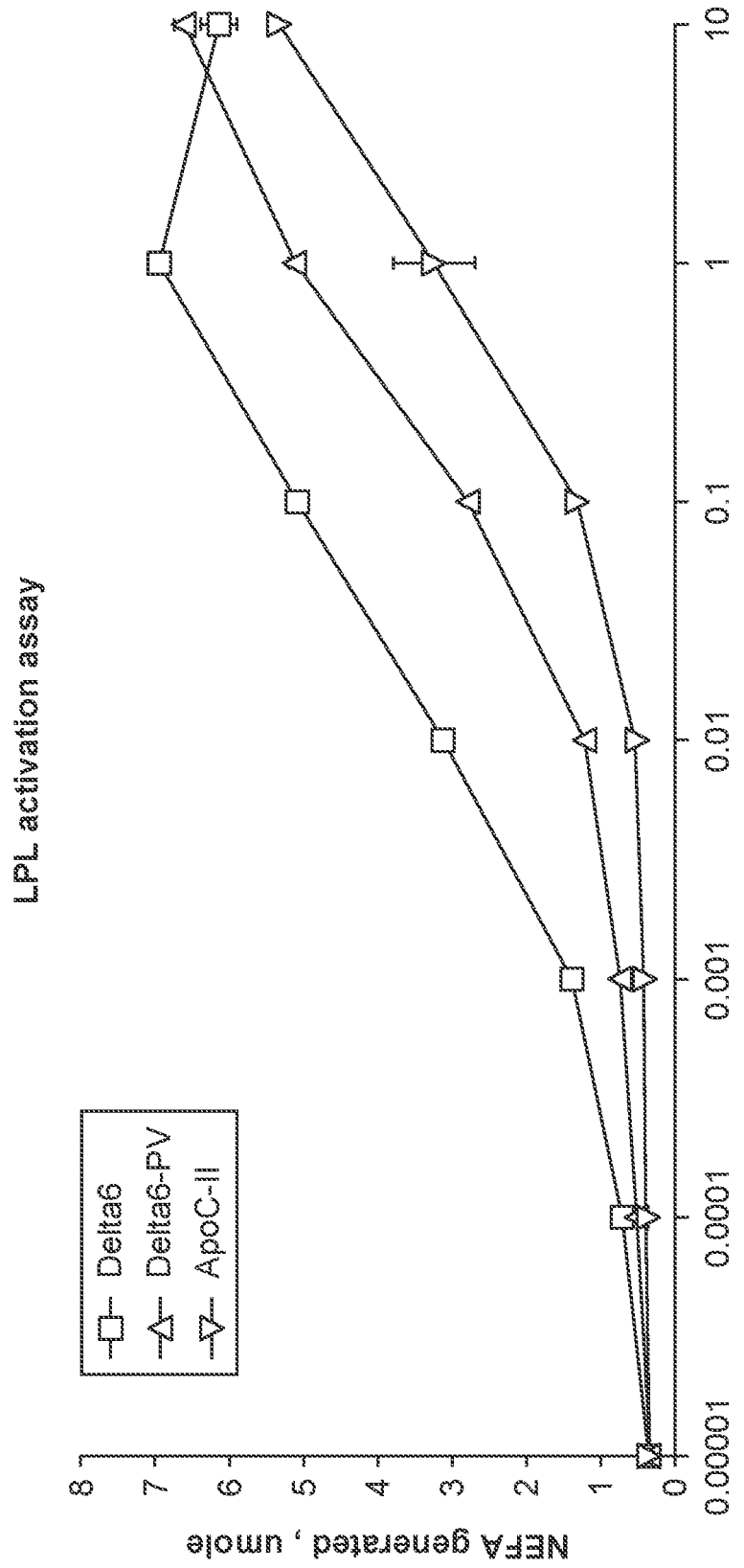
LPL activation assay
NEFA generated , umole
Peptide/protein, uM
Delta6
Delta6-PV
ApoC-II
0
1
2
3
4
5
6
7
8
0.00001
0.0001
0.001
0.01
0.1
1
10

FIG. 51

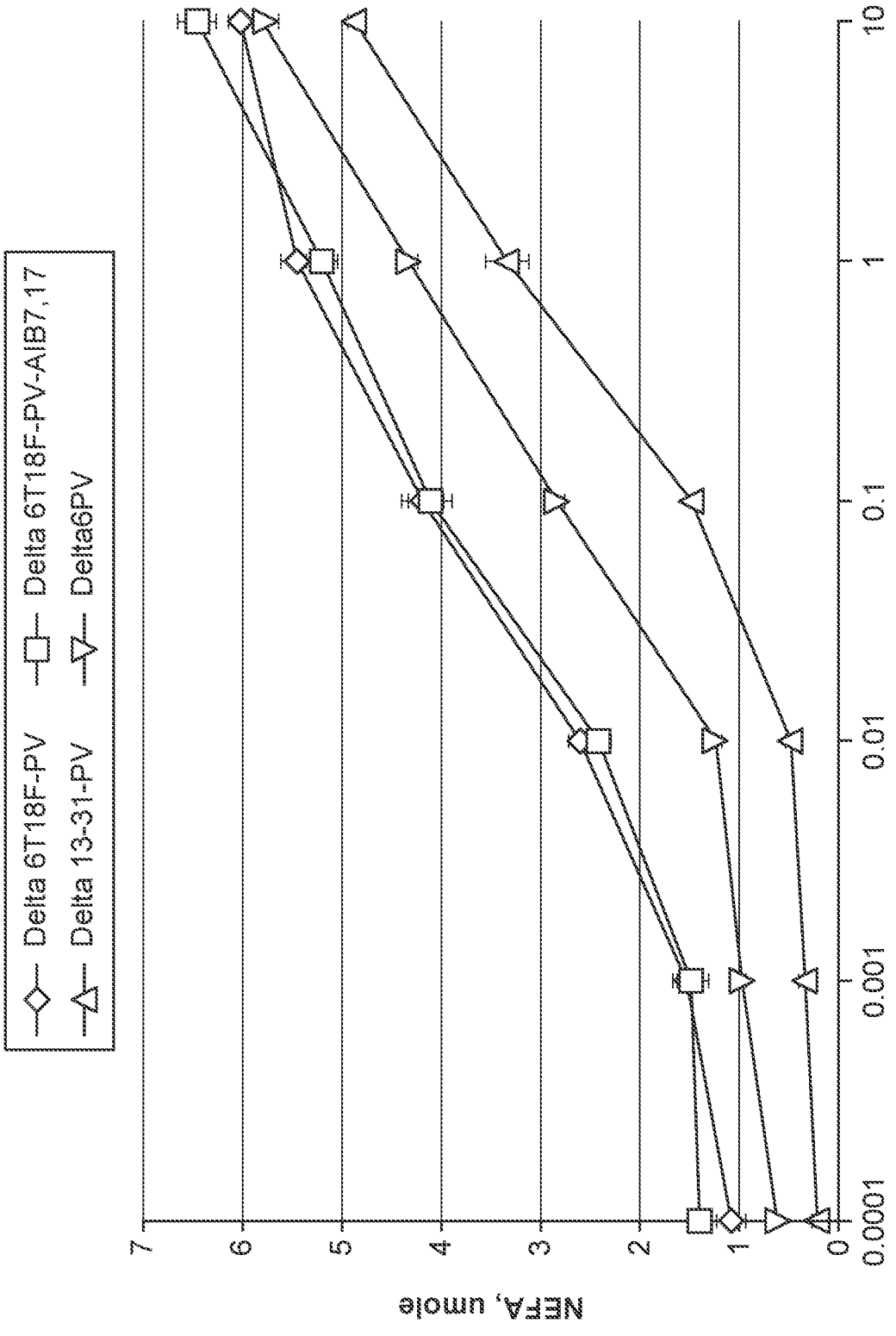
Delta 6T18F-PV
Delta 6T18F-PV-AIB7,17
Delta 13-31-PV
Delta6PV
NEFA, umole
0
1
2
3
4
5
6
7
0.0001
0.001
0.01
0.1
1
10
Peptide, uM

FIG. 52

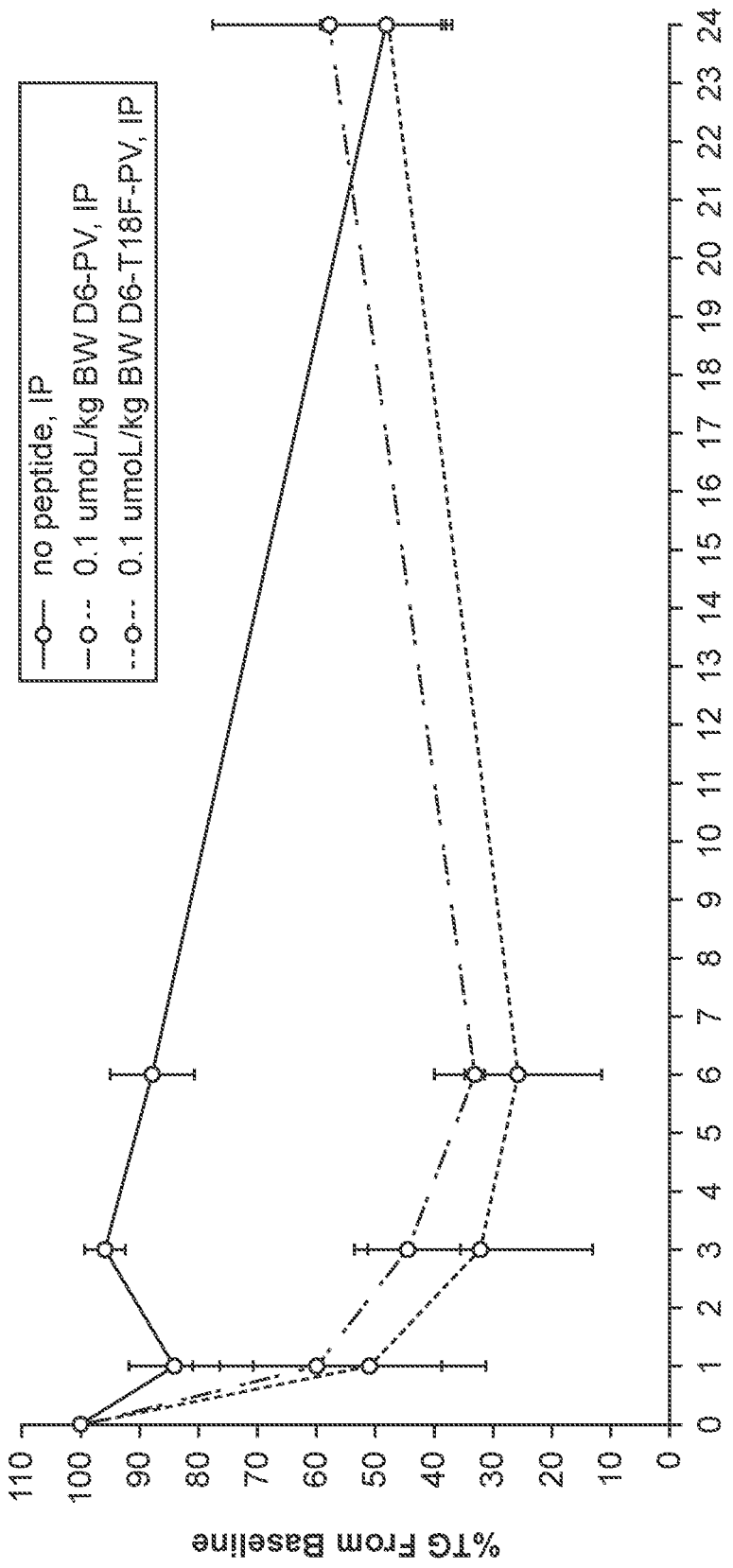
no peptide, IP
0.1 umoL/kg BW D6-PV, IP
0.1 umoL/kg BW D6-T18F-PV, IP
%TG From Baseline
Hour
110
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

FIG. 53

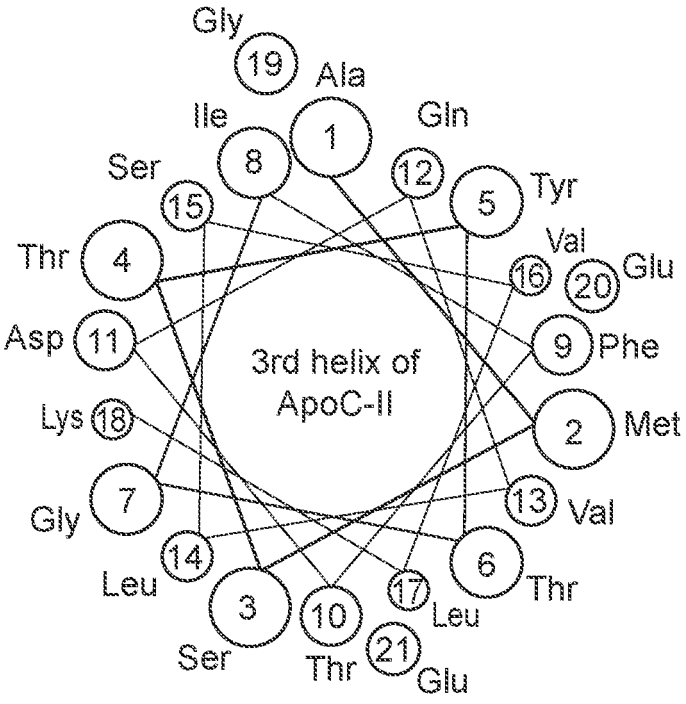

FIG. 54B

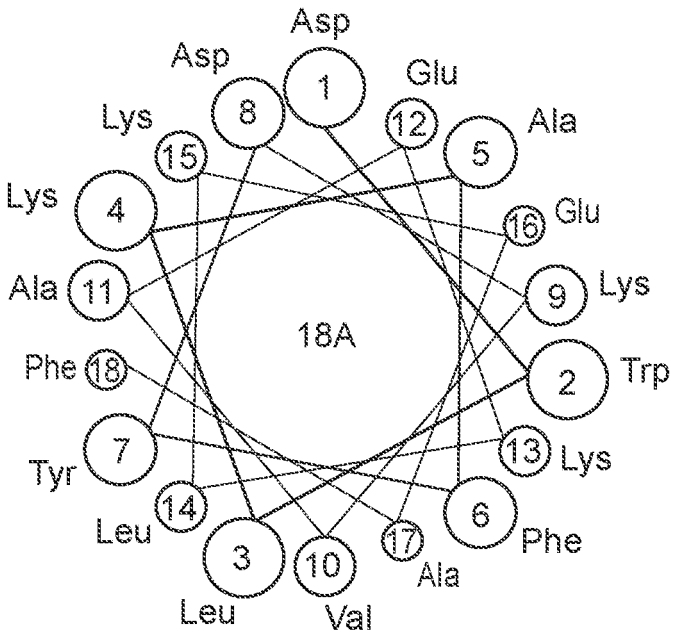

FIG. 54A

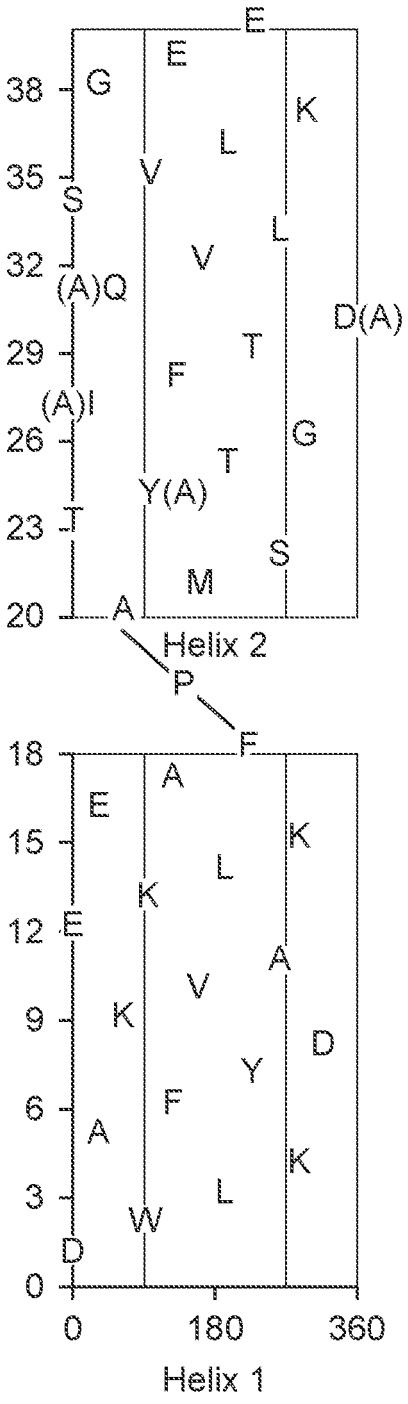

FIG. 54C

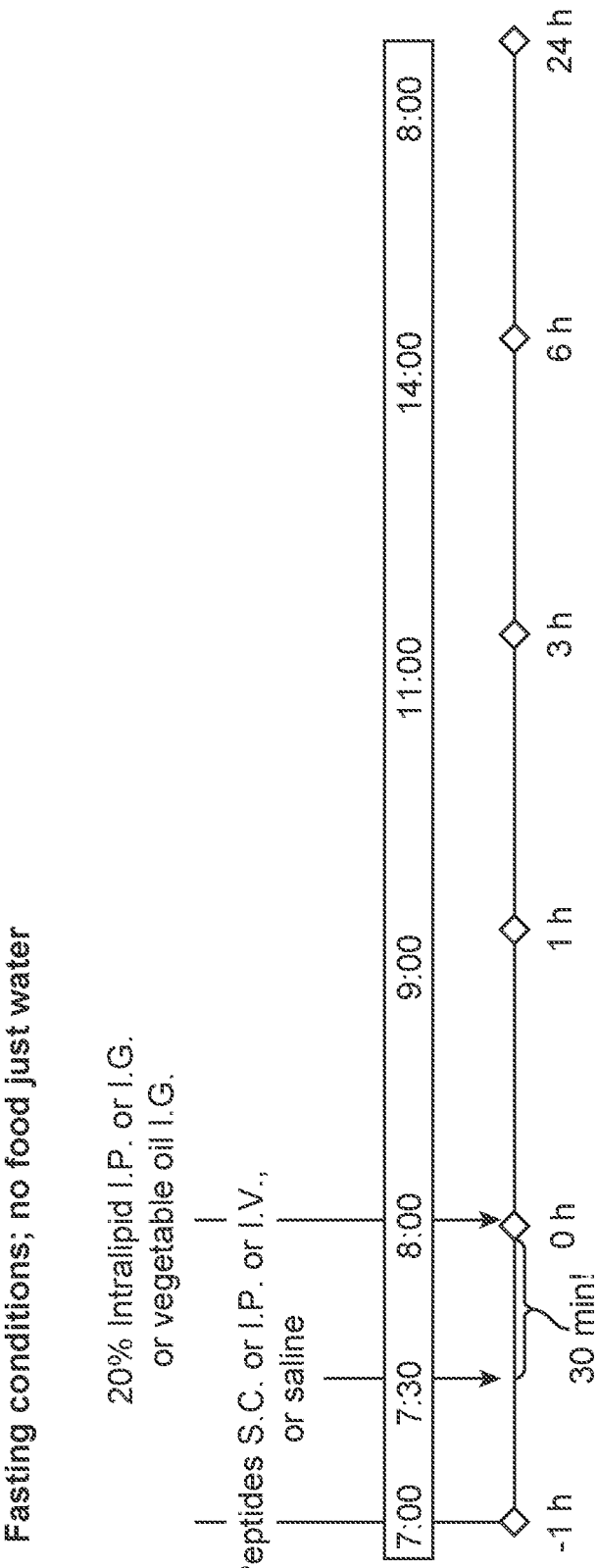
Fasting conditions; no food just water
20% Intralipid I.P. or I.G.
or vegetable oil I.G.
Peptides S.C. or I.P. or I.V.,
or saline
7:00
7:30
8:00
9:00
11:00
14:00
8:00
-1 h
30 min!
0 h
1 h
3 h
6 h
24 h

FIG. 55

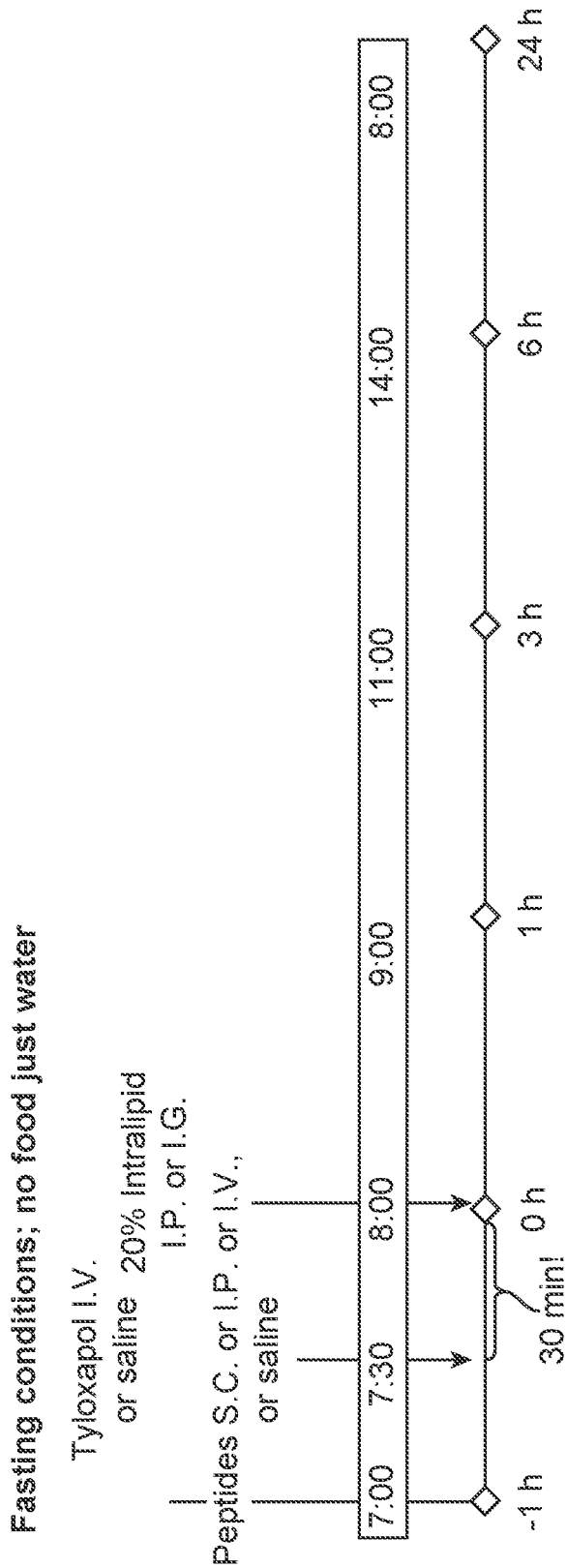
Fasting conditions; no food just water
Tyloxapol I.V.
or saline
20% Intralipid
I.P. or I.G.
Peptides S.C. or I.P. or I.V.,
or saline
7:00
7:30
8:00
9:00
11:00
14:00
8:00
-1 h
30 min
0 h
1 h
3 h
6 h
24 h

FIG. 56