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Defective Natriuretic Peptide Receptor Signaling in Skeletal Muscle Links Obesity to Type 2 Diabetes

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Circulating natriuretic peptide (NP) levels are reduced in obesity and predict the risk of type 2 diabetes (T2D). Since skeletal muscle was recently shown as a key target tissue of NP, we aimed to investigate muscle NP receptor (NPR) expression in the context of obesity and T2D. Muscle NPRA correlated positively with whole-body insulin sensitivity in humans and was strikingly downregulated in obese subjects and recovered in response to diet-induced weight loss. In addition, muscle NP clearance receptor (NPRC) increased in individuals with impaired glucose tolerance and T2D. Similar results were found in obese diabetic mice. Although no acute effect of brain NP (BNP) on insulin sensitivity was observed in lean mice, chronic BNP infusion improved blood glucose control and insulin sensitivity in skeletal muscle of obese and diabetic mice. This occurred in parallel with a reduced lipotoxic pressure in skeletal muscle due to an upregulation of lipid oxidative capacity. In addition, chronic NP treatment in human primary myotubes increased lipid oxidation in a PGC1α-dependent manner and reduced palmitate-induced lipotoxicity. Collectively, our data show that activation of NPRA signaling in skeletal muscle is important for the maintenance of long-term insulin sensitivity and has the potential to treat obesity-related metabolic disorders.

Obesity is a major risk factor of type 2 diabetes (T2D) and cardiovascular diseases (1,2). Although multiple hypotheses have been proposed, the link between obesity and the risk of T2D is still poorly understood. Over the last decade, several large cohort studies reported an inverse association between plasma natriuretic peptide (NP) levels and BMI (3,4), and the risk of T2D (5,6). Therefore, dysregulation of the NP system, referred to as the "NP handicap," might be an important factor in the initiation and progression of metabolic dysfunction, making NPs potential candidates linking obesity and T2D (7–10).

NPs, including atrial NP (ANP) and brain NP (BNP), are mainly known as heart hormones secreted in response to cardiac overload and mechanical stretch in order to regulate blood volume and pressure (11,12). ANP and BNP classically bind to a biologically active receptor A (NP receptor A [NPRA]) that promotes cGMP signaling (13). They are also quickly cleared from the circulation and degraded through NP clearance receptor (NPRC). The NPRA-to-NPRC ratio therefore controls the biological activity of NP at the target tissue level (14).

Besides their well-documented role in the cardiovascular system, several studies revealed a metabolic role of NP (15,16). Pioneering studies demonstrated a potent lipolytic role of these peptides in human adipose tissue (17,18), and more recent studies indicated they may play a role in the "browning" of human white fat cells (19) as well as in favoring fat oxidative capacity in human skeletal muscle cells (20). The underlying mechanism involves activation of cGMP signaling, induction of PGC1α (peroxisome

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proliferator–activated receptor γ coactivator-1α), and enhancement of mitochondrial respiration. Together these studies argue for an important role of NP in the regulation of whole-body energy metabolism. The lipolytic effect of NP is absent in mice naturally expressing high levels of NPRC in adipose tissue (19,21). However mice overexpressing BNP are protected from diet-induced obesity and insulin resistance, which suggests that the protective effect of NP is achieved by targeting other metabolic tissues such as skeletal muscles (22). We therefore hypothesized that a downregulation of NPRA and/or an upregulation of NPRC in skeletal muscle could contribute to the "NP handicap" and provide a novel pathophysiological and mechanistic link between obesity and T2D.

In the current study, through a comprehensive set of experiments in humans, mouse models of obesity and T2D, and human primary skeletal muscle cells, we demonstrated a pathophysiological link between obesity-induced insulin resistance and T2D and defective skeletal muscle NPR signaling. In addition, increasing circulating BNP levels in diabetic and high-fat diet (HFD)–fed mice improved blood glucose control and insulin sensitivity. These effects were accompanied by improved muscle insulin signaling resulting from reduced lipotoxic lipid pressure and elevated lipid oxidative capacity.

RESEARCH DESIGN AND METHODS

Clinical Studies and Human Subjects

Muscle biopsy samples from lean, obese with normal glucose tolerance, obese with impaired glucose tolerance (IGT), and obese subjects with T2D were obtained from three independent clinical studies. Study 1 included nine young lean and nine young obese subjects (Fig. 1*A–D*) (23). Study 2 included four middle-aged obese subjects with T2D and six with IGT at baseline and in response to 12 weeks of calorie restriction to induce weight loss and improve metabolic health (Fig. 1*E* and *F*) (24). Study 3 included 21 subjects with normal glucose tolerance but a wide range of body fat (Supplementary Fig. 1) (25). The clinical characteristics of the subjects are summarized in Supplementary Table 1. All volunteers gave written informed consent and the protocol was approved by an institutional review board. Studies were performed according to the latest version of the Declaration of Helsinki and the Current International Conference on Harmonization guidelines. Samples of vastus lateralis weighing 60–100 mg were obtained by muscle biopsy using the Bergström technique, blotted, cleaned, and snap frozen in liquid nitrogen (26). Insulin sensitivity was measured by hyperinsulinemic-euglycemic clamp after an overnight fast (27). An intravenous catheter was placed in an antecubital vein for infusion

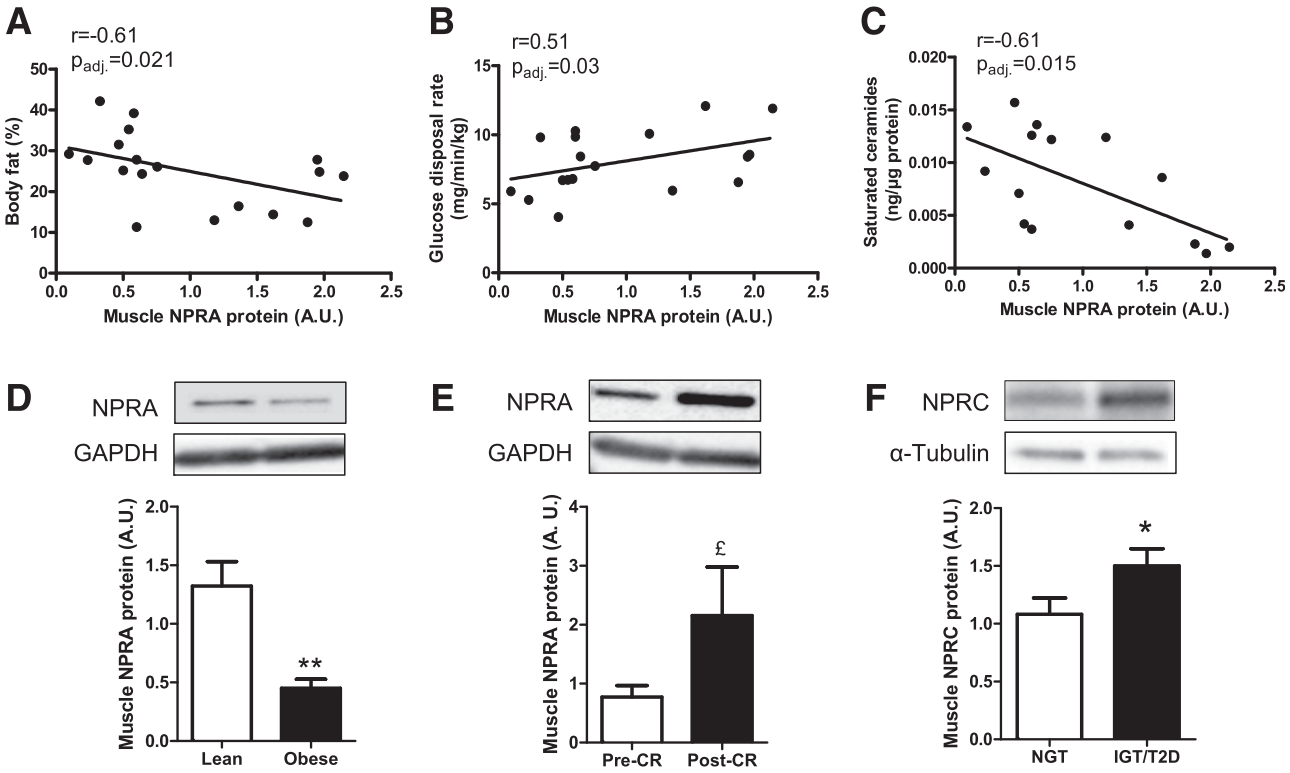

Figure 1—Skeletal muscle NPR expression relates to insulin sensitivity in humans. Correlation between vastus lateralis NPRA protein expression and percent body fat (*A*), glucose disposal rate measured by euglycemic-hyperinsulinemic clamp (*B*), and muscle-saturated ceramide content (*C*) (n = 15–20). *D*: NPRA protein levels in skeletal muscle of lean and obese subjects (*D*) and in obese subjects pre- and postcalorie restriction (pre-CR and post-CR) (*E*). *F*: NPRC protein levels in skeletal muscle of obese subjects with normal glucose tolerance (NGT) and with IGT and T2D. $^{**}P < 0.01$ vs. lean; £$P = 0.06$ vs. pre-CR; $^{*}P < 0.05$ vs. NGT (n = 6–10 per group).

of glucose and insulin during the clamp. A second catheter was placed retrograde in a dorsal vein of the contralateral hand for blood withdrawal. The hand was placed in a plastic heated box at ~60°C for arterialization of venous blood. Three blood samples were drawn before the initiation of insulin and glucose for the clamp and during the last 30 min of the clamp. A primed infusion of regular insulin (80 mU · min^{-1} · m^{-2}) was initiated and continued for 2 h. Plasma glucose was clamped at 90 mg/dL in all participants. Arterialized plasma glucose was measured at 5-min intervals and a variable infusion of exogenous glucose (20% solution) was given to maintain plasma glucose concentration. Plasma glucose was analyzed with a YSI 2300 STAT glucose analyzer (YSI Inc., Yellow Springs, OH), and plasma insulin was measured using an ultrasensitive ELISA kit (ALPCO Diagnostics, Salem, NH). Glucose disposal rate was adjusted by kilograms of fat-free mass. Body composition (considering a three-compartment model) was determined using a total-body dual-energy X-ray absorptiometer (DPX, Software 3.6; Lunar Radiation Corp., Madison, WI).

Mice and Diets

Five-week-old male diabetes-prone, obese *db/db* mice of the C57BL/KsJ-lept^{db}-lept^{db} strain with their nondiabetic lean littermate control *db/+* were used. For HFD studies, we used regular C57BL/6J male mice (JANVIER LABS). The mice were housed in a pathogen-free barrier facility (12-h light/dark cycle) with ad libitum access to water and food. After weaning, *db/db* and *db/+* mice were fed a normal chow diet (A04; SAFE Diets) for 4 weeks. C57BL/6J mice were fed for 16 weeks either a normal chow diet (10% energy as fat, D12450J; Research Diets, Inc., New Brunswick, NJ) or HFD containing 60% kcal from fat (D12492; Research Diets, Inc.). All experimental procedures were approved by a local institutional animal care and use committee and performed according to INSERM guidelines for the care and use of laboratory animals.

BNP Infusion Studies

Mice were randomly assigned to receive a saline vehicle (NaCl 0.9%) and/or chronic rat/mouse BNP1-32 (B9901; Sigma-Aldrich) at a rate of 5 or 10 ng/kg/min. Treatments were chronically administered intraperitoneally with mini-osmotic pumps (model 1004; Alzet, Cupertino, CA) (28). Mini-pumps were placed after 12 weeks of HFD and treatment was administered for 4 weeks in C57BL/6J mice and at 6 weeks of age in *db/db* mice. Body weight was measured weekly and body composition was assessed by quantitative nuclear magnetic resonance imaging (EchoMRI 3-in-1 system; Echo Medical Systems).

Glucose and Insulin Tolerance Tests

Six hour–fasted mice were injected intraperitoneally with a bolus of D-glucose at 2 g/kg (Sigma-Aldrich, Saint-Quentin Fallavier, France) and insulin 0.5 units/kg (Insuman Rapid; Sanofi, Paris, France) for glucose and insulin tolerance tests (GTT and ITT), respectively (29). Blood glucose levels were monitored from the tip of the tail with a glucometer (Accucheck; Roche, Meylan, France) at 0, 15, 30, 45, 60, and 90 min after injection. Radiolabeled GTTs were performed as previously described (29).

Blood Analyses and Tissue Collection

After an overnight fast, mice were decapitated and blood was collected into tubes containing EDTA and protease inhibitors. Organs and tissues were rapidly excised and snap frozen in liquid nitrogen before being stored at −80°C. Blood glucose was assayed using the glucose oxidase technique (Biomérieux, Paris, France), and plasma insulin was measured using an ultrasensitive ELISA kit (ALPCO Diagnostics). Plasma BNP was measured using the RayBio BNP Enzyme Immunoassay Kit (RayBiotech, Inc., Norcross, GA). HbA_{1c} and fructosamines were determined using a PENTRA 400 multianalyzer.

Human Skeletal Muscle Cell Culture

Satellite cells from rectus abdominis biopsies of healthy subjects with normal glucose tolerance (age 34.3 ± 2.5 years, BMI 26.0 ± 1.4 kg/m^2, fasting glucose 5.0 ± 0.2 mmol/L) were grown in DMEM supplemented with 10% FBS and growth factors (human epidermal growth factor, BSA, dexamethasone, gentamycin, fungizone, and fetuin) as previously described (23,30). Myotubes were differentiated up to 5 days and were treated with 100 nmol/L human ANP (A1663; Sigma-Aldrich) or BNP (B5900; Sigma-Aldrich) every day for the last 3 days.

Determination of Fatty Acid Metabolism

Pulse-chase experiments to determine lipolytic flux and oleate incorporation into total lipids, triacylglycerols (TAGs), and diacylglycerols (DAGs) by thin-layer chromatography were performed as previously described (31). Incorporation rates were normalized to total protein content in each well. Palmitate oxidation rates were measured as described previously (25).

Lipid Intermediate Determination

TAG and DAG content were measured by gas chromatography and ceramide and sphingomyelin species by high-performance liquid chromatography–mass spectrometry after total lipid extraction as previously described for mouse and human muscle tissues (25,29).

Western Blot Analysis

Soleus and gastrocnemius skeletal muscles, white and brown adipose tissues, and myotubes were homogenized in a buffer containing 50 mmol/L HEPES, pH 7.4, 2 mmol/L EDTA, 150 mmol/L NaCl, 30 mmol/L $NaPO_4$, 10 mmol/L NaF, 1% Triton X-100, 10 μL/mL protease inhibitor (Sigma-Aldrich), 10 μL/mL phosphatase I inhibitor (Sigma-Aldrich), 10 μL/mL phosphatase II inhibitor (Sigma-Aldrich), and 1.5 mg/mL benzamidine HCl. Tissue homogenates were centrifuged for 25 min at 15,000*g*, and supernatants were stored at −80°C. Solubilized proteins (30–40 μg) were run on a 4–20% SDS-PAGE (Bio-Rad, Hercules, CA), transferred onto nitrocellulose membrane (Hybond ECL; Amersham Biosciences), and blotted with the following primary antibodies: NPRA (Abcam), NPRC (Sigma-Aldrich), Akt, phospho-Akt

Ser473, p-Thr180/Tyr182-p38 MAPK, p38 MAPK, HSL, phospho-HSL Ser660, and phospho-HSL Ser565 (all Cell Signaling Technology, Beverly, MA). Subsequently, immunoreactive proteins were blotted with secondary horseradish peroxidase–coupled antibodies and revealed by enhanced chemiluminescence reagent (SuperSignal West Dura or SuperSignal West Femto; Thermo Scientific), visualized using the ChemiDoc MP Imaging System, and data analyzed using the Image Laboratory 4.1 version software (Bio-Rad). GAPDH (Cell Signaling Technology) and α-tubulin (Sigma-Aldrich) were used as internal controls for skeletal muscle and myotubes, and β-actin (Cell Signaling Technology) was used as internal control for adipose tissues.

Real-Time qRT-PCR

Total from tissues and primary myotubes were processed for RNA extraction using the RNeasy RNA mini kit (Qiagen GmbH, Hilden, Germany). After reverse transcription of total RNA (1 μg), samples were analyzed on a StepOnePLus real-time PCR system (Applied Biosystems). All primers were obtained from Applied Biosystems and presented in Supplementary Table 5. All expression data were normalized by the $2^{(\Delta Ct)}$ method using 18S as internal control.

Statistics

Statistical analyses were performed using GraphPad Prism 5.0 for Windows (GraphPad Software Inc.). Normal distribution and homogeneity of variance of the data were tested using Shapiro-Wilk and F tests, respectively. One-way ANOVA followed by Tukey post hoc tests and Student *t* tests were performed to determine differences between groups, interventions, and treatments. Two-way ANOVA followed by Bonferonni post hoc tests were applied when appropriate. Linear regression was performed after log transformation of nonparametric data. The false discovery rate for multiple testing was controlled by the Benjamini-Hochberg procedure with $P_{adj.}$ values ≤ 0.05 as threshold. All values in figures and tables are presented as mean ± SEM. Statistical significance was set at $P < 0.05$.

RESULTS

Muscle NPRA and NPRC Proteins Relate to Insulin Sensitivity in Humans

Muscle NPRA protein expression was investigated in human vastus lateralis biopsies of healthy volunteers with varying degrees of body fat and insulin sensitivity. We observed that muscle NPRA protein was inversely related to body fat (Fig. 1*A* and Supplementary Fig. 1*A*), BMI, fasting insulin, and indices of insulin resistance (Supplementary Table 2). In addition, muscle NPRA correlated positively with whole-body insulin sensitivity measured by euglycemic-hyperinsulinemic clamp (Fig. 1*B*) and the insulin sensitivity index (Supplementary Fig. 1*B*) and negatively with total muscle saturated ceramide content (Fig. 1*C*). Importantly, muscle NPRA protein content was significantly reduced (−65%) in obese subjects when compared with age-matched lean subjects (Fig. 1*D*). Conversely, muscle NPRA protein was upregulated (1.8-fold) together with insulin sensitivity (+37%, 5.4 ± 0.6 vs. 7.4 ± 1.1 mg/min/kg for pre- and postcalorie restriction, respectively, $P = 0.03$) in obese subjects with IGT in response to diet-induced weight loss (Fig. 1*E*). Finally, muscle NPRC protein content was unchanged in obese versus lean individuals with normal glucose tolerance (0.41 ± 0.08 vs. 0.29 ± 0.07 arbitrary units [A.U.], not significant) but increased significantly in obese individuals with IGT and T2D (Fig. 1*F*). The ratio of NPRA to NPRC protein was significantly reduced in obese versus lean subjects (2.1 ± 0.3 vs. 3.6 ± 0.2 A.U., respectively, $P = 0.0005$) and increased in obese subjects in response to calorie restriction (0.38 ± 0.16 vs. 0.14 ± 0.04 A.U., respectively, $P = 0.08$). Together this suggests that skeletal muscle NPR expression relates to insulin sensitivity in humans and is altered in obesity and T2D.

Impaired NPRA Expression in Skeletal Muscle and Fat of Diet-Induced Obese Mice

Since both skeletal muscle and adipose tissue are known as key target tissues of NP, both in humans and mice, we further examined NPR expression in metabolic tissues of chow-fed versus HFD-fed mice. In line with human data, we found a significant downregulation of NPRA protein in skeletal muscle (Fig. 2*A* and *D*), as well as in white (Fig. 2*B* and *D*) and brown fat (Fig. 2*C* and *D*) of HFD-fed mice. No significant change in NPRC protein content was found in skeletal muscle and brown fat, whereas NPRC protein decreased significantly in white fat (0.48 ± 0.08 vs. 0.14 ± 0.04 A.U. for chow and HFD, respectively, $P < 0.05$). Plasma BNP levels were unchanged in HFD-fed mice compared with chow-fed mice (Fig. 2*E*). Collectively, as in humans, our data indicate a reduced NPRA expression in skeletal muscle of obese mice.

Chronic BNP Infusion Protects Against HFD-Mediated Obesity and Glucose Intolerance

Since muscle NPRA is associated with insulin sensitivity in humans, we assessed the effect of acute and chronic BNP infusions on glucose tolerance and insulin sensitivity in chow-fed and HFD-fed mice. Acute intraperitoneal BNP injection did not affect fasting blood glucose levels over a time course of 30 min (Supplementary Fig. 2*A*) and had no effect on glucose excursion during an intraperitoneal GTT (Supplementary Fig. 2*B*). No effect of acute BNP injection was also seen on glucose disposal in skeletal muscle (Supplementary Fig. 2*C*). We further assessed the influence of acute NP treatment on basal and insulin-stimulated glucose uptake in human primary skeletal muscle cells. No effect of increasing doses of ANP and BNP on glucose uptake was observed (Supplementary Fig. 3). From these data we concluded that NPRA signaling does not acutely modulate glucose uptake in skeletal muscle.

Based on a previous study (32), we next infused BNP at a dose of 5 ng/kg/min, which raised plasma BNP levels by ∼40% (data not shown). BNP-treated mice had a similar body weight (Fig. 3*A*) and body composition (Fig. 3*B*) after

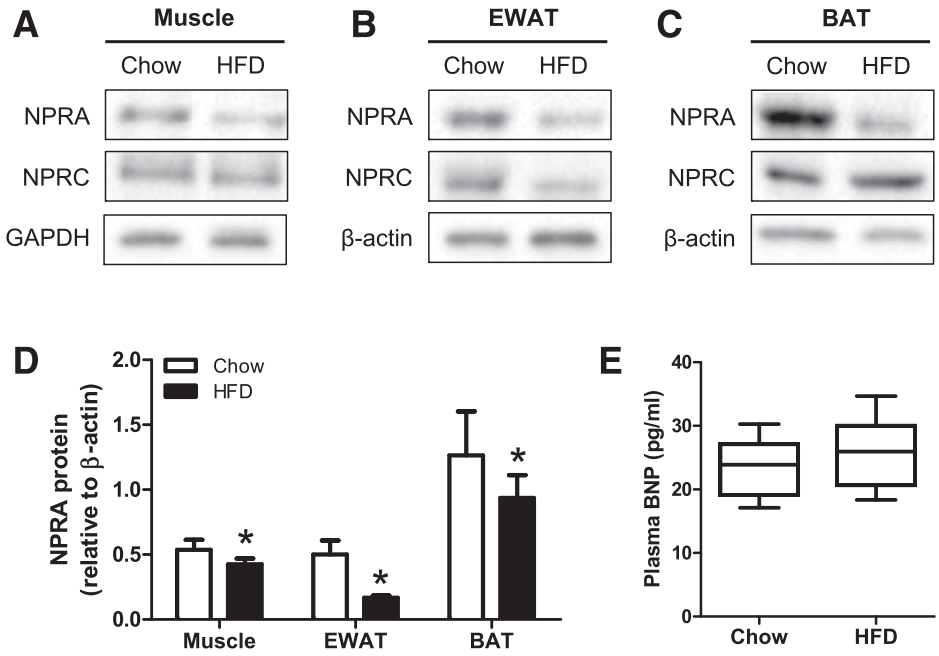

Figure 2—Defective NPR expression in metabolic tissues of diet-induced obese mice. Representative blots of NPRA and NPRC proteins in skeletal muscle (*A*), epididymal white adipose tissue (EWAT) (*B*), and brown adipose tissue (BAT) (*C*) of chow-fed and HFD-fed mice. Quantitative bar graph of NPRA protein (*D*) and overnight fasting plasma BNP (*E*) levels in chow and HFD-fed mice. *$P < 0.05$ vs. chow-fed mice (n = 8–10 per group).

saline and BNP treatment. Chronic BNP treatment significantly reduced fasting blood glucose levels in mice fed an HFD for 8 or 12 weeks (Fig. 3*C*). Lower blood glucose in the fasting state was also accompanied by improved glucose tolerance (Fig. 3*D*) despite no change in fasting and peak insulin at 15 min during the intraperitoneal GTT (Fig. 3*E*). In conclusion, whereas acute BNP treatment has no effect on insulin sensitivity, chronic BNP treatment improves glucose tolerance in HFD-fed mice.

Impaired NPR Signaling in Skeletal Muscle of Obese Diabetic Mice Contributes to the "NP Handicap"

We next examined NPR expression in metabolic tissues from leptin receptor–deficient mice (*db*/*db*) that become spontaneously obese and T2D by the age of 8 weeks. In line with data in human skeletal muscle and HFD-fed mice, NPRA protein was downregulated in white (Fig. 4*B–D*) and brown fat (Fig. 4*C* and *D*) of *db*/*db* mice compared with control *db*/+ mice. In agreement with data in individuals with IGT/T2D (Fig. 1*E*), we noted a remarkable upregulation of NPRC in skeletal muscle (Fig. 4*A–E*), as well as in white (Fig. 4*B–E*) and brown (Fig. 4*C–E*) fat of *db*/*db* mice. Overall the NPRA-to-NPRC protein ratio was markedly downregulated in muscle and fat of *db*/*db* mice (Fig. 4*F*) and was associated with dramatically lower levels of plasma BNP in *db*/*db* mice (−80%, $P < 0.05$) (Fig. 4*G*). This was also associated with a remarkable downregulation of p38 MAPK phosphorylation in skeletal muscle of *db*/*db* mice (−55%, $P < 0.001$) (Supplementary Fig. 4). Importantly, muscle NPRC was negatively correlated with plasma BNP levels (Supplementary Table 3). These changes in NPR signaling and plasma NP characterized an "NP handicap" of *db*/*db* mice. No association was found between white and brown fat NPRC protein and plasma BNP levels (data not shown). However, muscle NPRC was positively related to fasting blood glucose, insulin, and HbA_{1c} (Supplementary Table 4), again suggesting a link between defective skeletal muscle NPR signaling and impaired glucose control. Collectively, these data suggest that obesity and T2D are accompanied by profound changes in NPR expression and signaling in skeletal muscle, which may contribute to reduced plasma BNP levels.

Chronic BNP Infusion Improves Blood Glucose Control in Obese Diabetic Mice

We next studied the influence of chronic (4 weeks) BNP infusion on blood glucose control in *db*/*db* mice. BNP was infused at a dose of 10 ng/kg/min to induce a nearly twofold increase in plasma BNP levels with the goal of rescuing the "NP handicap." Despite no change in body weight (Fig. 5*A*) and composition (Fig. 5*B*), BNP-treated *db*/*db* mice displayed significantly improved blood glucose control, with reduced fasting plasma glucose (−21%) (Fig. 5*C*) and HbA_{1c} (−17%) (Fig. 5*D*). This improved blood glucose control occurred in the absence of noticeable changes in fasting insulin (Fig. 5*E*). In addition, insulin tolerance (Fig. 5*F*) and insulin responsiveness (area above the curve during the ITT, +36%, $P = 0.08$) were improved in BNP-treated mice. In summary, chronic BNP treatment improves blood glucose control and peripheral insulin sensitivity in obese diabetic mice independently of changes in body weight, thus suggesting a direct effect of NP on metabolic organs.

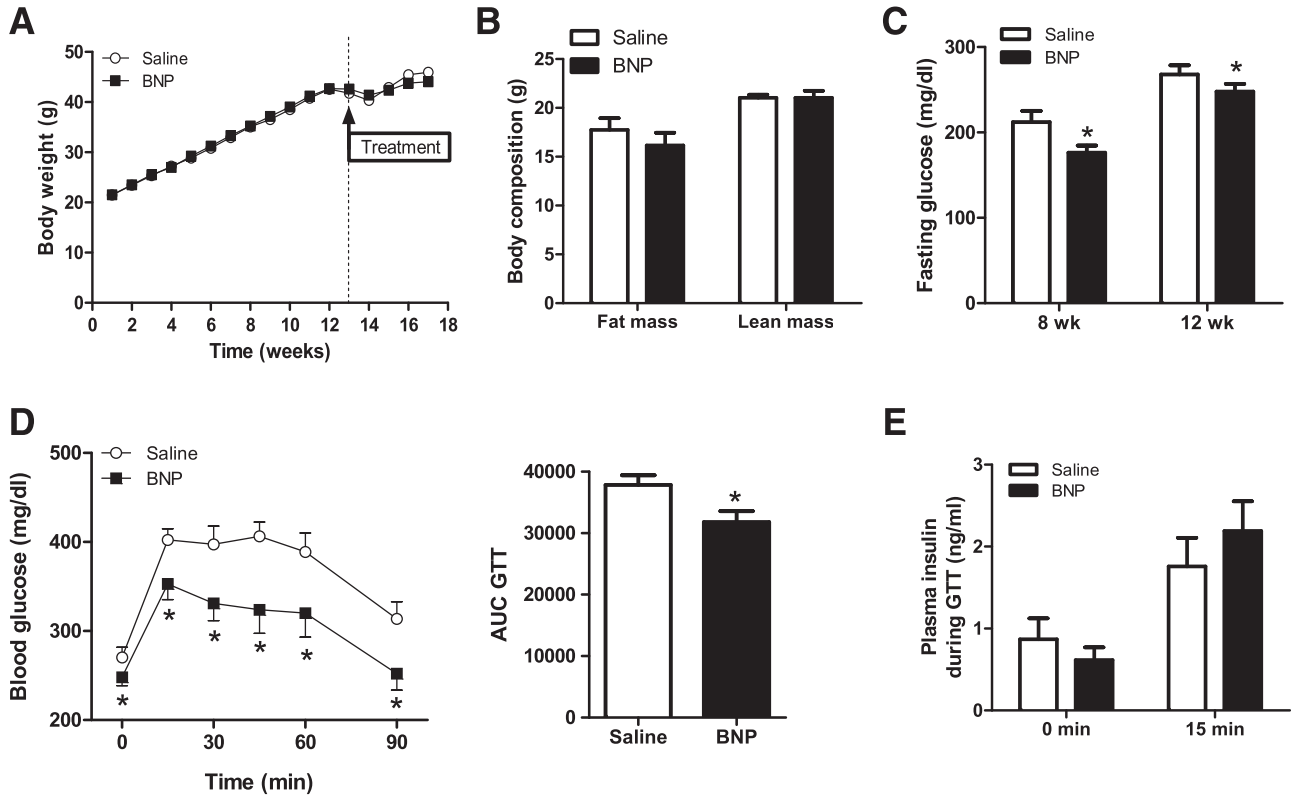

Figure 3—Chronic BNP infusion protects from HFD-induced obesity and glucose intolerance. C57BL/6J mice were treated for 4 weeks with saline (0.9% NaCl) or with BNP (5 ng/kg/min) via mini-osmotic pumps after 12 weeks of HFD. *A*: Follow-up of body weight during HFD and after mini-pump was placed. *B*: Body composition at the end of treatment in saline- and BNP-treated obese mice. *C*: Overnight fasting blood glucose in BNP-treated mice after 8 and 12 weeks of HFD. *D*: Time course of blood glucose levels during an intraperitoneal GTT and corresponding area under the curve (AUC). *E*: Plasma insulin after a 6-h fast (0 min) and 15 min after glucose bolus injection. *$P < 0.05$ vs. saline (n = 8–10).

Enhanced Insulin Signaling, Reduced Lipotoxicity, and Increased Lipid Oxidative Capacity in Skeletal Muscle of BNP-Treated Mice

We next studied the mechanism by which chronic BNP treatment improved blood glucose control and muscle insulin sensitivity in both HFD-fed and *db/db* mice. Insulin sensitivity is inhibited by the accumulation of toxic lipids such as DAGs and ceramides in skeletal muscle and liver (33,34). No significant change in total DAG and ceramides was found in the liver of BNP-treated *db/db* (Supplementary Fig. 5*A* and *B*) and HFD-fed mice (Supplementary Fig. 6*A* and *B*). No change as well in mRNA levels of genes involved in fat oxidation and glucose metabolism was observed after BNP infusion in the liver of *db/db* (Supplementary Fig. 5*C*) and HFD-fed mice (Supplementary Fig. 6*C*). Similarly, no change in the expression level of classical thermogenic genes in brown and white fat depots was observed in BNP-treated *db/db* (Supplementary Fig. 7) and HFD-fed mice (Supplementary Fig. 8). No change in *Ucp1* mRNA levels was noted as well in inguinal white adipose tissue (data not shown). However, we observed a muscle-autonomous improvement of insulin-mediated Akt (46%, P = 0.02) and p38MAPK phosphorylation (278%, P = 0.06) (Fig. 6*A*), which was paralleled by a reduced content of total and main species (data not shown) of ceramides (−17%) (Fig. 6*B*) as well as total and main species (data not shown) of sphingomyelin (−19%) (Fig. 6*C*) in skeletal muscle of BNP-treated HFD-fed mice, as well as a reduced content of total ceramides in *db/db* mice (52.4 ± 4.4 vs. 40.0 ± 5.1 ng/μg protein for *db/+* and *db/db* mice, respectively, $P < 0.05$). The content of total and subspecies of DAGs was also reduced in BNP-treated HFD-fed mice (ANOVA $P < 0.05$) (Fig. 6*D*). This lower lipotoxic pressure was paralleled by an upregulation of muscle palmitate oxidation rate (+46%) (Fig. 6*E*) and of *PGC1α* mRNA levels in HFD-fed mice (Fig. 6*F*) and in *db/db* mice (+32%, P = 0.08). Collectively, the data indicate that chronic BNP treatment improves insulin sensitivity in skeletal muscle by reducing lipotoxicity and upregulating fat oxidative capacity in a PGC1α-dependent manner in obese and diabetic mice.

Chronic NP Treatment Reduces Lipotoxicity and Enhances Lipid Oxidative Capacity in Human Primary Myotubes

We previously demonstrated a functional NPR signaling in human primary myotubes (20). Because NP are known to activate lipolysis in human adipocytes (35,36), we

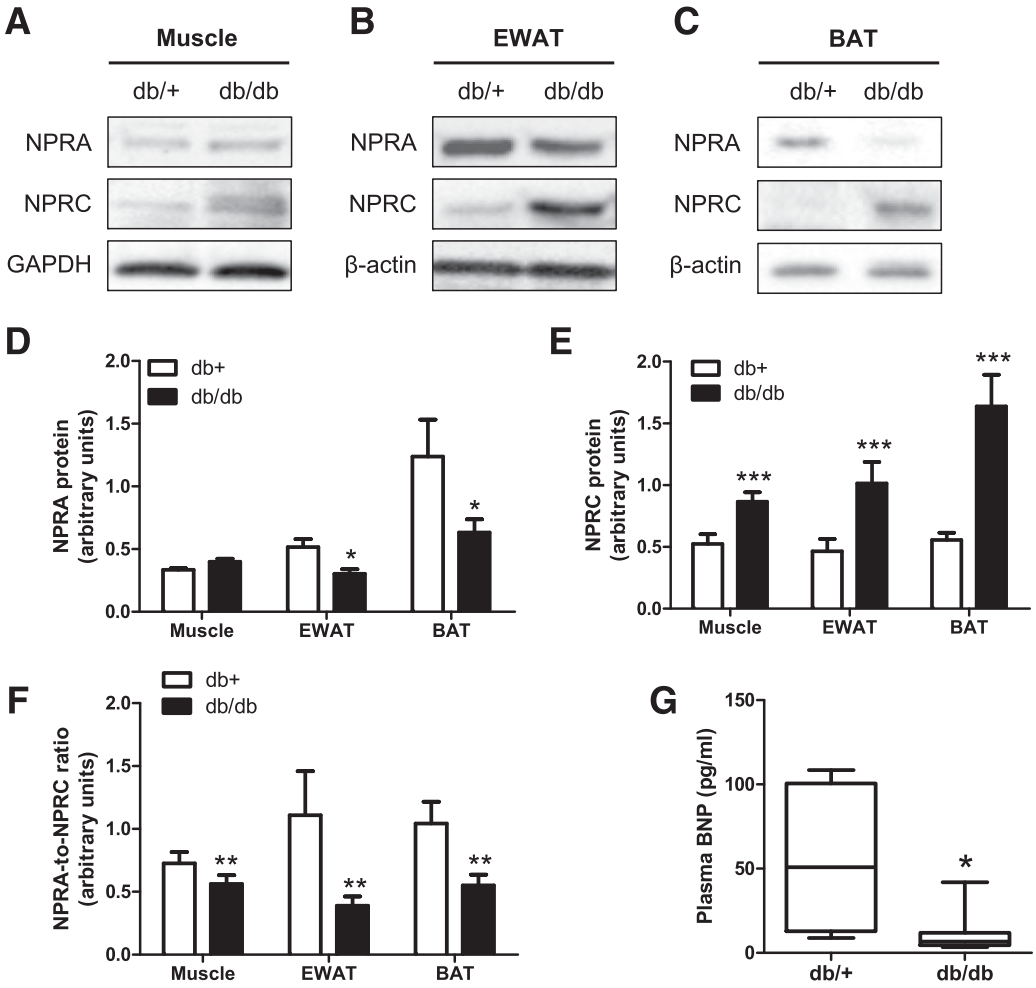

Figure 4—Defective NPR signaling in metabolic tissues of obese diabetic mice. Representative blots of NPRA and NPRC proteins in skeletal muscle (*A*), epididymal white adipose tissue (EWAT) (*B*), and brown adipose tissue (BAT) (*C*) of *db*/*db* and *db*/+ mice. Quantitative bar graph of NPRA (*D*), NPRC protein (*E*), NPRA-to-NPRC protein ratio (*F*), and overnight fasting plasma BNP levels in *db*/*db* and *db*/+ mice (*G*). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$ vs. *db*/+ mice (n = 8–10 per group).

studied here the acute effect of NP treatment on lipid metabolism. Acute treatment of myotubes with BNP did not influence lipid storage, endogenous TAG-derived fatty acid (FA) release (i.e., lipolysis) (Supplementary Fig. 9*A*), and endogenous TAG-derived FA oxidation (Supplementary Fig. 9*B*). We further tested whether NP could activate one of the rate-limiting enzymes of lipolysis. Acute BNP treatment of human myotubes did not influence hormone-sensitive lipase phosphorylation either on the activating Ser660 residue (Supplementary Fig. 9*C*) or on the inhibitory Ser565 residue (Supplementary Fig. 9*D*). In contrast, chronic treatment with NP for 3 days robustly reduced total lipid accumulation and total TAG and DAG content (one-way ANOVA $P < 0.001$) (Fig. 7*A*–*C*). In line with ex vivo muscle data in mice (Fig. 6), reduced lipid accumulation was concomitant with an upregulation of palmitate oxidation rate (+27 and +19%, respectively, for ANP and BNP treatment) (Fig. 7*D*), and a significant induction of *PGC1α* gene expression (Fig. 7*E*), which was independent of PPARδ activation (Fig. 7*F*). No change in myogenic gene expression and differentiation of myoblasts into myotubes was observed in response to chronic NP treatment (data not shown). Based on the findings that muscle NPRA protein relates inversely to saturated ceramide content in human skeletal muscle (Fig. 1*C*) and that chronic BNP treatment reduces ceramide content in skeletal muscle of HFD-fed mice (Fig. 6*B*), we assessed the influence of chronic NP treatment on ceramide content in human primary myotubes. No significant effect of NP treatment on the content of total ceramides and various ceramide species (Supplementary Fig. 10) was noticed in the basal condition with FA-free BSA treatment. When myotubes were challenged overnight with 500 μmol/L of palmitate/BSA to induce ceramide production (2.7-fold, $P = 0.001$), we observed a significant decrease of ~30% in total and various ceramide species analyzed in response to chronic ANP and BNP treatment (Fig. 7*G*). In summary, chronic NP treatment protects against lipotoxicity by upregulating lipid oxidative capacity in human primary myotubes.

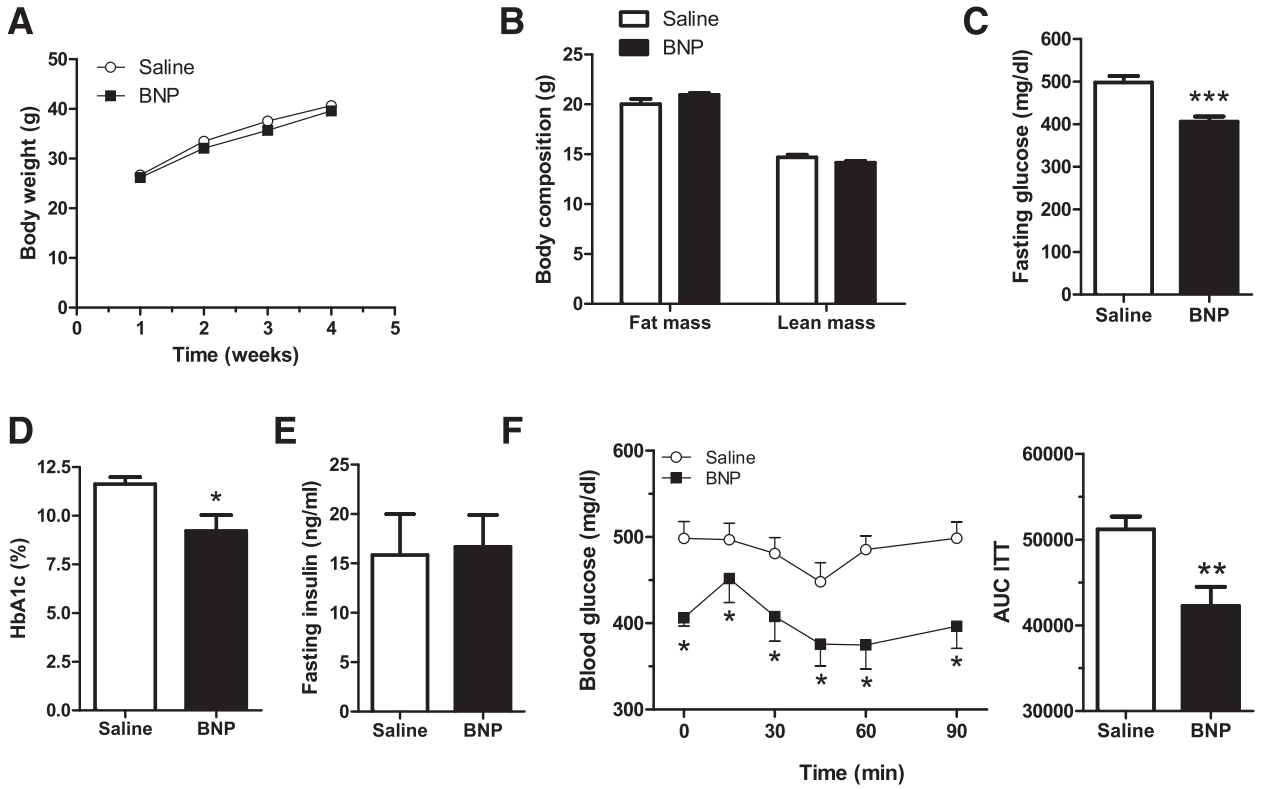

Figure 5—Chronic BNP infusion improves blood glucose control in obese diabetic mice. Five-week-old *db/db* mice were chronically treated for 4 weeks with saline (0.9% NaCl) or with BNP (10 ng/kg/min) via mini-osmotic pump. *A*: Follow-up of body weight over 4 weeks of treatment with saline or BNP. *B*: Body composition at the end of treatment. Overnight fasting blood glucose (*C*), HbA_{1c} (*D*), and overnight fasting insulin (*E*) were measured after 4 weeks of BNP treatment. *F*: Time course of blood glucose levels during an intraperitoneal ITT and corresponding area under the curve (AUC) after 4 weeks of treatment. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. saline-treated *db/db* mice (n = 8–10).

DISCUSSION

Although longitudinal prospective studies indicated that high baseline levels of plasma NP confer a reduced risk of developing T2D (5,6), no study so far had demonstrated a mechanistic link between NP biological activity and T2D. We believe our data provide the first evidence that NPRA signaling in skeletal muscle is necessary for the maintenance of long-term insulin sensitivity by regulating lipid oxidative capacity and metabolism (Fig. 8). Our data show for the first time that muscle NPRA signaling is impaired in the context of obesity and glucose intolerance in humans and mice. We also provide evidence that upregulation of NPRC in muscle tissue can contribute to the "NP handicap" observed in T2D. Last but not least, increasing NP levels in obese and diabetic mice, with the goal of rescuing the "NP handicap" and so a normal NPRA signaling tissue response, markedly improves blood glucose control and insulin sensitivity in skeletal muscle.

We first observed a significant positive association between muscle NPRA protein and insulin sensitivity measured by clamp in humans, at a dose that mainly reflects skeletal muscle insulin sensitivity. This observation is consistent with the negative association that we found between muscle NPRA and body fat, and between muscle NPRA and muscle total saturated ceramide content, two factors negatively influencing whole-body and muscle insulin sensitivity (33,34). To our knowledge, this is the first study reporting an association between skeletal muscle NPRA signaling and insulin sensitivity. This indicates that besides plasma NP levels, NPR signaling in skeletal muscle may influence insulin sensitivity. Additionally, muscle NPRA protein was dramatically downregulated in obese individuals while increased in response to diet-induced weight loss and related improvement in insulin sensitivity. Although the biological factors modulating muscle NPRA protein content were not investigated in the current study, the data suggest that muscle NPRA behaves as a determinant of insulin sensitivity. Moreover, upregulation of muscle NPRC as glucose tolerance deteriorates in obese subjects with IGT and T2D can further repress biological activation of muscle NPRA and contribute to the "NP handicap" in the long-term. Considering that muscle mass represents up to 40% of total body weight, even a moderate increase in muscle NPRC expression could largely reduce plasma NP levels by an increased rate of clearance. Muscle NPRC might be induced by high blood insulin levels in obese subjects as

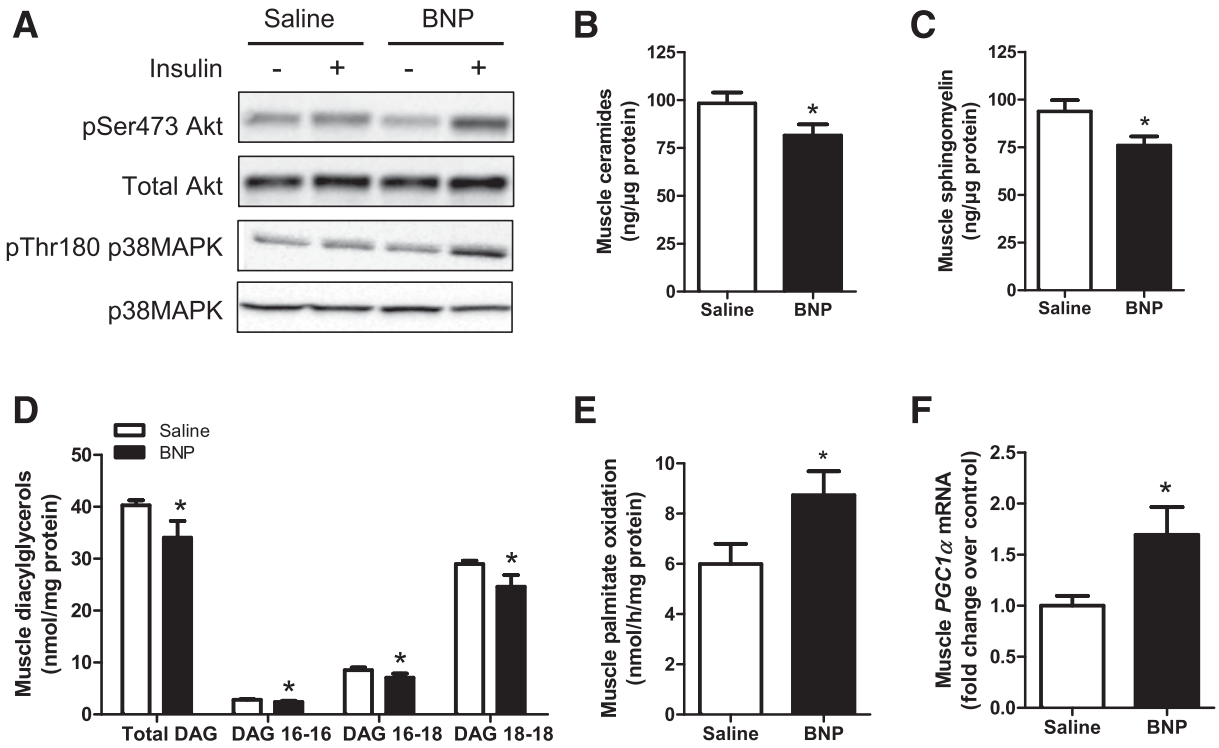

Figure 6—Muscle-autonomous improvement of insulin signaling and reduced lipotoxicity in skeletal muscle of BNP-treated obese mice. *A*: Extensor digitorum longus muscles were incubated ex vivo in absence (−) or presence of 100 nmol/L of insulin (+), and phosphorylated and total Akt and p38 MAPK were measured by Western blot. Total ceramides (*B*), total sphingomyelin (*C*), total and DAG subspecies content (*D*), ex vivo palmitate oxidation rate (*E*), and *PGC1α* gene expression (*F*) in skeletal muscle of HFD-fed mice treated with saline and BNP 5 ng/kg/min. *$P < 0.05$ vs. saline (n = 8–10).

glucose tolerance worsens independently of blood glucose concentrations as previously shown in adipose tissue (37). Although obese control and IGT/T2D were not age matched, increased expression of NPRC in skeletal muscle appeared independent of age since no correlation between age and muscle NPRC protein was found. Importantly, these findings in human muscle were largely replicated in obese diabetic mice. NPRC protein content was increased in skeletal muscle, white fat, and brown fat of obese diabetic mice, but only muscle NPRC protein negatively correlated with plasma BNP levels, reflecting that an increased plasma BNP clearance by the muscle can contribute to the "NP handicap" observed in these mice. Our data are in line with other studies demonstrating that elevated *NPRC* mRNA levels in white fat relate to metabolic dysfunction in mice and humans (22,38,39). Our data also provide a mechanistic understanding of the tight link observed between the NP handicap and insulin resistance independently of obesity in humans (40). The "NP handicap" concept is supported by the fact that the half-life of NP in the blood circulation is substantially increased in NPRC knockout mice and the biological activity of NP significantly increased in target tissues (41). Importantly, the altered NPRA-to-NPRC protein ratio in skeletal muscle was accompanied by a marked alteration of p38 MAPK phosphorylation in *db*/*db* vs. *db*/+ mice, thus indicating a potential signaling defect, considering that p38 MAPK is recognized as a canonical downstream molecular effector of the NPR signaling pathway (19).

Despite the observed link between muscle NPRA and insulin sensitivity, acute injection of BNP had no impact on fasting blood glucose, glucose tolerance, and muscle insulin sensitivity in mice. Furthermore, no acute effect of NP on glucose uptake was observed in human primary myotubes. These findings are in agreement with at least one human study reporting no acute effect of BNP on insulin sensitivity and insulin secretion (42). Altogether these data indicate that NP signaling does not acutely modulate skeletal muscle glucose uptake in vivo. We therefore performed chronic BNP infusion studies in HFD-fed and obese diabetic *db*/*db* mice to assess the long-term influence of BNP treatment on blood glucose control and insulin sensitivity. BNP was preferred for infusion studies as it has a higher half-life than ANP (14). Strikingly, chronic BNP infusion, at doses mimicking a physiological increase of the peptide and targeted to rescue the "BNP handicap" and/or a normal tissue NPRA signaling response, very significantly improved blood glucose control in both mouse models of obesity-induced glucose intolerance and T2D. We observed >20% reduction in fasting blood glucose levels as well as >15% decrease in HbA_{1c}, which is clinically meaningful and

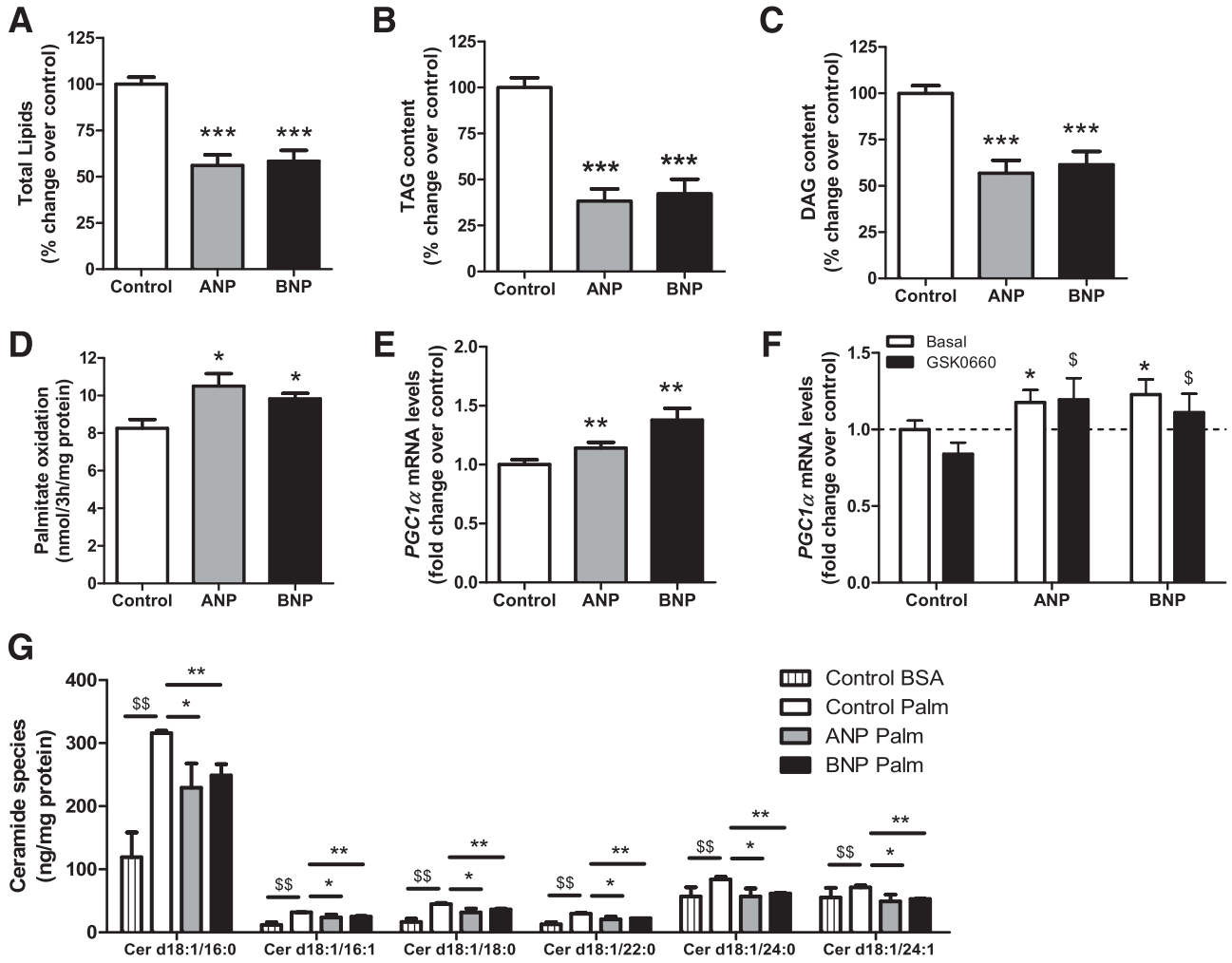

Figure 7—Chronic NP treatment reduces lipotoxicity and increases lipid oxidative capacity in human primary myotubes. Total lipid accumulation (*A*), TAG (*B*), and DAG (*C*) content were determined with [1-^{14}C]oleate after 3-day chronic treatment with 100 nmol/L of ANP and BNP in human differentiated myotubes. *D*: Total palmitate oxidation rate was also measured in response to chronic ANP and BNP treatment. *PGC1α* gene expression in response to 3-day treatment with ANP and BNP (*E*) and in presence or absence of 500 nmol/L of the selective PPARδ antagonist GSK0660 (*F*). **P* <0.05, ***P* < 0.01, ****P* < 0.0001 vs. control (*A–E*); **P* < 0.05 vs. basal; $*P* < 0.05 vs. GSK0660 (*n* = 4–10). *G*: Ceramide species content in human primary myotubes in basal condition (BSA), and in response to overnight treatment with 500 μmol/L of palmitate/BSA (Palm) in control myotubes and in response to 3-day treatment with ANP or BNP. $$*P* < 0.01 vs. control BSA; **P* < 0.05; ***P* < 0.01 vs. control palm (*n* = 4).

strongly reduces the risk of T2D complications (43). Reduced blood glucose levels during fasting and upon oral glucose challenge occurred in the absence of changes in blood insulin levels, indicating an improved metabolic clearance of glucose and insulin sensitivity. These findings are in agreement with other studies showing that increasing plasma BNP levels either pharmacologically (32) and/or genetically (22) improves glucose tolerance in obese mice. Preliminary evidence from our laboratory indicates that ANP-knockout mice are insulin resistant under normal chow diet compared with their wild-type littermates (data not shown), again arguing for a direct physiological link between NP signaling and insulin sensitivity.

Improved blood glucose control and insulin sensitivity were independent of significant changes in total DAGs and ceramides in liver, neither with noticeable changes in expression level of key metabolic genes in liver and thermogenic genes in white and brown fat of BNP-treated *db/db* and HFD-fed mice. However BNP-treated obese mice had an increased insulin-mediated Akt activation in skeletal muscle. Because Akt activation and phosphorylation are inhibited by lipotoxic lipids such as ceramides and DAGs (33,34), we measured ceramides and DAGs in skeletal muscle. In agreement with the negative correlation found in humans between muscle NPRA and ceramide content, we found a reduced level of total and main species of ceramides as well as main species of sphingomyelin in muscle of both HFD-fed and *db/db* mice chronically treated with BNP. Ceramides inhibit Akt activation and are produced de novo from saturated FAs and from sphingomyelin degradation (33). We also observed reduced

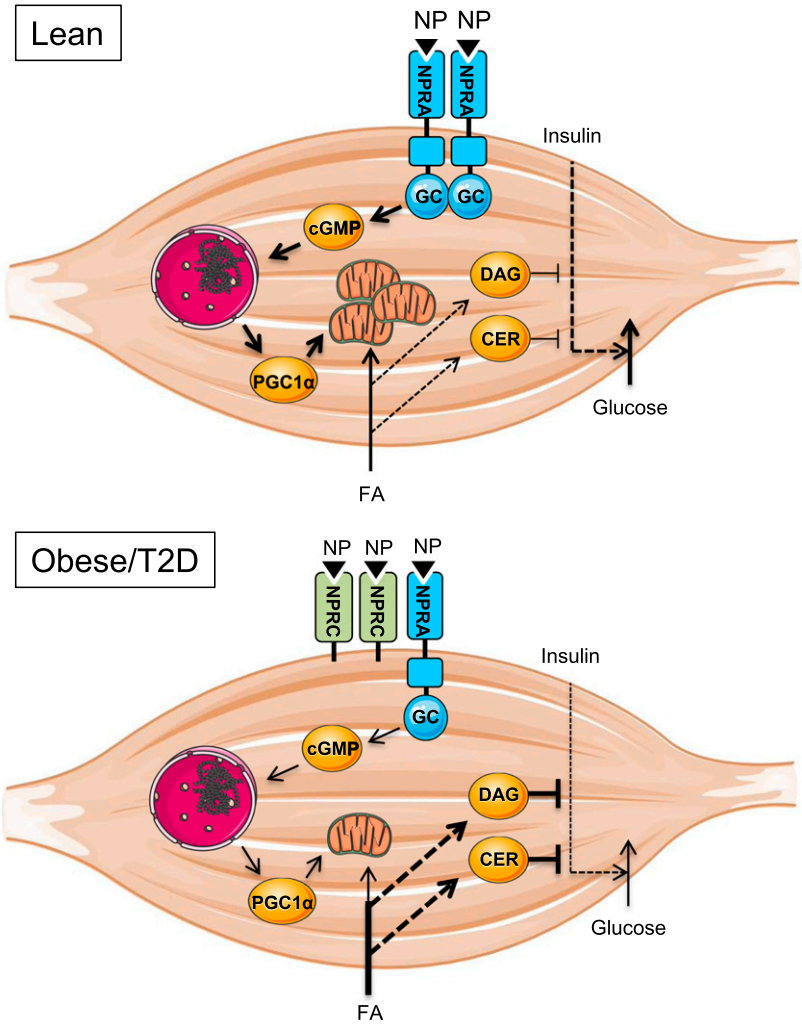

Figure 8—Model linking natriuretic peptide signaling in skeletal muscle and insulin sensitivity. In lean individuals, NPRA activation (bearing an intrinsic guanylyl cyclase activity [GC]) by circulating NP induces PGC1α expression in a cGMP-dependent manner that leads to increased fat oxidation rates and low levels of lipotoxic DAGs and ceramides (CER), which maintain a normal insulin responsiveness in skeletal muscle. Defective NPR signaling in skeletal muscle during obesity contributes to reduced fat oxidative capacity, increased lipotoxicity, and insulin resistance. Upregulation of NPRC in skeletal muscle as glucose tolerance deteriorates with obesity further inhibits the biological activation of NPRA by circulating NP and reduces NP circulating levels. Solid arrow line, direct effect; dashed arrow line, indirect effect.

muscle total DAG levels in BNP-treated mice. Interestingly, the reduced muscle lipotoxic lipid level was accompanied by a significant upregulation of muscle fat oxidative capacity and *PGC1α* gene expression. To demonstrate that elevated lipid oxidative capacity can reduce lipid accumulation, we chronically treated human primary myotubes with NP and showed increased palmitate oxidation rates and robustly reduced total lipid, TAG, and DAG accumulation. Chronic NP treatment also prevented palmitate-induced ceramide production in human primary myotubes. Although the precise mechanism was not investigated, it is likely that NP treatment reduces de novo ceramide production by increasing palmitate oxidation. We also show that NP-mediated elevated lipid oxidation involved the induction of PGC1α, which was independent of PPARδ activation. We and others previously described a cGMP-dependent induction of PGC1α gene expression by NP in white fat and skeletal muscle cells (19,20). PPARδ can be activated by lipid ligands derived from endogenous TAG lipolysis (31,44). In contrast to what has been shown in human fat cells (35,36), acute NP treatment of human primary myotubes did not influence the rate of lipolysis and TAG-derived FA oxidation or HSL phosphorylation at key regulatory sites.

Our findings provide the first evidence that NPRA signaling in skeletal muscle is pivotal for the maintenance of long-term insulin sensitivity by regulating lipid oxidative capacity through a PGC1α-dependent pathway. We also provide strong evidence that NPR signaling in skeletal muscle relates to insulin sensitivity and is disrupted in humans and mice with obesity and diabetes. Increasing plasma BNP

levels in obese diabetic mice remarkably improves blood glucose control and could prove a novel therapeutic avenue to alleviate obesity-related insulin resistance.

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References

1. Arnlöv J, Ingelsson E, Sundström J, Lind L. Impact of body mass index and the metabolic syndrome on the risk of cardiovascular disease and death in middle-aged men. Circulation 2010;121:230–236
2. Arnlöv J, Sundström J, Ingelsson E, Lind L. Impact of BMI and the metabolic syndrome on the risk of diabetes in middle-aged men. Diabetes Care 2011;34:61–65
3. Wang TJ, Larson MG, Keyes MJ, Levy D, Benjamin EJ, Vasan RS. Association of plasma natriuretic peptide levels with metabolic risk factors in ambulatory individuals. Circulation 2007;115:1345–1353
4. Wang TJ, Larson MG, Levy D, et al. Impact of obesity on plasma natriuretic peptide levels. Circulation 2004;109:594–600
5. Magnusson M, Jujic A, Hedblad B, et al. Low plasma level of atrial natriuretic peptide predicts development of diabetes: the prospective Malmo Diet and Cancer study. J Clin Endocrinol Metab 2012;97:638–645
6. Lazo M, Young JH, Brancati FL, et al. NH2-terminal pro-brain natriuretic peptide and risk of diabetes. Diabetes 2013;62:3189–3193
7. Moro C. Natriuretic peptides and fat metabolism. Curr Opin Clin Nutr Metab Care 2013;16:645–649
8. Wang TJ. The natriuretic peptides and fat metabolism. N Engl J Med 2012; 367:377–378
9. Moro C, Smith SR. Natriuretic peptides: new players in energy homeostasis. Diabetes 2009;58:2726–2728
10. Coué M, Moro C. Natriuretic peptide control of energy balance and glucose homeostasis. Biochimie. 30 May 2015 [Epub ahead of print]
11. Potter LR, Abbey-Hosch S, Dickey DM. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. Endocr Rev 2006;27:47–72
12. Gardner DG. Natriuretic peptides: markers or modulators of cardiac hypertrophy? Trends Endocrinol Metab 2003;14:411–416
13. Kuhn M. Structure, regulation, and function of mammalian membrane guanylyl cyclase receptors, with a focus on guanylyl cyclase-A. Circ Res 2003;93: 700–709
14. Potter LR. Natriuretic peptide metabolism, clearance and degradation. FEBS J 2011;278:1808–1817
15. Moro C, Lafontan M. Natriuretic peptides and cGMP signaling control of energy homeostasis. Am J Physiol Heart Circ Physiol 2013;304:H358–H368
16. Collins S. A heart-adipose tissue connection in the regulation of energy metabolism. Nat Rev Endocrinol 2014;10:157–163
17. Moro C, Crampes F, Sengenes C, et al. Atrial natriuretic peptide contributes to physiological control of lipid mobilization in humans. FASEB J 2004;18:908–910
18. Sengenes C, Berlan M, De Glisezinski I, Lafontan M, and Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. FASEB J 2000; 14:1345–1351
19. Bordicchia M, Liu D, Amri EZ, et al. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. J Clin Invest 2012;122:1022–1036
20. Engeli S, Birkenfeld AL, Badin PM, et al. Natriuretic peptides enhance the oxidative capacity of human skeletal muscle. J Clin Invest 2012;122:4675–4679
21. Sengenès C, Zakaroff-Girard A, Moulin A, et al. Natriuretic peptide-dependent lipolysis in fat cells is a primate specificity. Am J Physiol Regul Integr Comp Physiol 2002;283:R257–R265
22. Miyashita K, Itoh H, Tsujimoto H, et al. Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. Diabetes 2009;58:2880–2892
23. Badin PM, Louche K, Mairal A, et al. Altered skeletal muscle lipase expression and activity contribute to insulin resistance in humans. Diabetes 2011; 60:1734–1742
24. Riou ME, Pigeon E, St-Onge J, et al. Predictors of cardiovascular fitness in sedentary men. Appl Physiol Nutr Metab 2009;34:99–106
25. Galgani JE, Vasquez K, Watkins G, et al. Enhanced skeletal muscle lipid oxidative efficiency in insulin-resistant vs insulin-sensitive nondiabetic, nonobese humans. J Clin Endocrinol Metab 2013;98:E646–E653
26. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. Scand J Clin Lab Invest 1975;35:609–616
27. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–E223
28. Vila IK, Badin PM, Marques MA, et al. Immune cell Toll-like receptor 4 mediates the development of obesity- and endotoxemia-associated adipose tissue fibrosis. Cell Reports 2014;7:1116–1129
29. Badin PM, Vila IK, Louche K, et al. High-fat diet-mediated lipotoxicity and insulin resistance is related to impaired lipase expression in mouse skeletal muscle. Endocrinology 2013;154:1444–1453
30. Ukropcova B, McNeil M, Sereda O, et al. Dynamic changes in fat oxidation in human primary myocytes mirror metabolic characteristics of the donor. J Clin Invest 2005;115:1934–1941
31. Badin PM, Loubière C, Coonen M, et al. Regulation of skeletal muscle lipolysis and oxidative metabolism by the co-lipase CGI-58. J Lipid Res 2012;53: 839–848
32. Plante E, Menaouar A, Danalache BA, Broderick TL, Jankowski M, Gutkowska J. Treatment with brain natriuretic peptide prevents the development of cardiac dysfunction in obese diabetic *db*/*db* mice. Diabetologia 2014;57:1257–1267
33. Chavez JA, Summers SA. A ceramide-centric view of insulin resistance. Cell Metab 2012;15:585–594
34. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell 2012;148:852–871
35. Moro C, Galitzky J, Sengenes C, Crampes F, Lafontan M, Berlan M. Functional and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway in human fat cells. J Pharmacol Exp Ther 2004;308: 984–992
36. Sengenes C, Bouloumie A, Hauner H, et al. Involvement of a cGMP-dependent pathway in the natriuretic peptide-mediated hormone-sensitive lipase phosphorylation in human adipocytes. J Biol Chem 2003;278:48617–48626
37. Pivovarova O, Gögebakan Ö, Klöting N, et al. Insulin up-regulates natriuretic peptide clearance receptor expression in the subcutaneous fat depot in obese subjects: a missing link between CVD risk and obesity? J Clin Endocrinol Metab 2012;97:E731–E739
38. Sarzani R, Dessì-Fulgheri P, Salvi F, et al. A novel promoter variant of the natriuretic peptide clearance receptor gene is associated with lower atrial

natriuretic peptide and higher blood pressure in obese hypertensives. J Hypertens 1999;17:1301–1305
39. Sarzani R, Strazzullo P, Salvi F, et al. Natriuretic peptide clearance receptor alleles and susceptibility to abdominal adiposity. Obes Res 2004;12:351–356
40. Khan AM, Cheng S, Magnusson M, et al. Cardiac natriuretic peptides, obesity, and insulin resistance: evidence from two community-based studies. J Clin Endocrinol Metab 2011;96:3242–3249
41. Matsukawa N, Grzesik WJ, Takahashi N, et al. The natriuretic peptide clearance receptor locally modulates the physiological effects of the natriuretic peptide system. Proc Natl Acad Sci U S A 1999;96:7403–7408
42. Heinisch BB, Vila G, Resl M, et al. B-type natriuretic peptide (BNP) affects the initial response to intravenous glucose: a randomised placebo-controlled cross-over study in healthy men. Diabetologia 2012;55:1400–1405
43. DeFronzo RA, Abdul-Ghani M. Assessment and treatment of cardiovascular risk in prediabetes: impaired glucose tolerance and impaired fasting glucose. Am J Cardiol 2011;108(Suppl.):3B–24B
44. Tang T, Abbott MJ, Ahmadian M, Lopes AB, Wang Y, Sul HS. Desnutrin/ATGL activates PPARδ to promote mitochondrial function for insulin secretion in islet β cells. Cell Metab 2013;18:883–895