**Supplemental Material**

# SUPPLEMENTAL TEXT

## Supplemental Methods

***Euglycemic Hyperinsulinemic Clamp***

Following a 12-hour fast, patients received a priming dose of regular insulin (400 mU x $m^2$ x $min^{-1}$) for 2 minutes followed by a continuous infusion of 80 mU x $m^2$ x $min^{-1}$ regular insulin for the next 118 minutes. An infusion of 20% dextrose was adjusted to maintain plasma glucose concentrations at the euglycemic value of 5 mmol/L (90 mg/dl). Blood glucose was determined every 5 minutes using a B-Glucose Analyzer (Hemocue, Lake Forest, CA). Insulin samples were collected every 20 minutes. Insulin stimulated glucose disposal (M) was determined using the method of DeFronzo et al. [1] for the interval between 100-120 minutes. In addition, analyses were performed with M corrected for steady state insulin level (M/I x 100) and, at baseline and 6 months, indexed to fat-free mass as measured by DXA (M/I per LBM, mg/kg of FFM/ min per μU/mL insulin x 100). Insulin-stimulated glucose disposal (M) correlated strongly with M/I ($r = 0.94$, $P < 0.0001$) and with M/I per LBM ($r = 0.95$, $P < 0.0001$).

A single investigator performed all of the clamp procedures. Glucose values during the steady-state phase were 88 ± 6mg/dL (mean ± SD); the coefficient of variation of glucose during the steady-state phase was 6.8%. Serum insulin concentrations measured during the steady state phase were 123 ± 38 μU/mL (mean ± SD).

## Supplemental Results

***Relationship between Insulin Sensitivity by Clamp and Other Measures of Diabetes Risk***

At baseline, insulin sensitivity (M) was significantly correlated with BMI ($\rho = -0.49$, $P = 0.03$), waist circumference ($\rho = -0.66$, $P = 0.002$), and fasting insulin ($\rho = -0.72$, $P = 0.0005$). There was a trend toward correlation with $\log_{10}$ triglyceride ($r = -0.45$, $P = 0.05$), and no correlation with fasting glucose ($r = -0.07$, $P = 0.79$).

***Dose Reductions***

Two patients underwent IRB-approved dose reduction (with study blind maintained) to 1mg daily due to paresthesias, with significant improvement in symptoms. IGF-1 levels in these two patients, shown in Supplemental Table 6, were not analyzed until the end of the study to maintain blinding. After breaking the blind, it was determined that these two patients were in the active treatment arm, and in each case, IGF-1 levels decreased commensurate with improvement in symptoms and dose reduction.

***Sensitivity Analyses***

The effects of tesamorelin vs. placebo on hepatic fat ($P = 0.001$) and VAT ($P = 0.006$) remained highly significant in analyses excluding the two patients in whom dose reductions were performed.

## Supplemental References

**1.** DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: A method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237:E214-223.

**Supplemental Table 1: Statistical Analyses and Treatment of Missing Data**

| Analysis | Method |
|---|---|
| *All available data* | *Analyses performed using all available data, with missing data treated as missing. Performed for all endpoints.* |
| *Imputation Analyses* | *For repeated measures analysis and for normally distributed endpoints obtained at 0 & 6 months, analyses performed by replacing missing values with imputed values calculated over 100 iterations, using longitudinal mixed effects modeling. First 10 iterations discarded.* |
| | *For non-normally distributed endpoints obtained at 0 & 6 months, analyses performed by replacing the missing data for the 0 to 6 month changes using the median of the change in the combined groups as the imputed values.* |

## Supplemental Table 2: Lipids, Transaminases, and Cardiovascular Risk Markers

| | Baseline | | 3 Months | | 6 Months | | Δ after 6months | | Treatment effect – all available data‡ | P-value - all available data‡ | P-value - imputation‡‡ (range of estimates) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tesamorelin | Placebo | Tesamorelin | Placebo | Tesamorelin | Placebo | Tesamorelin | Placebo | | | |
| **Lipid Parameters** | | | | | | | | | | | |
| Total Chol (mg/dl) | 176 ± 37 n=28 | 174 ± 43 n=22 | 186 ± 35 n=25 | 193 ± 46 n=20 | 175 ± 40 n=22 | 186 ± 52 n=20 | -5 [-16, 7] n=22 | 7 [-6, 20] n=20 | -10 [-26, 6] | 0.22 | 0.16 (-21, -6) |
| HDL (mg/dl) | 40 (33, 49) n=28 | 43 (36, 52) n=22 | 37 (30, 51) n=25 | 41 (35, 51) n=20 | 40 (33, 50) n=22 | 43 (38, 54) n=20 | 0 (-3, 6) n=22 | 1 (-3, 6) n=20 | 1 [-4, 6] | 0.67 | 0.68 (-2, 3) |
| LDL (mg/dl) | 110 ± 32 n=28 | 106 ± 29 n=22 | 106 ± 38 n=24 | 108 ± 34 n=20 | 116 ± 27 n=21 | 117 ± 35 n=20 | -1 [-12, 9] n=21 | 7 [-3, 17] n=20 | -8 [-22, 6] | 0.24 | 0.26 (-17, -1) |
| TGL (mg/dl) | 161 (90, 220) n=28 | 127 (89 , 181 ) n=22 | 139 (111, 249 ) n=25 | 158 (94 , 202 ) n=20 | 109 (75, 181) n=22 | 121 (87 , 187 ) n=20 | -25 (-68, 8) n=22 | -10 (-33 , 8) n=20 | -13 [-86, 61] | 0.73 | 0.69 (-40, 13) |
| **Liver Transaminases** | | | | | | | | | | | |
| ALT (U/L) | 20 (16, 32 ) n=28 | 19 (15, 29) n=22 | 22 (16, 31 ) n=24 | 20 (16, 24) n=19 | 20 (15, 25) n=22 | 17 (13, 21) n=20 | -5 (-12, 2) n=22 | -3 (-7, 3) n=20 | -6 [-23, 10] | 0.44 | 0.36 (-10, -5) |
| AST (U/L) | 25 (20, 32) n=28 | 25 (16, 32) n=22 | 23 (20, 31) n=24 | 25 (19, 30) n=19 | 23 (16, 29) n=22 | 22 (15, 31) n=20 | -4 (-12 , 2) n=22 | 0 (-6, 5) n=20 | -7 [-16, 1] | 0.10 | 0.046 (-11, -6) |
| **Inflammatory Markers** | | | | | | | | | | | |
| CRP (mg/L)* | 1.8 (1.4, 3.0) n=28 | 2.7 (1.2, 6.6) n=22 | 1.3 (1.0, 2.4) n=25 | 2.3 (0.9, 3.7) n=20 | 2.0 (0.7, 3.7) n=22 | 2.3 (1.5, 3.9) n=20 | -0.1 (-0.8, 0.5) n=22 | -0.3 (-2.3, 0.6) n=20 | 0.02 [-0.45, 0.49] | 0.94 | 0.66 (-0.17, 0.33) |
| Adiponectin (ng/ml) | 3348 (2576, 5022) n=28 | 3091 (2447, 4250) n=22 | 3606 (2318, 5151) n=25 | 2833 (2060, 3992) n=20 | 3606 (2447, 5280) n=22 | 2833 (2060, 4121) n=20 | 0 (-515, 1159) n=22 | 0 (-1030, 515) n=20 | 850 [-38, 1738] | 0.06 | 0.07 (554, 1140) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Blood Pressure** | | | | | | | | | | | |
| Systolic BP (mm Hg) | 130 (119, 136) n=28 | 127 (118, 135) n=22 | 120 (113, 129) n=25 | 127 (112, 139) n=20 | 125 (116, 133) n=23 | 123 (118, 138) n=20 | 0 (-16, 6) n=23 | -3 (-11, 10) n=20 | -1 [-9, 7] | 0.81 | 0.75 (-3, 3) |
| Diastolic BP (mm Hg) | 80 ± 10 n=28 | 81 ± 7 n=22 | 75 ± 11 n=25 | 77 ± 8 n=20 | 78 ± 8 n=23 | 76 ± 7 n=20 | -2 [-8, 3] n=23 | -4 [-9, 0] n=20 | 2 [-4, 7] | 0.49 | 0.47 (0, 4) |

No statistically significant differences between groups at baseline. Results for normally distributed data are presented as the mean ± SD at each timepoint and as mean [95% CI] for change after 6 months. Non-normally distributed data are presented as median with interquartile range (25%, 75%).

‡Treatment effect and p-value for mixed effects model (time×randomization) using all available data over six months.

‡‡P-value for imputation analyses. Multiple imputation was performed by replacing missing values with imputed values calculated over 100 iterations, using longitudinal mixed effects modeling, and discarding the first 10 iterations. The p-value is the average of the p-values from the individual runs of the multiply imputed data sets. The values in parentheses provide a range ($2.5^{th}$ percentile, $97.5^{th}$ percentile) of the estimated effect sizes for the imputation analyses.

*Raw data are shown for CRP to provide clinical context. Log CRP was used for analysis due to significant outliers, and the p-values, treatment effect, and range of estimates shown are for analysis of Log CRP.

Abbreviations: HOMA-IR: homeostasis model assessment of insulin resistance; Chol: cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TGL: triglycerides; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CRP: c-reactive protein.

SI conversion factors: To convert cholesterol to mmol/L, multiple values by 0.0259. To convert triglyceride to mmol/L multiple by 0.0113. To convert AST and ALT to μkat/L, multiple by 0.0167. To convert CRP to nmol/L, multiply by 9.524.

## Supplemental Table 3: Nutrition and Activity

| | Baseline | | Six Months | | Δ over Six Months | | Treatment effect – all available data‡ | P-value – all available data‡ | P-value – imputation‡‡ (range of estimates) |
|---|---|---|---|---|---|---|---|---|---|
| | Tesamorelin | Placebo | Tesamorelin | Placebo | Tesamorelin | Placebo | | | |
| **Total Calories (kcal/d)** | 2100 ± 684 n=25 | 2151 ± 733 n=21 | 2127 ± 800 n=21 | 1975 ± 865 n=16 | 1 [-309, 312] n=20 | -154 [-436, 129] n=15 | 155 [-249, 558] | 0.44 | 0.40 (-57, 403) |
| **Carbohydrates (g/d)** | 243 ± 82 n=25 | 250 ± 92 n=21 | 241 ± 82 n=21 | 229 ±89 n=16 | -3 [-41, 35] n=20 | -31 [-73, 11] n=15 | 28 [-26, 83] | 0.29 | 0.46 (-11, 49) |
| **Fat (g/d)** | 85 ± 35 n=25 | 87 ± 35 n=21 | 89 ± 43 n=21 | 80 ± 47 n=16 | 1 [-15, 17] n=20 | -3 [-18, 11] n=15 | 5 [-16, 25] | 0.65 | 0.46 (-4, 19) |
| **Protein (g/d)** | 93 ± 29 n=25 | 98 ± 35 n=21 | 87 ± 34 n=21 | 88 ± 43 n=16 | -6 [-21, 9] n=20 | -2 [-15, 11] n=15 | -5 [-24, 15] | 0.63 | 0.60 (-14, 8) |
| **Total Weekly Activity (METs)** | 106 (29, 165) n=27 | 46 (15, 63) n=21 | 82 (32, 148) n=23 | 37 (26, 73) n=19 | -13 (-81, 39) n=23 | 8 (-12, 45) n=19 | -5 | 0.12 | 0.11 (N/A) |
| **Television (hours/day)** | 3.0 (2.0, 6.0) n=27 | 2.0 (1.8, 3.0) n=21 | 3.0 (2.0, 5.0) n=23 | 2.0 (1.5, 3.0) n=20 | -0.5 (-1.8, 0.5) n=23 | 0.0 (-0.5, 0.4) n=20 | -0.5 | 0.33 | 0.43 (N/A) |

No statistically significant differences between groups at baseline. Results for normally distributed data are presented as the mean ± SD at each timepoint and as mean [95% CI] for change after 6 months. Non-normally distributed data are presented as median with interquartile range (25%, 75%).

‡p-value for a modified intention to treat analysis using all available data. Student's t-test was used to compare changes between groups for normally distributed variables, and Wilcoxon rank sum test was used to compare changes between groups for variables that were not normally distributed. Treatment effect is mean [95% CI] for normally distributed endpoints and the net difference between median changes in each group for endpoints that were not normally distributed.

‡‡p-value for imputation analyses. For normally distributed variables, multiple imputation was performed by replacing missing values with imputed values calculated over 100 iterations, using longitudinal mixed effects modeling, and discarding the first 10 iterations. The p-value is the average of the p-values from the individual runs of the multiply imputed data sets. The values in parentheses provide a range ($2.5^{th}$ percentile, $97.5^{th}$ percentile) of the estimated effect sizes for the imputation analyses. For non-normally distributed endpoints, imputation analysis was performed by replacing the missing data for the 0 to 6 month

changes using the median of the change in the combined groups. The p-value given is for Wilcoxon rank sum test for comparison of change between groups using the imputed data set. For these data, range of estimates is not available.

**Supplemental Table 4: Relationships between VAT, Liver Fat and Metabolic Indices**

| | VAT | | Liver Fat | |
|---|---|---|---|---|
| | R | P-Value | R | P-Value |
| Liver Fat | 0.42<br>n=49 | 0.003 | -- | -- |
| Fasting Glucose | 0.05<br>n=50 | 0.73[A] | 0.04<br>n=49 | 0.76 |
| 2 Hr Glucose | 0.37<br>n=50 | 0.007[A] | 0.28<br>n=49 | 0.05 |
| HOMA-IR | 0.43<br>n=50 | 0.002 | 0.48<br>n=49 | 0.0006 |
| M (clamp)* | -0.43<br>n=19 | 0.07[A] | -0.70<br>n=19 | 0.0009 |
| $Log_{10}$ TGL | 0.18<br>n=50 | 0.20[A] | 0.44<br>n=49 | 0.002 |
| HDL | 0.03<br>n=50 | 0.85 | -0.31<br>n=49 | 0.03 |
| ALT | 0.13<br>n=50 | 0.37 | 0.36<br>n=49 | 0.01 |
| AST | -0.06<br>n=50 | 0.69 | 0.02<br>n=49 | 0.88 |
| IGF-1 | 0.13<br>n=50 | 0.38 | 0.05<br>n=49 | 0.72 |
| Overnight mean GH | -0.43<br>n=45 | 0.003 | -0.44<br>n=44 | 0.003 |

Relationships between two continuous variables were assessed using Pearson correlation coefficient when both variables were normally distributed ([A]) and Spearman's rank correlation coefficient when one or both variables were not normally distributed. Sample sizes for each analysis are shown.

*M is insulin stimulated glucose uptake (mg/kg/min) during steady state (100-120 minutes) during euglycemic hyperinsulinemic clamp. Higher values indicate greater insulin sensitivity.

Abbreviations: VAT: visceral adipose tissue; HOMA-IR: homeostasis model assessment of insulin resistance; TGL: triglycerides; HDL: high density lipoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; IGF-1: insulin-like growth factor 1; GH: growth hormone.

**Supplemental Table 5: Distribution of Glucose Abnormalities by Glucose Category**

| | Baseline Visit | | 2 Week Visit | | 3 Month Visit | | 6 Month Visit | |
|---|---|---|---|---|---|---|---|---|
| | Tesamorelin n=28 | Placebo n=22 | Tesamorelin n=26 | Placebo n=21 | Tesamorelin n=25 | Placebo n=20 | Tesamorelin n=23* | Placebo n=20* |
| Fasting Glucose | | | | | | | | |
| Normal | 25 | 16 | 20 | 14 | 23 | 17 | 20 | 16 |
| Impaired | 3 | 6 | 5 | 7 | 1 | 3 | 2 | 3 |
| Diabetes | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 |
| 2 Hour OGTT Glucose | | | | | | | | |
| Normal | 24 | 12 | Not performed | | 18 | 15 | 17 | 14 |
| Impaired | 3 | 10 | | | 5 | 5 | 3 | 3 |
| Diabetes | 1 | 0 | | | 2 | 0 | 2 | 1 |

American Diabetes Association definitions used: Normal fasting glucose: <100 mg/dl; Impaired fasting glucose: ≥ 100 and <126 mg/dl; Diabetes: fasting glucose ≥ 126; Normal 2 hour OGTT glucose: <140 mg/dl; Impaired 2 hour OGTT glucose: ≥ 140 and <200 mg/dl; Diabetes: 2 hour OGTT glucose ≥ 200 mg/dl

*One patient in the Tesamorelin group and two patients in the placebo group did not complete 2 hour OGTT at the 6 Month Visit.

Abbreviations: OGTT: Oral Glucose Tolerance Test

**Supplemental Table 6: IGF-1 Z-scores and IGF-1 Concentrations**

| | Baseline | | 2 Week | | 3 Month | | 6 Month | |
|---|---|---|---|---|---|---|---|---|
| | Z-score | IGF-1 (ng/mL) | Z-score | IGF-1 (ng/mL) | Z-score | IGF-1 (ng/mL) | Z-score | IGF-1 (ng/mL) |
| Placebo | -0.3 ± 0.8 n=22 | 123 ± 42 n=22 | -0.2 ± 0.9† n=21 | 134 ± 52† n=21 | -0.3 ± 1.0† n=20 | 131 ± 60† n=20 | -0.3 ± 1.2† n=20 | 135 ± 68† n=20 |
| Tesamorelin | -0.2 ± 1.1 n=28 | 139 ± 72 n=28 | 1.4 ± 0.9† n=26 | 275 ± 108† n=26 | 1.2 ± 1.0† n=24 | 251 ± 111† n=24 | 0.9 ± 0.9† n=22 | 221 ± 87† n=22 |
| Dose Reduction #1 | -0.4 | 115 | 2.3 | 372 | 2.9 | 463 | 1.5* | 272* |
| Dose Reduction #2 | -0.3 | 126 | 2.0 | 334 | 1.6 | 279 | 0.5* | 181* |

Mean±SD IGF-1 Z-scores and IGF-1 levels (ng/mL) at each timepoint in the placebo group and the tesamorelin group (with sample sizes shown), along with IGF-1 Z-scores and levels at each timepoint for the two patients who received blinded dose reduction between the 3 and 6 month visits. IGF-1 levels were analyzed only after breaking the blind at the completion of the randomized study, when it was determined that both of these patients were in the tesamorelin group.

There were no significant differences between treatment groups in IGF-1 or IGF-1 Z-score at baseline.

†Indicates change from baseline significantly different ($p<0.05$) between treatment groups at specified timepoint by Student's t-test.

*Indicates IGF-1 Z-score/level after dose reduction to 1mg SC daily.

SI conversion factors: To convert IGF-1 to nmol/L, multiply by 0.131.

**Supplemental Table 7: Relationships between change in Liver Fat and Change in Metabolic Variables**

| | Δ HCL/W% | | | | | |
|---|---|---|---|---|---|---|
| | **Entire Cohort** | | **Tesamorelin** | | **Placebo** | |
| | ρ | P-value | ρ | P-value | ρ | P-value |
| ΔM (clamp)* | -0.17<br>n=19 | 0.48 | -0.73<br>n=10 | 0.02 | -0.32<br>n=9 | 0.41 |
| $\Delta Log_{10}$ Triglyceride | 0.24<br>n=39 | 0.14 | -0.02<br>n=20 | 0.93 | 0.47<br>n=19 | 0.04 |
| ΔAST | 0.20<br>n=39 | 0.22 | 0.27<br>n=20 | 0.24 | 0.13<br>n=19 | 0.60 |
| ΔALT | 0.24<br>n=39 | 0.15 | 0.42<br>n=20 | 0.07 | 0.05<br>n=19 | 0.83 |

Bivariate relationships were assessed using Spearman's rank correlation coefficient, as change in liver fat is not normally distributed. Multivariable regression analysis was performed to assess for significant differences in the slope of the relationship between the tesamorelin vs. placebo groups (i.e., randomization × x-variable term), and no significant interactions were found.

*M is insulin stimulated glucose uptake (mg/kg/min) during steady state (100-120 minutes) during euglycemic hyperinsulinemic clamp.

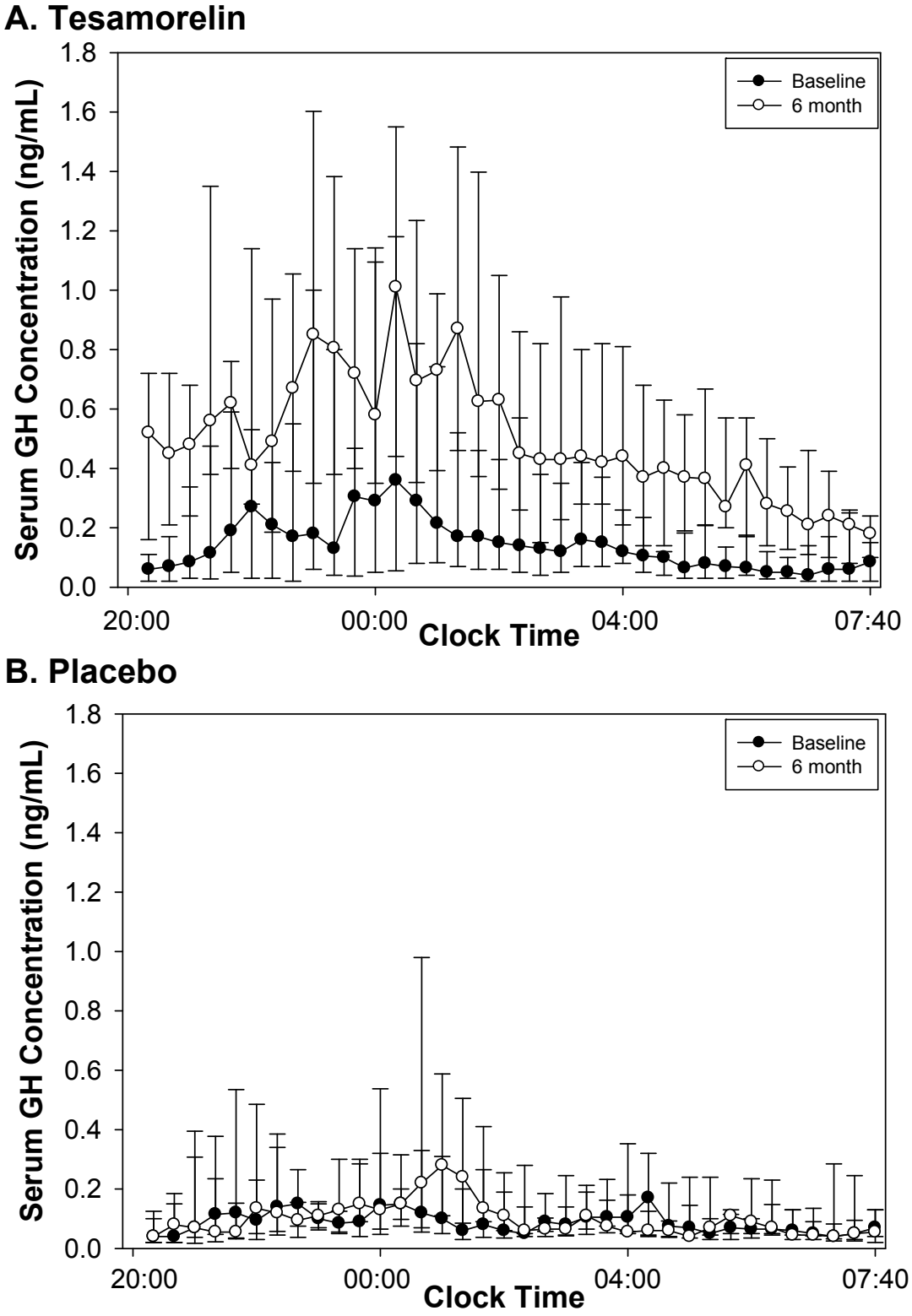
Abbreviations: HCL/W%: hepatic lipid-to-water percent; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

## Supplemental Figure Legends

**Supplemental Figure 1:** Median overnight growth hormone at each measured timepoint at baseline (black circles) and 6 months (white circles) in the tesamorelin (A) and placebo (B) groups. Error bars are IQR. Sampling occurred every 20 minutes from 8pm to 7:40am. Sample size in the tesamorelin group is 25 at baseline and 21 at 6 months. Sample size in the placebo group is 20 at baseline and 15 at 6 months.

**Supplemental Figure 2:** Relationship between change in liver fat and change in VAT. Black circles indicate patients in the tesamorelin group and white circles those in the placebo group. $\rho$ and P-value for Spearman regression. Thicker line represents Pearson regression line among all subjects. Thinner regression lines labeled (T) and (P) represent Pearson regression lines within each treatment group (tesamorelin and placebo, respectively). Multivariable regression analysis was performed to assess for significant differences in the slope of the relationship between the tesamorelin vs. placebo groups (i.e., randomization $\times$ $\Delta$ liver fat term), and the interaction was not significant.

**Supplemental Figure 1**



**Supplemental Figure 2**