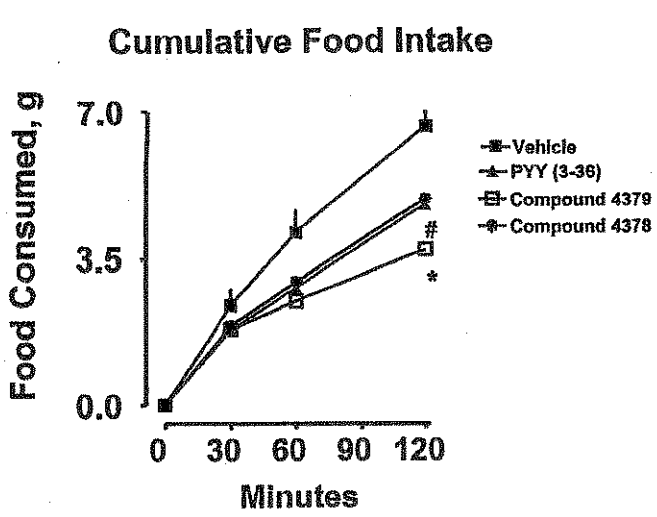
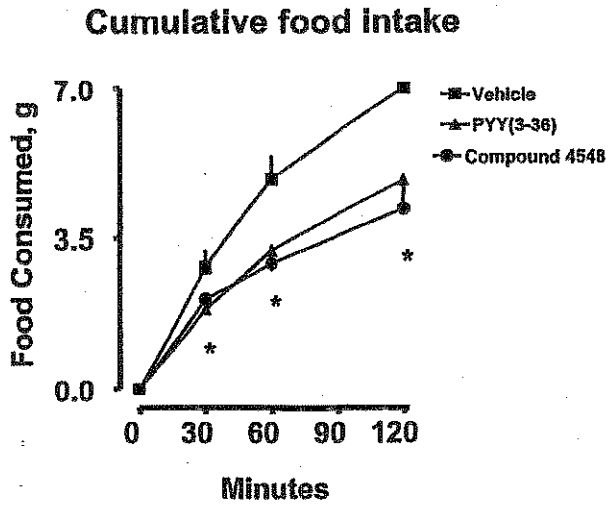
Peptides with Combination of Substitutions Are Active in Appetite Suppression



* P<0.01; # P<0.05; ANOVA vs HF vehicle;
N=4 cages; 3 animals/cage

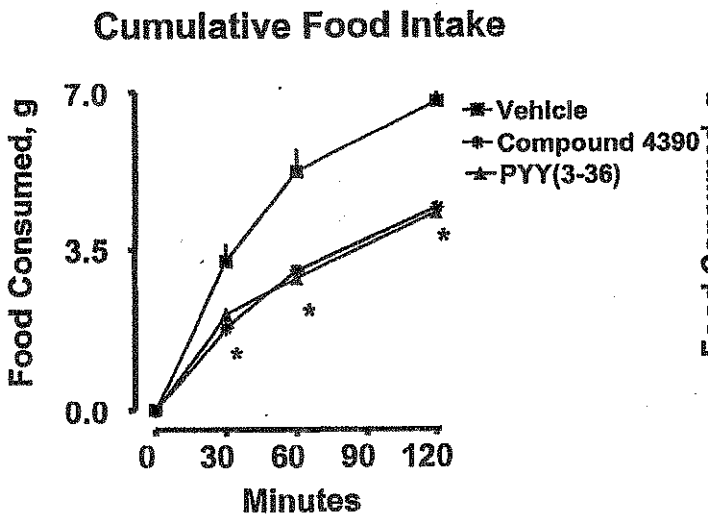


* P<0.01; ANOVA vs HF Vehicle;
N=4 cages; 3 animals/cage

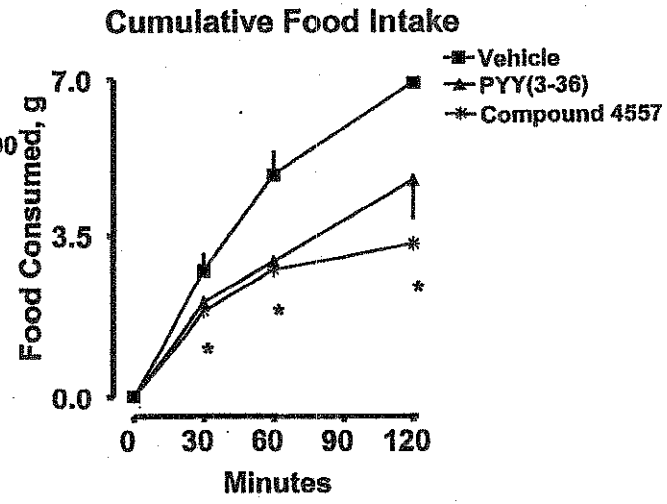
Food Intake Assay, Mouse, Dose: 10 nmol/kg i.p.

FIG. 1

PPF Polypeptides with Unnatural AA Substitution or N-Terminus Modification Retain Anorectic Effect



*P<0.01; ANOVA vs HF Vehicle
N = 4 cages; 3 animals/cage

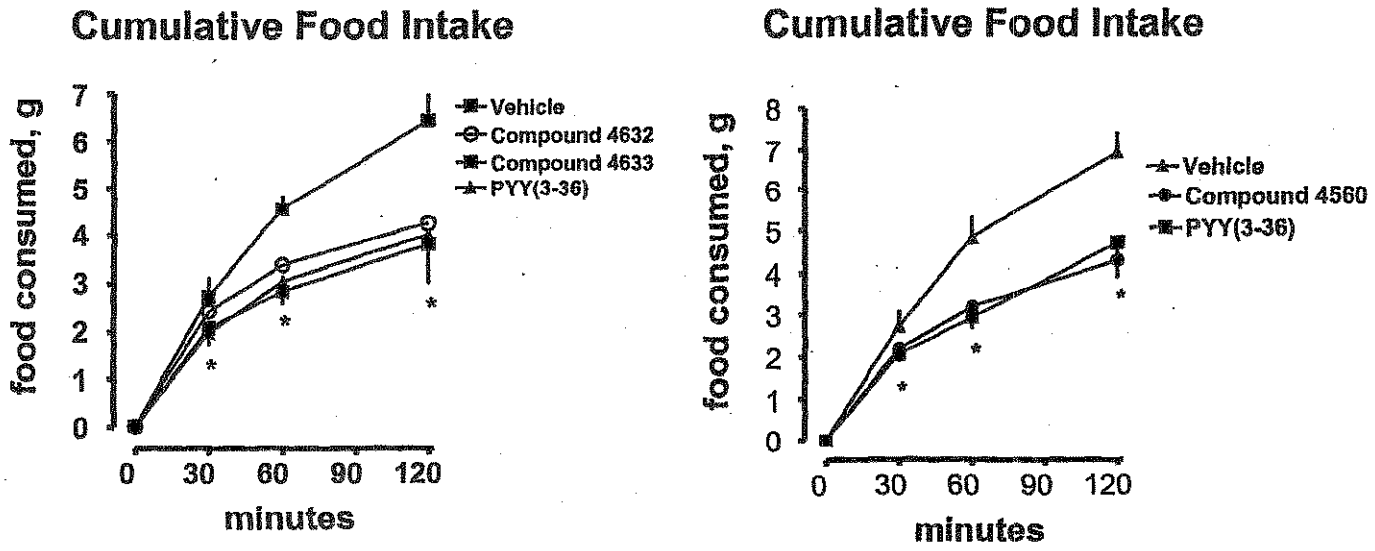


* P<0.01; ANOVA vs HF Vehicle
N = 4 cages; 3 animals/cage

Food Intake Assay, Mouse, Dose: 10 nmol/kg i.p.

FIG. 2

PPF Chimeric Polypeptides Are Active in Appetite Suppression



*P<0.01; ANOVA vs HF Vehicle

N = 4 cages; 3 animals/cage

Food Intake Assay, Mouse, Dose: 10 nmol/kg i.p.

FIG. 3

PPF Chimeric Polypeptides Are Active in Appetite Suppression

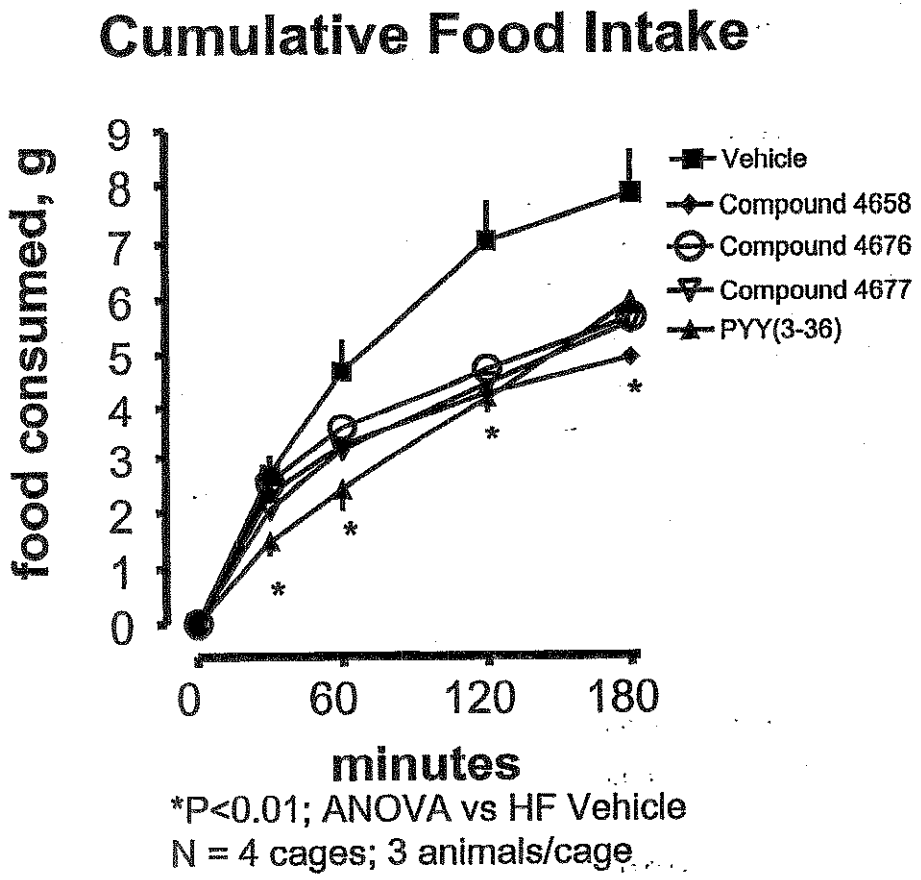
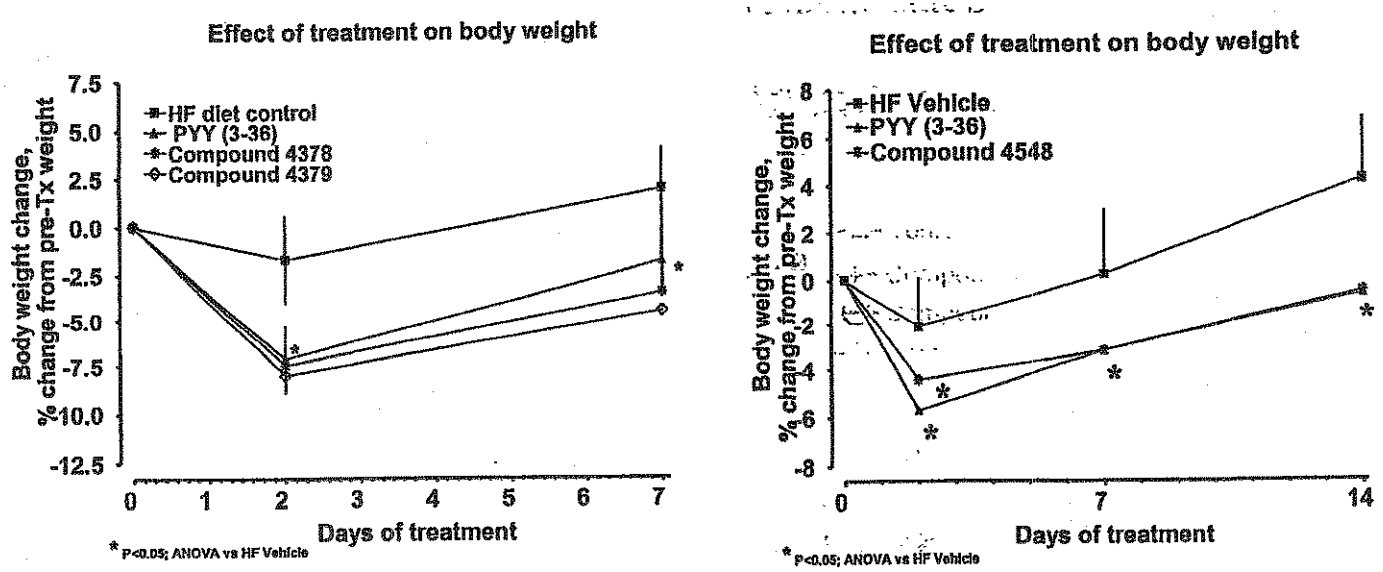


FIG. 4

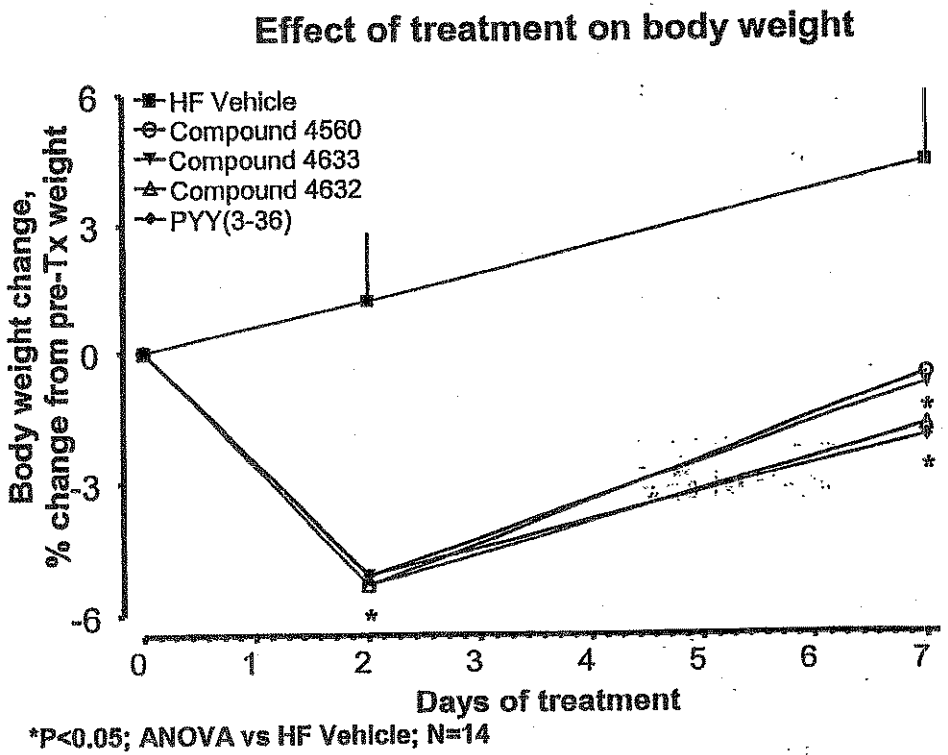
Peptides with Combination of Substitutions Are Active in DIO Mouse Model



DIO Assay, Fattened C57BL/6Mouse Dose: 75 nmol/kg/day, s.c. pump infusion

FIG. 5

PPF Chimeric Polypeptides Are Active in DIO Assay



DIO Assay, Fattened C57BL/6Mouse Dose: 75 nmol/kg/day, s.c. pump infusion

FIG. 6

Weight Gain Following Once Daily IP Injection in Rats: Comparison of PYY [3-36] and PPF Polypeptides

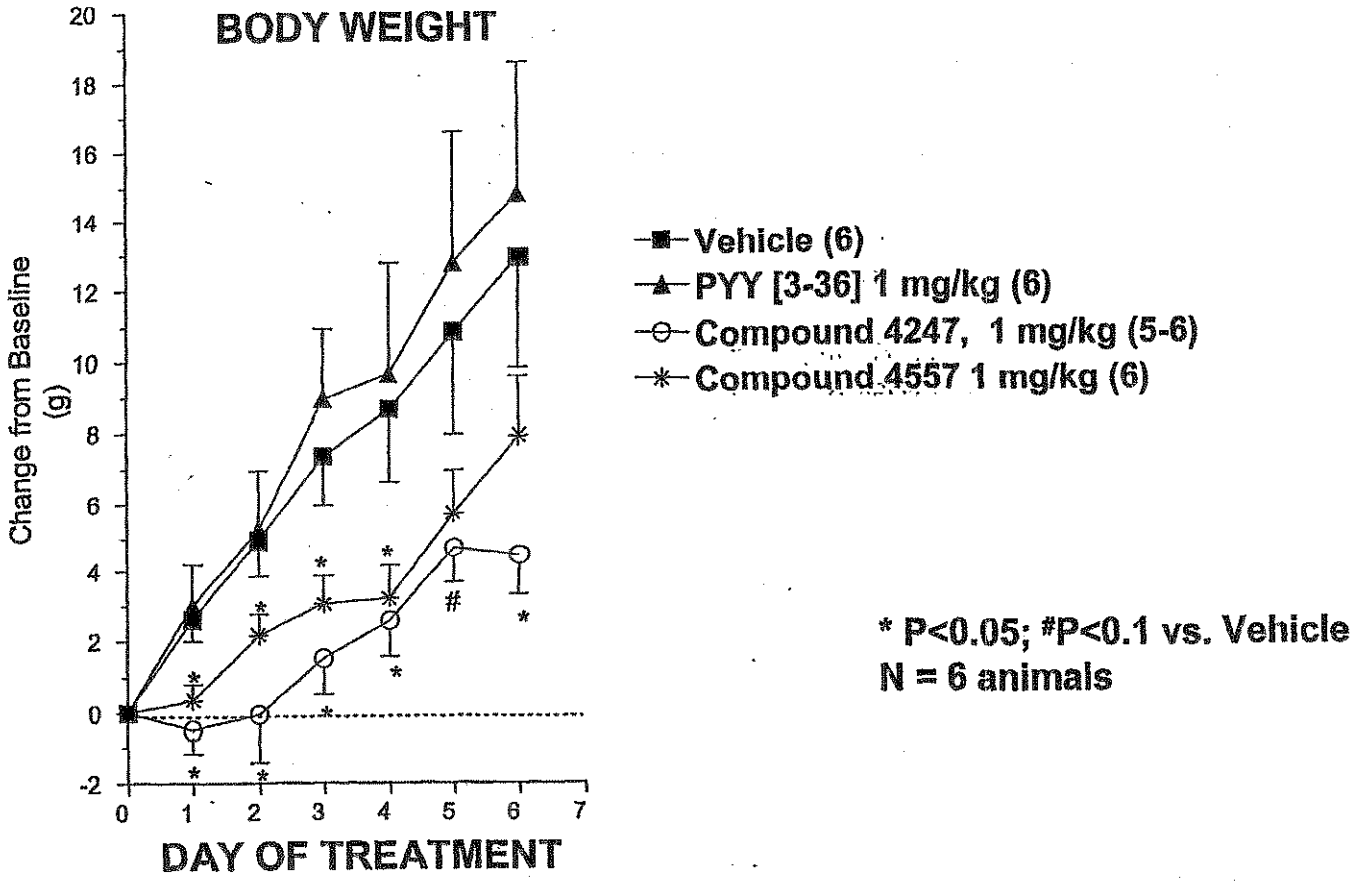
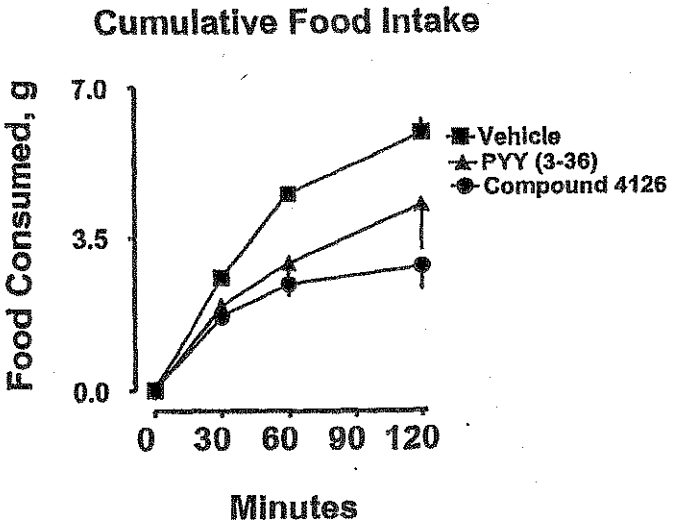
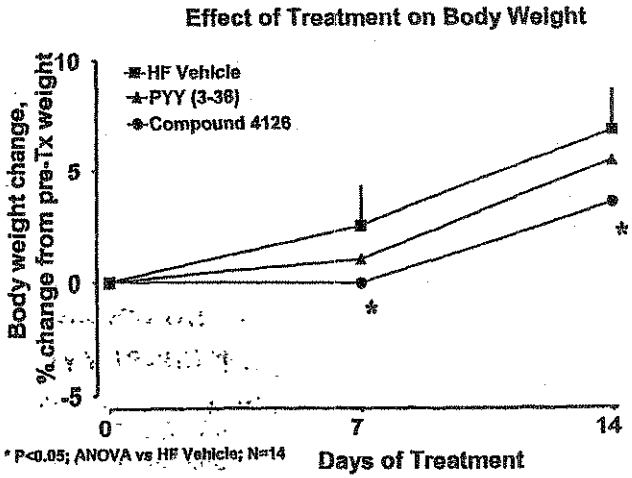


FIG. 7

PPF Polypeptide Shows efficacy in *in-vivo* Assays



Food Intake Assay, Mouse
Dose: 10 nmol/kg i.p.



DIO Assay, Fattened C57BL/6Mouse
Dose: 25 nmol/kg/day s.c. pump infusion

FIG. 8

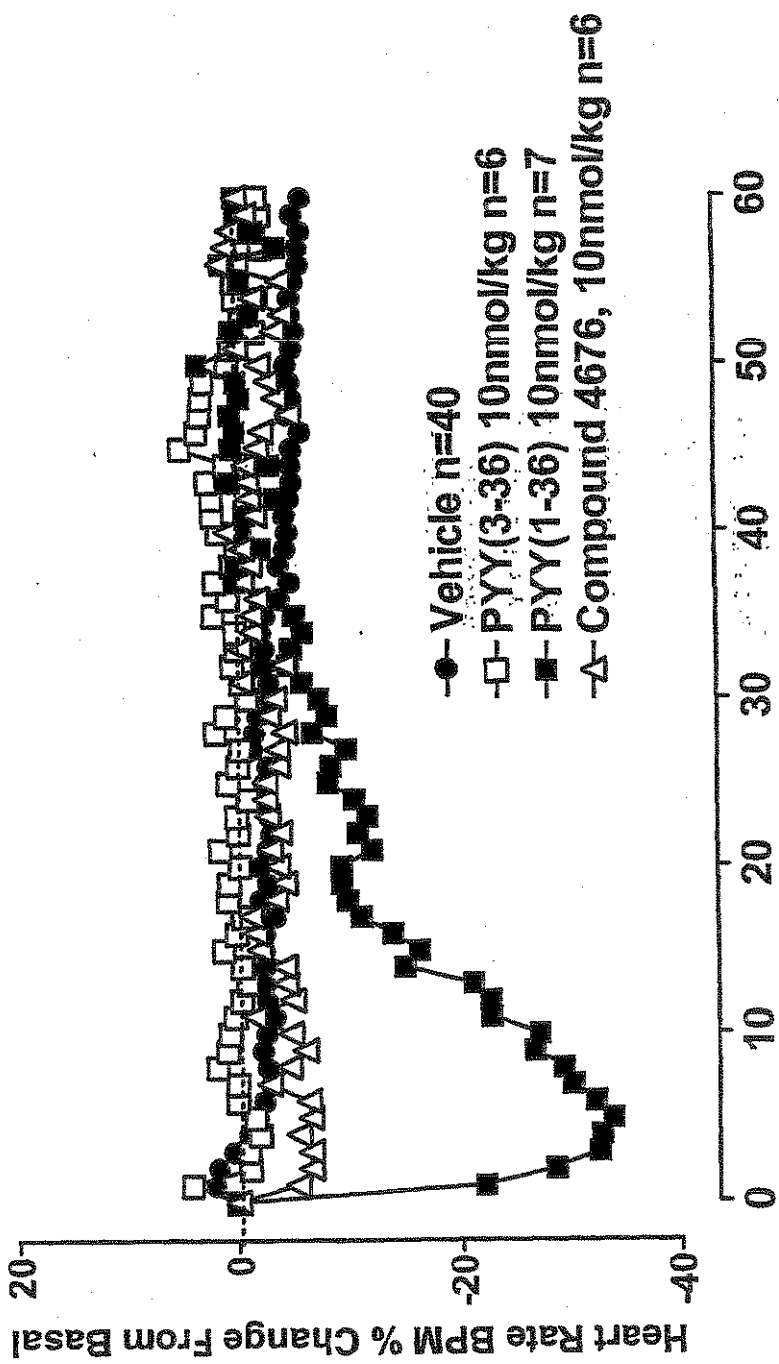

Heart Rate
Heart Rate BPM % Change From Basal
20
0
-20
-40
0
10
20
30
40
50
60
Minutes After IV Bolus
Vehicle n=40
PYY(3-36) 10nmol/kg n=6
PYY(1-36) 10nmol/kg n=7
Compound 4676, 10nmol/kg n=6

FIG. 9-A

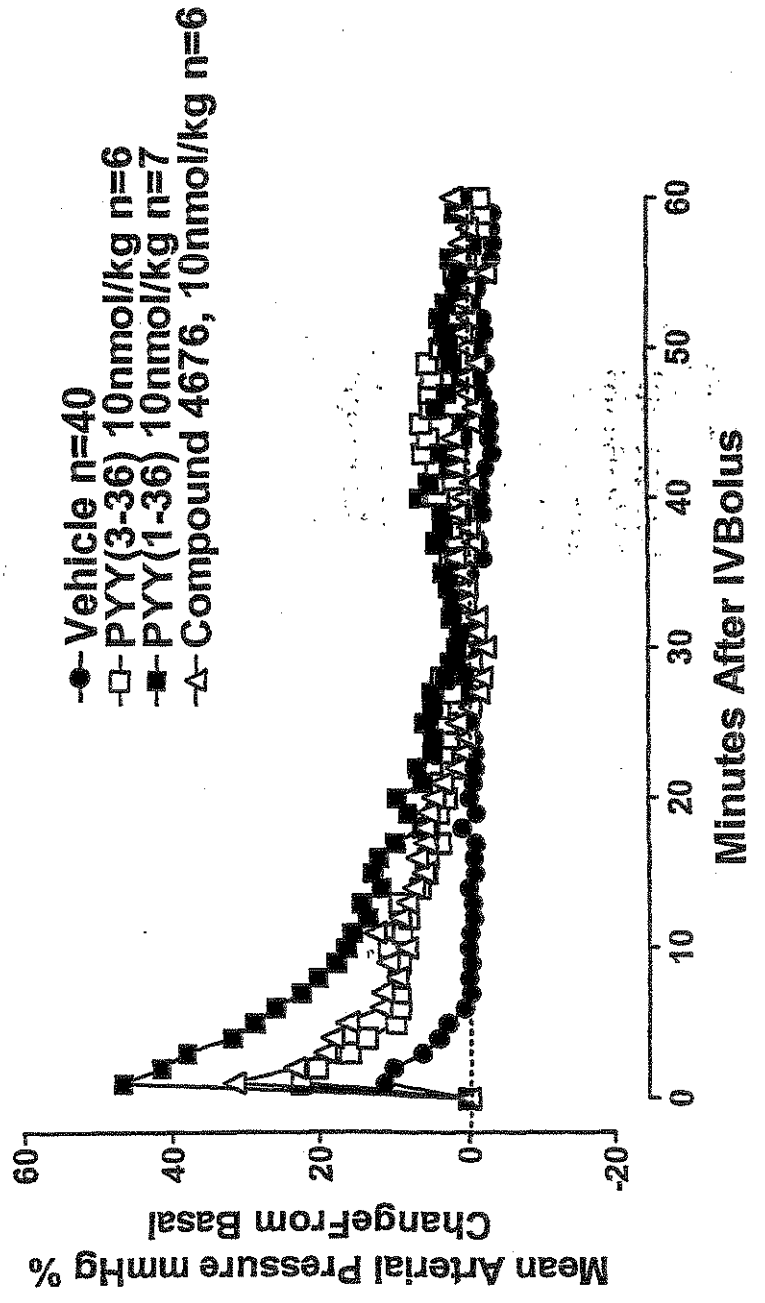

Mean Arterial Pressure
Vehicle n=40
PYY(3-36) 10nmol/kg n=6
PYY(1-36) 10nmol/kg n=7
Compound 4676, 10nmol/kg n=6
Mean Arterial Pressure mmHg %
ChangeFrom Basal
60
40
20
0
-20
0
10
20
30
40
50
60
Minutes After IVBolus

FIG. 9-B

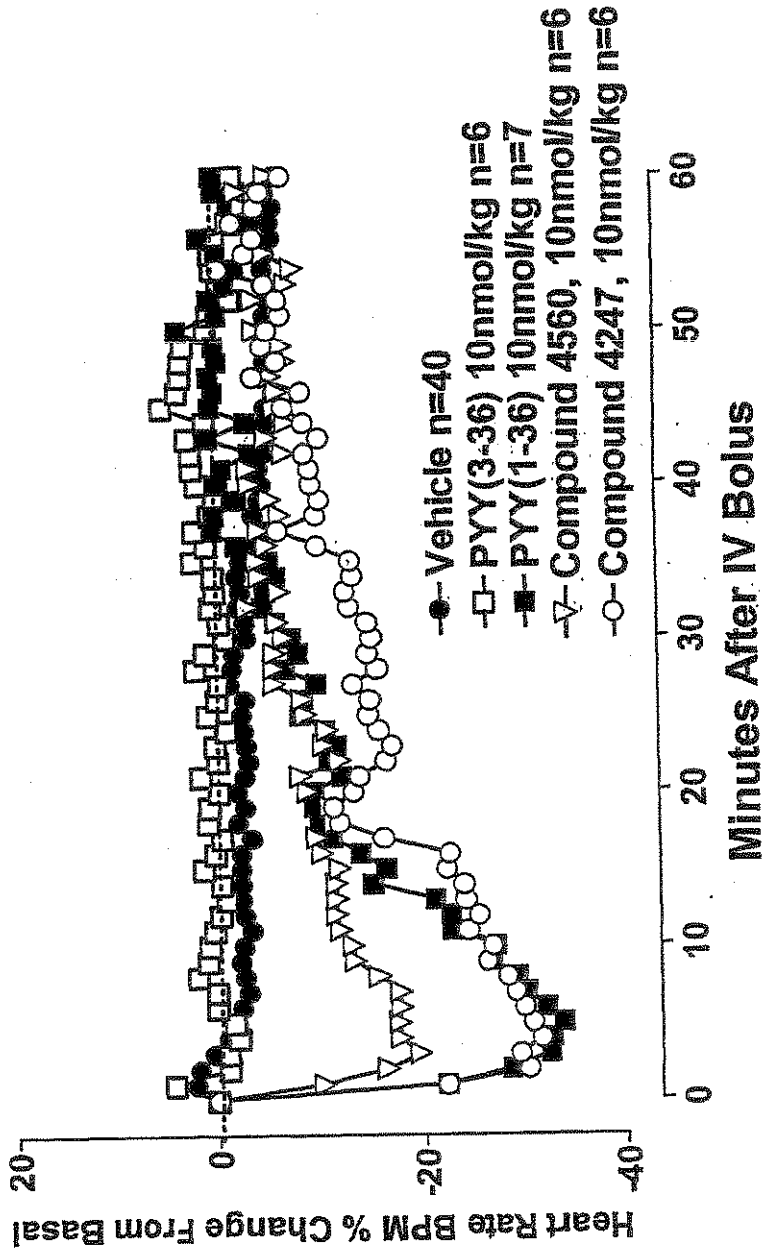

Heart Rate
Heart Rate BPM % Change From Basal
20
0
-20
-40
0
10
20
30
40
50
60
Minutes After IV Bolus
Vehicle n=40
PYY(3-36) 10nmol/kg n=6
PYY(1-36) 10nmol/kg n=7
Compound 4560, 10nmol/kg n=6
Compound 4247, 10nmol/kg n=6

FIG. 9-C

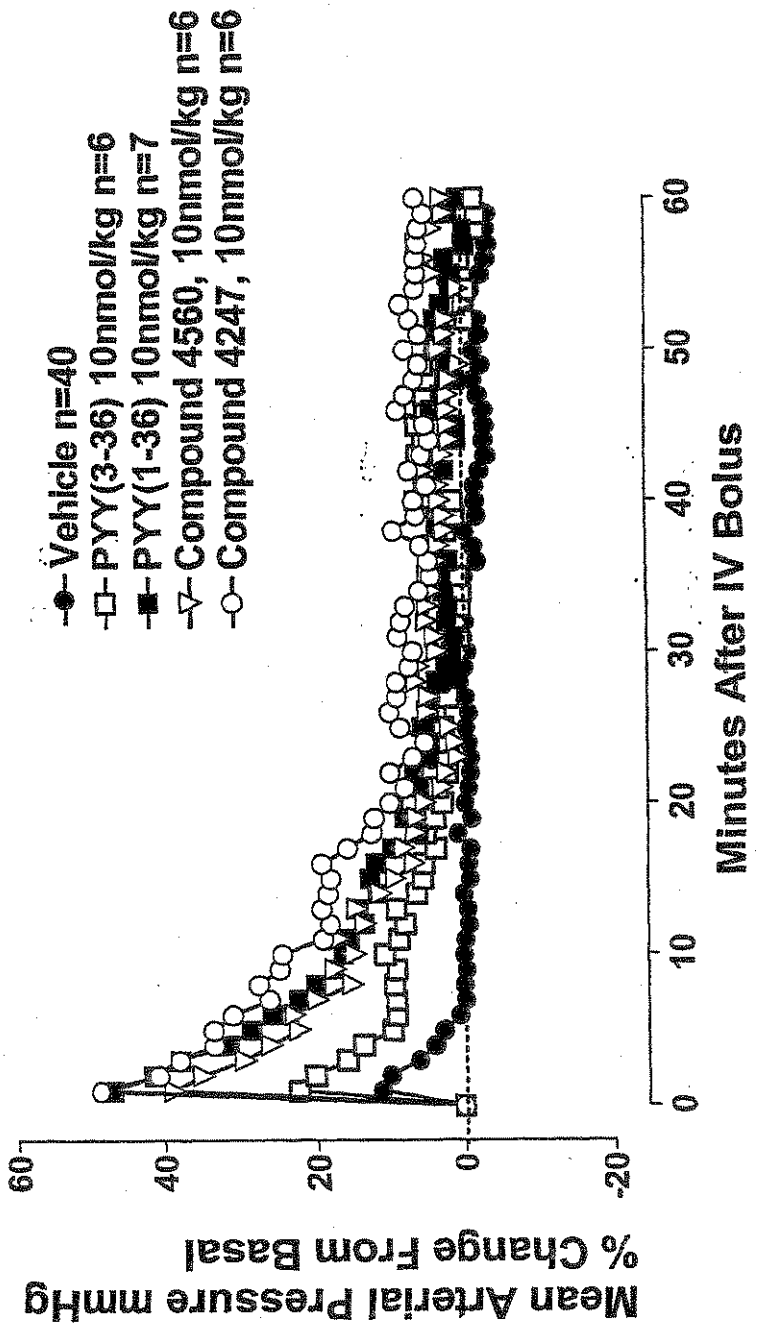

Mean Arterial Pressure
Vehicle n=40
PYY(3-36) 10nmol/kg n=6
PYY(1-36) 10nmol/kg n=7
Compound 4560, 10nmol/kg n=6
Compound 4247, 10nmol/kg n=6
Mean Arterial Pressure mmHg
% Change From Basal
60
40
20
0
-20
0
10
20
30
40
50
60
Minutes After IV Bolus

FIG. 9-D

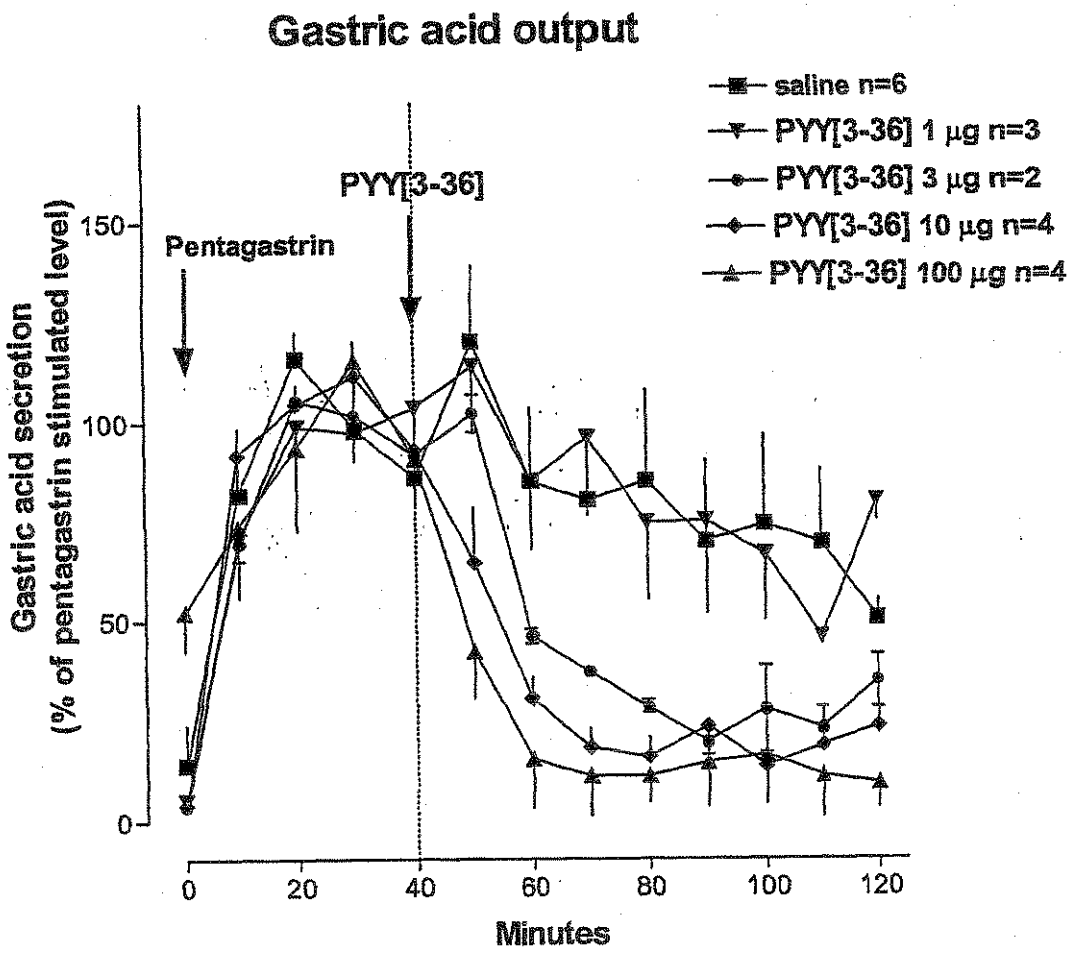

Gastric acid output
saline n=6
PYY[3-36] 1 µg n=3
PYY[3-36] 3 µg n=2
PYY[3-36] 10 µg n=4
PYY[3-36] 100 µg n=4
PYY[3-36]
Pentagastrin
Gastric acid secretion
(% of pentagastrin stimulated level)
150
100
50
0
0
20
40
60
80
100
120
Minutes

FIG. 10

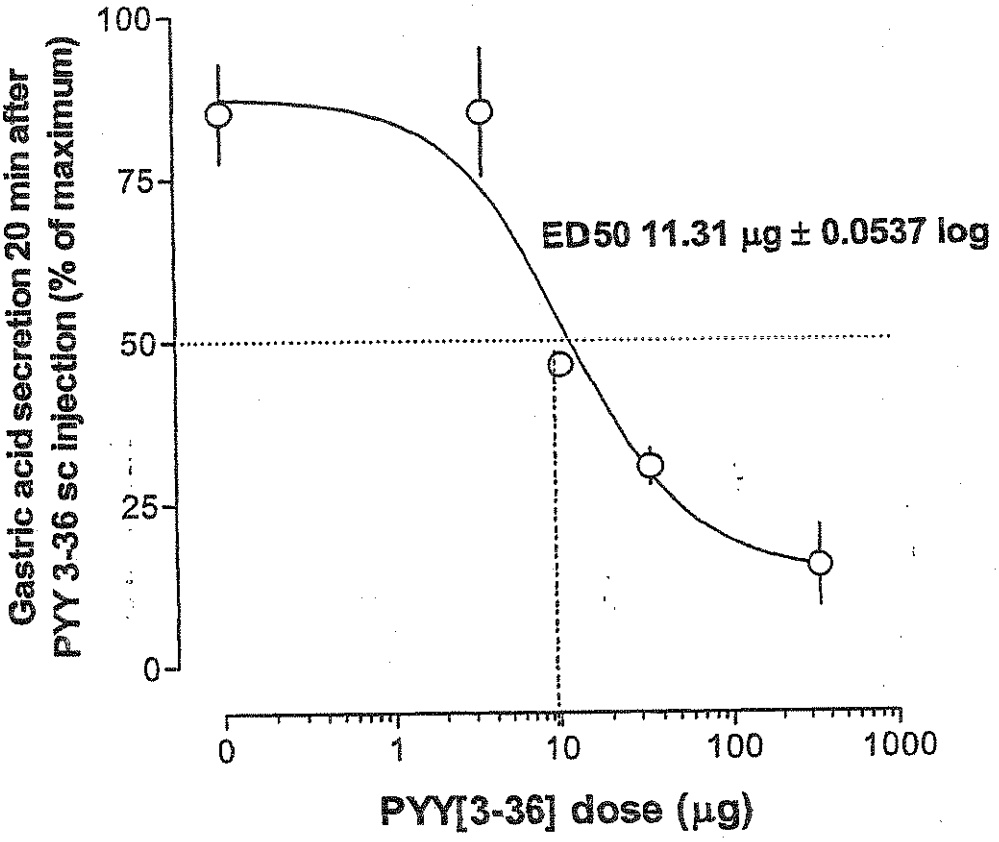

Gastric acid secretion 20 min after PYY 3-36 sc injection (% of maximum)
100
75
50
25
0
ED50 11.31 µg ± 0.0537 log
0
1
10
100
1000
PYY[3-36] dose (µg)

FIG. 11

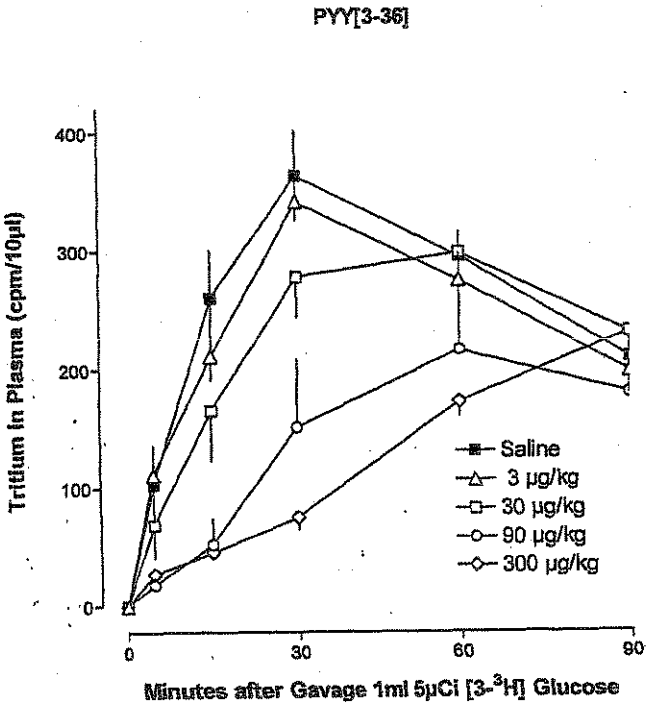

PYY[3-36]
Tritium in Plasma (cpm/10µl)
400
300
200
100
0
0
30
60
90
Minutes after Gavage 1ml 5µCi [3-3H] Glucose
Saline
3 µg/kg
30 µg/kg
90 µg/kg
300 µg/kg

FIG. 12

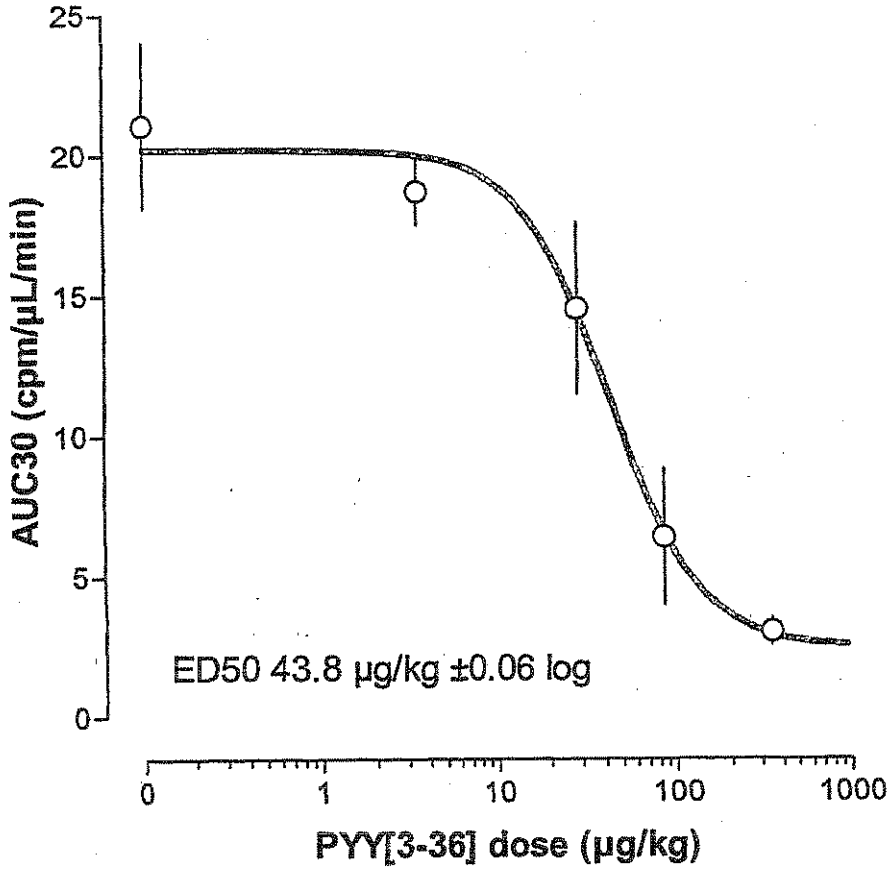

25
20
15
10
5
0
AUC30 (cpm/µL/min)
ED50 43.8 µg/kg ±0.06 log
0
1
10
100
1000
PYY[3-36] dose (µg/kg)

FIG. 13

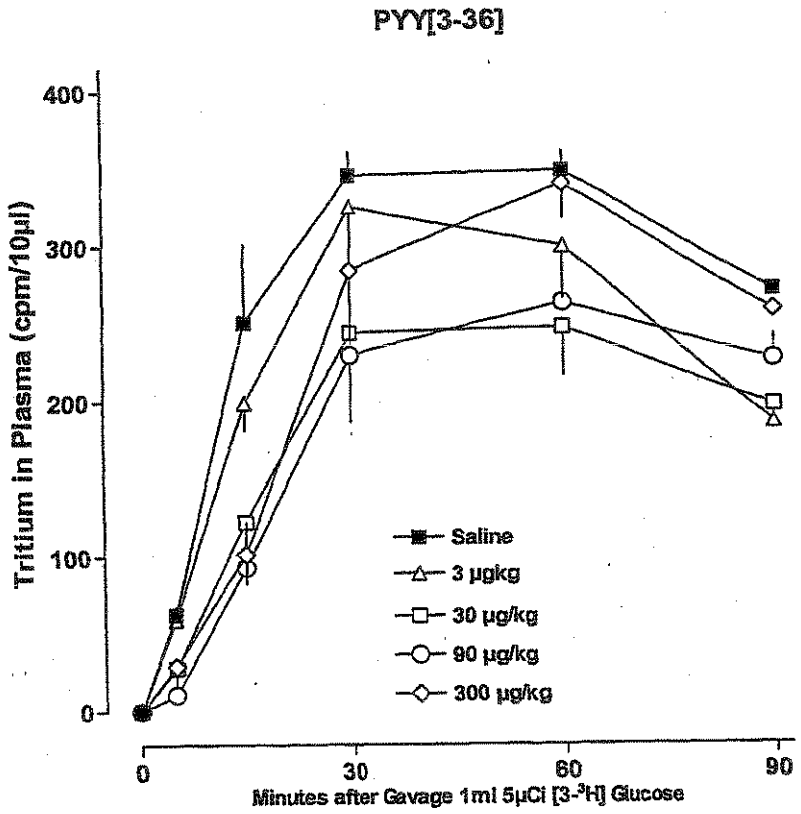

PYY[3-36]
Tritium in Plasma (cpm/10µl)
400
300
200
100
0
Saline
3 µgkg
30 µg/kg
90 µg/kg
300 µg/kg
0
30
60
90
Minutes after Gavage 1ml 5µCi [3-3H] Glucose

FIG. 14

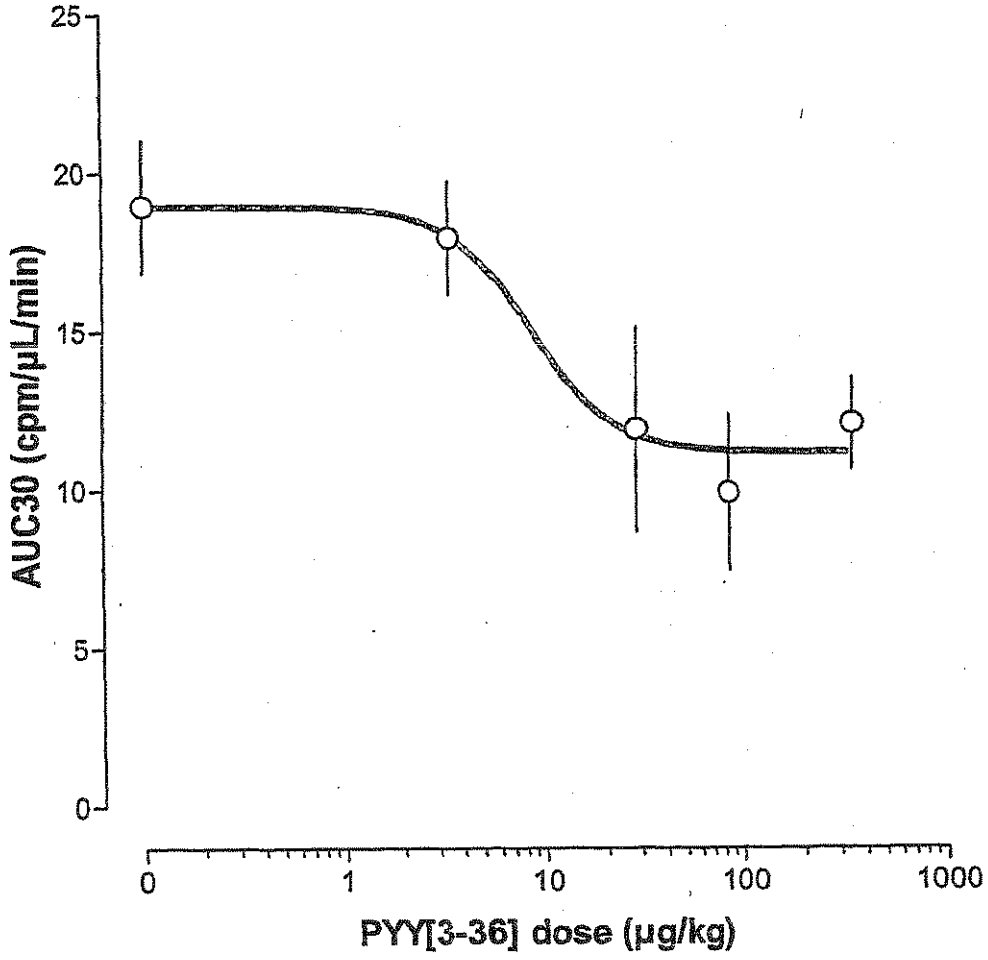


FIG. 15

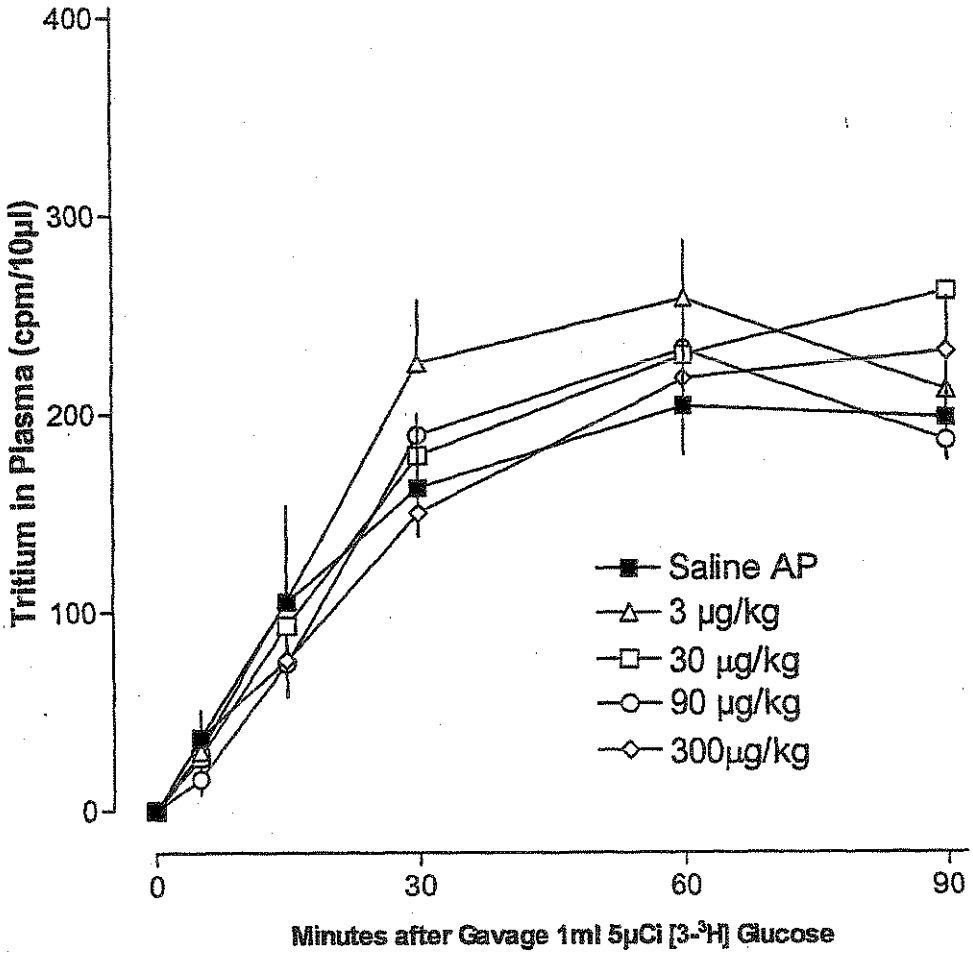

400
300
200
100
0
Tritium in Plasma (cpm/10µl)
0
30
60
90
Minutes after Gavage 1ml 5µCi [3-3H] Glucose
Saline AP
3 µg/kg
30 µg/kg
90 µg/kg
300µg/kg

FIG. 16

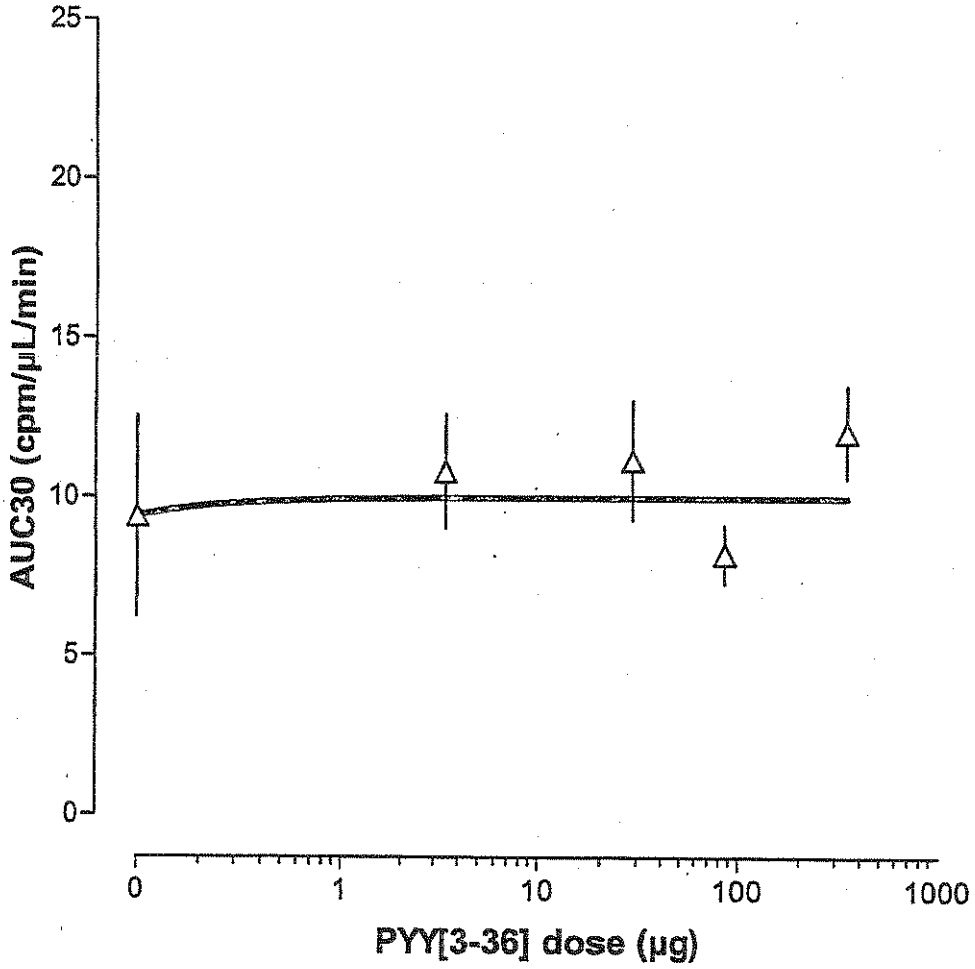


FIG. 17

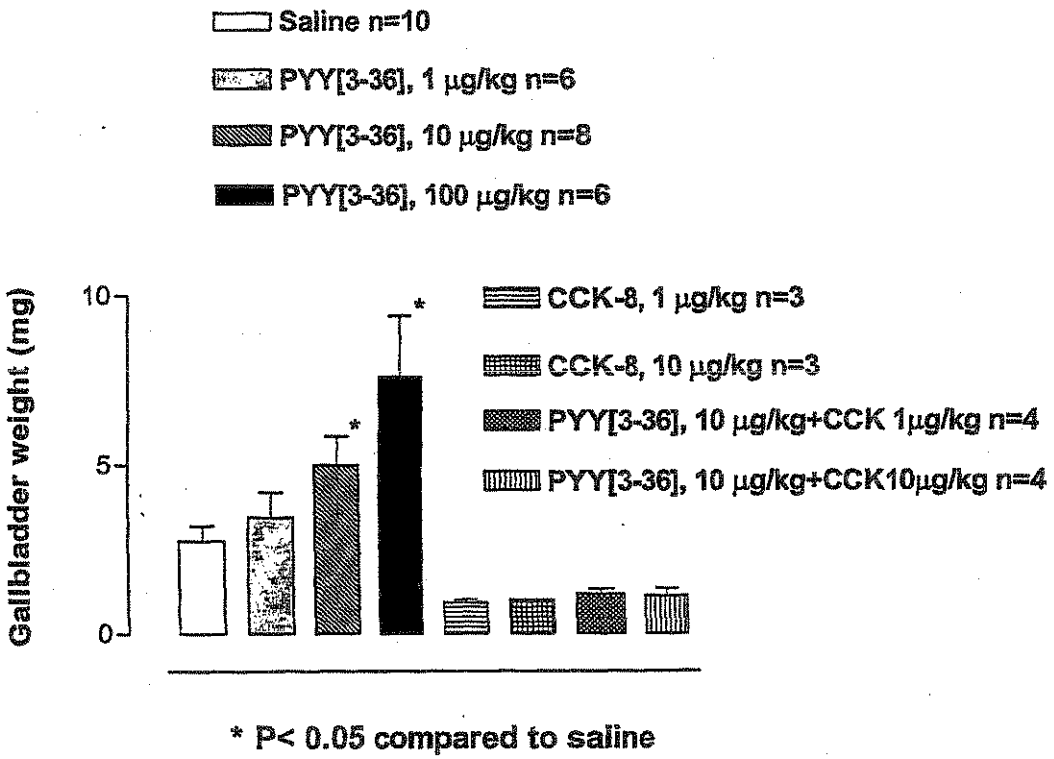

Saline n=10
PYY[3-36], 1 μg/kg n=6
PYY[3-36], 10 μg/kg n=8
PYY[3-36], 100 μg/kg n=6
CCK-8, 1 μg/kg n=3
CCK-8, 10 μg/kg n=3
PYY[3-36], 10 μg/kg+CCK 1μg/kg n=4
PYY[3-36], 10 μg/kg+CCK10μg/kg n=4
Gallbladder weight (mg)
10
5
0
*
*
* P< 0.05 compared to saline

FIG. 18

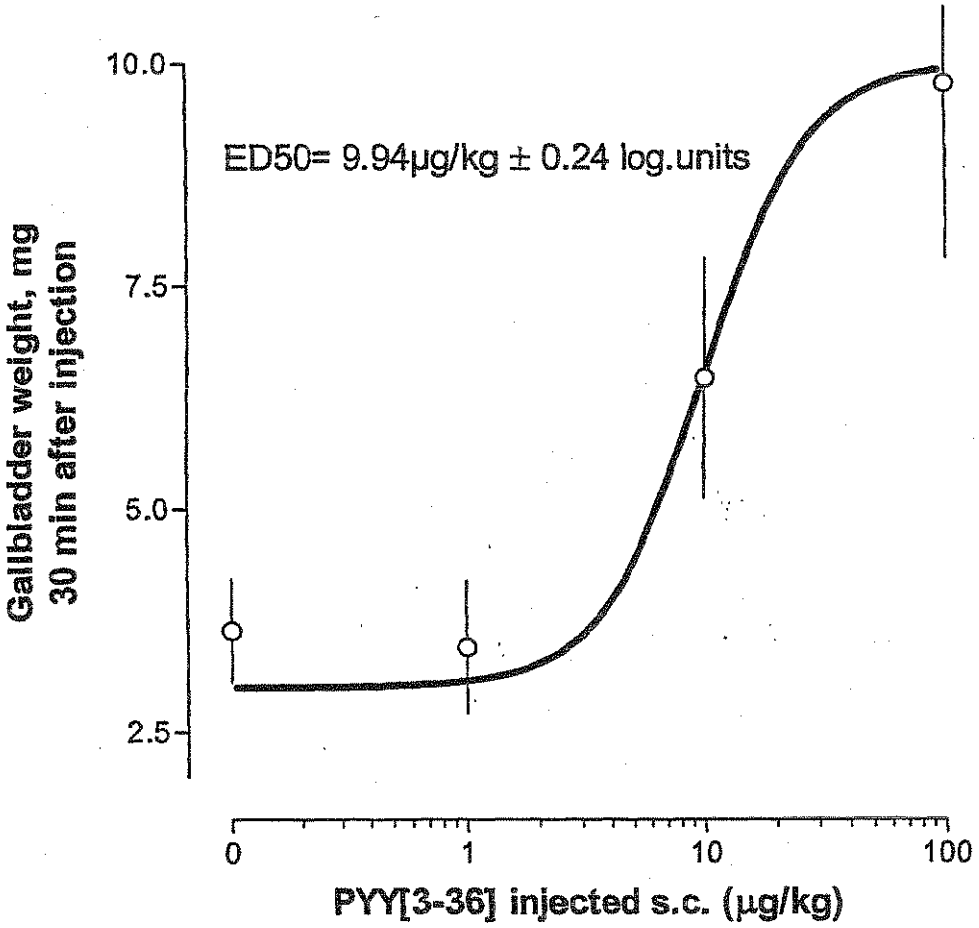

10.0
7.5
5.0
2.5
ED50= 9.94µg/kg ± 0.24 log.units
Gallbladder weight, mg
30 min after injection
0
1
10
100
PYY[3-36] injected s.c. (µg/kg)

FIG. 19

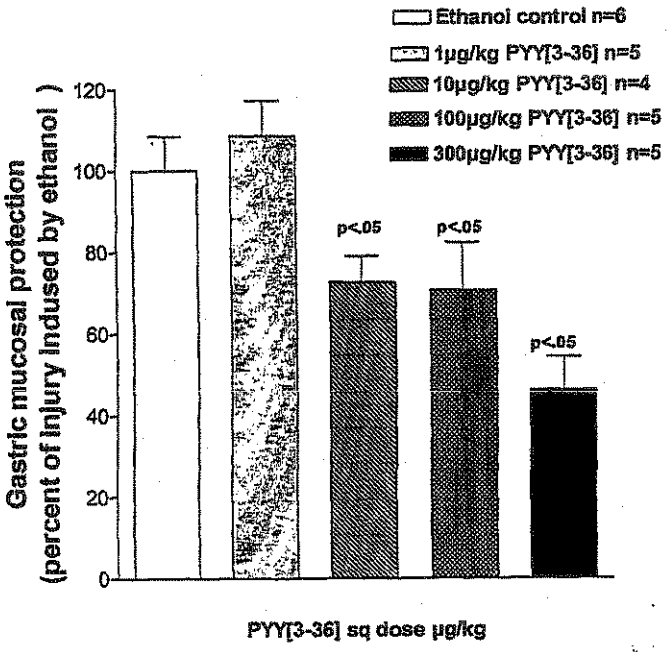

Ethanol control n=6
1µg/kg PYY[3-36] n=5
10µg/kg PYY[3-36] n=4
100µg/kg PYY[3-36] n=5
300µg/kg PYY[3-36] n=5
Gastric mucosal protection
(percent of injury Indused by ethanol)
120
100
80
60
40
20
0
p<.05
p<.05
p<.05
PYY[3-36] sq dose µg/kg

FIG. 20

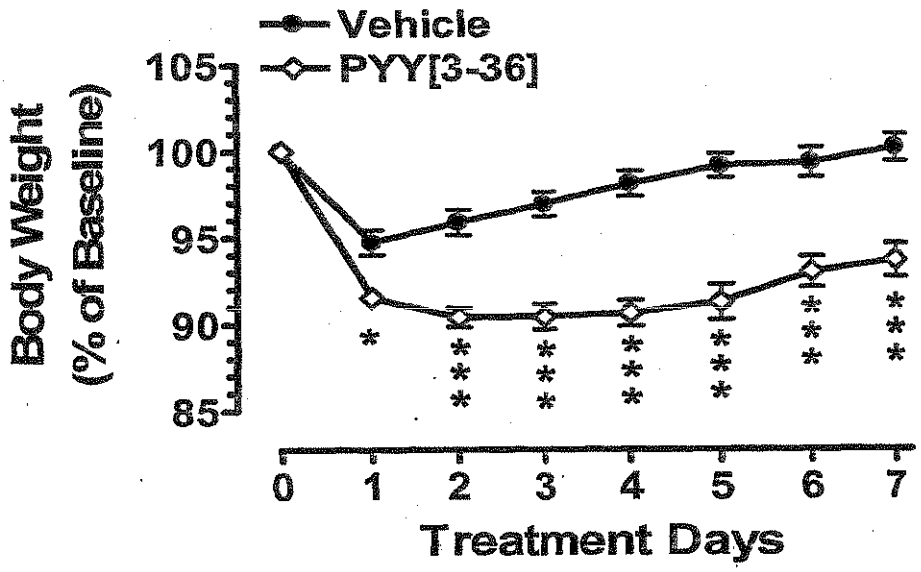


FIG. 21A

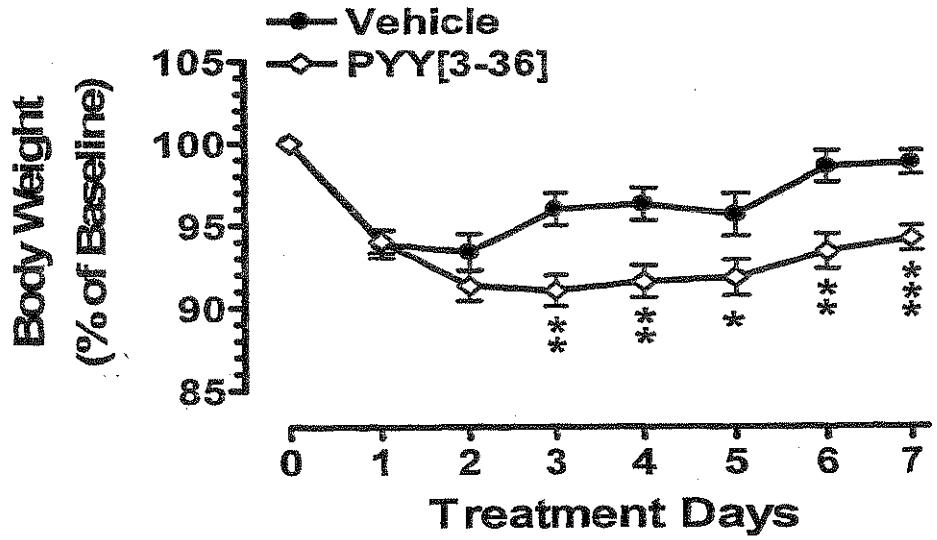


FIG. 21B

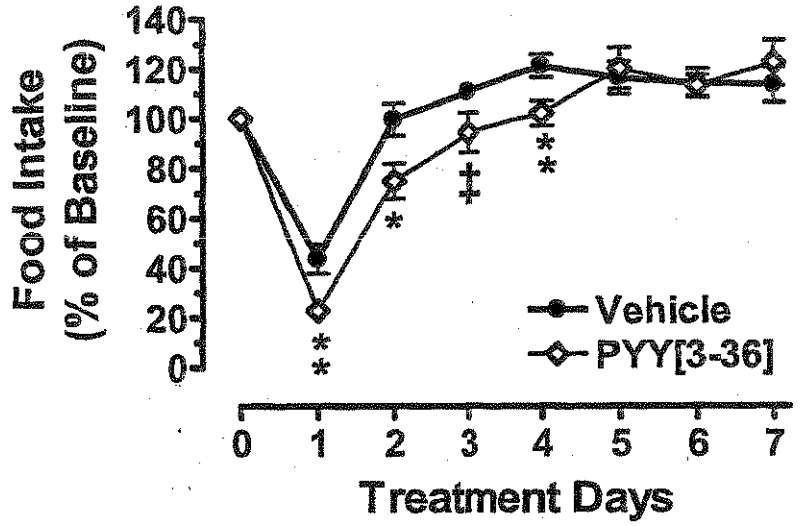


FIG. 22A

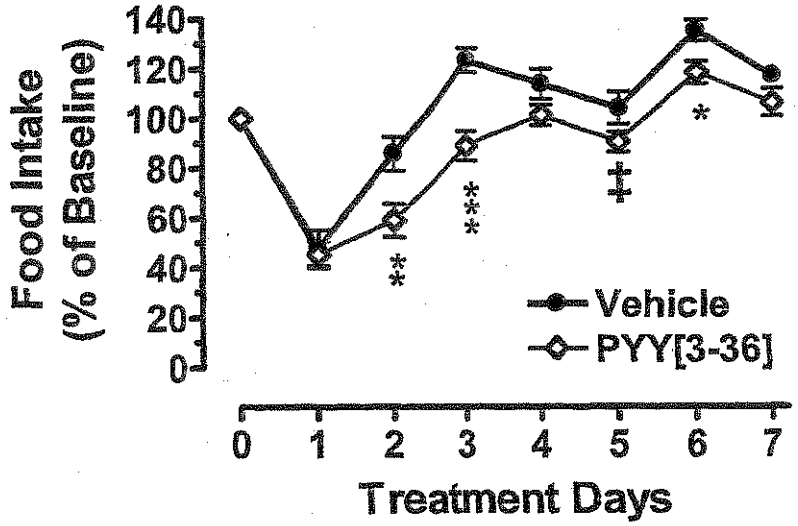


FIG. 22B

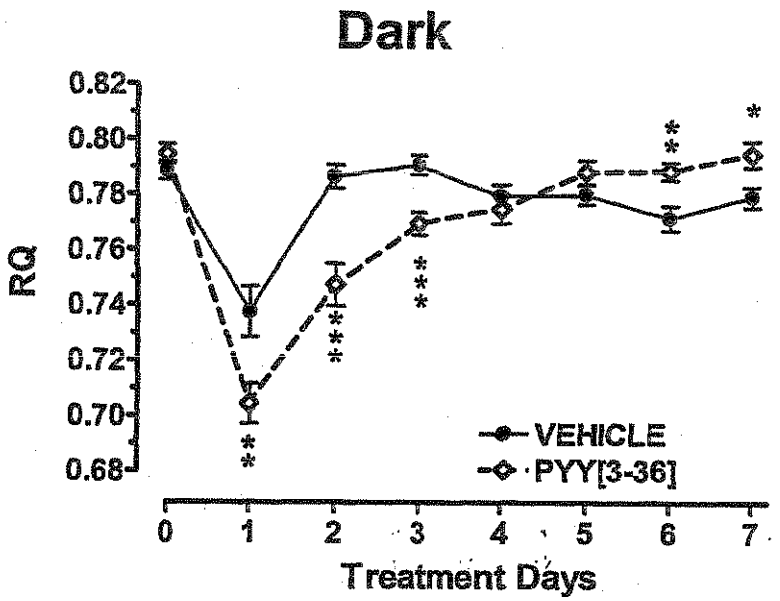


FIG. 23A

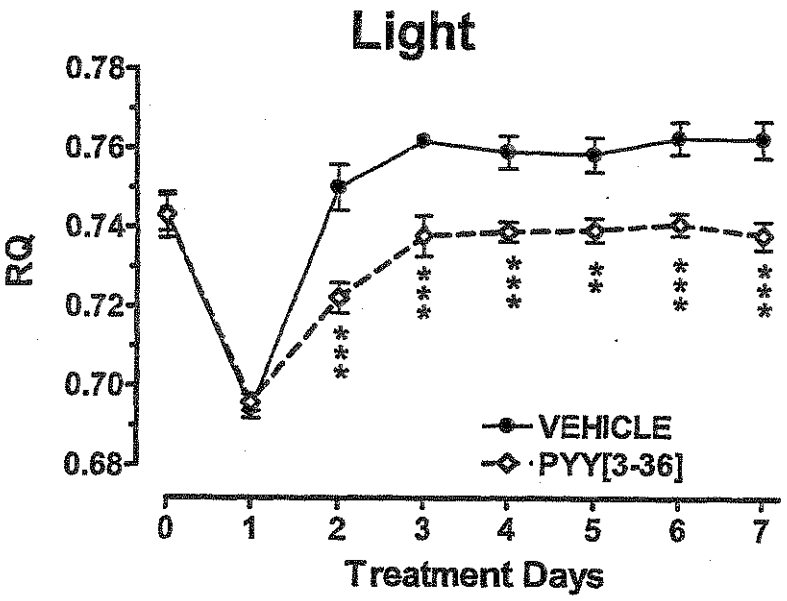


FIG. 23B

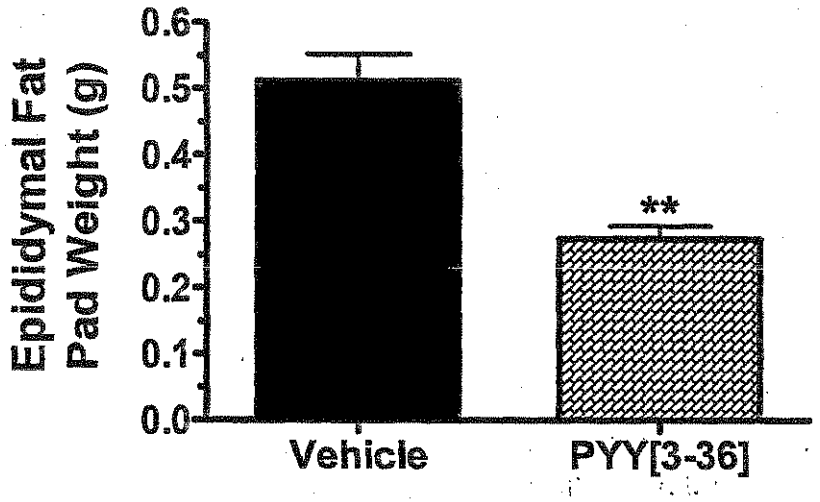


FIG. 24A

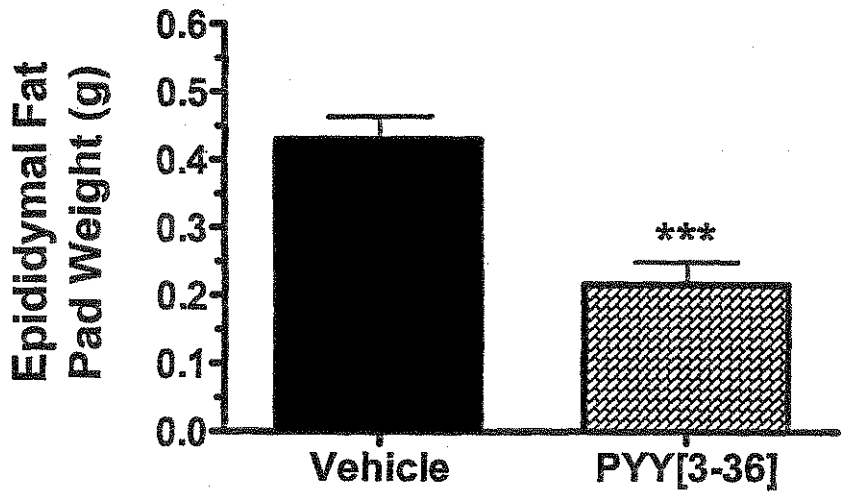


FIG. 24B

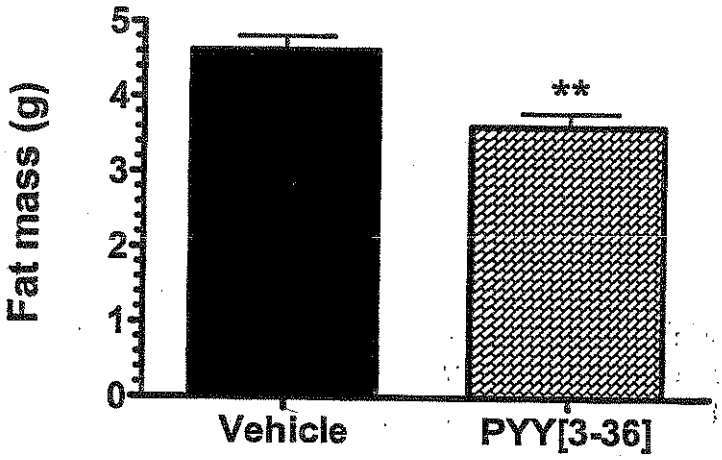


FIG. 25A

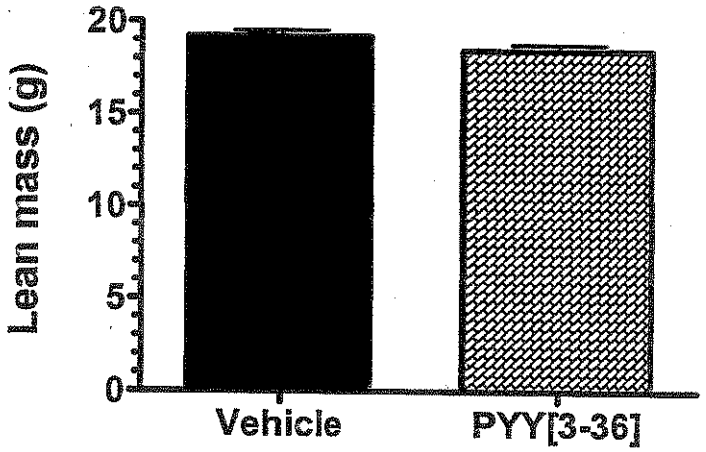


FIG. 25B

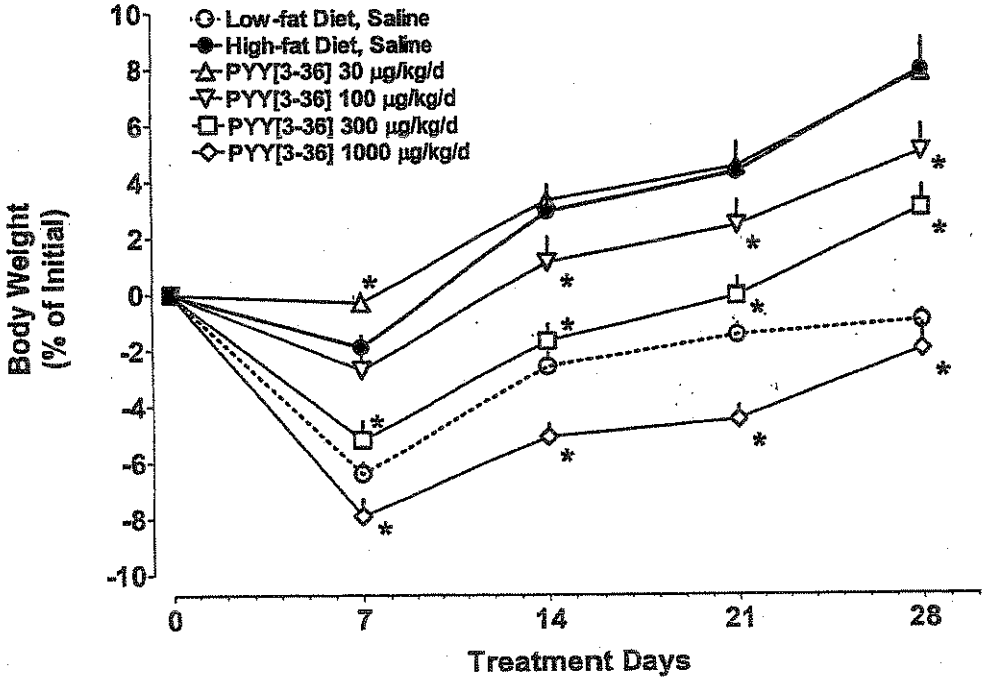

Low-fat Diet, Saline
High-fat Diet, Saline
PYY[3-36] 30 µg/kg/d
PYY[3-36] 100 µg/kg/d
PYY[3-36] 300 µg/kg/d
PYY[3-36] 1000 µg/kg/d
Body Weight (% of Initial)
10
8
6
4
2
0
-2
-4
-6
-8
-10
0
7
14
21
28
Treatment Days

FIG. 26

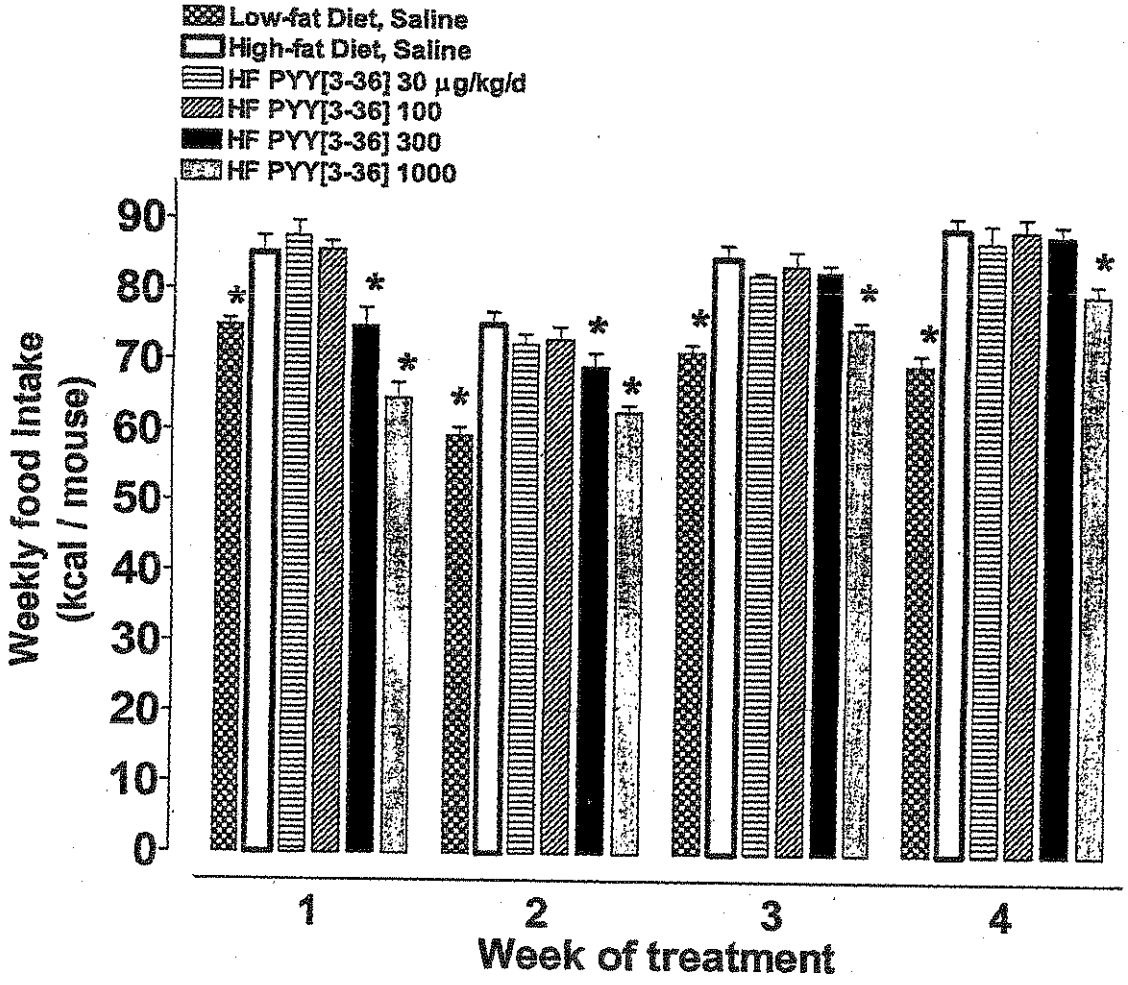

Low-fat Diet, Saline
High-fat Diet, Saline
HF PYY[3-36] 30 µg/kg/d
HF PYY[3-36] 100
HF PYY[3-36] 300
HF PYY[3-36] 1000
Weekly food intake (kcal / mouse)
0
10
20
30
40
50
60
70
80
90
1
2
3
4
Week of treatment

FIG. 27

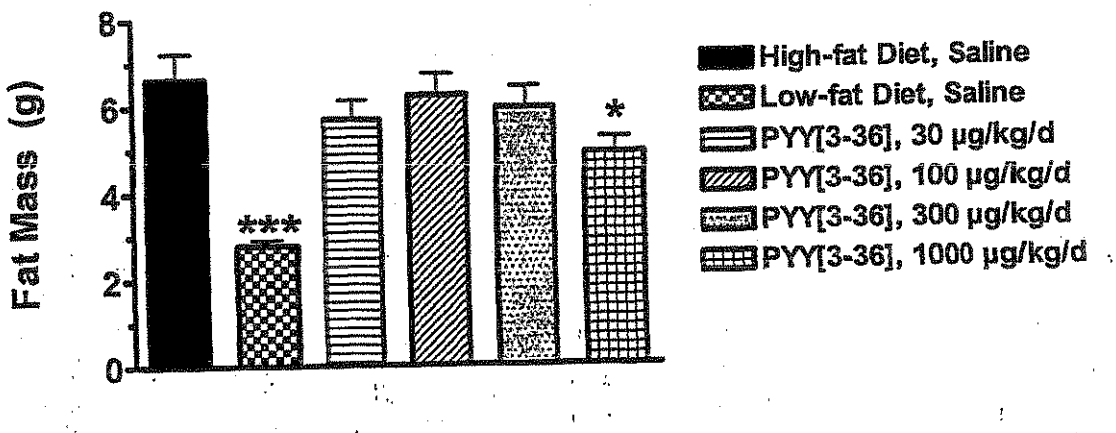


FIG. 28A

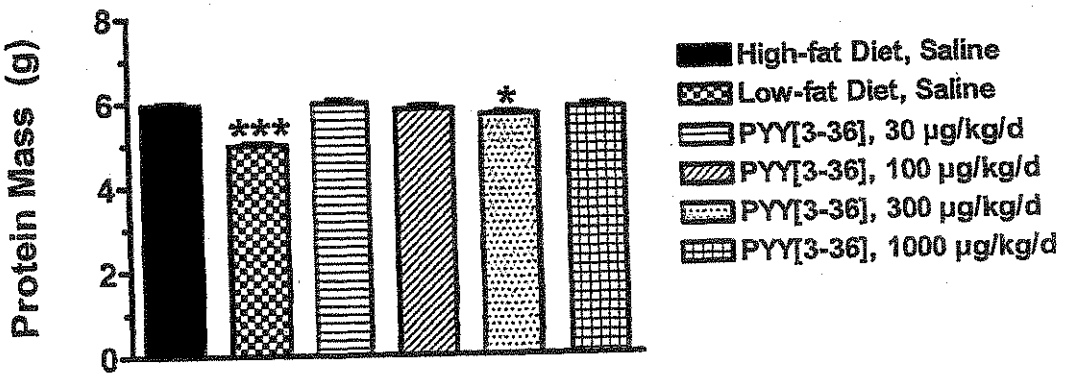


FIG. 28B

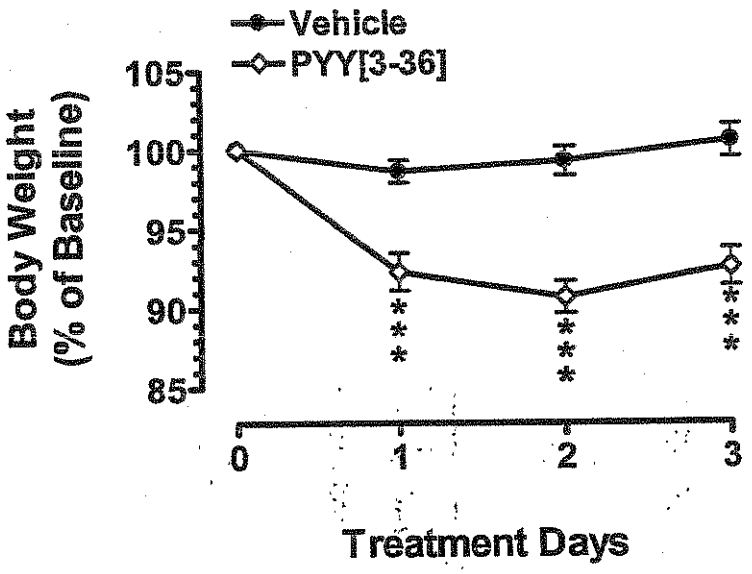


FIG. 29A

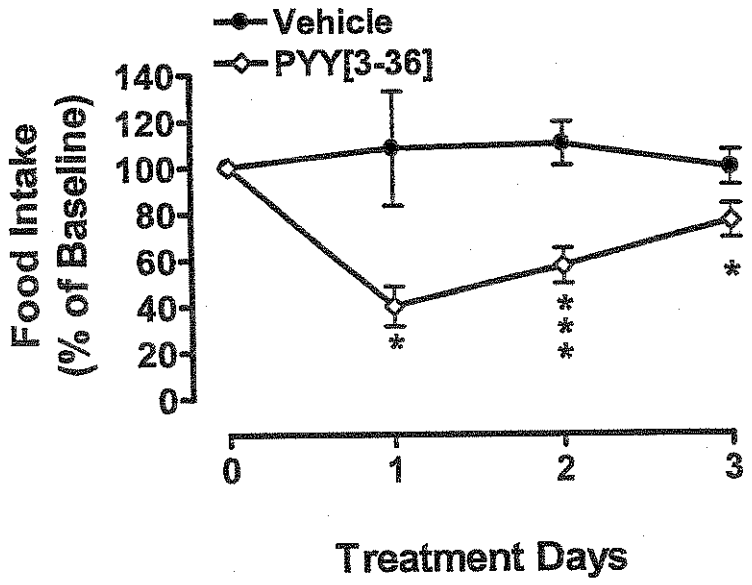


FIG. 29B

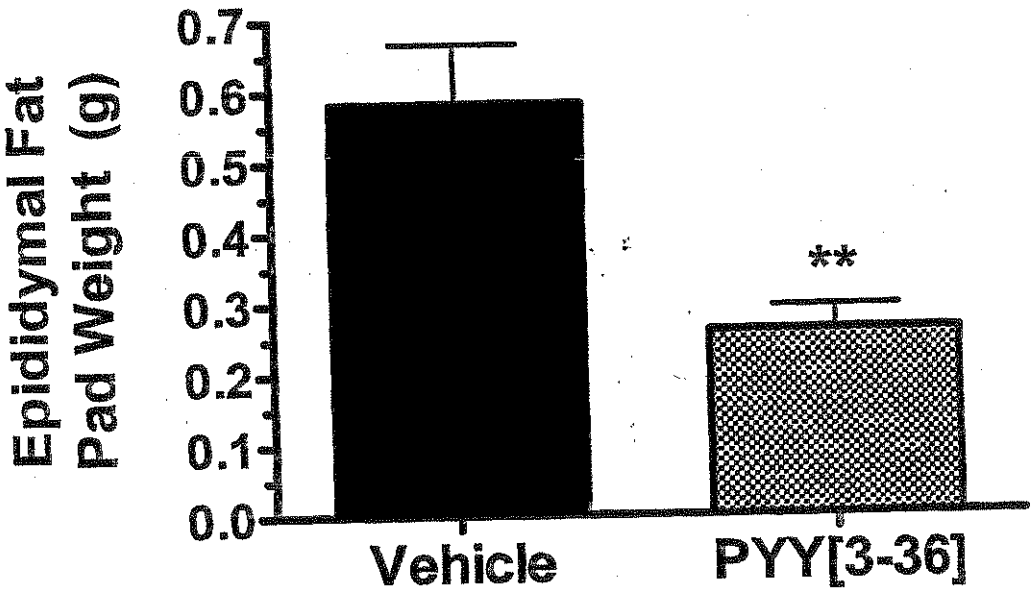

Epididymal Fat
Pad Weight (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
**
Vehicle
PYY[3-36]

FIG. 30

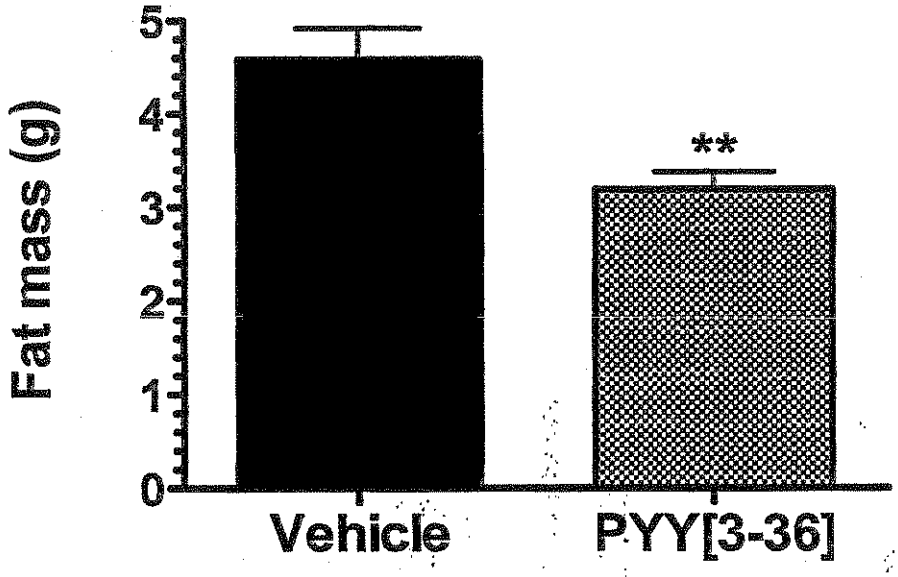


FIG. 31A

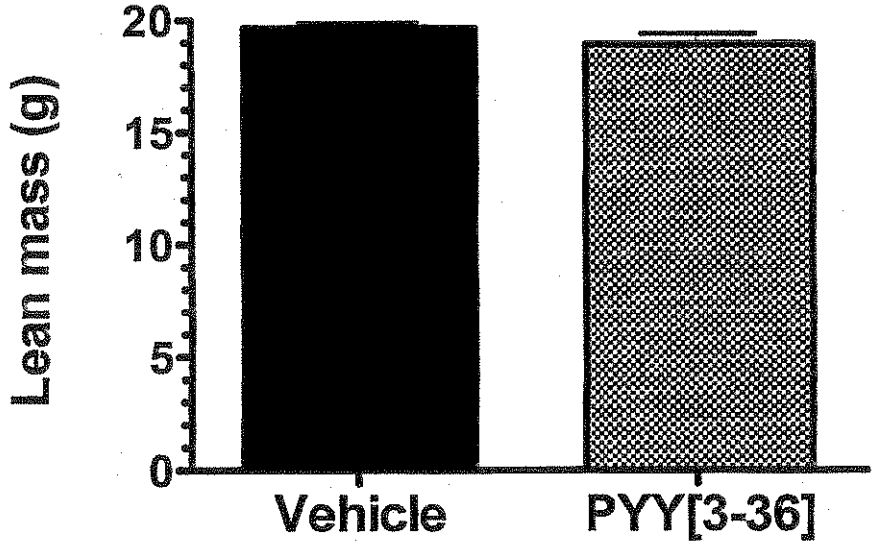


FIG. 31B

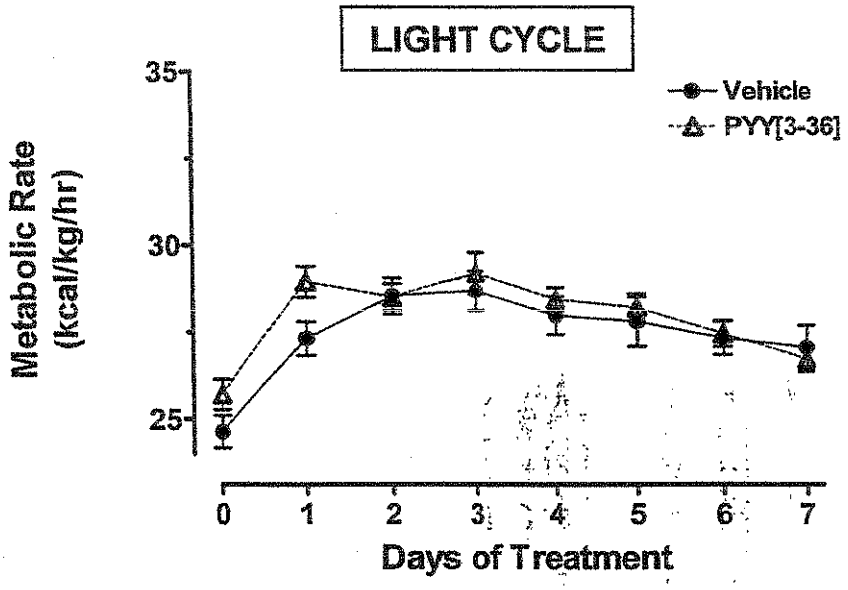


FIG. 32A

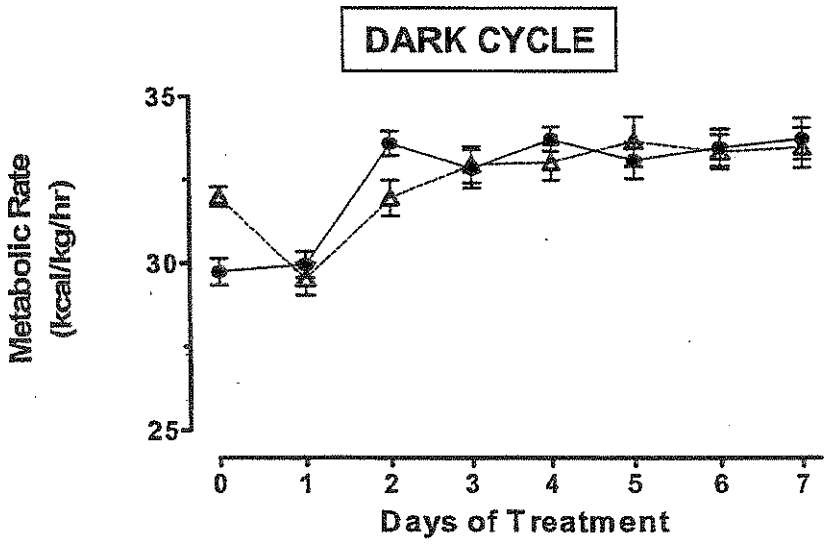


FIG. 32B

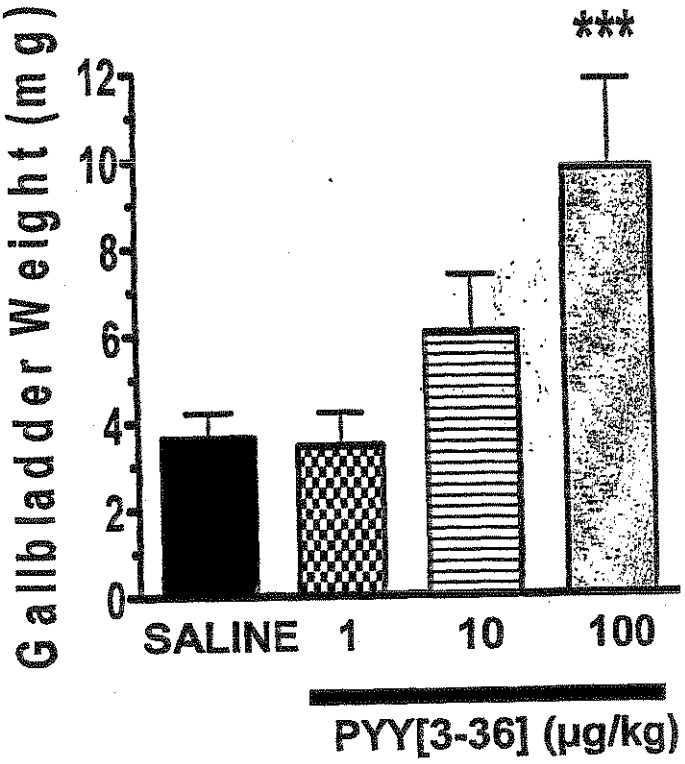

Gallbladder Weight (mg)
12
10
8
6
4
2
0
***
SALINE
1
10
100
PYY[3-36] (µg/kg)

FIG. 33

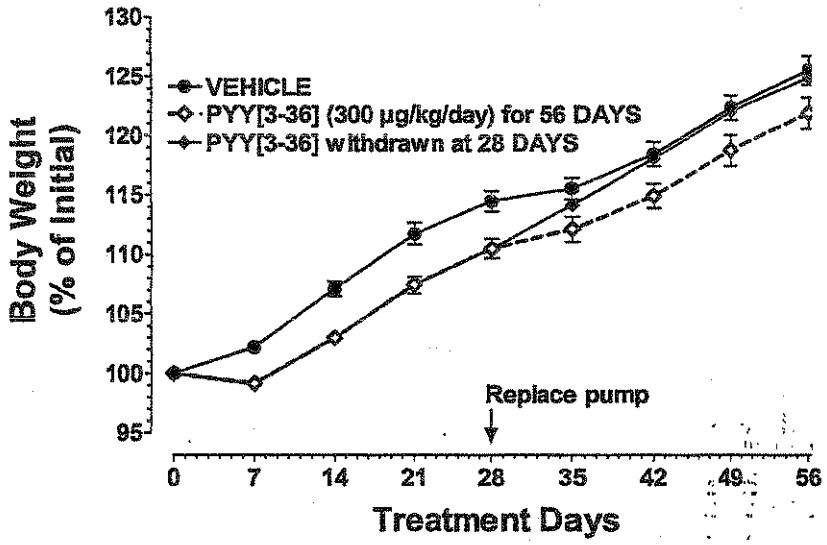


FIG. 34A

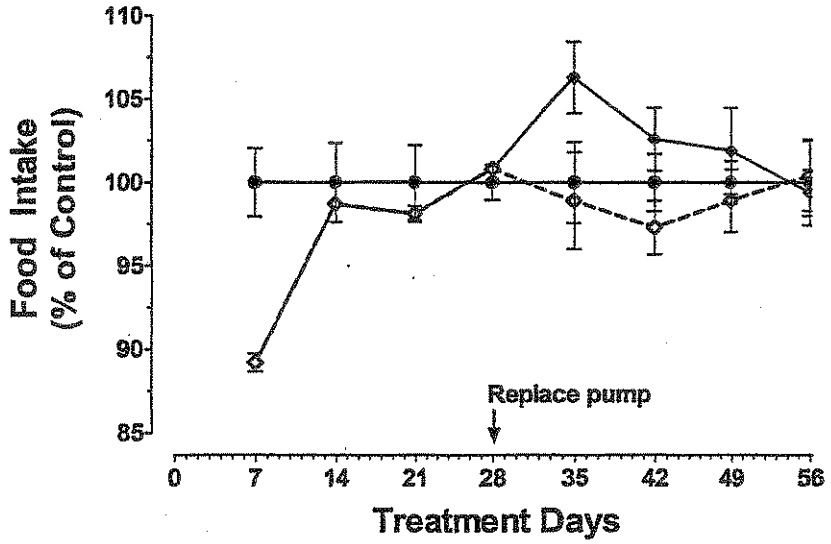


FIG. 34B

Dose dependent decrease in food intake and body weight in DIO prone rats

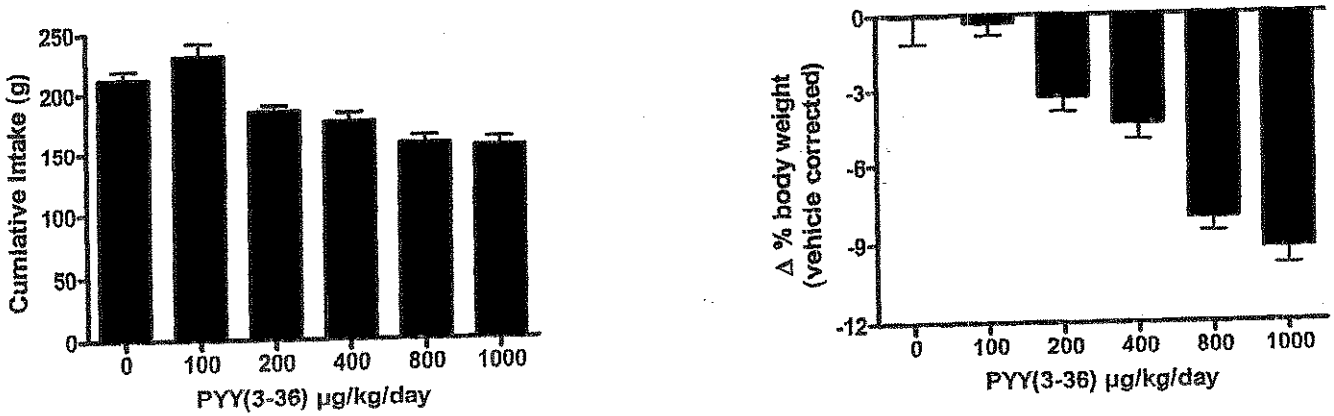
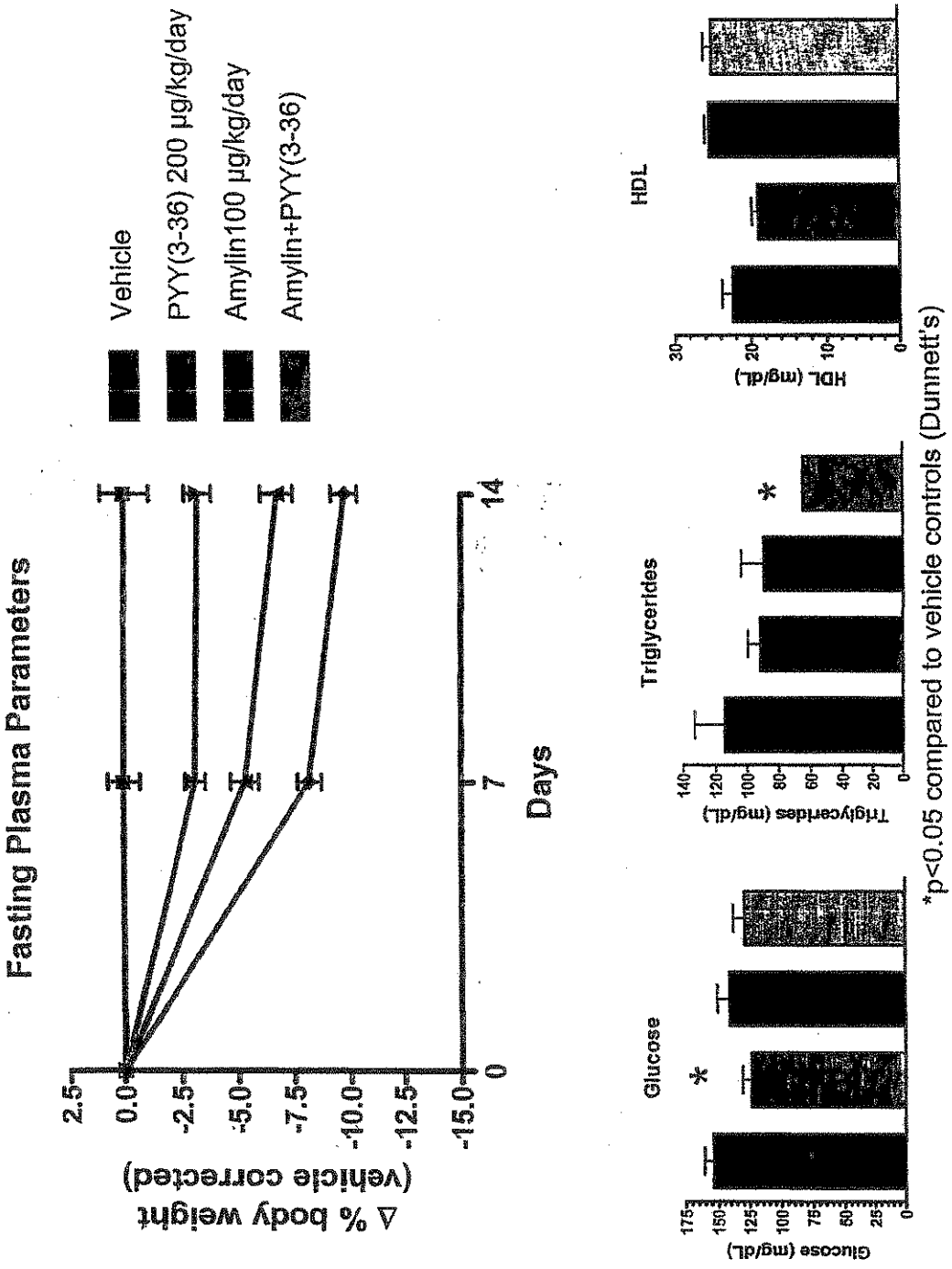
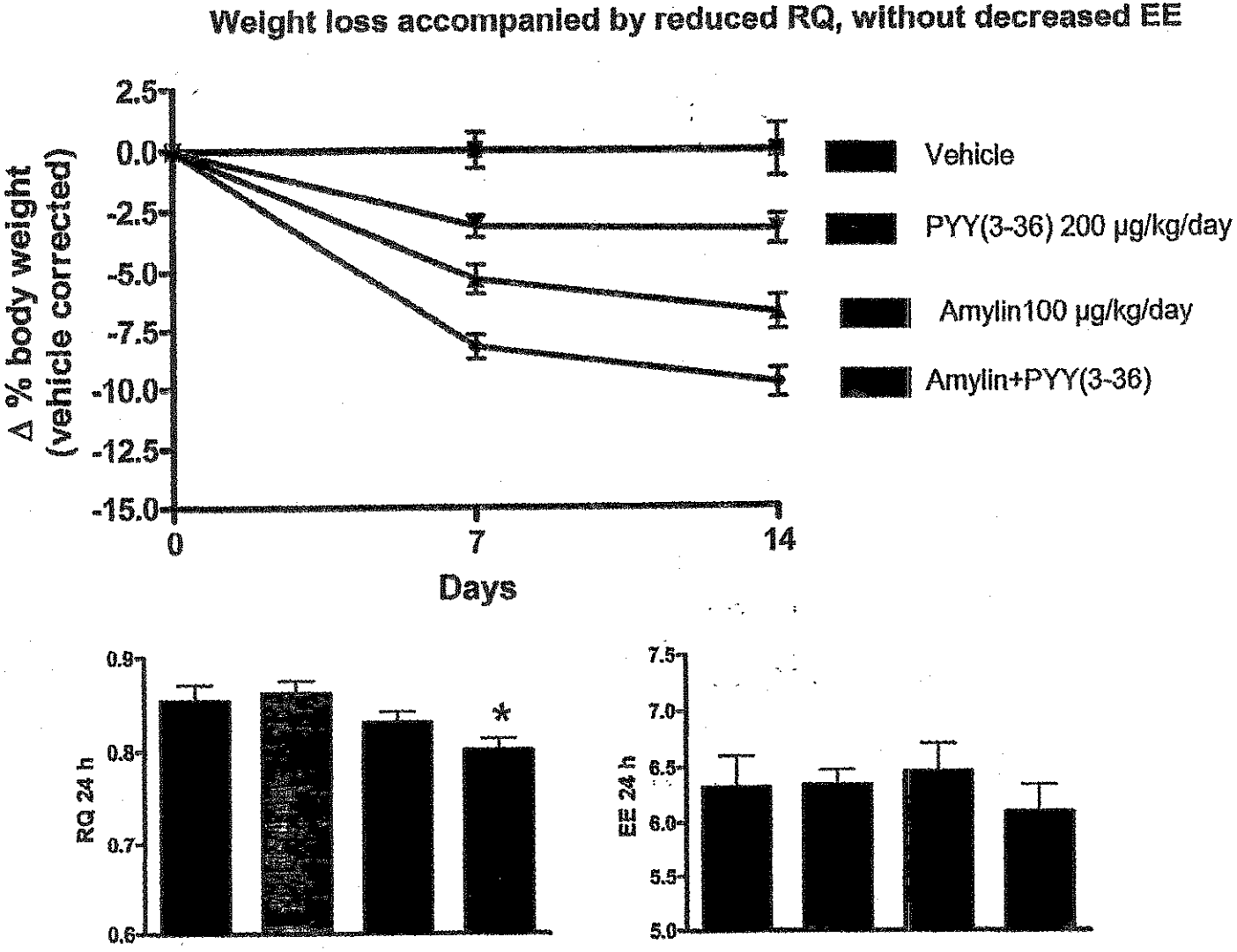


FIG. 35



*p<0.05 compared to vehicle controls (Dunnett's)

FIG. 36



*p<0.05 compared to vehicle controls (Dunnett's). Calorimetry performed @ Week 1

FIG. 37

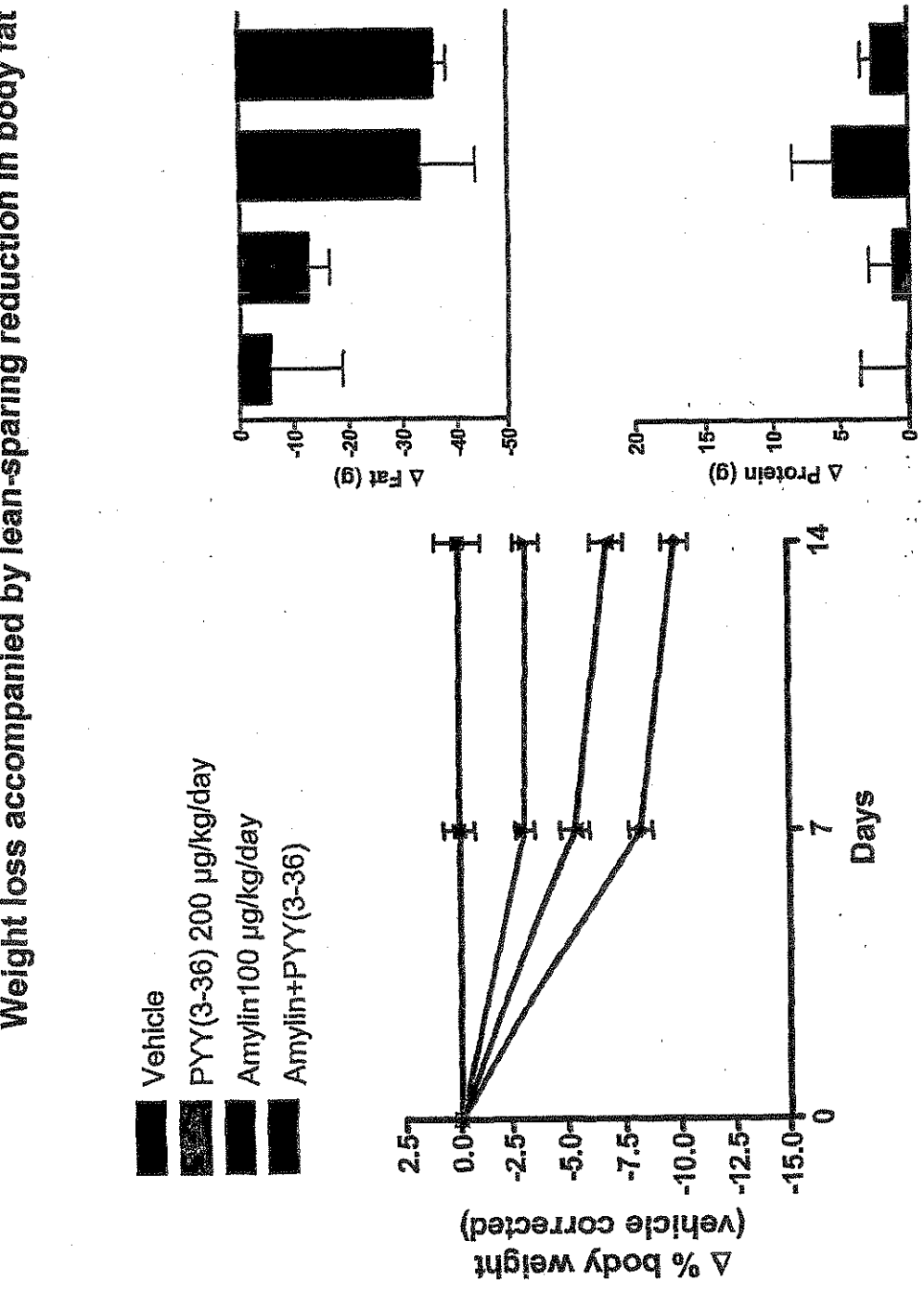

Weight loss accompanied by lean-sparing reduction in body fat
Vehicle
PYY(3-36) 200 µg/kg/day
Amylin100 µg/kg/day
Amylin+PYY(3-36)
Δ % body weight
(vehicle corrected)
2.5
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
-15.0
0
7
14
Days
Δ Fat (g)
0
-10
-20
-30
-40
-50
Δ Protein (g)
20
15
10
5
0

FIG. 38

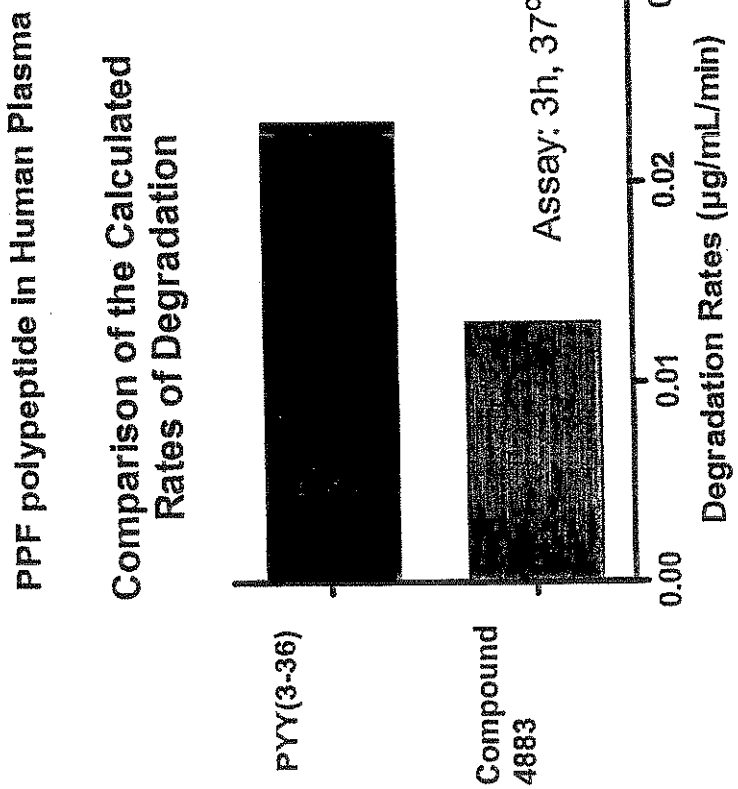
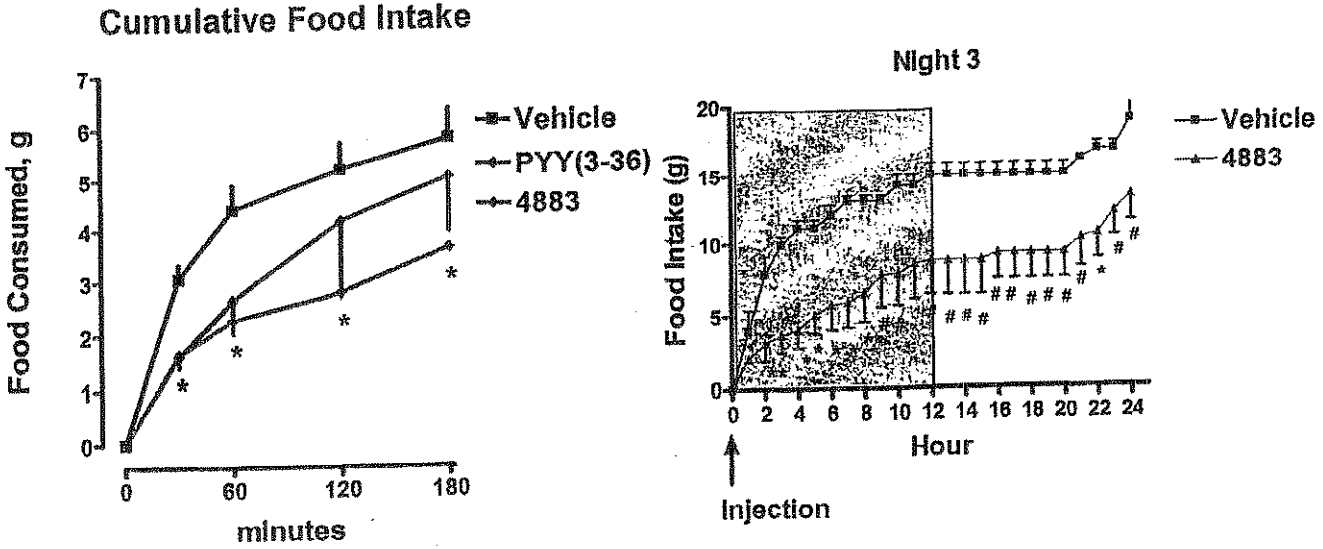

PPF polypeptide in Human Plasma
Comparison of the Calculated Rates of Degradation
PYY(3-36)
Compound 4883
Assay: 3h, 37°C
0.00
0.01
0.02
0.03
Degradation Rates (µg/mL/min)

FIG. 39

PPF polypeptide Compound 4883 is more efficacious than PYY(3-36) in Rodent Food Intake Models (Acute)



*P<0.05; ANOVA vs. HF Vehicle
N= 4 Cages; 3 animals/ Cage

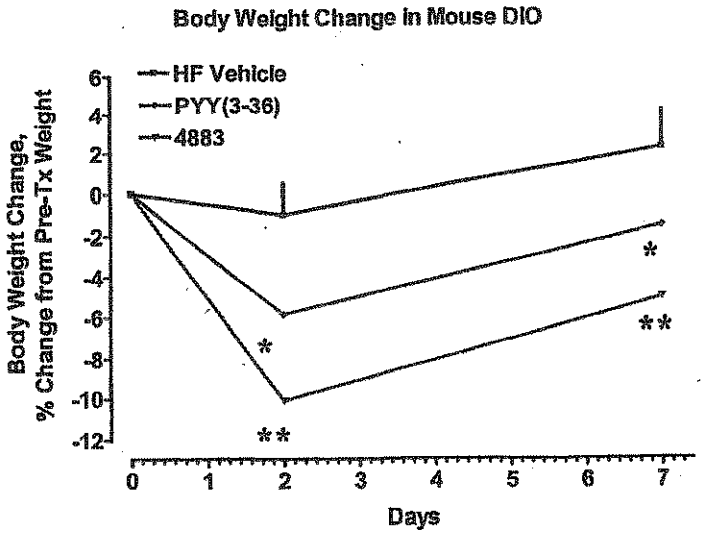
Food Intake Assay, NIH/Swiss Mouse
Dose: 8 nmol/kg i.p.

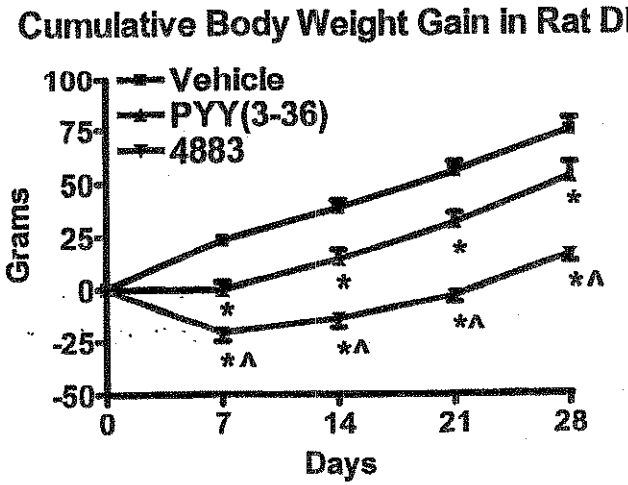
*P<0.05 compared to vehicle
#P<0.10 compared to vehicle

Feeding Pattern, HSD Rat
Dose: 1 mg/kg i.p.

FIG. 40

PPF polypeptide Compound 4883 is More Effective than PYY(3-36) in Rodent DIO Models (Chronic)





% BW loss mouse DIO day 7
PYY(3-36): 3.8
Compound 4883: 7.2

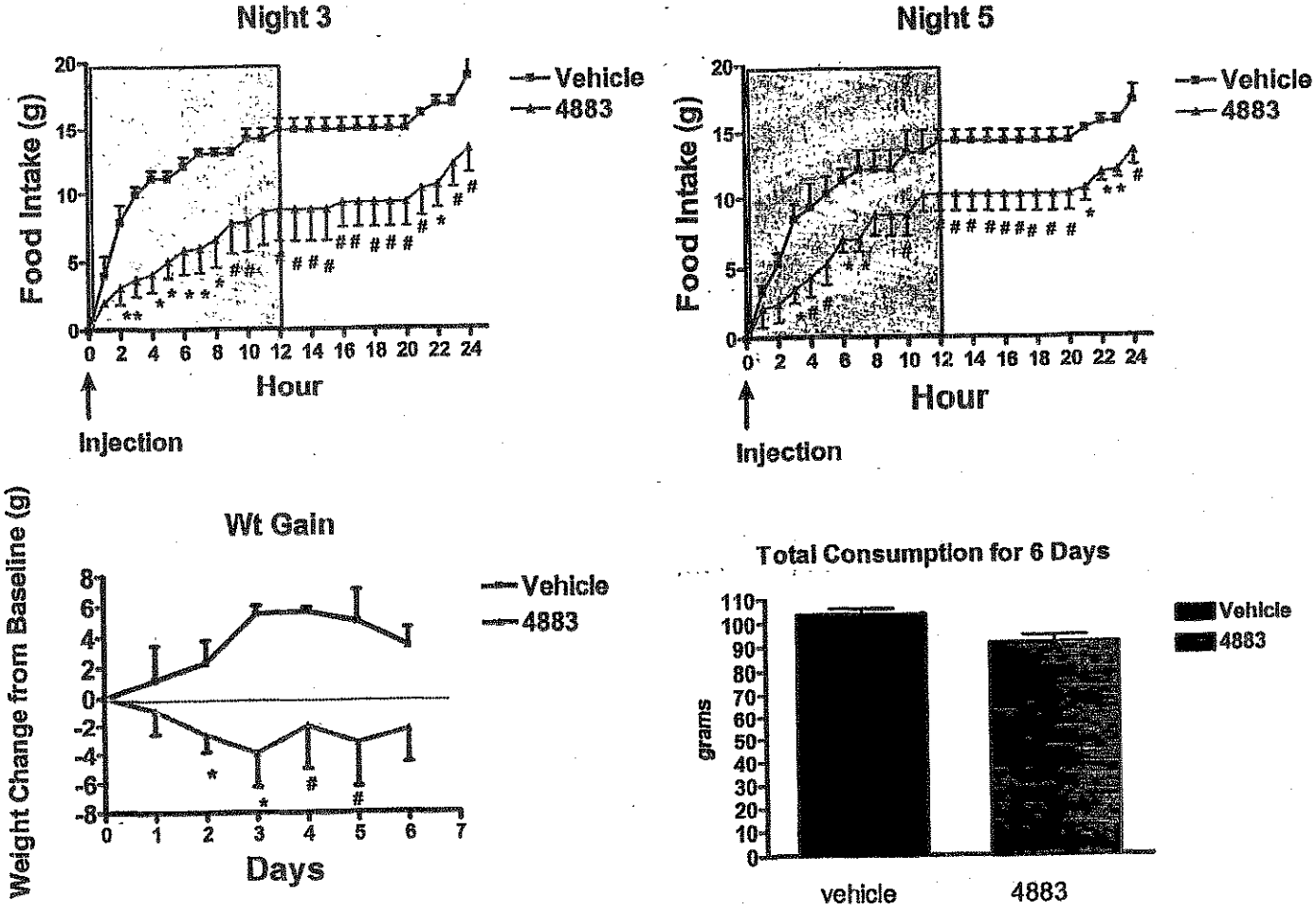
* P<0.05; compared to Vehicle
** P<0.05 compared to Vehicle and PYY(3-36)
Dose: 75 nmol/kg/day s.c. pump infusion

% BW loss rat DIO day 28
PYY(3-36): 4.6
Compound 4883: 12.4

*P<.05 compared to Vehicle
^P<.05 compared to PYY(3-36)
Dose: 67 nmol/kg/day, s.c. pump infusion

FIG. 41

Feeding Pattern Analysis (Rat):
PPF polypeptide Compound 4883 is More Efficacious than PYY(3-36)



*P<0.05; #P<0.1 vs. Vehicle
Dose: 1 mg/ Kg i.p.

FIG. 42

PPF polypeptide Compound 4883 in DIO Rats:
Body Composition Changes Similar to PYY(3-36)

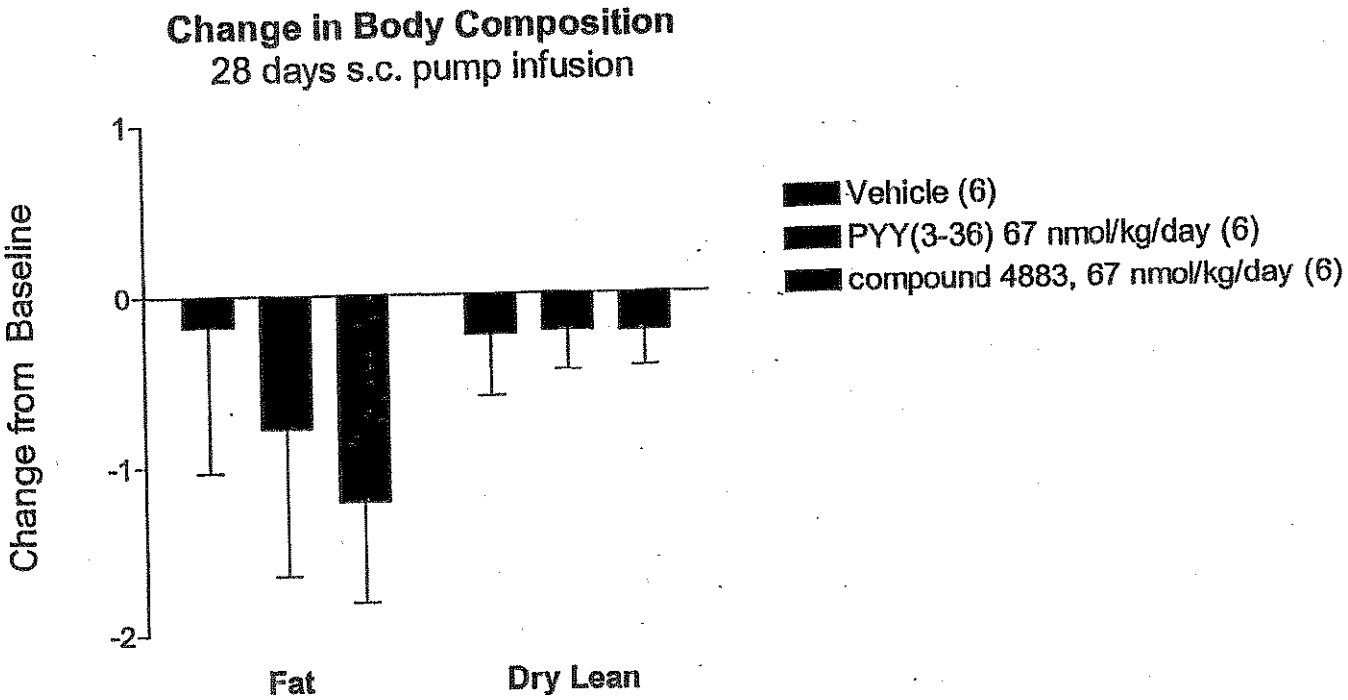
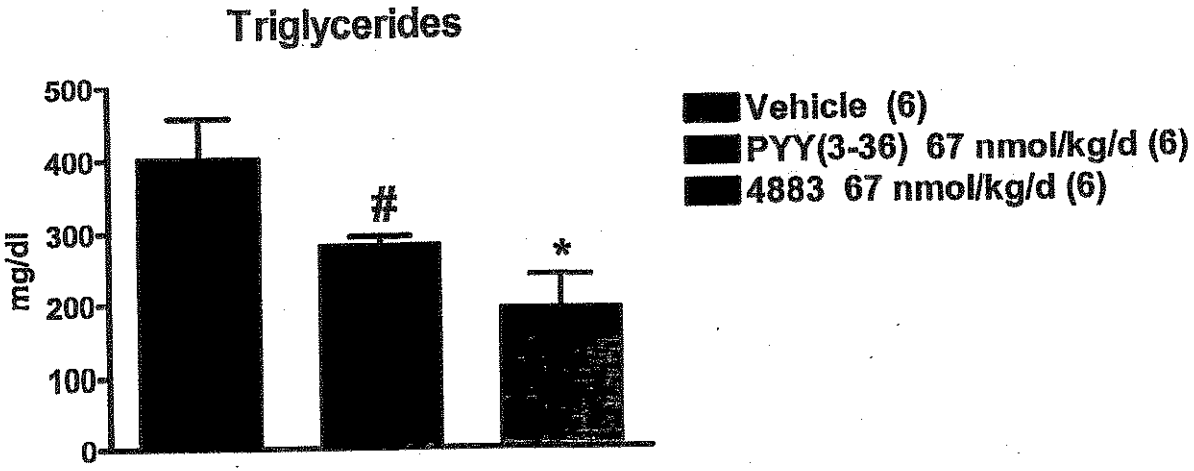


FIG. 43

PPF polypeptide Compound 4883 in DIO Rats: Preferential lowering of Triglycerides



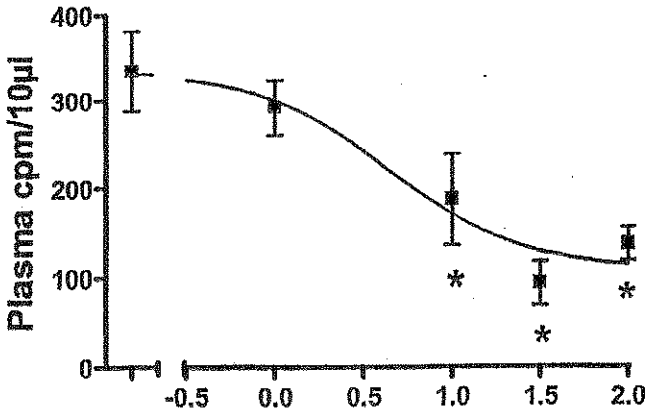
*P<0.05 compared to vehicle
#P<1.0 compared to vehicle

- No Changes in other Plasma Analytes Compared to PYY(3-36): Cholesterol, HDL, Glucose, HbA1C
- Lower Amylase level compared to PYY(3-36)

FIG. 44

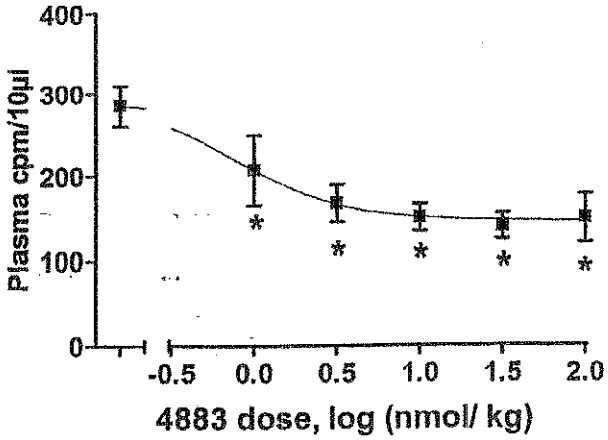
PPF polypeptide Compound 4883 is More Potent than PYY(3-36) for Inhibition of Gastric Emptying

PYY(3-36) Dose Response:
Gastric Emptying Assay



ED_{50} = 4.3nmol/kg = 17 µg/kg
Maximum decrease in plasma cpm:-72%
Goodness of fit: r^2 =0.8655 AR2521-12

4883 Dose Response:
Gastric Emptying Assay



ED_{50} = 0.8nmol/kg = 4 µg/kg
Maximum decrease in plasma cpm:-51%
Goodness of fit: r^2 =0.8645 AR2660-29

Points represent mean ± sd of 3-8 SD rats (fed, conscious). The indicated dose of peptide was injected subcutaneously at t=0. Blood was collected 45 minutes later for cpm analysis.

FIG. 45

Telemetry Studies (Rat):

Effect of PPF polypeptide Compound 4753 on Heart Rate

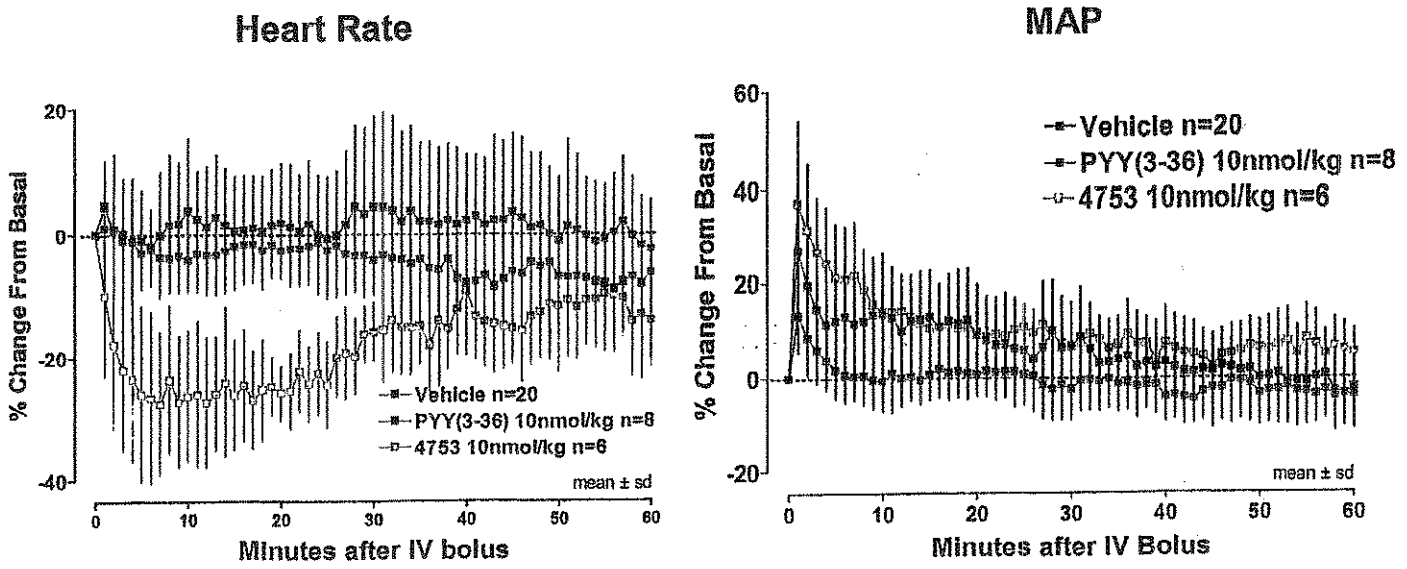


FIG. 46

Telemetry Studies (Rat):

Effect of PPF polypeptide Compound 4883 on Heart Rate

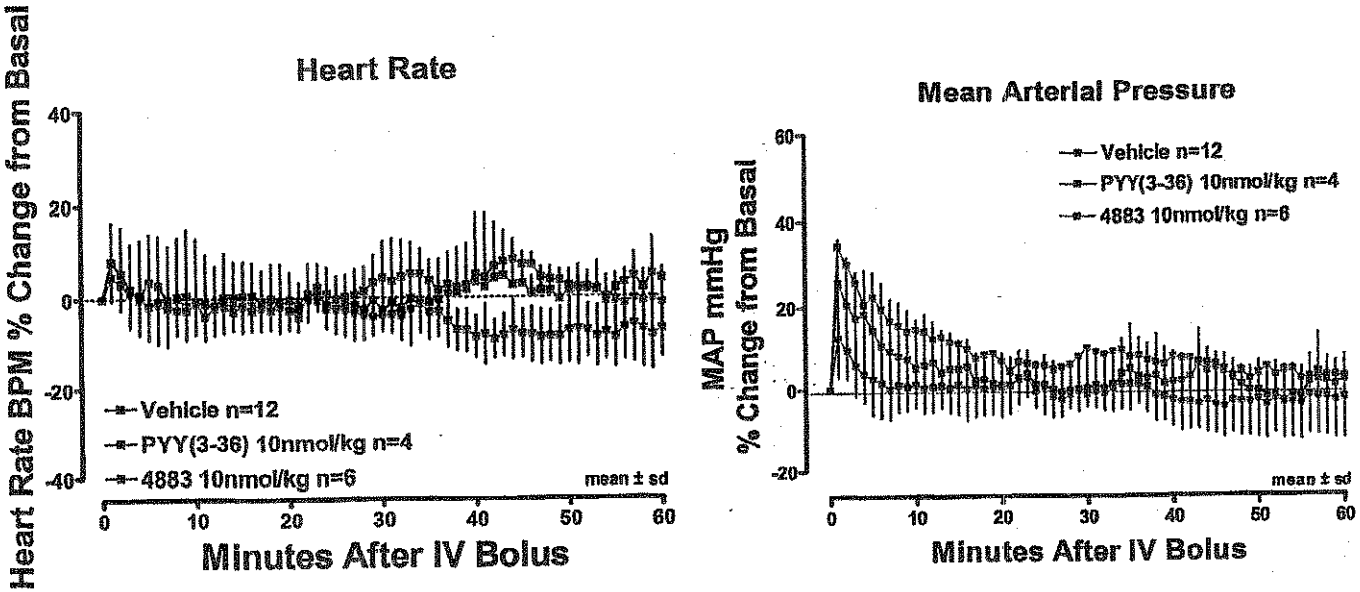
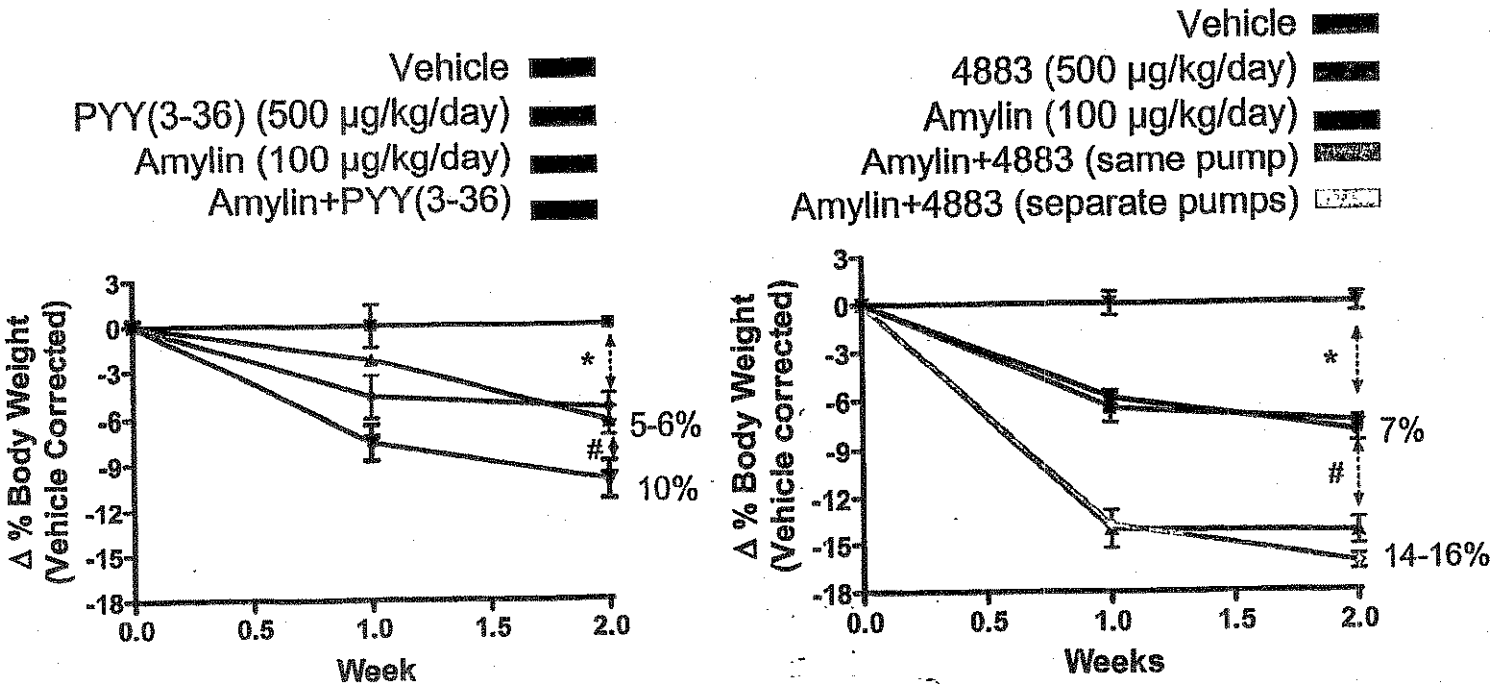


FIG. 47

Effect of co-adminstration of Amylin + Compound 4883 in DIO Rats



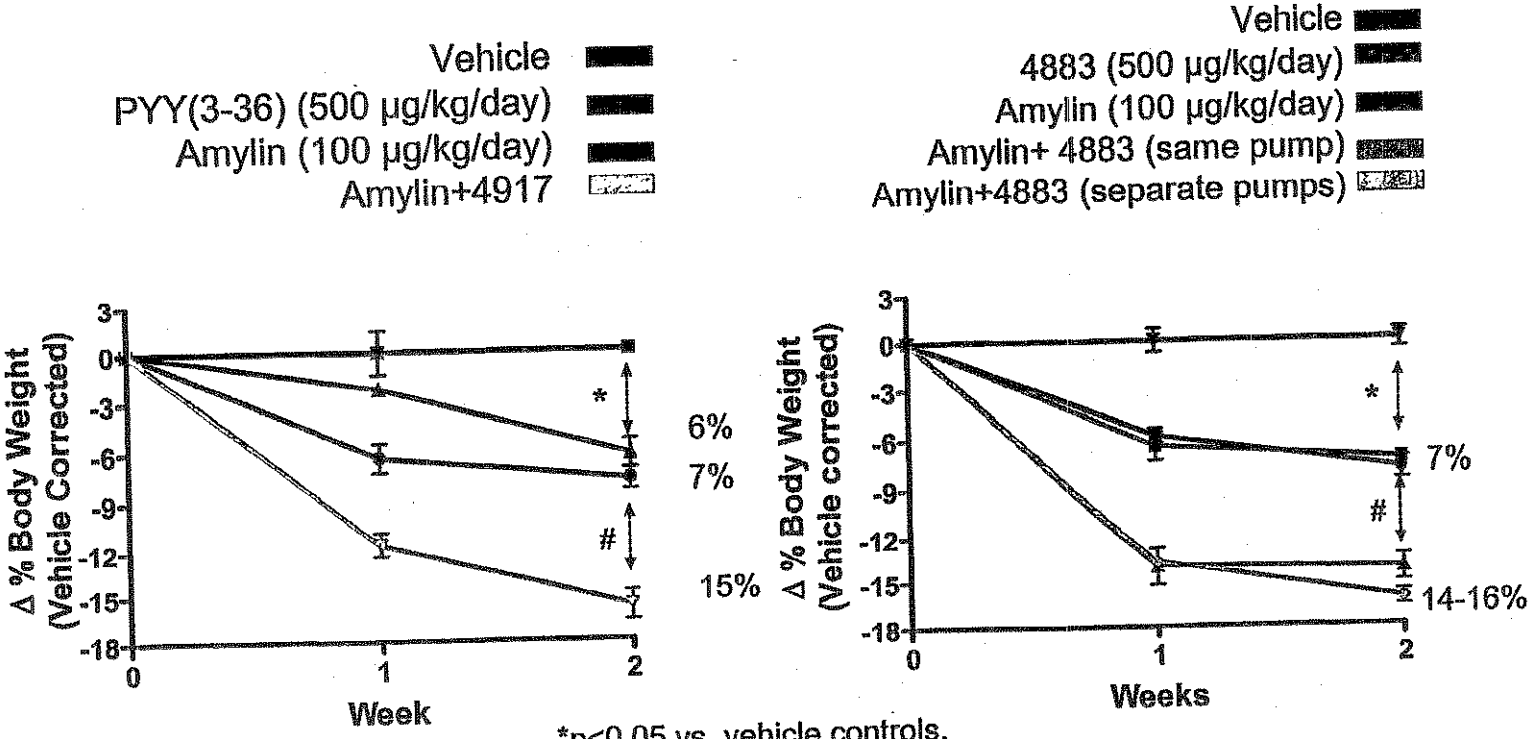
*p<0.05 vs. vehicle controls,

#p<0.05 vs. vehicle controls or mono-therapy

n=4-5/group, in-bred DIO prone rats

FIG. 48

Effect of co-administration of Amylin with Compound 4917 or Compound 4883 on weight loss



*p<0.05 vs. vehicle controls,

#p<0.05 vs. vehicle controls or mono-therapy

n=4-5/group, in-bred DIO prone rats

FIG. 49

Effect of Amylin & Compound 4917 on body composition

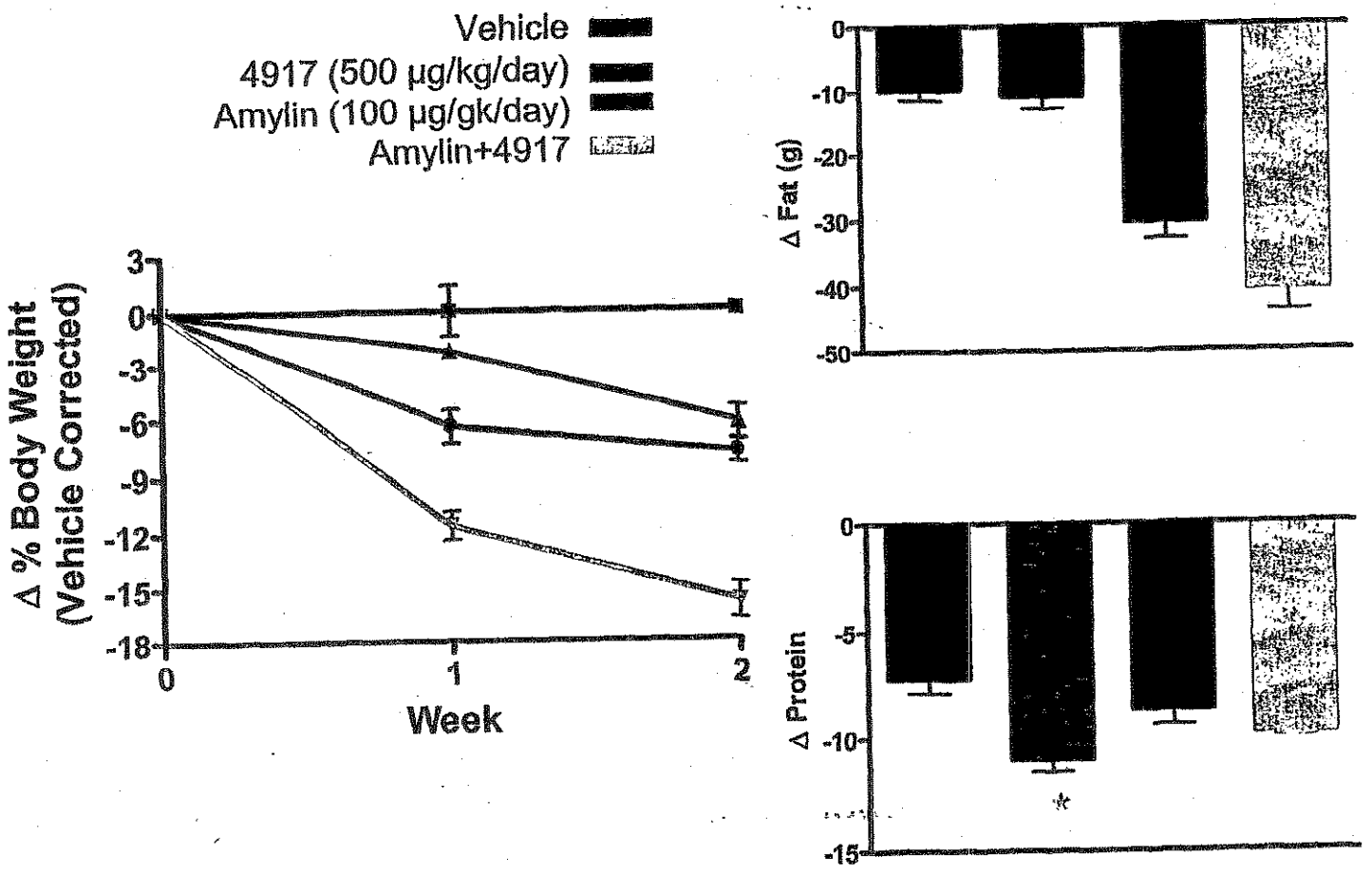


FIG. 50

Effect of Amylin & Compound 4883 on body composition

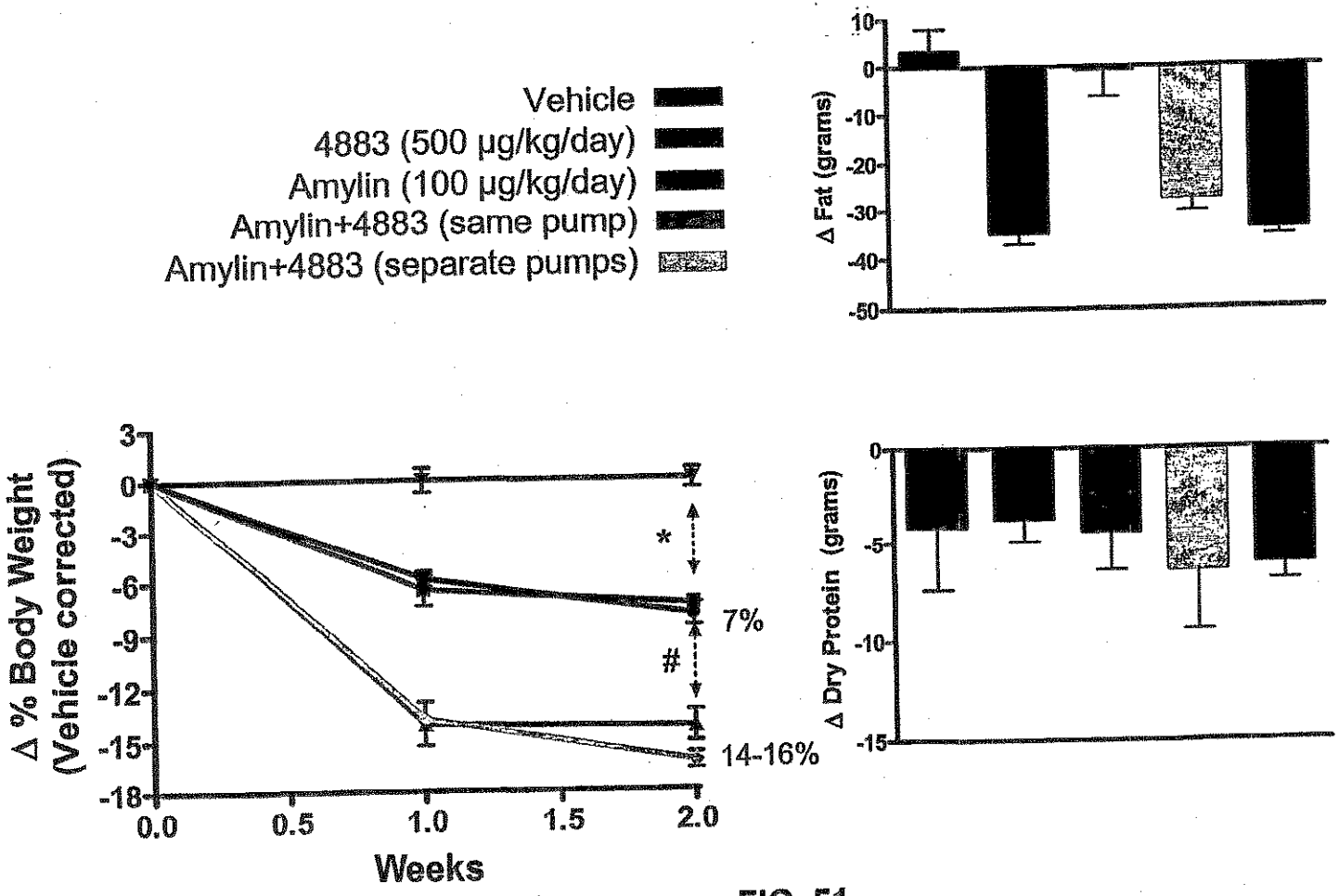


FIG. 51

Effects of 4917 on Fasting Insulin Levels

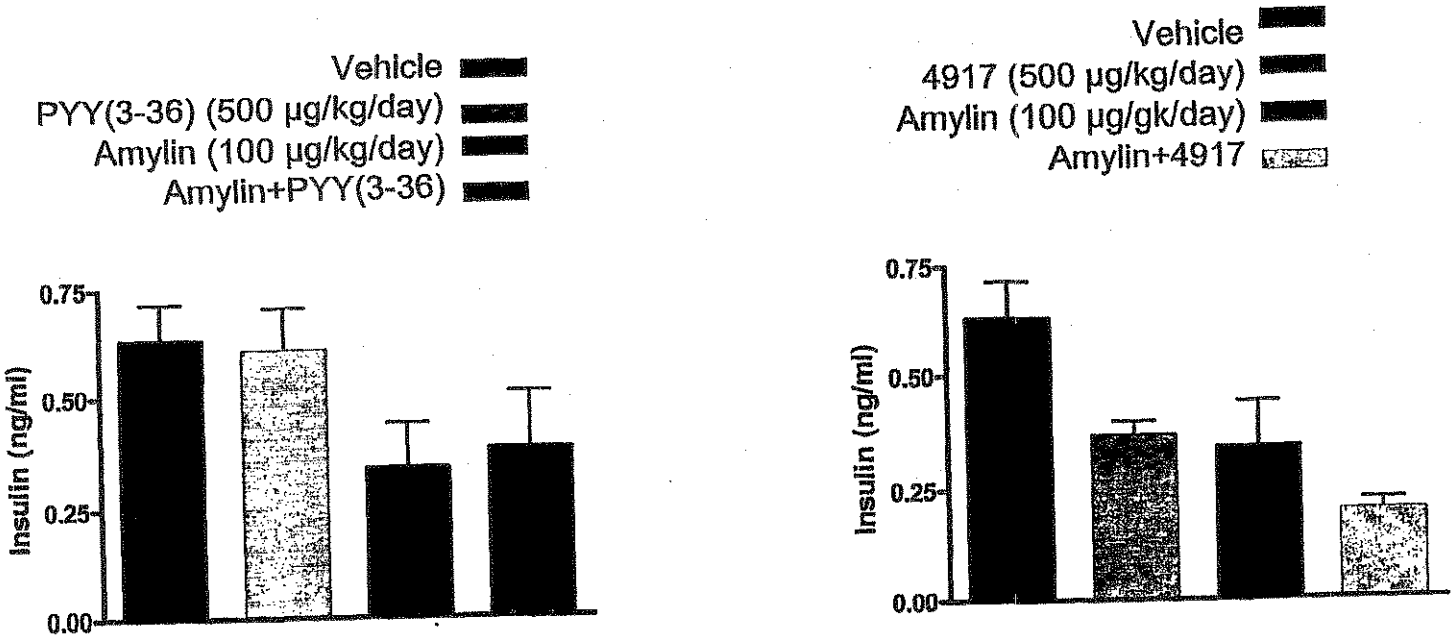
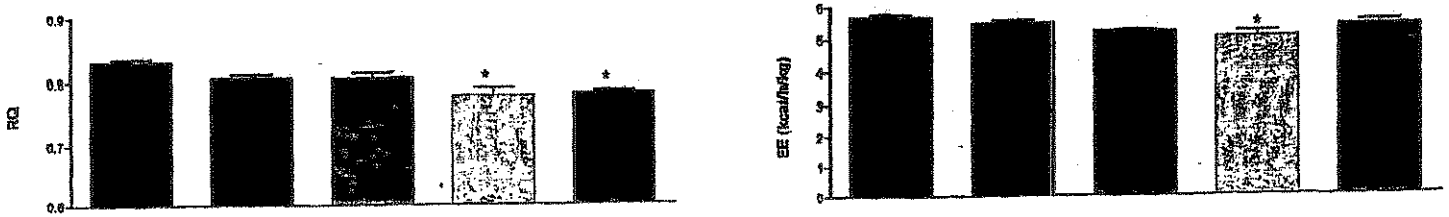


FIG. 52

Co-administration of Amylin & Compound 4883 on Body Weight is accompanied by Decreases in RQ and EE

Vehicle
4883 (500 µg/kg/day)
Amylin (100 µg/kg/day)
Amylin+4883 (same pump)
Amylin+4883 (separate pumps)



Indirect calorimetry was performed during the 1st week of treatment
Rats (n=4-5/group) had ad-lib access to food
* p<0.05 vs. vehicle controls

FIG. 53

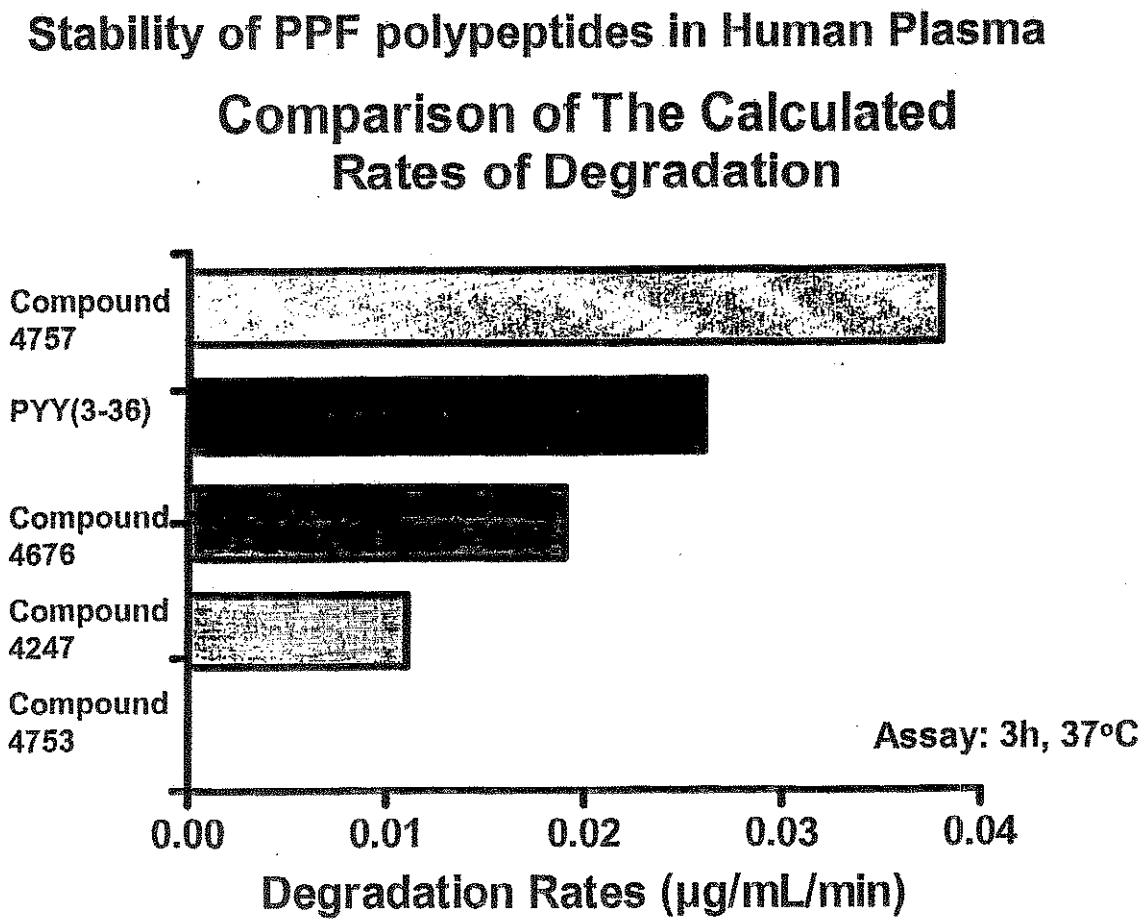

Stability of PPF polypeptides in Human Plasma
Comparison of The Calculated
Rates of Degradation
Compound
4757
PYY(3-36)
Compound
4676
Compound
4247
Compound
4753
Assay: 3h, 37°C
0.00
0.01
0.02
0.03
0.04
Degradation Rates (µg/mL/min)

FIG. 54