

SUPPLEMENTARY DATA

Supplementary Table 1. Baseline comparison of study completers (per-protocol population) and drop-outs. Two men randomized to testosterone therapy dropped out. The reasons were: moved out of area after 2 months of therapy (one subject); withdrew consent at week 1 due to lack of time (one subject). Eight subjects randomized to placebo group dropped out. The reasons were: coronary stent placement at week 1 (one subject), developed neuropathic wound infection on foot (week 2, one subject), perceived lack of benefit (week 9, one subject). Five subjects withdrew consent after 2 weeks of starting the study (lack of time to follow the study protocol in entirety). Thus 20 subjects in testosterone group and 14 subjects in placebo group completed the study. At the end of the study, we asked the participants if they could guess whether they had received testosterone or placebo. 44% of subjects guessed their randomization correctly. The rest either guessed incorrectly or said “I don’t know”. 55% of subjects in testosterone group and 29% in placebo group guessed their treatment correctly (p=0.13 for comparison amongst groups).

Supplementary Table 1	Study population	Drop-out	P
Number of subjects	34	10	
Age (years)	54±8	57±8	0.39
Duration of diabetes (years)	9.7±9.3	8.4±6.0	0.72
BMI (kg/m ²)	39±8	43±9	0.19
Total testosterone (ng/dl)	253±83	248±82	0.86
Free testosterone (ng/dl)	4.4±1.3	4.3±1.1	0.89
Calculated Free testosterone (ng/dl)	5.4±1.0	5.5±1.1	0.77
SHBG (nmol/L)	27 ±13	25±12	0.67
HbA1c% (mmol/mol)	7.0±1.1 (53±12)	7.4±1.1 (57±12)	0.24
Total Cholesterol (mg/dl)	157±36	149±58	0.70
HDL cholesterol (mg/dl)	37±9	44±8	0.06
LDL cholesterol (mg/dl)	85±30	98±24	0.23
Triglycerides (mg/dl)	195±162	140±47	0.31
Hemoglobin (g/dl)	13.9±0.9	13.4±2.3	0.31
Waist circumference (cm)	126±19	121±33	0.62
Waist/hip ratio	1.05±0.07	1.08±0.06	0.44
trunk fat mass by DEXA (kg)	27±8	32±6	0.06
Total body subcutaneous fat mass (kg)	44±14	52±10	0.12
Visceral fat (L)	7.78±2.88	9.79±4.39	0.12
Hepatic fat (%)	5.29±6.39	7.68±9.09	0.42
Total body lean mass (kg)	70±11	74±13	0.36
GIR (mg/kg fat free mass/min)	6.32±4.00	7.89±4.52	0.42

SUPPLEMENTARY DATA

Supplementary Table 2. Baseline comparison of men randomized to testosterone or placebo therapy.

Supplementary Table 2	Testosterone	Placebo	<i>P</i>
Age (years)	54.7 ±7.8	54.5 ±8.7	0.96
Duration of diabetes (years)	9.4 ±8.2	9.9 ±9.2	0.85
Medications			
Metformin	90%	86%	0.92
Sulfonylureas	25%	50%	0.60
Insulin	50%	50%	0.71
GLP-1 agonists	10%	7%	0.62
DPP-4 inhibitors	15%	14%	0.92
Thiazolidinediones	15%	14%	0.92

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Supplementary Figure 1 A-C. Testosterone concentrations (mean \pm S.D.) in placebo and testosterone treatment groups (black circles= testosterone therapy group, white circles= placebo group).

1A: total testosterone

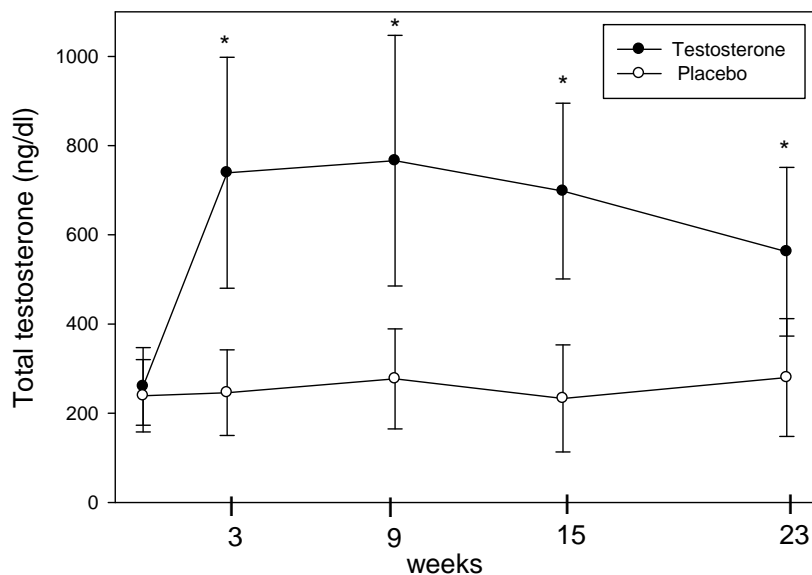
1B: free testosterone

1C: calculated free testosterone

* $p < 0.001$ for comparison with placebo.

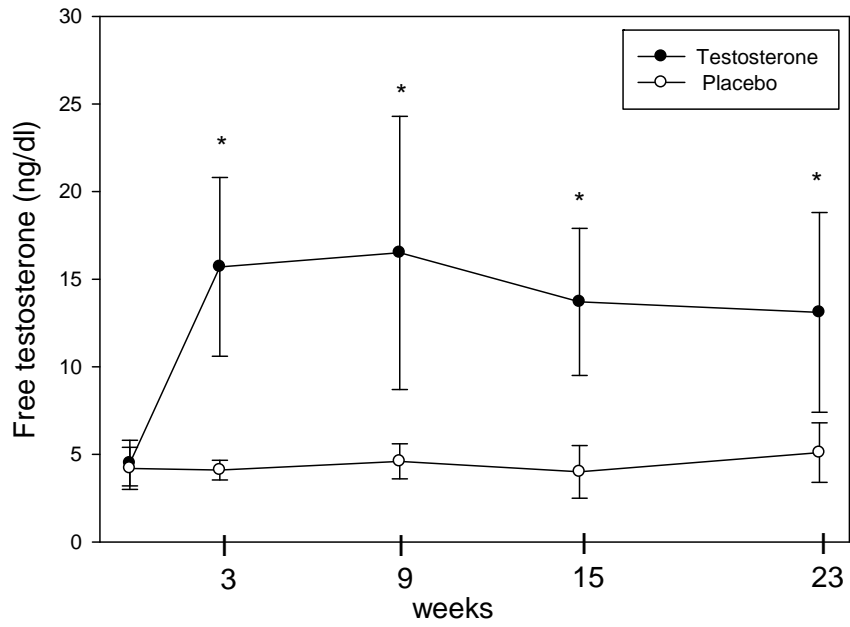
Serum testosterone and SHBG concentrations in both groups were measured at weeks 3, 9 and 15 (always one week after the injection). Dose of testosterone was adjusted to keep cFT concentrations in normal range (6.5-25 ng/dl). If cFT was < 10 or 10-14 ng/dl, then the dose of testosterone was increased by 100 or 50mg/injection respectively. If cFT was > 17 ng/dl, then the dose was reduced by 50mg/injection. Patients and study investigators except one research nurse were blinded to the study treatment. The mean dose of testosterone given over 24 weeks to subjects randomized to testosterone therapy was 216 ± 40 mg every 2 weeks. The mean cFT concentration of those subjects during the trial was 18.2 ± 7.1 ng/dl. Few men in the testosterone group exceeded the upper limit of normal for cFT (> 25 ng/dl): two men at week 3, three men at week 9 and one man at week 23. All these men had supranormal cFT concentrations for less than 6 weeks.

1A

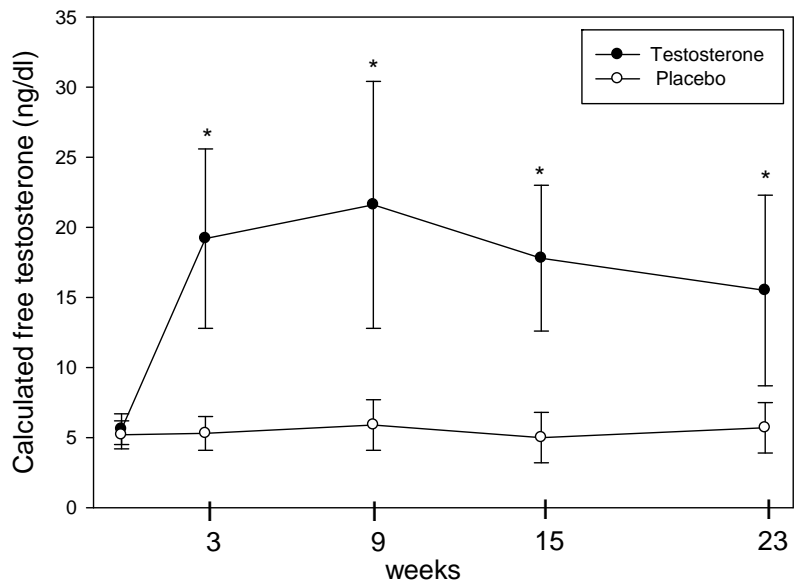


SUPPLEMENTARY DATA

1B

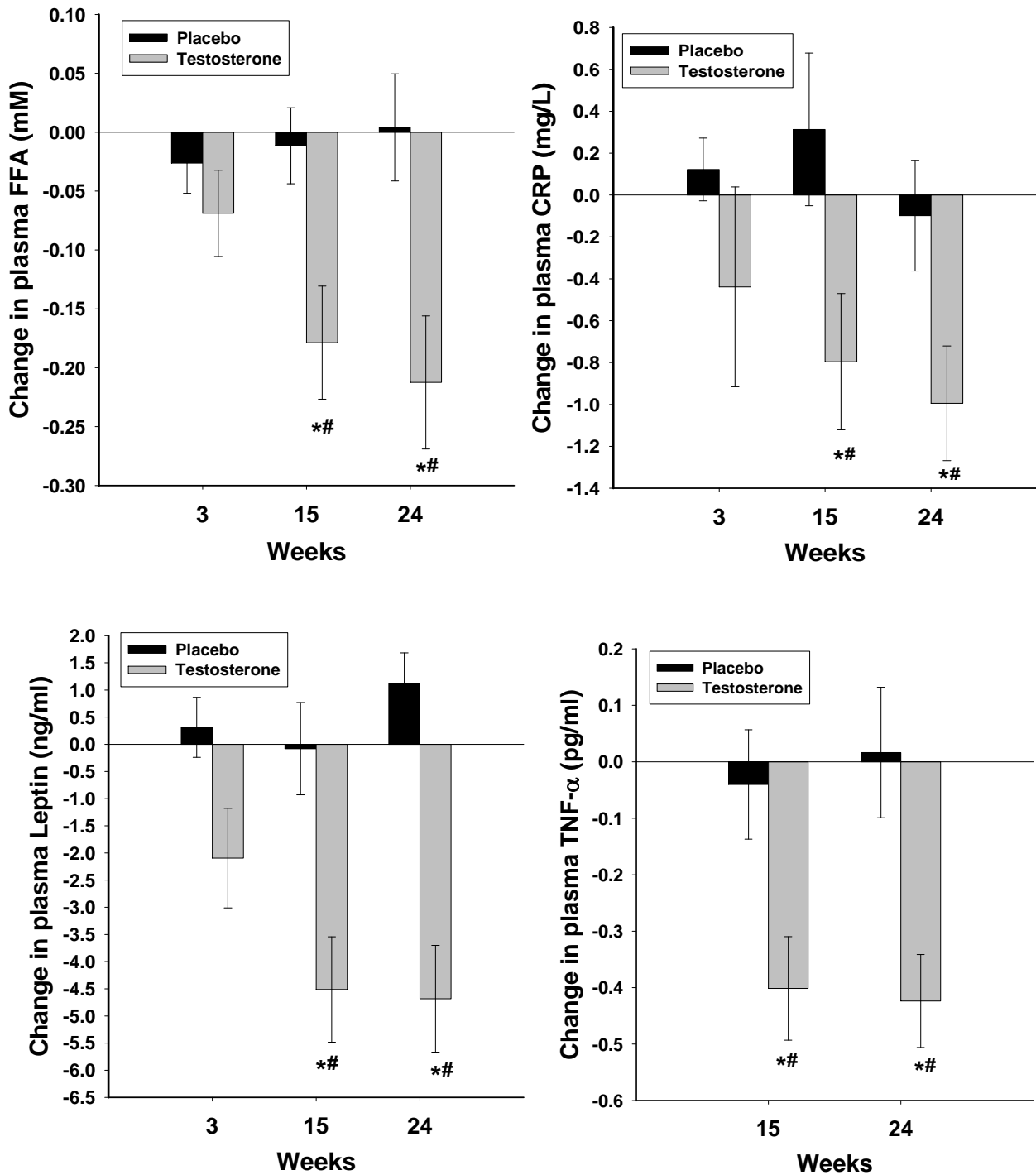


1C

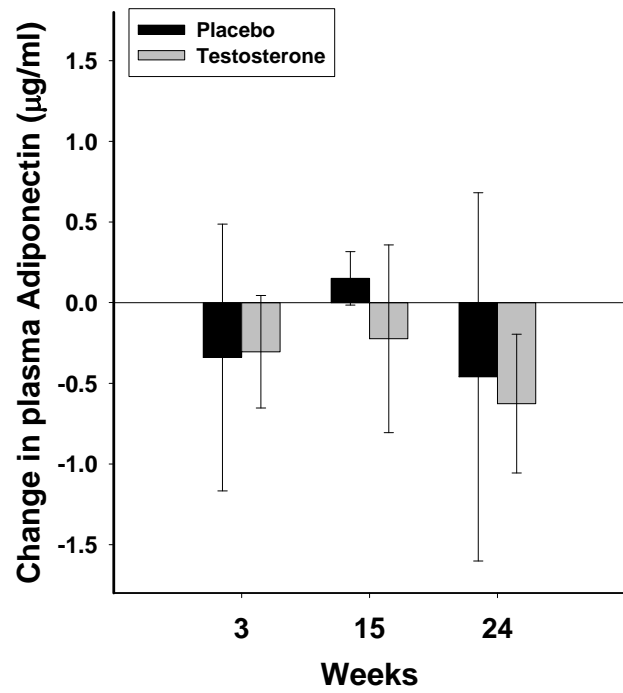
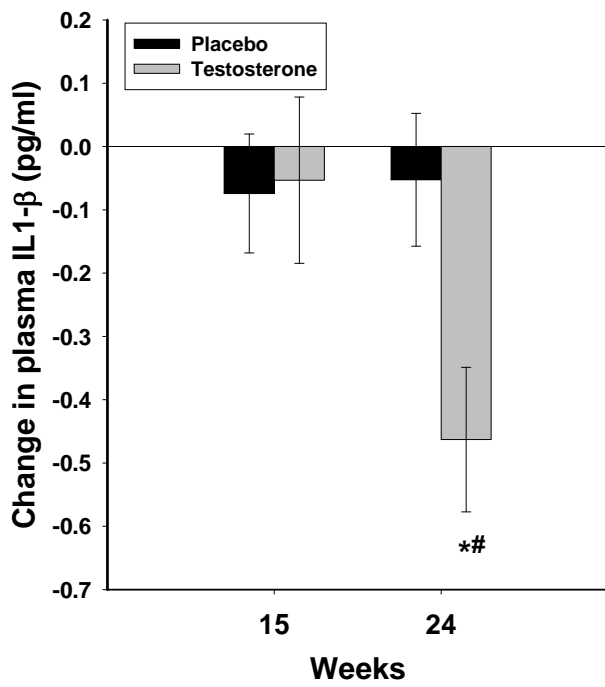


SUPPLEMENTARY DATA

Supplementary Figure 2. Change in plasma FFA(2A), CRP(2B), leptin(2C), TNF- α (2D), IL-1 β (2E) and adiponectin(2F) concentrations after testosterone or placebo treatment. Change in testosterone group was compared by t-test to baseline (*P<0.05) and to placebo (#P<0.05).



SUPPLEMENTARY DATA



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Supplementary Figure 3A-C.

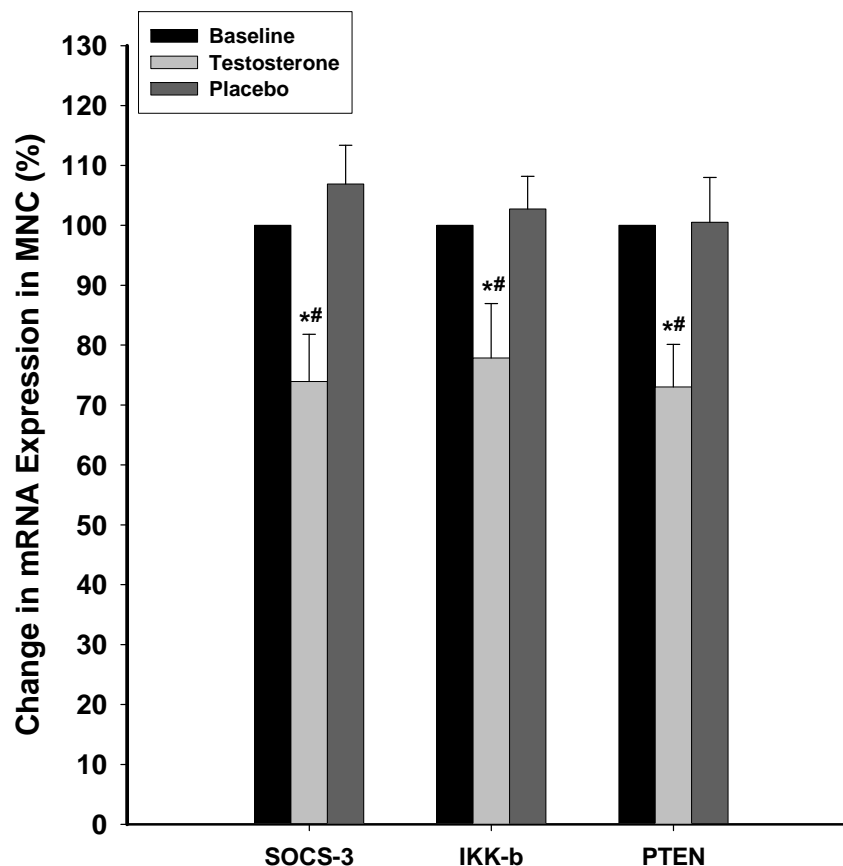
3A: Percent change in mRNA expression of SOCS-3, IKK- β and PTEN in MNC following 24 weeks of testosterone or placebo treatment. N= 18 vs. 14 (Testosterone vs placebo). Testosterone therapy suppressed the expression of SOCS-3 by 25 \pm 6%, IKK- β by 23 \pm 8% and PTEN by 28 \pm 7% as compared to placebo treatment in HH men (p<0.05 for all).

3B: Representative western blot showing protein levels of SOCS-3 in MNC before and after treatment with testosterone or placebo.

3C: Percent change in protein levels of SOCS-3 (decrease by 21 \pm 8%) in MNC following 24 weeks of testosterone or placebo treatment. N= 18 vs. 14 (Testosterone vs placebo).

Data are represented as mean \pm SE. Protein density was measured and corrected for loading with actin. Baseline values were normalized to 100% and percent changes from baselines calculated; therefore the first bar in each group is the baseline for both testosterone and placebo treatment. Change in testosterone group was compared by t-test to baseline (*P<0.05) and to placebo (#P<0.05).

Figure 3A



SUPPLEMENTARY DATA

Figure 3B

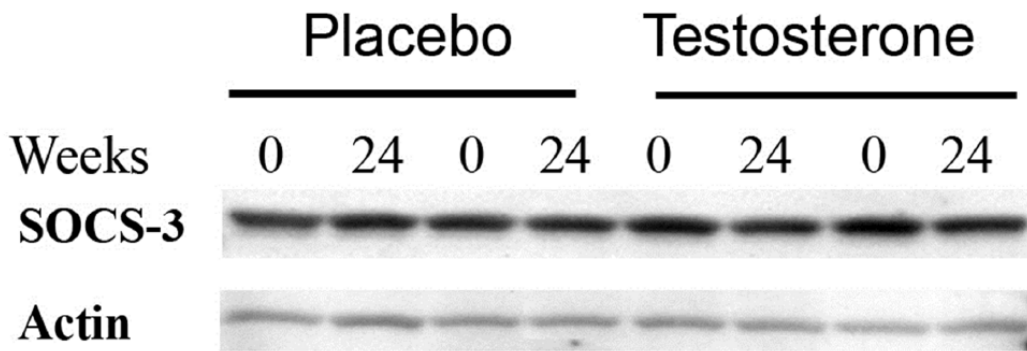
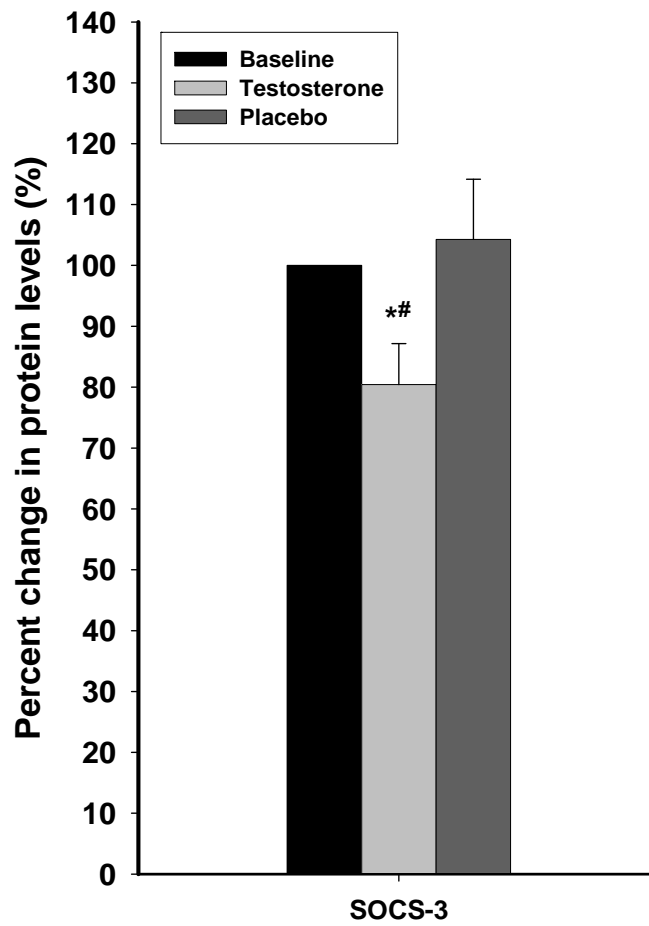


Figure 3C



SUPPLEMENTARY DATA

Supplementary Figure 4A-C.

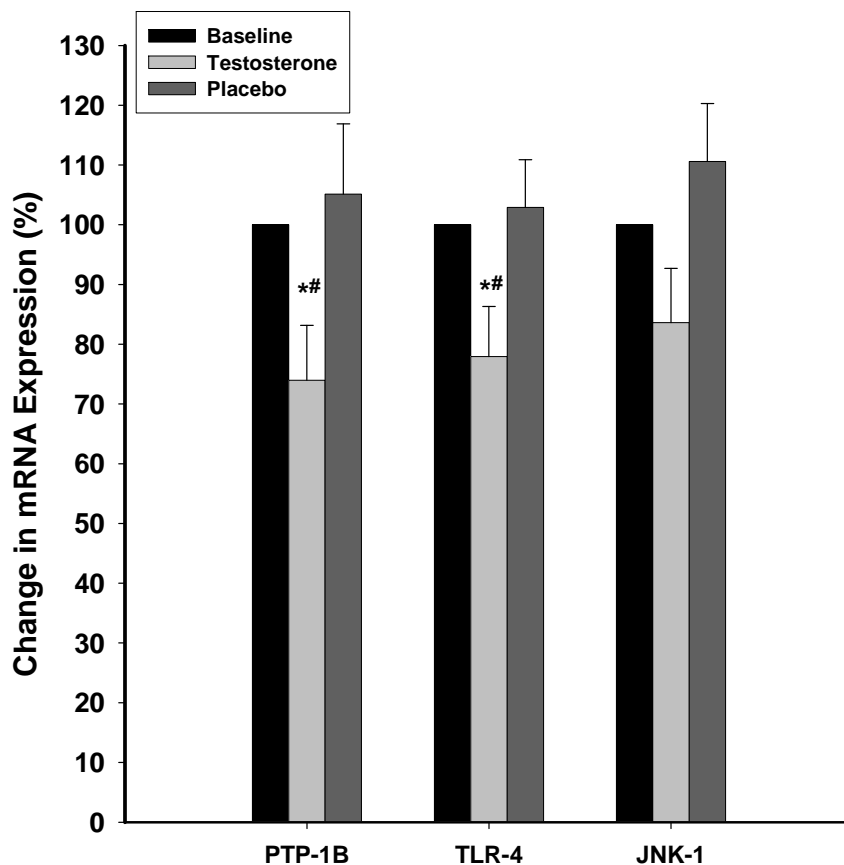
4A: Percent change in mRNA expression of PTP-1B, TLR-4 and JNK-1 in adipose tissue following 24 weeks of testosterone or placebo treatment. N= 16 vs. 10 (Testosterone vs placebo). Testosterone treatment reduced the mRNA expression of PTP-1B by $26\pm 8\%$ and TLR4 by $23\pm 7\%$.

4B: Representative western blot showing protein levels of PTP-1-B in adipose tissue before and after treatment with testosterone or placebo.

4C: Percent change in protein levels of PTP-1B (decrease of $28\pm 7\%$) in adipose tissue following 24 weeks of testosterone or placebo treatment. N= 16 vs. 10 (Testosterone vs placebo).

Data are represented as mean \pm SE. Protein density was measured and corrected for loading with actin. Baseline values were normalized to 100% and percent changes from baselines calculated; therefore the first bar in each group is the baseline for both testosterone and placebo treatment. Change in testosterone group was compared by t-test to baseline (*P<0.05) and to placebo (#P<0.05).

Figure 4A



SUPPLEMENTARY DATA

Figure 4B

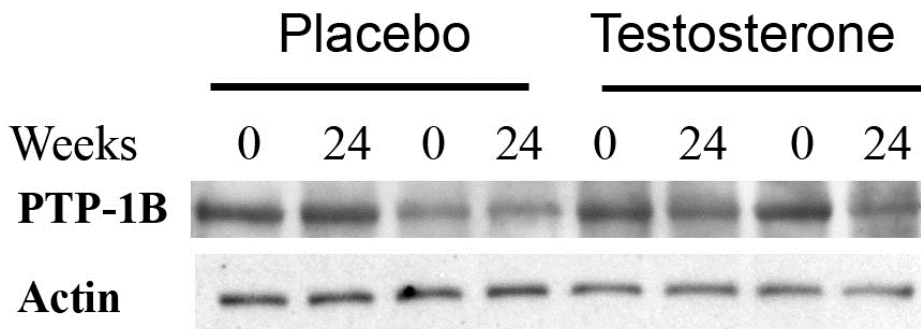


Figure 4C

