Published in final edited form as:
Circ Res. 2009 November 6; 105(10): 1013–1022. doi:10.1161/CIRCRESAHA.109.206318.

DELETION OF PROTEIN TYROSINE PHOSPHATASE 1B IMPROVES PERIPHERAL INSULIN RESISTANCE AND VASCULAR FUNCTION IN OBESE, LEPTIN RESISTANT MICE VIA REDUCED OXIDANT TONE

M. Irfan Ali[3,*], **Pimonrat Ketsawatsomkron**[3,*], **Eric J. Belin de Chantelemele**[3], **James D. Mintz**[3], **Kenjiro Muta**[3], **Christina Salet**[3], **Stephen M. Black**[3], **Michel L. Tremblay**[4], **David J. Fulton, Ph.D.**[2,3], **Mario B. Marrero, Ph.D**[2,3,**], and **David W. Stepp, Ph.D.**[1,3,**]

[1]Department of Physiology of the Medical College of Georgia, Augusta, Georgia, 30912

[2]Department of Pharmacology of the Medical College of Georgia, Augusta, Georgia, 30912

[3]Vascular Biology Center of the Medical College of Georgia, Augusta, Georgia, 30912

[4]Goodman Cancer Center and Department of Biochemistry, McGill University, Montreal, Quebec H3G 1Y6, Canada

Abstract

Rationale—Obesity is a risk factor for cardiovascular dysfunction, yet the underlying factors driving this impaired function remain poorly understood. Insulin resistance is a common pathology in obese patients and has been shown to impair vascular function. Whether insulin resistance or obesity, itself, is causal remains unclear.

Objective—The current study tested the hypothesis that insulin resistance is the underlying mediator for impaired nitric oxide mediated dilation in obesity by genetic deletion of the insulin-desensitizing enzyme protein tyrosine phosphatase 1B (PTP1B) in *db/db* mice.

Methods and Results—The *db/db* mouse is morbidly obese, insulin resistant and has tissue-specific elevation in PTP1B expression compared to lean controls. In *db/db* mice, PTP1B deletion improved glucose clearance, dyslipidemia, and insulin receptor signaling in muscle and fat. Hepatic insulin signaling in *db/db* mice was not improved by deletion of PTP1B, indicating specific amelioration of peripheral insulin resistance. Additionally, obese mice demonstrate an impaired endothelium dependent and independent vasodilation to acetylcholine and sodium nitroprusside, respectively. This impairment, which correlated with increased superoxide in the *db/db* mice, was corrected by superoxide scavenging. Increased superoxide production was associated with increased expression of NAD(P)H Oxidase 1 and its molecular regulators, Noxo1 and Noxa1.

Conclusion—Deletion of PTP1B improved both endothelium dependent and independent nitric oxide mediated dilation and reduced superoxide generation in *db/db* mice. PTP1B deletion did not affect any vascular function in lean mice. Taken together, these data reveal a role for peripheral insulin resistance as the mediator of vascular dysfunction in obesity.

Address for Correspondence: David W. Stepp, Ph.D. Vascular Biology Center Medical College of Georgia 1459 Laney Walker Blvd Augusta, GA 30912 Phone: 706-721-1949 FAX: 706-721-9799 dstepp@mcg.edu.
*These authors contributed equally to this work.
**These authors share senior authorship

DISCLOSURES The authors have nothing to disclose.

Keywords

Obesity; leptin resistance; PTP1B

INTRODUCTION

The prevalence of obesity and its cardiovascular complications represents a significant health concern in Western societies [1, 2], but the root causes of cardiovascular dysfunction in obese individuals remain unclear. Metabolic dysfunction, notably insulin resistance, is evident in obesity [3, 4]. It has been speculated that insulin resistance, rather than other aspects of obesity, is the underlying cause of cardiovascular injury in obese patients [5–9]. This hypothesis has been difficult to test since insulin-sensitizing drugs have off-target effects [4, 10] and non-obese models of insulin resistance do not evaluate the relative importance of obesity versus insulin resistance [11–17].

The insulin receptor is a classic receptor tyrosine kinase [18] and as such, is de-activated by protein tyrosine phosphatases, notably protein tyrosine phosphatase 1B (PTP1B) [19–21]. Deletion of PTP1B improves insulin sensitivity in mouse models of obesity [22] and putative PTP1B antagonists have been used pharmacologically to improve glucose tolerance [23–25]. Increases in the activity and/or expression of PTP1B correlate with blunted insulin signaling in a variety of tissue types [26–28]. Whether PTP1B deletion and amelioration of insulin resistance improves cardiovascular dysfunction associated with obesity remains unknown.

The current study tested the hypothesis that PTP1B deletion attenuates vascular dysfunction in a model of obesity-induced insulin resistance. Four experimental genotypes were generated through breeding of *db/db*$^{+/-}$ and PTP1B$^{-/-}$ mice to produce double knockout PTP1B null, obese mice. Metabolic profiling, insulin receptor phosphorylation, and PTP1B gene expression were used to assess insulin sensitivity in target tissues. Endothelium-dependent and -independent vascular function were determined *in vitro*. Molecular techniques examined the mechanism whereby deletion of PTP1B improved vascular function. Taken together, these studies critically test the hypothesis that insulin resistance in obesity is the underlying risk factor driving vascular dysfunction in obese individuals.

MATERIALS AND METHODS

Animal Models

Two parental strains of mice were used in these studies: leptin receptor mutant *db/db* mice bred on a C57BL/6 background (Jackson Laboratories) and PTP1B null mice bred on a BALB/c background (Michel Tremblay, Ph.D. at the Cancer Institute of McGill University (Montreal, Canada)). Since *db/db* mice are sterile, progeny were generated from dual heterozygotes (H_{db}, heterozygous for mutant leptin receptor; H_{PTP}, PTP1B gene deletion). Dual heterozygotes were interbred, producing obese, PTP1B gene null, and dual KO mice at 1:4, 1:4, and 1:16 ratios, respectively. In the F_4 generation, dual heterozygotes were bred to heterozygotes for the leptin receptor mutation and PTP1B gene null mice. This breeding strategy yielded obese and dual KO mice at 1:4 and 1:8 ratios, respectively. Dual heterozygous littermates were used as lean controls and littermates heterozygous for *db* and PTP1B gene deletion were used as lean PTP1B null controls. All experiments were conducted in male progeny. In all cases, mice are designated as H or K, indicating heterozygote or knockout. The *db* gene is designated first and the PTP1B second. Thus, $H_{db}H_{PTP}$ are heterozygous for both genes, $H_{db}K_{PTP}$ are lean PTP1B KO mice, $K_{db}H_{PTP}$ are obese mice with intact PTP1B, and $K_{db}K_{PTP}$ are deficient in both leptin receptors and PTP1B. Mice were genotyped by PCR of

genomic DNA. Metabolic phenotyping was accomplished by assessment of glucose tolerance and plasma chemistry (Online Supplement).

Insulin signaling

To determine the effects of PTP1B deletion on insulin receptor phosphorylation, mice were subjected to an insulin stimulation protocol *in vivo* 29. Briefly, mice were anesthetized with isoflurane, and either saline or insulin (1 mU/g) was injected into a jugular vein catheter. After 12 minutes, mice were euthanized by isoflurane overdose and samples of liver, skeletal muscle, and adipose tissue were obtained and snap-frozen in liquid nitrogen. The time period between overdose and tissue harvesting was less than 2 minutes total and samples were obtained in differing order to avoid collection bias.

Western Blotting

Tissue homogenates (20–50 μg) were separated via SDS-PAGE and transferred to Immobilon–P PVDF membranes. In order to determine the expression of relevant proteins, immunoblots were probed with antibodies for PTP1B (Upstate), actin (Calbiochem), insulin receptor-β (IRβ, Santa Cruz), endothelial nitric oxide synthase (eNOS), and phosphorylated eNOS 1177/79 (BD Transduction Laboratories).

Insulin receptor phosphorylation

An anti-IRβ antibody was used to immunoprecipitate insulin receptor proteins from 400–1000 μg of tissue lysates and phosphorylation status assess with phosphor-tyrosine antibody (PY4G10, Upstate). (Online Supplement)

In Vitro Microvessel Preparation

Small mesenteric arteries (SMA) are defined in this study as the 2nd and 3rd distal arcuate artery branches secondary to the conduit superior mesenteric artery ranging from 50 to 150 μm in internal diameter. SMA were dissected and segments (0.25 − 1 mm in length) were mounted in a vessel bath between two glass micropipettes (25 μm-diameter tip) and secured with 10–0 silk ophthalmic suture. SMA were then placed in a chilled, oxygenated (21% O_2, 5% CO_2, and 74% N_2) Krebs-Ringer bicarbonate solution composed of (in mM) 118.3 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO4_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11.1 D-glucose prior to analysis to hibernate physiological activity. The lumen of the vessel was filled with Krebs buffer through the micropipette and maintained at a constant pressure of 60 mmHg. Vessels were monitored under a Nikon inverted light microscope (Melville, NY) connected to a video monitor. Internal diameter was continually measured using video calipers and expressed in micrometers. Buffer temperature was increased to 37°C, and microvessels were allowed to develop spontaneous myogenic tone. After tone was developed, vasodilator responses were measured with sequential doses of: acetylcholine (1×10^{-10} mol/L to 1×10^{-5} mol/L), sodium nitroprusside (1×10^{-9} mol/L to 1×10^{-4} mol/L), or papaverine (1×10^{-9} mol/L to 1×10^{-4} mol/L). Superoxide dismutase (SOD) was used to scavenge superoxide (100 U/mL). ω-Nitro-L-arginine methyl ester (L-NAME) was used to inhibit nitric oxide synthase (100 μM). Dose responses are expressed as a percentage of dilation compared to initial diameter and maximum passive diameter. One dose response curve was performed per vessel per mouse.

Assesment of Superoxide

The quantitative abundance of super-oxide was assessed using Electron Paramagnetic Resonance (EPR) Spectroscopy. Qualitative assessment of super-oxide localization was made using dihydroethidium staining (Online Supplement).

Real Time RT-PCR

Mesenteric arterial cascades were harvested from euthanized animals, removed of non-vascular tissue, and snap-frozen in liquid nitrogen. Total RNA was extracted using Trizol Plus RNA (Invitrogen) and cDNA synthesized using the iScript cDNA Synthesis Kit (Biorad). cDNA was then used to assess relative gene expression using real time RT-PCR (Bio Rad iQ SYBR Green). Primer sequences for the selected genes are described in Table ST1.

Statistics

All data are expressed as mean ± SEM. Differences among all four genotypes were compared by One Way ANOVA or by Student's t-test with Bonferroni correction test used as the post-hoc test. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Weight gain

Increases in body mass are depicted in Supplemental Figure 2 (S2). Data are presented as a scatter plot of 5 male mice of each genotype. Summary data at age 12 weeks are shown in Table 1. Consistent with the inactivating mutation in the leptin receptor gene, $K_{db}H_{PTP}$ mice displayed morbid obesity compared to $H_{db}H_{PTP}$. Deletion of PTP1B did not affect weight gain in either $H_{db}K_{PTP}$ or $K_{db}K_{PTP}$ mice compared to PTP1B intact controls.

$H_{db}K_{PTP}$ mice had modest reductions in plasma leptin versus $H_{db}H_{PTP}$ mice, consistent with sensitization of the leptin receptor, which is also a substrate of PTP1B. In *db/db* mice, plasma leptin levels were markedly increased along with body weight and were unaffected by deletion of PTP1B (Table 1).

Food and water intake and urine output are also summarized in Table 1. $H_{db}H_{PTP}$ and $H_{db}K_{PTP}$ mice exhibited normal and similar food and water intake and urine output. $K_{db}H_{PTP}$ mice displayed hyperphagia, polydypsia, and polyuria, consistent with obesity. Deletion of PTP1B on the obese background did not affect food and water intake.

In sum, these data indicate that the fundamental defects in leptin signaling that drive obesity in *db/db* mice are not moderated by the deletion of PTP1B. Thus, the metabolic improvements arising from PTP1B deletion must be attributed to changes in insulin receptor signaling and not modification of obesity.

Glucose Metabolism

Baseline levels of plasma glucose and insulin are shown in Table 1. Fasting glucose in $H_{db}H_{PTP}$ mice was euglycemic and deletion of PTP1B in $H_{db}K_{PTP}$ mice did not alter fasting glucose. The obesity observed in $K_{db}H_{PTP}$ mice was associated with moderate hyperglycemia. Deletion of PTP1B in $K_{db}K_{PTP}$ mice did not reduce fasting blood glucose, suggesting persistent hepatic insulin resistance in the $K_{db}K_{PTP}$ mice. Consistent with these observations, $H_{db}H_{PTP}$ mice were euinsulinemic but $K_{db}H_{PTP}$ and $K_{db}K_{PTP}$ mice had persistent hyperinsulinemia. Insulin levels in $H_{db}K_{PTP}$ mice were similar to $H_{db}H_{PTP}$ mice.

In vivo clearance of a glucose bolus is shown in Supplemental Figure 3 (S3). $H_{db}H_{PTP}$ mice displayed rapid glucose disposal, and clearance of glucose in $H_{db}K_{PTP}$ mice was similar. $K_{db}H_{PTP}$ mice showed markedly blunted glucose clearance, but $K_{db}K_{PTP}$ mice showed normalization of glucose clearance despite obesity.

HbA_{1c} levels, an index of total glycemic load, are shown in Table 1. $H_{db}H_{PTP}$ and $H_{db}K_{PTP}$ mice showed HbA_{1C} levels lower than 5%, consistent with euglycemic control. In contrast,

$K_{db}H_{PTP}$ mice showed markedly elevated HbA_{1c} levels, consistent with their observed glucose intolerance and fasting hyperglycemia. Although not completely normalized, HbA_{1c} levels were significantly reduced in $K_{db}K_{PTP}$ mice compared to $K_{db}H_{PTP}$ mice, despite equivalent food intake.

Lipid Metabolism

Plasma concentration of FFAs, triglycerides, and cholesterol are shown in Table 1. $H_{db}H_{PTP}$ and $H_{db}K_{PTP}$ mice show normal levels of all three lipid compounds. $K_{db}H_{PTP}$ mice had elevated fasting FFAs and increased triglyceride levels, consistent with the loss of insulin sensitivity in fat cells. $K_{db}K_{PTP}$ mice displayed largely normal levels of FFAs and triglycerides, suggesting a normalization of adipocyte insulin resistance by deletion of PTP1B. In contrast, total cholesterol was elevated to a similar extent in both $K_{db}H_{PTP}$ and $K_{db}K_{PTP}$ mice.

Expression of PTP1B

To determine the effects of obesity on the tissue expression of PTP1B, Western blotting was performed on extracts from liver, muscle, and fat, which are the three major targets of the metabolic actions of insulin. The results are shown in Figure 1A–C. PTP1B expression was heterogeneous with marked increases in expression in the skeletal muscle (Figure 1B) and adipose tissue (Figure 1C) of obese mice. Expression of PTP1B in the liver (Figure 1A) was not statistically different between lean $H_{db}H_{PTP}$ and obese $K_{db}H_{PTP}$ mice.

Insulin signaling

Phosphorylation of the insulin receptor was used as a molecular readout of insulin signaling capacity. In skeletal muscle, adipose, and liver tissue samples, insulin provoked a marked increase in receptor tyrosine phosphorylation that was similar in $H_{db}H_{PTP}$ and $H_{db}K_{PTP}$ mice. In contrast, in $K_{db}H_{PTP}$ mice, the tyrosine phosphorylation of the insulin receptor was markedly reduced, consistent with obesity-induced insulin resistance. In skeletal muscle (Figure 1E) and adipose tissue (Figure 1F), insulin receptor phosphorylation was markedly increased in $K_{db}K_{PTP}$ mice, suggesting that deletion of PTP1B improved insulin signaling. In contrast, insulin receptor phosphorylation remained depressed in the liver (Figure 1D) of $K_{db}K_{PTP}$ mice, suggesting persistent hepatic insulin insensitivity in these animals.

Vascular Reactivity

Endothelium-dependent, acetylcholine-mediated vasodilation in the SMA from all mice is shown in Figure 2A. Smooth muscle reactivity to nitric oxide (NO) was determined using sodium nitroprusside (SNP, Figure 2B). Lean mice that are PTP1B deficient ($H_{db}K_{PTP}$) do not have differences in maximum dilation to acetylcholine (70% vs. 68 %, p<NS) or sodium nitroprusside (96% vs. 94%, p<NS) from $H_{db}H_{PTP}$ mice, indicating that PTP1B deletion does not affect endothelium-dependent or - independent vasodilation. The maximum vasodilator response to acetylcholine was reduced markedly in $K_{db}H_{PTP}$ compared to $H_{db}H_{PTP}$ (50% vs. 70 %, $p<0.05$), indicating impairment of endothelial function in $K_{db}H_{PTP}$ mice. A significant deficiency in reactivity to exogenous NO was also detected in $K_{db}H_{PTP}$ mice (68% vs. 94 %, $p<0.05$). The maximum vasodilator response to acetylcholine was markedly reduced in all mice following treatment with 100 μM L-NAME (Figure 2C), indicating NO as the primary dilator mediating the response to acetylcholine. Furthermore, the NO-independent component of acetylcholine-induced vasodilation was not different among all groups of mice. Taken together, these data indicate that vasodilator reactivity is compromised at the level of NO utilization in obese mice. In contrast to the findings observed in $K_{db}H_{PTP}$ mice, endothelium-dependent vasodilation in obese $K_{db}K_{PTP}$ was similar to that observed in lean $H_{db}H_{PTP}$ mice (Figure 2A). The impaired response to exogenous NO (SNP) was also restored by PTP1B deletion (Figure 2B). Vascular dysfunction was not due to loss of eNOS expression or phosphorylation as these

variables were similar in all strains of mice (Figure S4). Responses to the NO-independent vasodilator papaverine were similar across all mice (Figure S5), suggesting that the vasodilation deficiency in $K_{db}H_{PTP}$ mice is not due to a general deficit in vascular dilation, but is confined to NO-mediated dilation.

To determine whether elevated superoxide production was a mechanism of impaired vasodilation in obesity, vascular function was assessed in the presence of 100 U/mL PEG-SOD. PEG-SOD reversed the impaired dilation to acetylcholine (Figure 2D) and SNP (Figure 2E) in $K_{db}H_{PTP}$ mice with no effect on vascular function in the other genotypes. To further determine if PEG-SOD was indeed restoring NO bioactivity, endothelium-dependent dilation was assessed in the presence of PEG-SOD and both the presence and absence of 100 μM L-NAME (Figure S6). All mouse vessels exhibited equivalent degrees of L-NAME resistant dilation thus confirming that the main dilation mechanism in these microvessels is NO and further that scavenging of superoxide did not improve NO-independent dilation in $K_{db}H_{PTP}$ mice.

Passive mechanics were assessed in a zero-Ca^{2+} Krebs solution and results are shown in ST2. Vascular architectural changes were assessed as previously described. Maximal vessel wall thickness and wall to lumen ratio (at 120 mmHg of intraluminal pressure) were similar across all genotypes. Vascular compliance, as calculated by the exponential fit of a circumferential stress-strain plot (β-coefficient), also remained similar across all genotypes. Taken together, these data indicate that neither obesity nor deletion of PTP1B produce structural changes that could account for observed deficits in vasodilator function.

Superoxide production

EPR spectroscopy was employed to semi-quantitatively measure superoxide. The relative PEG-SOD inhibitable signal was 4 times higher in mesenteric vessels from $K_{db}H_{PTP}$ mice versus control $H_{db}H_{PTP}$ mice (Figure 3A) and reversed to control levels by deletion of PTP1B in obese $K_{db}K_{PTP}$. The increased superoxide signal in the $K_{db}H_{PTP}$ mice was nearly eliminated following acetovanillone (apocynin) incubation (Figure 3B), suggesting it derives from NAD(P)H oxidases. Taken together, peripheral insulin resistance increases vascular superoxide production that is corrected by PTP1B deletion. As a further measure of vascular ROS production, we also performed DHE staining of blood vessels. These results are in agreement with the EPR studies and demonstrate higher levels of superoxide in the blood vessels of $K_{db}H_{PTP}$ mice compared to controls (Figure 3C). The deletion of PTP1B in obese animals ($K_{db}K_{PTP}$) decreased DHE staining to control levels, and there was no difference in superoxide production due to the deletion of PTP1B in lean animals. DHE staining was most intense in the medial layer of mesenteric microvessels in all 4 groups of mice.

The source of elevated superoxide levels in $K_{db}H_{PTP}$ mice was addressed using real time quantitative RT-PCR. As shown in Figure 4, the expression levels for Nox1 and its novel activator and organizer (Noxa1 and Noxo1) are significantly elevated in $K_{db}H_{PTP}$ mice as compared to control ($2^{-\Delta\Delta Ct}$ ± SEM in $H_{db}H_{PTP}$ vs. $K_{db}H_{PTP}$; Nox1: 1.45 ± .23 vs. 3.52 ± 1.6; Noxa1: 1.17 ± .11 vs. 9.94 ± 1.5; Noxo1: 1.14 ± .23 vs. 8.81 ± 2.8). Nox2 and Nox4 are expressed similarly in all animals (Figure 4), as are p22phox, p47phox and p67phox (data not shown). Select anti-oxidant enzymes were also examined, demonstrating a statistically significant increase in SOD2 and SOD3 in $K_{db}K_{PTP}$ mice compared to both controls and $K_{db}H_{PTP}$ mice ($2^{-\Delta\Delta Ct}$ ± SEM in $K_{db}H_{PTP}$ vs. $K_{db}K_{PTP}$; SOD2: 1.00 ± .5 vs. 3.36 ± .52; SOD3: .68 ± .10 vs. 4.76 ± 1.6). A summary for all genes studied is shown in Supplemental Table 3.

DISCUSSION

The goal of the current study was to determine whether the metabolic consequences of obesity or the state of obesity itself results in vascular dysfunction. To test this hypothesis we generated obese mice harboring deletion of PTP1B, a tyrosine phosphatase that antagonizes insulin signaling. The key findings of this study are: 1) deletion of PTP1B in *db/db* mice does not affect obesity and corrects peripheral but not hepatic insulin resistance; 2) correction of peripheral insulin resistance improves NO-mediated dilation in SMA despite persistent obesity; 3) correcting peripheral insulin resistance mediates the improvement in NO dilation by decreasing superoxide levels, primarily through reduced expression of Nox1, Noxa1 and Noxo1.

Metabolic effects of PTP1B deletion in obese mice

Although PTP1B has attracted considerable attention as a target in the treatment of non-insulin dependent diabetes, the impact of obesity on the relative distribution of PTP1B expression in tissues central to insulin action is unclear. Moreover, expression of PTP1B in models of obesity and diabetes is complicated as it varies with the stage of diabetes and genetic background [30]. As shown in Figure 1A–C, obesity in the *db/db* mice used in these studies caused a differential increase in PTP1B expression, with the most prominent increases in muscle and fat and a statistically undetectable difference in the liver. The increases in PTP1B expression correlate with decreased insulin receptor phosphorylation and are reversed by PTP1B deletion.

Deletion of PTP1B did not affect weight gain in either lean or obese mice. Food and fluid intake, urine output, and plasma leptin levels also remained unchanged. These observations are consistent with those of Cheng et al. [31] in which *ob/ob* mice heterozygous for PTP1B and *ob/ob* mice with deletion PTP1B showed similarity in weight gain. In contrast, adenoviral delivery of PTP1B anti-sense RNA produces reductions in body weight and fat mass [32] and when *ob/ob* mice that lack PTP1B are compared to *ob/ob* mice with wild-type PTP1B expression, an approximately 15% weight difference is observed [31]. It is important to note that in this study, deletion of PTP1B improves glycemic control in *ob/ob* mice when wild type (2 copies) and knockout (no copies) are compared. However, one could not determine in this setting whether the improvement in glycemic control reflects the actions of PTP1B on insulin signaling or the weight loss in *ob/ob* mice. In the current study, we evaluated metabolic control between mice in which body weight is identical and the leptin receptor is completely missing. Thus, differences between $K_{db}H_{PTP}$ and $K_{db}K_{PTP}$, which are equally obese, reflect the effects of PTP1B deletion on improvements in the insulin signaling pathway.

To verify the functional importance of the increase in PTP1B expression in $K_{db}H_{PTP}$ mice, we used physiologic (plasma serum chemistry and glucose tolerance) and molecular (phosphorylation of the insulin receptor) measurements as indices of insulin signaling. Consistent with recent observations from Delibgovic et al [33], the elevated expression of PTP1B in skeletal muscle of obese mice correlated with marked impairment in muscle insulin receptor phosphorylation and significant impairment in glucose tolerance and increased HbA_{1c} percentage. These variables were markedly improved by the deletion of PTP1B. These findings, combined with the marked improvement in insulin receptor tyrosine phosphorylation in muscle, indicate that PTP1B is a key determinant of skeletal muscle insulin sensitivity in *db/db* mice.

Reductions in serum triglycerides and FFAs following deletion of PTP1B, combined with the marked increase in PTP1B expression in visceral adipose tissue and improvement in insulin receptor signaling in visceral adipose tissue, indicate that PTP1B also plays a critical role in the adipose tissue of obese mice. In this study, the lack of a leptin receptor results in similar body weight between $K_{db}H_{PTP}$ and $K_{db}K_{PTP}$ mice but marked differences in triglyceride and

FFA levels in the plasma, indicating an improvement of insulin signaling in fat tissue. Plasma cholesterol was elevated in $K_{db}H_{PTP}$ mice compared to $H_{db}H_{PTP}$ mice (Table 1), but was not affected by PTP1B deletion in $H_{db}K_{PTP}$ or $K_{db}K_{PTP}$ mice. Since both $K_{db}H_{PTP}$ and $K_{db}K_{PTP}$ mice retain the hyperphagic phenotype, the lack of a difference in plasma cholesterol likely indicates that elevated cholesterol in these animals reflects dietary intake or persistent hepatic insulin resistance.

Fasting blood glucose, primarily driven by hepatic gluconeogenesis, was similar in $K_{db}H_{PTP}$ and $K_{db}K_{PTP}$ mice. Plasma insulin levels are also elevated, consistent with the loss of insulin receptor function in the liver [34, 35]. PTP1B expression was not significantly increased in the liver and insulin receptor phosphorylation remains depressed in $K_{db}K_{PTP}$mice despite deletion of PTP1B. Although previous studies have described a role for PTP1B in hepatic insulin signaling in lean mice [36]37 or with non-genomic methods [38], our model does not reflect this outcome, likely due to the background of these mice[30] . Nevertheless, the lack of improvement in hepatic insulin signaling and moderate fasting hyperglycemia indicate that hepatic insulin resistance cannot explain observed impairments in cardiovascular function.

Effect of deletion of PTP1B on vasodilation

Previous studies in obese rodents have indicated that obesity is a risk factor for vascular dysfunction [11, 39, 40], but the culpable component of obesity has remained elusive. As described above, the dual KO mice developed for these studies remain obese, but peripheral insulin resistance is improved when PTP1B is deleted. Moreover, the results described in these studies indicate that when PTP1B is deleted in obese mice, vasodilation to NO is improved.

The role of PTP1B in improving NO-mediated dilation could be attributed to: 1) a direct effect of PTP1B on endothelial function or 2) an improvement in vasodilation secondary to correction of peripheral insulin resistance. A direct effect of PTP1B deletion is refuted by the lack of vascular outcomes in lean $H_{db}K_{PTP}$ mice, consistent with previous work in which over-expression of PTP1B in cultured endothelial cells did not influence the function of eNOS [41]. These observations preclude PTP1B as a direct modulator of vasodilation.

Our observations are more consistent with the hypothesis that insulin resistance is the causal factor in vessel dysfunction in obesity. To date, this hypothesis has been primarily based on studies in non-obese models of insulin resistance [11, 13, 42, 43] and studies with pharmacological compounds [4, 10, 42]. Clear interpretation of these studies is confounded by off-target effects of drugs and because they lack the physiologic context of obesity. In the current study, we have developed a novel double knockout model and characterized in detail the metabolic parameters relevant to insulin resistance. The outcome is that we have described a model in which improvement of peripheral insulin resistance improves vascular function, despite persistent obesity and modest hyperglycemia. This normalization provides strong evidence that obesity has minimal impact on vasodilation mediated by NO in the absence of insulin resistance. Since the deletion of PTP1B in this model improves peripheral but not hepatic insulin resistance, we can refine the metabolic hypothesis of vessel dysfunction beyond whole body insulin resistance to specific compartments. Impairment of vascular function correlates with markers of metabolic dysfunction in muscle and fat but not the liver. To the authors' knowledge, this is the first study to localize vascular dysfunction in obesity to peripheral insulin resistance.

Effect of PTP1B deletion on reactive oxygen species

The mechanisms by which insulin resistance impairs NO-mediated vasodilation are incompletely understood. In the current study, we present evidence that the primary mechanism is an increase in reactive oxygen species (ROS). In obese mice, the level of superoxide is increased versus lean controls (Figure 3A, 3C); vasodilation is restored by oxidant scavenging

and components of the NAD(P)H oxidase pathway are increased (Figure 2D,E; Figure 4; Table 2). Blockade of NAD(P)H oxidases normalizes oxidant load. Correction of insulin resistance by deletion of PTP1B in obese mice corrects the augmented ROS levels. Scavenging of superoxide restores sensitivity to acetylcholine as does deletion of PTP1B in $K_{db}K_{PTP}$ mice. The L-NAME resistant component of endothelium-dependent dilation remains the same in all mice, ruling out differences in NO-independent endothelial vasodilation. Taken *in toto*, these data suggest that insulin resistance corrupts endothelial vasodilation by NAD(P)H oxidase-derived oxidants.

Previous studies have attributed increases in superoxide levels to an increase in NAD(P)H oxidase activity [44, 45]. Nox2 ($gp91_{phox}$) was originally identified as the primary source of pathological superoxide production in insulin resistant states, but more recently roles for Nox1 and Nox4 have been identified [46–48]. A key finding in the current study is that the expression levels of Nox1 and its regulatory enzymes Noxa1/Noxo1 in the vasculature correlate with obesity-induced insulin resistance. In obese mice, the expression of Nox1, Noxa1, and Noxo1 are markedly increased and these levels are reversed by the deletion of PTP1B. Co-expression of Nox1 with Noxo1 and Noxa1 results in the constitutive and high-level production of superoxide [49, 50]. The significance of increased Nox1 activity in insulin resistant states in not clear. The loss of Nox1 attenuates, while an increased expression of Nox1 potentiates, angiotensin-induced hypertension [51, 52]. In large conduit blood vessels, Nox1 expression is unchanged in *db/db* mice [53]; however, our study is the first to measure expression levels of these proteins in microvessels. The expression level of other Nox isoforms was examined in addition to Nox1, and in contrast to other studies [53] there were no changes with obesity.

In summary, these experiments provide new evidence that in the context of obesity, the underlying risk factor that impairs both endothelium-dependent and - independent NO dilation is peripheral insulin resistance. Moderate hyperglycemia or morbid obesity does not cause endothelial dysfunction when the enzyme PTP1B is absent. These data indicate that PTP1B may represent an important therapeutic target for not only the metabolic but also cardiovascular therapy of obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge the assistance of Dr. Brenda Lilly, MCG, in developing a PCR screening strategy for PTP1B. We would also acknowledge Jessica Osmond for her helpful review of the text. Finally, the authors would like to thank Dr. David M. Stern for initial guidance and encouragement.

Sources of Funding This work was supported by NIH grants to DWS (5R01HL076533, 1R01HL092446) DJF (5R01HL085827, 1R01HL092446) and MBM (5R01HL058139, 5R01DK061687) and an AHA Established Investigator Award to DJF.

Non-Standard Abbreviations and Acronyms

The abbreviations used are:

PTP1B, protein tyrosine phosphatase 1B
H_{db}, heterozygous for mutant leptin receptor
H_{PTP}, heterozygous for PTP1B gene deletion
K_{db}, homozygous for mutant leptin receptor
K_{PTP}, homozygous for PTP1B gene deletion
HbA_{1c}, Hemoglobin A1c

FFA, free fatty acids
eNOS, endothelial nitric oxide synthase
IRβ, insulin receptor-β
SMA, small mesenteric arteries
SOD, Superoxide dismutase
L-NAME, ω-Nitro-L-arginine methyl ester
CMH, methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine
DHE, Dihydroethidium
Nox, NAD(P)H oxidase
Noxa1, NAD(P)H oxidase novel activator
NAD(P)H oxidase novel organizer,

References

1. Ritchie SA, Connell JM. The link between abdominal obesity, metabolic syndrome and cardiovascular disease. Nutr Metab Cardiovasc Dis May;2007 17(4):319–326. [PubMed: 17110092]
2. Morisco C, Lembo G, Trimarco B. Insulin resistance and cardiovascular risk: New insights from molecular and cellular biology. Trends in cardiovascular medicine Aug;2006 16(6):183–188. [PubMed: 16839860]
3. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nature reviews May;2008 9(5):367–377.
4. Goldstein BJ. Insulin resistance: from benign to type 2 diabetes mellitus. Rev Cardiovasc Med 2003;4 (Suppl 6):S3–10. [PubMed: 14668698]
5. Verma S, Leung YM, Yao L, Battell M, Dumont AS, McNeill JH. Hyperinsulinemia superimposed on insulin resistance does not elevate blood pressure. Am J Hypertens 2001;14(5 Pt 1):429–432. [PubMed: 11368463]
6. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. The Journal of clinical investigation 1996;97(11):2601–2610. [PubMed: 8647954]
7. Katakam PV, Ujhelyi MR, Hoenig ME, Miller AW. Endothelial dysfunction precedes hypertension in diet-induced insulin resistance. The American journal of physiology 1998;275(3 Pt 2):R788–792. [PubMed: 9728076]
8. Dimitropoulou C, Han G, Miller AW, Molero M, Fuchs LC, White RE, Carrier GO. Potassium (BK (Ca)) currents are reduced in microvascular smooth muscle cells from insulin-resistant rats. American journal of physiology 2002;282(3):H908–917. [PubMed: 11834486]
9. Baron AD. Insulin resistance and vascular function. J Diabetes Complications 2002;16(1):92–102. [PubMed: 11872375]
10. Chen ZP, Mitchelhill KI, Michell BJ, Stapleton D, Rodriguez-Crespo I, Witters LA, Power DA, Ortiz de Montellano PR, Kemp BE. AMP-activated protein kinase phosphorylation of endothelial NO synthase. FEBS Lett Jan 29;1999 443(3):285–289. [PubMed: 10025949]
11. Romanko OP, Stepp DW. Reduced constrictor reactivity balances impaired vasodilation in the mesenteric circulation of the obese Zucker rat. American journal of physiology Nov;2005 289 (5):H2097–2102. [PubMed: 15951349]
12. D'Angelo G, Elmarakby AA, Pollock DM, Stepp DW. Fructose feeding increases insulin resistance but not blood pressure in Sprague-Dawley rats. Hypertension Oct;2005 46(4):806–811. [PubMed: 16157789]
13. Verma S, Bhanot S, Yao L, McNeill JH. Defective endothelium-dependent relaxation in fructose-hypertensive rats. Am J Hypertens Apr;1996 9(4 Pt 1):370–376. [PubMed: 8722439]
14. Shinozaki K, Ayajiki K, Nishio Y, Sugaya T, Kashiwagi A, Okamura T. Evidence for a causal role of the renin-angiotensin system in vascular dysfunction associated with insulin resistance. Hypertension Feb;2004 43(2):255–262. [PubMed: 14698997]
15. Lee DH, Lee JU, Kang DG, Paek YW, Chung DJ, Chung MY. Increased vascular endothelin-1 gene expression with unaltered nitric oxide synthase levels in fructose-induced hypertensive rats. Metabolism: clinical and experimental Jan;2001 50(1):74–78. [PubMed: 11172478]

16. Kamata K, Yamashita K. Insulin resistance and impaired endothelium-dependent renal vasodilatation in fructose-fed hypertensive rats. Res Commun Mol Pathol Pharmacol Feb;1999 103(2):195–210. [PubMed: 10461686]

17. Zavaroni I, Sander S, Scott S, Reaven GM. Effect of fructose feeding on insulin secretion and insulin action in the rat. Metabolism: clinical and experimental Oct;1980 29(10):970–973. [PubMed: 6999292]

18. White MF, Kahn CR. The insulin signaling system. The Journal of biological chemistry Jan 7;1994 269(1):1–4. [PubMed: 8276779]

19. Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, Saltiel AR, Kusari J, Olefsky JM. Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. Diabetes Oct;1996 45(10): 1379–1385. [PubMed: 8826975]

20. Bandyopadhyay D, Kusari A, Kenner KA, Liu F, Chernoff J, Gustafson TA, Kusari J. Protein-tyrosine phosphatase 1B complexes with the insulin receptor in vivo and is tyrosine-phosphorylated in the presence of insulin. The Journal of biological chemistry Jan 17;1997 272(3):1639–1645. [PubMed: 8999839]

21. Dadke S, Kusari J, Chernoff J. Down-regulation of insulin signaling by protein-tyrosine phosphatase 1B is mediated by an N-terminal binding region. The Journal of biological chemistry Aug 4;2000 275(31):23642–23647. [PubMed: 10807907]

22. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science Mar 5;1999 283(5407):1544–1548. [PubMed: 10066179]

23. Chen H, Cong LN, Li Y, Yao ZJ, Wu L, Zhang ZY, Burke TR Jr. Quon MJ. A phosphotyrosyl mimetic peptide reverses impairment of insulin-stimulated translocation of GLUT4 caused by overexpression of PTP1B in rat adipose cells. Biochemistry Jan 5;1999 38(1):384–389. [PubMed: 9890920]

24. Malamas MS, Sredy J, Moxham C, Katz A, Xu W, McDevitt R, Adebayo FO, Sawicki DR, Seestaller L, Sullivan D, Taylor JR. Novel benzofuran and benzothiophene biphenyls as inhibitors of protein tyrosine phosphatase 1B with antihyperglycemic properties. Journal of medicinal chemistry Apr 6;2000 43(7):1293–1310. [PubMed: 10753467]

25. Winter CL, Lange JS, Davis MG, Gerwe GS, Downs TR, Peters KG, Kasibhatla B. A nonspecific phosphotyrosine phosphatase inhibitor, bis(maltolato)oxovanadium(IV), improves glucose tolerance and prevents diabetes in Zucker diabetic fatty rats. Experimental biology and medicine (Maywood, N.J Mar;2005 230(3):207–216.

26. Ahmad F, Considine RV, Bauer TL, Ohannesian JP, Marco CC, Goldstein BJ. Improved sensitivity to insulin in obese subjects following weight loss is accompanied by reduced protein-tyrosine phosphatases in adipose tissue. Metabolism: clinical and experimental Oct;1997 46(10):1140–1145. [PubMed: 9322796]

27. Wu X, Hardy VE, Joseph JI, Jabbour S, Mahadev K, Zhu L, Goldstein BJ. Protein-tyrosine phosphatase activity in human adipocytes is strongly correlated with insulin-stimulated glucose uptake and is a target of insulin-induced oxidative inhibition. Metabolism: clinical and experimental Jun;2003 52(6):705–712. [PubMed: 12800095]

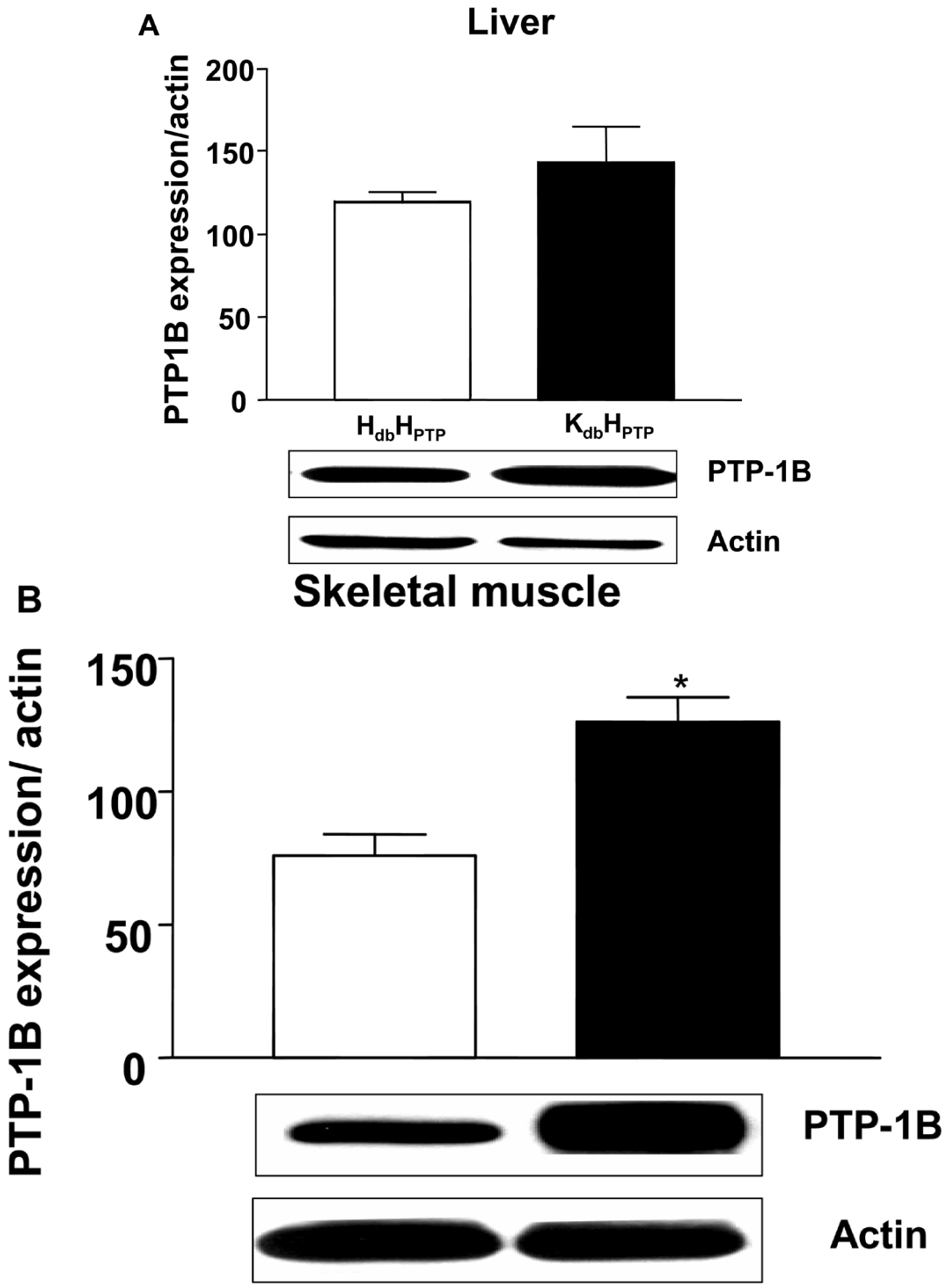
28. Venable CL, Frevert EU, Kim YB, Fischer BM, Kamatkar S, Neel BG, Kahn BB. Overexpression of protein-tyrosine phosphatase-1B in adipocytes inhibits insulin-stimulated phosphoinositide 3-kinase activity without altering glucose transport or Akt/Protein kinase B activation. The Journal of biological chemistry Jun 16;2000 275(24):18318–18326. [PubMed: 10751417]

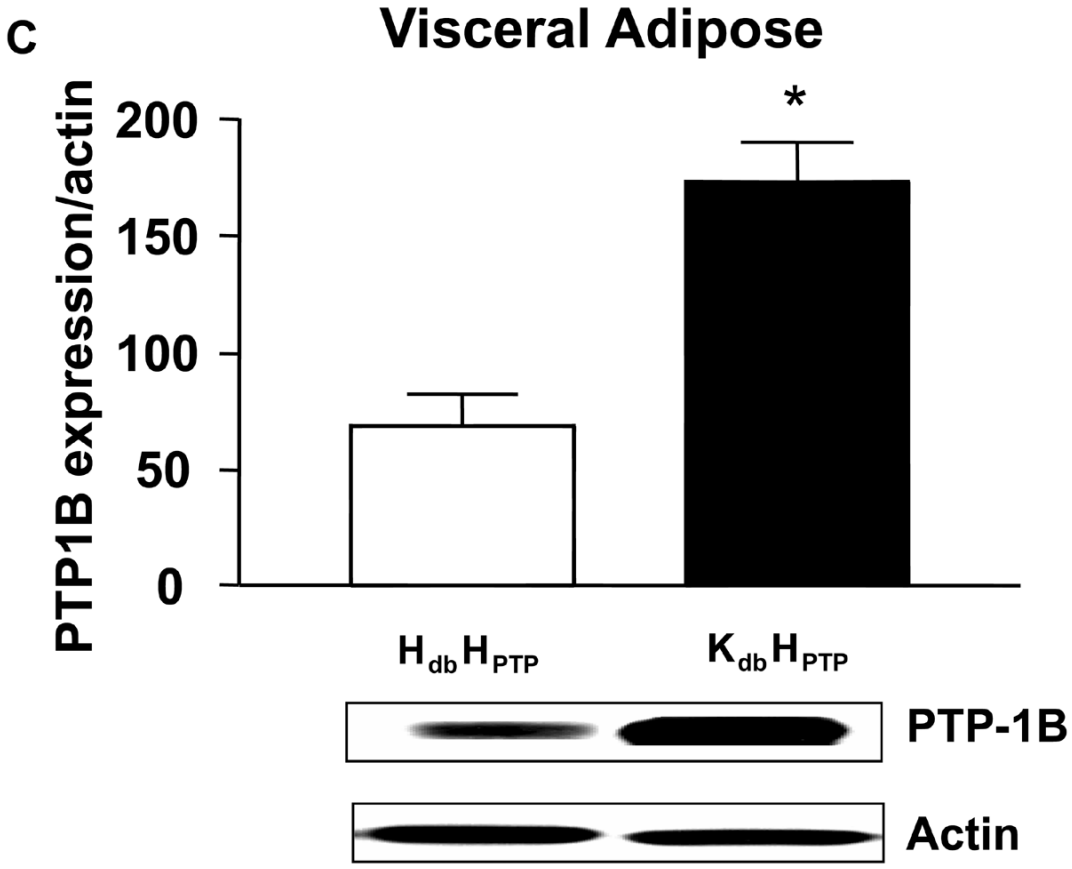
29. Dummler B, Tschopp O, Hynx D, Yang ZZ, Dirnhofer S, Hemmings BA. Life with a single isoform of Akt: mice lacking Akt2 and Akt3 are viable but display impaired glucose homeostasis and growth deficiencies. Molecular and cellular biology Nov;2006 26(21):8042–8051. [PubMed: 16923958]

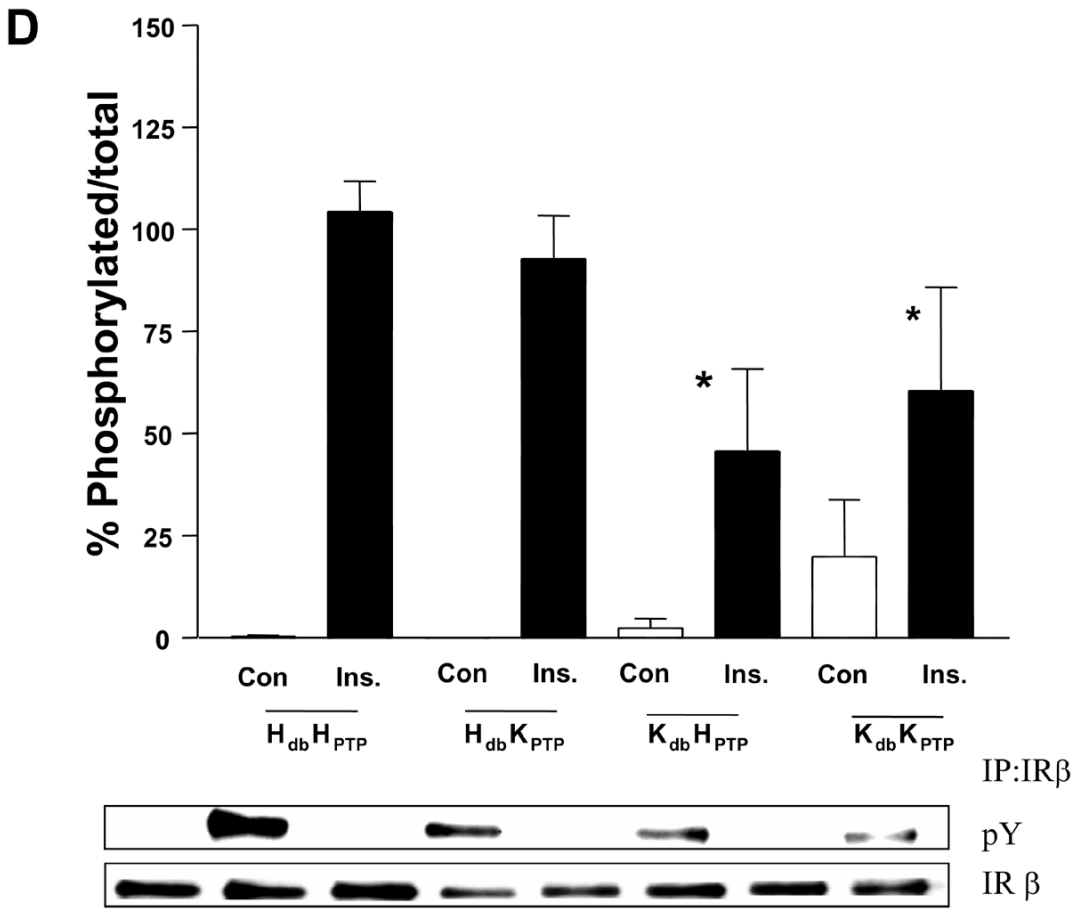
30. Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB. Protein tyrosine phosphatase 1B (PTP1B) expression is induced by inflammation in vivo. The Journal of biological chemistry. Feb 14;2008

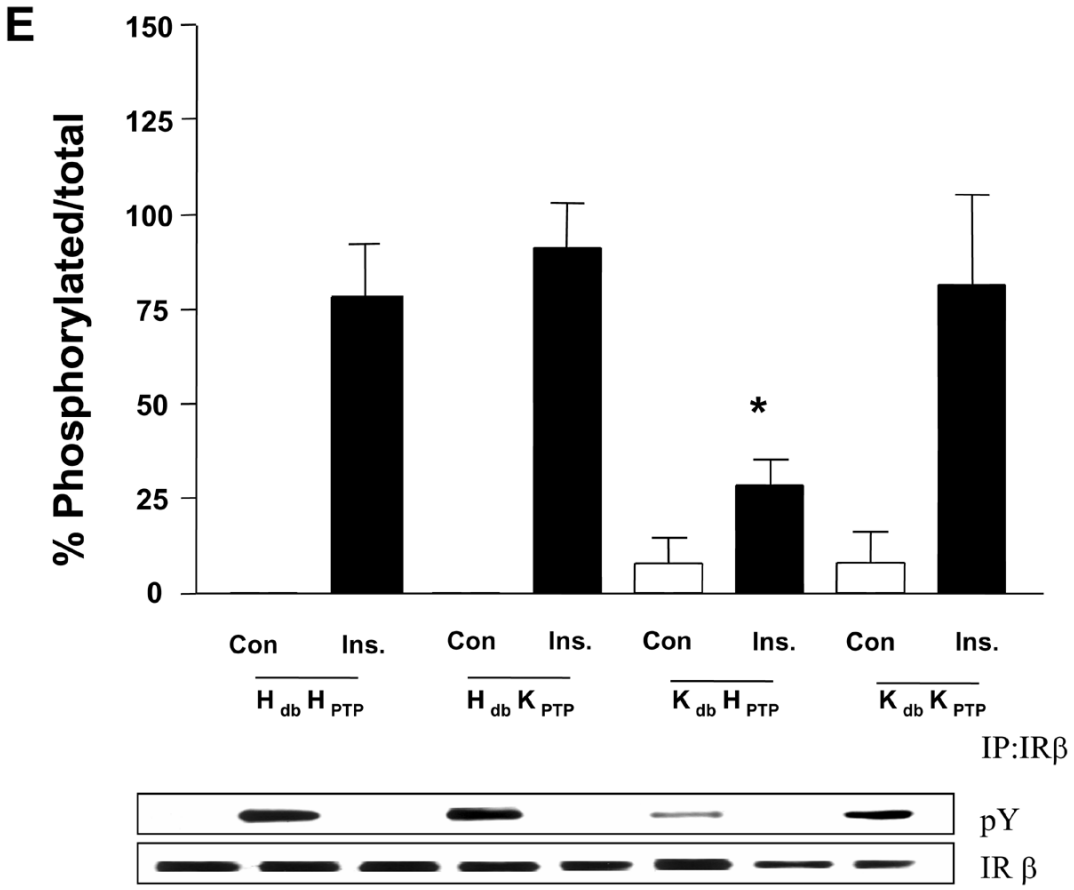
31. Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ, Kennedy BP, Tremblay ML. Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. Developmental cell Apr;2002 2(4):497–503. [PubMed: 11970899]

32. Rondinone CM, Trevillyan JM, Clampit J, Gum RJ, Berg C, Kroeger P, Frost L, Zinker BA, Reilly R, Ulrich R, Butler M, Monia BP, Jirousek MR, Waring JF. Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. Diabetes Aug;2002 51(8):2405–2411. [PubMed: 12145151]
33. Delibegovic M, Bence KK, Mody N, Hong EG, Ko HJ, Kim JK, Kahn BB, Neel BG. Improved glucose homeostasis in mice with muscle-specific deletion of protein-tyrosine phosphatase 1B. Molecular and cellular biology Nov;2007 27(21):7727–7734. [PubMed: 17724080]
34. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Molecular cell Jul;2000 6(1):87–97. [PubMed: 10949030]
35. Fisher SJ, Kahn CR. Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. The Journal of clinical investigation Feb;2003 111(4):463–468. [PubMed: 12588884]
36. Xue B, Kim YB, Lee A, Toschi E, Bonner-Weir S, Kahn CR, Neel BG, Kahn BB. Protein-tyrosine phosphatase 1B deficiency reduces insulin resistance and the diabetic phenotype in mice with polygenic insulin resistance. The Journal of biological chemistry Aug 17;2007 282(33):23829–23840. [PubMed: 17545163]
37. Haj FG, Zabolotny JM, Kim YB, Kahn BB, Neel BG. Liver-specific protein-tyrosine phosphatase 1B (PTP1B) re-expression alters glucose homeostasis of PTP1B−/− mice. The Journal of biological chemistry Apr 15;2005 280(15):15038–15046. [PubMed: 15699041]
38. Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, Xie N, Wilcox D, Jacobson P, Frost L, Kroeger PE, Reilly RM, Koterski S, Opgenorth TJ, Ulrich RG, Crosby S, Butler M, Murray SF, McKay RA, Bhanot S, Monia BP, Jirousek MR. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proceedings of the National Academy of Sciences of the United States of America Aug 20;2002 99 (17):11357–11362. [PubMed: 12169659]
39. Oltman CL, Richou LL, Davidson EP, Coppey LJ, Lund DD, Yorek MA. Progression of coronary and mesenteric vascular dysfunction in Zucker obese and Zucker diabetic fatty rats. American journal of physiology Oct;2006 291(4):H1780–1787. [PubMed: 16714356]
40. Frisbee JC. Impaired dilation of skeletal muscle microvessels to reduced oxygen tension in diabetic obese Zucker rats. American journal of physiology Oct;2001 281(4):H1568–1574. [PubMed: 11557545]
41. Fulton D, Harris MB, Kemp BE, Venema RC, Marrero MB, Stepp DW. Insulin resistance does not diminish eNOS expression, phosphorylation, or binding to HSP-90. American journal of physiology Dec;2004 287(6):H2384–2393. [PubMed: 15271670]
42. Katakam PV, Ujhelyi MR, Hoenig M, Miller AW. Metformin improves vascular function in insulin-resistant rats. Hypertension Jan;2000 35(1 Pt 1):108–112. [PubMed: 10642283]
43. Takagawa Y, Berger ME, Hori MT, Tuck ML, Golub MS. Long-term fructose feeding impairs vascular relaxation in rat mesenteric arteries. Am J Hypertens Sep;2001 14(8 Pt 1):811–817. [PubMed: 11497199]









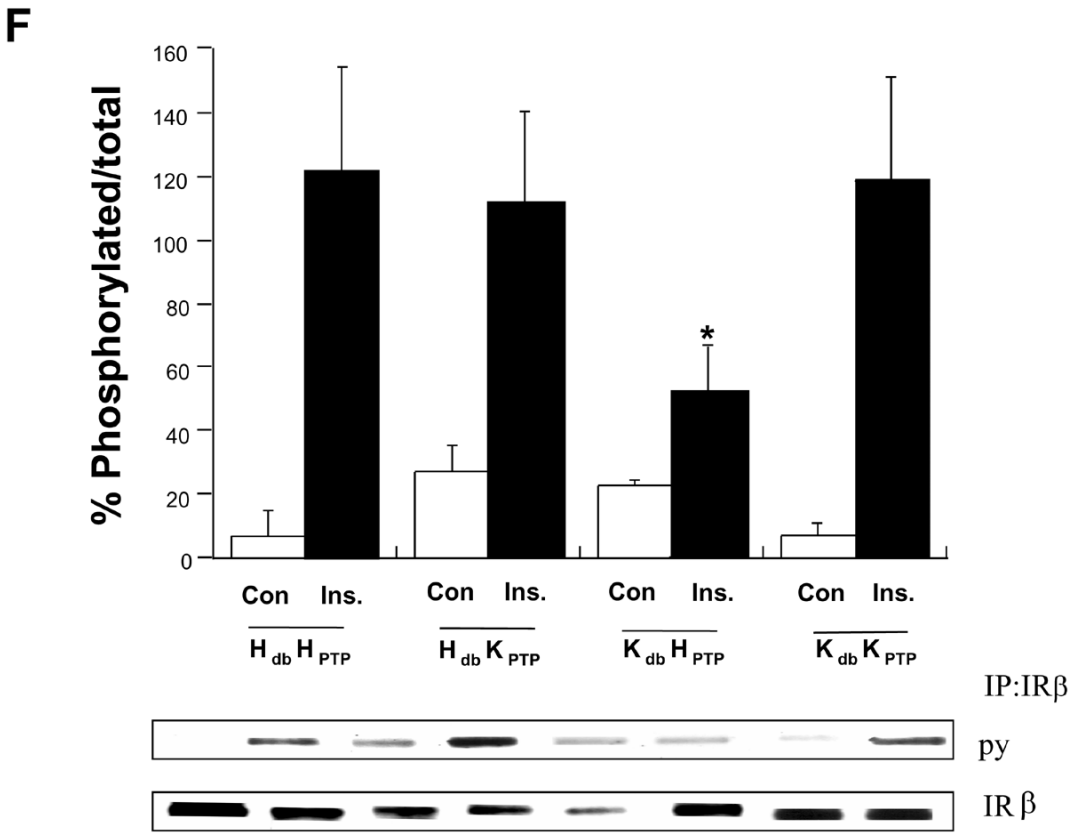
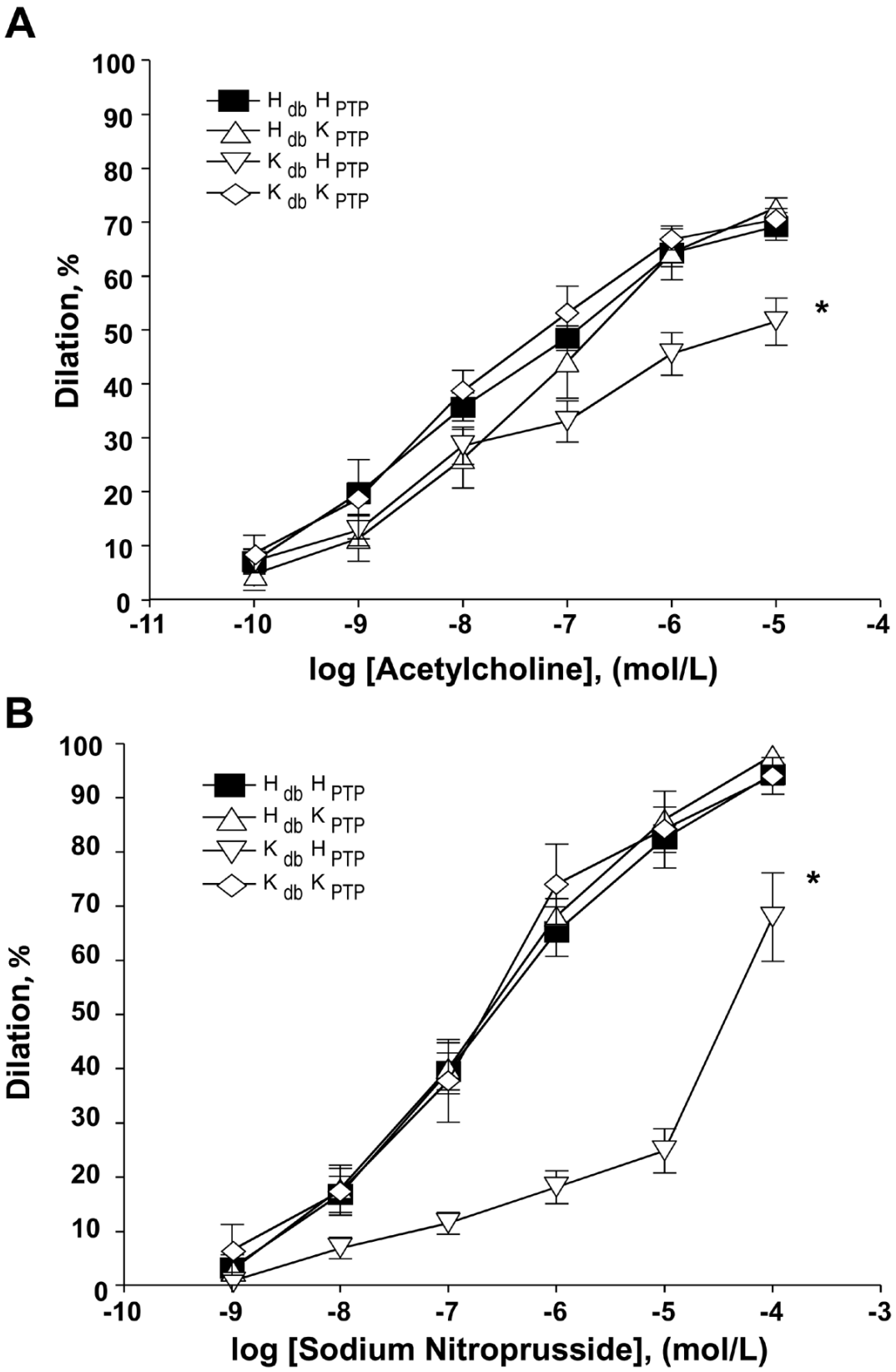
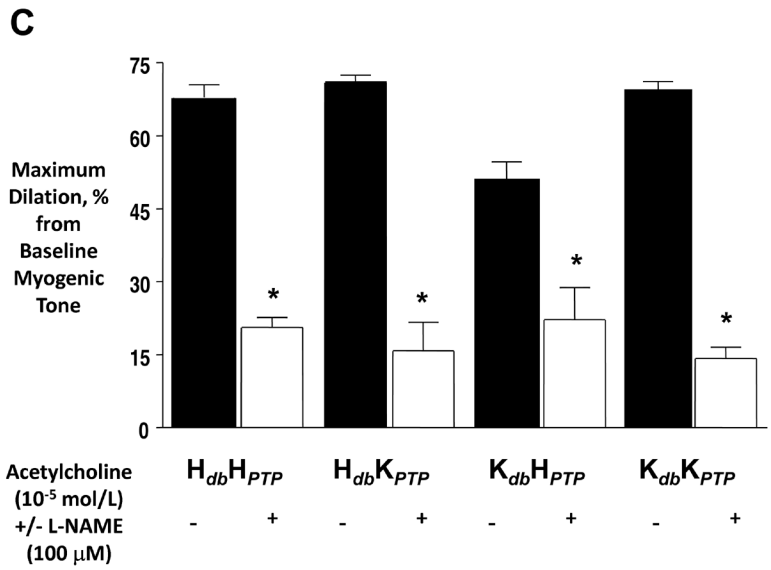


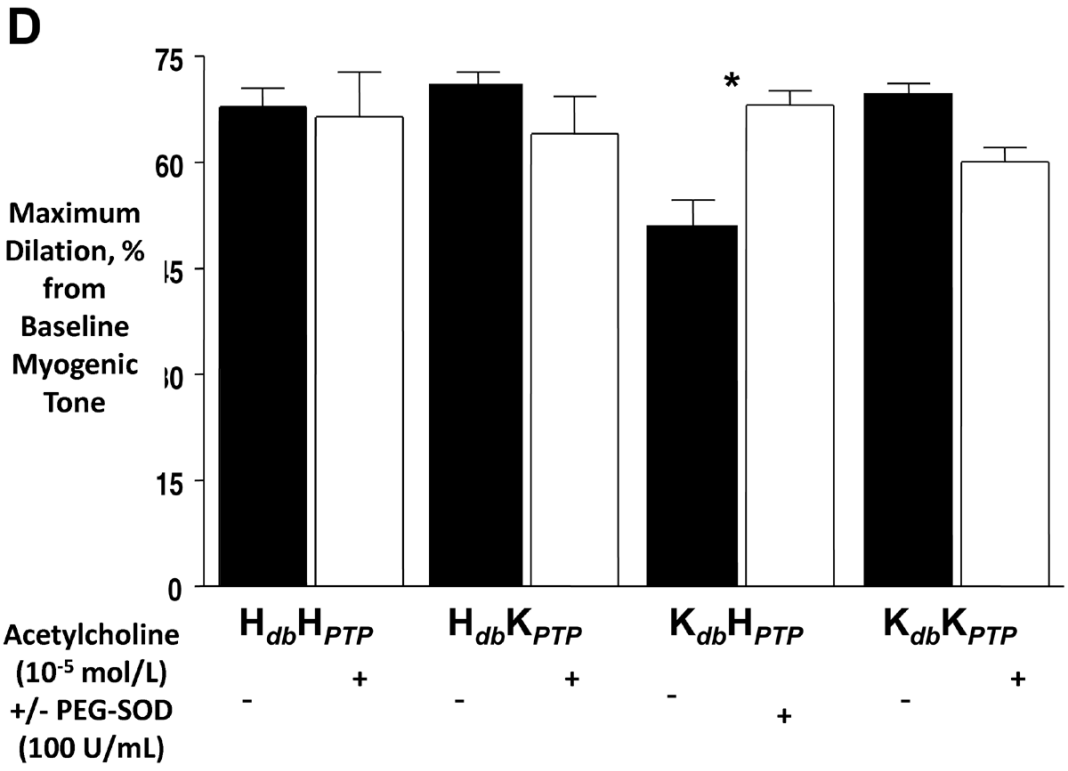
Figure 1.

The PTP1B gene was significantly upregulated in obese, insulin resistant mice compared to control in muscle (1B) and fat (1C) but not hepatic tissues (1A). PTP1B gene deletion markedly improves insulin receptor tyrosine phosphorylation in muscle (1E) and fat (1F) of dual KO animals compared to obese, insulin resistant animals. Dual KO hepatic tissue insulin resistance persisted (1D). Control animals and PTP1B KO lean animals displayed similar in insulin receptor signaling in all tissues.

[A-F: * = $p < 0.05$ vs. $H_{db}H_{PTP}$, $n > 5$]







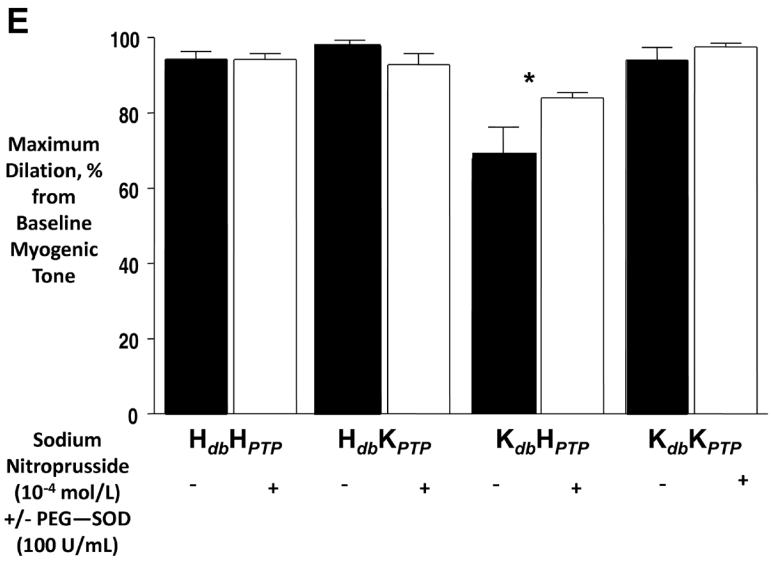
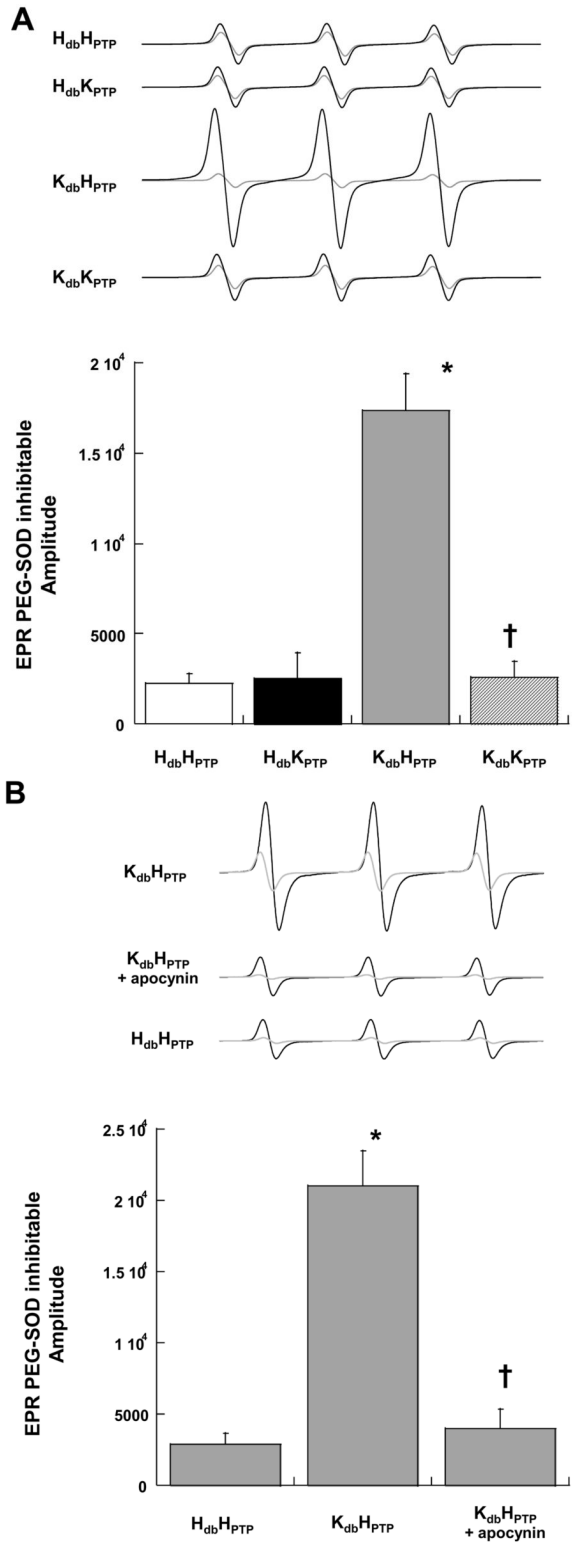


Figure 2.

Obese, insulin resistant mice ($K_{db}H_{PTP}$) demonstrated a significant decrease in endothelium dependent, acetylcholine-mediated dilation (2A) and endothelium independent, SNP-mediated dilation (2B) that is ameliorated by PTP1B gene deletion. Endothelium dependent dilation was nearly abolished by L-NAME (2C). Superoxide scavenging produced a complete restoration of acetylcholine-mediated dilation in obese, insulin resistant mice (2D). Superoxide dismutase incubation improves endothelium independent nitric oxide-mediated dilation in $K_{db}H_{PTP}$ (2E). [A, B: * = $p < 0.05$ $K_{db}H_{PTP}$ vs. $H_{db}H_{PTP}$, $n > 5$; C: * = $p < 0.05$ (+) L-NAME vs. (−) L-NAME for each mouse, $n > 5$; D, E: * = $p < 0.05$ (+) PEG-SOD vs. (−) PEG- SOD, $n > 5$]



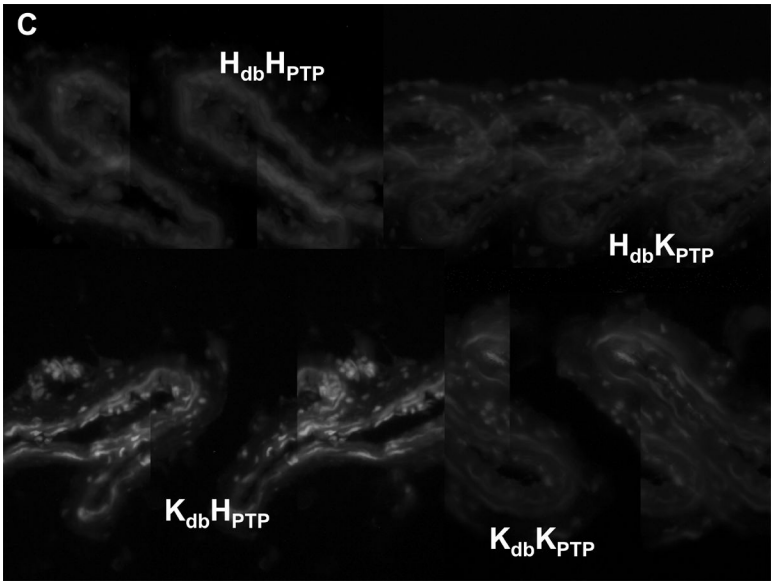


Figure 3.

EPR spectroscopy revealed a 5-fold increase in superoxide signal from obese insulin resistant mice, while PTP1B gene deletion ameliorates this increase (3A). Acetovanillone (apocynin) incubation of $K_{db}H_{PTP}$ samples decreased the superoxide signal to levels seen in controls (3B). Dihydroethidium staining revealed broad localization of superoxide signal throughout the arteries of $K_{db}H_{PTP}$ mice that is absent in all other mice.

[A, B: * = $p < 0.05$ $K_{db}H_{PTP}$ vs. $H_{db}H_{PTP}$, $n > 6$; † = $p < 0.05$ $K_{db}K_{PTP}$ vs. $K_{db}H_{PTP}$, $n > 6$]

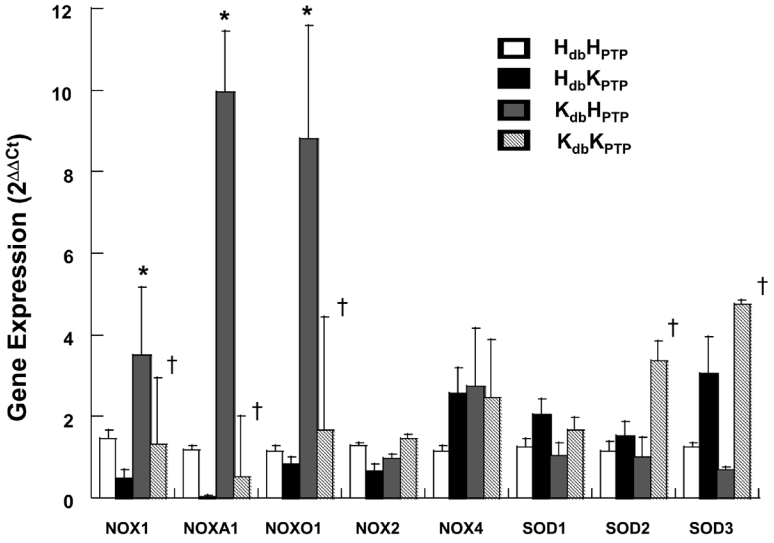


Figure 4.

Gene expression of superoxide-generating and anti-oxidant defense enzymes were assessed using quantitative Real-Time RT-PCR. $K_{db}H_{PTP}$ mice showed major increases in Nox1, Noxa1 and Noxo1 gene expression.

[* = $p < 0.05$ all genotypes vs. $H_{db}H_{PTP}$, $n > 6$; † = $p < 0.05$ $K_{db}K_{PTP}$ vs. $K_{db}H_{PTP}$, $n > 6$]

Table 1

Metabolic phenotyping of fasted male dual KO mice yielded marked improvements in several indices of insulin sensitivity. n > 10 for each measurement.

Parameter	$H_{db}H_{PTP}$	$K_{db}H_{PTP}$	$H_{db}K_{PTP}$	$K_{db}K_{PTP}$
Body Weight (gms)	*29±1*	*57±2*[*]	*26±1*	*53±3*[*]
Food Intake(gms/day)	*2.5±0.4*	*5.3±0.7*[*]	*2.4±0.5*	*4.6±0.8*[*]
Water Intake(ml/day)	*4±1*	*9±2*[*]	*4±1*	*8±1*[*]
Urine Output (ml/day)	*1±.02*	*3.2±.07*[*]	*0.8±.01*	*3.0±.07*[*]
Plasma Glucose (mg/dl)	*108±9*	*170±27*[*]	*120±7*	*172±13*[*]
HbA1c (%)	*4.8±.01*	*10.2±0.2*[*]	*4.9±0.07*	*7.6±0.2*[*,**]
% of glucose bolus cleared in 30 minutes (%)	*68±6*	*24±10*[*]	*87±6*	*87±10*[**]
Plasma Insulin (ng/ml)	*0.48±0.07*	*5.8±0.8*[*]	*0.29±0.08*[*]	*5.1±0.6*[*]
Plasma Cholesterol (mg/dl)	*73±5*	*143±13*[*]	*78±7*	*124±20*[*]
Plasma Triglyerides (mg/dl)	*47±5*	*145±31*[*]	*35±12*	*51±11*[**]
Non-Esterified Fatty Acids(mEq/ml)	*0.49±0.06*	*0.81±0.1*[*]	*0.51±0.07*	*0.34±0.05*[**]
Plasma Leptin (pg/ml)	*147±38*	*1075±300*[*]	*80±21*[*]	*1105±215*[*]

[*] = $p < 0.05$ all other genotypes vs. lean controls (HdbHPTP)

[**] = $p < 0.05$ vs. KdbHPTP