HHS Public Access

Author manuscript

*Neurourol Urodyn*. Author manuscript; available in PMC 2020 August 01.

Published in final edited form as:

*Neurourol Urodyn*. 2019 August ; 38(6): 1524–1532. doi:10.1002/nau.24015.

# Chronic high fat diet decreased detrusor mitochondrial respiration and increased nerve-mediated contractions

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## Abstract

**Aims:** To assess the impact of chronic high fat diet (HFD) on behavioral voiding patterns, detrusor contractility and smooth muscle mitochondrial function in male mice.

**Methods:** Male C57BL/6J mice (6 weeks) were fed a control or HFD for 20 weeks. Bladder function was assessed by void spot assays. Bladders were collected and detrusor contractility to carbachol ($10^{-9}$-$10^{-5}$M), and electrical field stimulation (EFS, 0.5–32 Hz) in the presence and absence of atropine was measured. Homogenized detrusor samples were placed in oxygraphs to assess the rate of oxygen consumption of the mitochondria within the detrusor in the presence of different substrates. Mitochondrial hydrogen peroxide ($H_2O_2$) emission was measured fluorometrically. Detrusor citrate synthase activity was measured via enzyme activity kit and Western blots assessed the electron transport chain (ETC) protein content.

**Results:** HFD significantly increased body weight, adiposity and blood glucose levels. HFD mice demonstrated increased voiding frequency, and increased EFS-induced detrusor contractility. There were no changes in detrusor relaxation or cholinergic-medicated contraction. Mitochondrial respiration was decreased with HFD and $H_2O_2$ emission was increased. The relative amount of mitochondria in the detrusor was similar between groups. However, ETC complexes V and III were increased following HFD.

**Conclusions:** Chronic HFD increased adiposity, lead to more frequent voiding and enhanced EFS-mediated detrusor contractions. Mitochondrial respiration was decreased and $H_2O_2$ emission

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Ethics Statement

All animal use and experimental procedures were approved by the Brody School of Medicine Institutional Animal Care and Use Committee and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

increased following HFD. Further research is required to determine if alterations in mitochondrial function could play a role in the development of HFD-induced bladder dysfunction.

## 1. Introduction

Over 40% of adults in the United States are obese.[1] Current ease and availability of fast food high in saturated fat and sedentary lifestyles are driving this epidemic. Obesity is a risk factor for cardiovascular disease, cancer, fatty liver disease, and the development of type 2 diabetes mellitus (T2DM). Being overweight or obese is an independent risk factor for urinary incontinence.[2] Each 5-unit increase in body mass index (BMI) is associated with a 20–70% increased risk of urinary incontinence.[2] Alterations in the physiology of the mucosal layer (lamina propria, urothelium) or the detrusor smooth muscle layer as well as changes to innervation can contribute to bladder dysfunction. The symptoms of obesity-induced bladder dysfunction range from overactive bladder to stress and/or urge incontinence.[3] While it's speculated that obesity increases abdominal pressure and contributes to incontinence, the mechanisms underlying the pathological changes leading to bladder dysfunction remain unknown.

A common model of obesity used to study bladder dysfunction is mice fed a high fat diet (HFD). This mouse model is valuable in understanding the development of obesity-induced bladder dysfunction. Previous studies have established that male mice fed a HFD for 10–12 weeks develop increased body weight, hyperlipidemia, and insulin resistance.[4,5] Additionally, anesthetized cystometry indicate increased voiding frequency, non-voiding contractions, and ex vivo bladder contractility while bladder relaxation is impaired.[4,5] Most of these studies describe overall bladder physiology in models of HFD-induced obesity; however, few have determined what underlying mechanisms are leading to bladder dysfunction.

Obesity and HFD frequently lead to increased oxidative stress and inflammation; both systemically and in the bladder. A source of HFD-induced inflammation is free radical reactive oxygen species (ROS) produced by the mitochondria which can oxidize both lipids and proteins.[7] Mitochondria produce ATP, which is necessary for bladder energy expenditure, is critical to the maintenance of cellular homeostasis, and is a signaling model involved in bladder contraction. In rats given an energy rich, high fructose diet, decreased in vitro bladder contraction was evident, and swollen, degenerating mitochondria were found in bladder smooth muscle cells.[8] Markers of bladder mitochondrial dysfunction have been shown in rabbit models of partial bladder outlet obstruction and in a rat HFD model; however, mitochondrial function and mitochondrial-derived ROS production have not been measured in mice with HFD-induced bladder dysfunction.[9,10]

Following 20 weeks of HFD, the current study assessed behavioral voiding in mice and in vitro bladder smooth muscle contractility. We are specifically interested in changes to bladder detrusor smooth muscle function; therefore, the mucosal layer has been removed for all experiments. Detrusor mitochondrial respiration to different substrates and hydrogen peroxide ($H_2O_2$) emission is measured, and protein abundance of mitochondrial complexes in the electron transport chain are quantified. Citrate synthase activity is utilized to

determine detrusor mitochondrial content. The goal of this study is to elucidate whether a HFD changes detrusor mitochondrial respiration in the presence of different substrates in order to further our understanding of the pathological mechanisms leading to the development of HFD-associated bladder dysfunction in mice.

## 2. Materials and Methods

### 2.1 Animals

All experiments were performed in accordance with Brody School of Medicine Institutional Animal Care and Use Committee guidelines for animal care and use. Six week old C57BL/6J mice (n=32, Jackson Laboratory) were housed on a 12hr light/dark cycle with free access to food and water. Mice were provided with either a standard diet (RMH3000:14% fat, 60% carbohydrates, 26% protein; Lab Diet, St Louis, MO, USA) or a HFD (D12451: 45% fat, 35% carbohydrates, 20% protein; Research Diets, New Brunswick, NJ, USA) for 20 weeks.

### 2.2 In vivo urinary function - void spot assay (VSA)

One week prior to sacrifice, each mouse was placed into a standard cage with a wire mesh bottom over three-ply filter paper (Fisher Scientific, Pittsburgh, PA, USA).[11] Mice were left undisturbed to void normally for 4 hours (8am-12pm) and the filter papers were collected. The void spots were visualized under ultraviolet light and photographed (UVP ChemiDoc-ITS3, Upland, CA, USA). Individual void spots were counted and their areas measured using ImageJ (NIH, Bethesda, MD, USA). Bright spots smaller than 0.06 $cm^2$ were eliminated to account for grid “spray” and non-urine particles.[12] The VSA parameters measured were: the number of voids, the total void area, the average area per void and the area of the primary (largest) void.

### 2.3 Measurement of physical and metabolic parameters

Following 20 weeks of HFD, mice were fasted overnight, and blood glucose was measured from the tail vein using a glucometer (Free Style Lite, Abbott Diabetes Care, Alameda, CA, USA). Mice were euthanized by ketamine/xylazine injection and thoracotomy. Bladders were carefully removed, drained of urine, and wet weight measured. Additionally, the visceral (retroperitoneal, mesenteric, and inguinal) fat depots were collected and weighed.

### 2.4 Functional studies of isolated detrusor

The bladders were collected from half of the mice (n=8/group) and placed in ice-cold physiological Krebs solution (in mM: 130 NaCl, 4.7 KCl, 14.9 $NaHCO_3$, 5.5 dextrose, 1.18 $KH_2PO_4$, 1.17 $MgSO_4$, 1.6 $CaCl_2$, pH 7.4). Using a high powered dissecting scope, the bladder was opened longitudinally, the dome and trigone regions removed, and the mucosal layer removed to assess detrusor smooth muscle response. The removal of the mucosa was confirmed by visualization of the detrusor surface and a lack of major vasculature. Two circumferential detrusor strips (2 × 6 mm) were mounted in muscle strip myographs (DMT 820M, Denmark) in Krebs solution maintained at 37°C and aerated with 95% $O_2$ and 5% $CO_2$. Strips were stretched to a resting tension of 2 mN and equilibrated for 45 minutes. Following equilibration, high concentration potassium chloride solution (KCl; 120 mM)

assessed detrusor contraction and tissue viability. Cholinergic contraction was evaluated via concentration-response curves to carbachol ($10^{-9}$ to $10^{-5}$ M). Following pre-contraction with carbachol, a concentration-response curve to norepinephrine ($10^{-8}$ to $10^{-4}$ M) measured adrenergic relaxation. Nerve-evoked contractions were measured via electric field stimulation (EFS: 30 V, 0.3 ms pulse width, 0.5–32 Hz) for 10 seconds at 2-minute intervals (Grass Instruments, Quincy, MA). Non-cholinergic EFS-mediated contractility was also measured in the presence of atropine ($10^{-5}$ M). Responses were recorded using Lab Chart 8 software and Power Lab acquisition hardware (AD Instruments, Colorado Springs, CO, USA). Data were measured as percentage of the maximal response to KCl or relaxation from contraction to carbachol. All compounds and drugs were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

### 2.5 Bladder mitochondrial respiration

Mitochondrial experiments were conducted on the remaining bladders (n=8/group). Bladders were denuded of the mucosal layer, as previously described, and the detrusor homogenized in a buffer solution (in mmol/l: 300 sucrose, 10 HEPES, 1 EGTA; pH 7.1) using a dounce homogenizer with Teflon pestle. High-resolution respirometry was performed using the Oroboros O2K Oxygraph in Buffer Z (in mM: 110 potassium MES, 35 KCl, 1 EGTA, 5 $K_2HPO_4$, 3 $MgCl_2 \cdot 6H_2O$, 0.5 mg/ml BSA; pH 7.1, 295 mOsm) at 37˚C. A total of 300 μg protein homogenate was loaded for each experiment. Complex I-supported respiration was assessed using 5 mM pyruvate + 0.5 mM malate; state 3 respiration was assessed following the addition of 4 mM ADP; complex I + II was next evaluated with the addition of 10 mM succinate; complex IV-supported respiration was measured using 2 mM ascorbate + 0.4 mM TMPD (N,N,N’,N’-tetramethyl-p-phenylenediamine).[13] Complex II-supported respiration was assessed in a parallel experiment with 10 mM succinate with 0.01 mM rotenone (to inhibit reverse electron flow to Complex I). The integrity of the outer mitochondrial membrane was assessed in each experiment by addition of exogenous cytochrome c and samples with an increase in oxygen consumption greater than 10% were excluded. All mitochondrial experiments were performed within ~2 hours of tissue homogenization. Oxygen consumption was corrected for mitochondrial content using a commercially available citrate synthase activity assay kit (Sigma CS0720) per the manufacturer’s instructions. The rate of mitochondrial respiration ($JO_2$) was expressed as picomoles per citrate synthase activity.

### 2.6 Bladder mitochondrial $H_2O_2$ emission

Bladder mitochondrial $H_2O_2$ emission was measured fluorometrically at 37˚C via Amplex Ultra Red (10 μmol/l)/horseradish peroxidase detection system.[13] Fluorescence was monitored using a fluorescent plate reader (BioTek Synergy, Winooski, VT, USA). Mitochondrial $H_2O_2$ emission was assessed in state 4 conditions with the addition of 10 mM succinate. For each experiment, resorufin fluorescence was converted to $H_2O_2$ picomoles via an $H_2O_2$ standard curve generated under identical substrate conditions and normalized to citrate synthase activity.

### 2.7 Western blot analysis

Total protein from detrusor muscle, denuded of the mucosal layers (n=8/group), was extracted using RIPA buffer (150 mM NaCl, 1% Triton, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris; pH 7.65) and protein (20 μg) was loaded on a 4–12% Bis-Tris SDS-polyacrylamide gel (Thermo Fisher, Waltham, MA, USA). Protein electrophoresis was run at 160 V (60 min), proteins were transferred to nitrocellulose membrane and incubated with total OXPHOS primary antibody cocktail (1:250; ab110413, Abcam) to measure all 5 ETC complexes. Bands were detected by chemiluminescence (Pierce ECL, Thermo Fisher) and imaged with Bio-Rad ChemiDoc Touch (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were stripped and ß-actin (1:500; PA1–183, Thermo Fisher) expression was used to normalize the protein expression of each complex. Densitometry of the bands were analyzed using ImageJ software (NIH).

### 2.8 Statistical analysis

Data were expressed as mean ± standard error of the mean. Groups were compared using a two-tailed Student t-test. Nonparametric Mann-Whitney test was used to determine differences between the numbers of voids between groups. For all tests, P values of less than 0.05 were considered statistical significant (Prism 5.0, GraphPad, La Jolla, CA, USA).

## 3. Results

### 3.1 Chronic HFD increased visceral adiposity and frequency of voids

Mice fed a 45% kcal HFD for 20 weeks had significantly higher body weights and visceral adiposity compared to mice fed a control diet (Table 1). Fasting blood glucose was elevated ~40% in HFD mice. A previous study by co-authors performed glucose and insulin tolerance tests in another cohort of mice at 16 weeks and demonstrated whole-body glucose intolerance and decreased insulin sensitivity.[13] Bladder weight was not different between the two groups, but was significantly lower relative to body weight with HFD. The void spot analysis showed a doubling in the number of voids and an increase in total overall void area in the HFD mice (Fig. 1C, E). Interestingly, there was no difference in the average area per void or the total void area normalized to body weight (Fig. 1D, F).

### 3.2 Increased EFS-mediated detrusor contraction and less cholinergic inhibition in HFD mice

Bladder strips were denuded of the mucosal layers to assess smooth muscle contractile and relaxation responses in control and HFD mice. Contractions induced by high potassium were no different in the detrusors from control and HFD mice (19.0±1.36 vs 22.4±2.53 mN, data not shown). Cholinergic-mediated contractions and norepinephrine-mediated relaxation were unchanged with chronic HFD (Fig. 2A, B). EFS-mediated contractile responses were considerably increased in HFD mice compared to controls (Fig. 2C). Furthermore, the control bladders demonstrated greater cholinergic inhibition compared to the HFD bladders (Fig. 2D, E). The contractile responses at 32 Hz stimulation were inhibited almost 80% in the control detrusor compared to only ~40% inhibition in the HFD detrusor (Fig. 2F).

### 3.4 Detrusor mitochondrial respiratory capacity is decreased while mitochondrial $H_2O_2$ emission is increased with chronic HFD

Chronic HFD decreased detrusor mitochondrial respiratory function when examined under multiple substrate conditions. Respiratory capacity was decreased under ADP-stimulated state 3 conditions in the presence of pyruvate/malate and succinate which activate complex I and II supported respiration, respectively (Fig. 3A). In contrast, under state 4 conditions (i.e. low energy demand), there was no difference in complex I- or complex II-supported respiration in HFD detrusors. Complex IV supported respiration by TMPD in the detrusor was also decreased with HFD. Mitochondrial density in the detrusor was consistent across control and HFD mice as indicated by the citrate synthase activity assay (Fig. 3B). Chronic HFD significantly elevated detrusor submaximal mitochondrial $H_2O_2$ emission under state 4 conditions (Fig. 3C). Interestingly, while citrate synthase activity was unaltered by HFD, the protein expression of complexes III and V were markedly elevated in the detrusor from chronically HFD fed mice (Fig. 4).

## 4. Discussion

Animals chronically fed a HFD demonstrated altered bladder function and decreased detrusor smooth muscle mitochondrial respiration. Following 20 weeks of HFD, mice have both increased body weight and elevated visceral adiposity. HFD leads to an increased number of voids but no change in overall total void area normalized to body weight; these symptoms indicate increased frequency of urination. Additionally, the HFD bladders have higher amplitude EFS-mediated atropine-insensitive contractions suggesting a switch to purinergic dominant responses. HFD detrusor mitochondrial respiration is decreased upon activation of complexes I, II and IV; in contrast HFD elevated the detrusor protein expression of both complexes III and V. In addition to decreased respiration, HFD bladders generated greater $H_2O_2$ emission. These data indicate that HFD leads to decreased mitochondrial respiration, elevated nerve-mediated contraction and increased voiding frequency.

HFD is a widely used model to study bladder dysfunction in mice; however, both the severity and type of detrusor dysfunction is often variable. The variability in HFD outcomes may be due to the different diet compositions, the length of diet, or the age, strain and sex of the animals at the diet initiation. Frequently the metabolic parameters are not assessed and hyperlipidemia is not distinguished from obesity. For example, mice fed a 55% HFD from 4 to 16 weeks of age demonstrated overactive bladder symptoms such as frequent voiding, increased non-voiding contractions and greater contraction to both EFS and muscarinic agonists.[14] In contrast, mice fed 35% HFD from 8 to 20 weeks of age had decreased contraction to high potassium and muscarinic stimulation.[15] In another study, 20 weeks of 32% HFD from 5–25 weeks of age led to decreased voiding frequency and total voiding volume in animals due to decreased water intake and EFS contractions were increased.[16] Similar to the 20 week HFD study, the current study showed increased bladder contractile responses to EFS. Additionally, we also demonstrated that HFD shifted the EFS-mediated contraction to less cholinergic. Other studies in type 1 diabetic rats have shown greater EFS contractions in the presence of atropine compared to controls with concomitant changes in

muscarinic and purinergic receptor populations.[17,18] We hypothesize that there is a greater purinergic response with high fat diet leading to greater EFS-mediated contraction and bladder overactivity. Limitations to the current study are a lack of assessment of the purinergic component of the detrusor's EFS-mediated contractile response, and in vivo bladder function was only assessed by void spot assay.

Over 50 years ago, mitochondria were first identified in bladder epithelial cells from toads and bullfrogs.[19] Since then, mitochondrial oxygen consumption has been measured during electrical stimulated contraction in rabbit bladder smooth muscle strips. In Wendt's study, the initiation of contraction in rabbit bladder lead to high mitochondrial respiration which dropped during the maintenance phase of contraction.[20] Wendt concluded that changes in mitochondrial respiration indicated greater energy requirements during the initiation of contraction.[20] Bladder mitochondrial function has been most commonly examined in both early and late stages of partial bladder outlet obstruction (PBOO). PBOO can lead to decreased smooth muscle contraction, lowered maximal oxygen consumption rates, and attenuated citrate synthase, complex IV and malate dehydrogenase activity.[21–23] All previous assessments of bladder oxygen consumption have been measured in whole bladder tissue or isolated bladder mitochondria using a Clark electrode. To our knowledge, this is the first use of high-resolution respirometry to measure bladder mitochondrial oxygen consumption. In the presence of substrates for multiple complexes, HFD decreased detrusor maximal mitochondrial respiration. Interestingly, we did not find a change in citrate synthase activity between the HFD and control bladders indicating that the same amount of mitochondria are present, but did observe increases in the protein expression of complexes III and V. This study is limited as we did not determine if the decreased respiration equated to decreased activity of the different complexes or a concomitant decrease in ATP production.

In addition to energy production, the mitochondria are also implicated in the maintenance of cellular homeostasis. They convert fuel to and electrochemical potential by passing electrons from carbon sources to the mitochondrial electron transport system (ETS), and subsequently utilize the electrochemical potential energy to generate ATP at the ATP synthase. However, it is well established that the ETS can also leak electrons creating ROS that can drive the development of inflammation. Inflammation has been widely studied in a variety of bladder dysfunction disease states. Pro-inflammatory cytokines such as TNF-alpha and interleukin-1β are elevated in HFD-induced bladder dysfunction.[15] We demonstrate that chronic HFD leads to an increase in detrusor mitochondrial $H_2O_2$ emission.

A recent editorial compared the progression of diabetic bladder dysfunction to the development of heart failure.[24] Both diseases undergo an initial compensation of the cardiac or smooth muscle followed by a decompensatory phase in which the muscle becomes acontractile. In heart failure research, many studies have examined rescuing mitochondrial respiration to improve heart function.[25] Using the mitochondrial antioxidant, MitoQ, heart failure in rats induced by pressure overload was improved by reducing $H_2O_2$ production, and restoring mitochondrial respiration.[26] In atherosclerotic rats with hypoxic bladder dysfunction due to chronic ischemia, treatment with mitochondrial antioxidants, coenzyme Q10 prevented reduced bladder contractility and preserved bladder capacity.[27] Clinically,

antioxidant or ROS scavenging agents have not proven efficacious in heart failure or bladder dysfunction and show no long-term benefit.[26] However, novel scavenging compounds, such as elamipretide, which are targeted and permeable to mitochondria are showing promise.[26] Our mice demonstrate increase frequency and EFS-mediated contractions which are representative of the compensatory phase of bladder dysfunction or overactive bladder dysfunction.[3] Our future studies will assess mitochondrial targeted antioxidants to determine if restoring mitochondrial function can recover bladder function in diseased states such as obesity or diabetes.

## 5. Conclusions

Chronic HFD leads increased adiposity, greater frequency of urination and elevated nerve-mediated detrusor contraction of likely purinergic origin. Additionally, detrusor mitochondrial respiration is decreased and mitochondrial derived $H_2O_2$ emission is high with HFD. These data suggest that alterations in mitochondrial bioenergetics could play a role in the development of HFD-induced bladder dysfunction. Future studies are required to confirm causation of mitochondrial dysfunction leading to impaired bladder physiology.

## Acknowledgements

This work was supported by grants from (NIH)/National Heart, Lung, and Blood Institute (NHLBI) R01-HL-125695 (JMM) and F32-HL-129632 (TER). Additional funding was provided from Brody School of Medicine Startup Funds (JLH).

## References

1. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of obesity among adults and youth: United States, 2015–2016 NCHS data brief, no 288. Hyattsville, MD: National Center for Health Statistics 2017.
2. Subak LL, Richter HE, Hunskaar S. Obesity and urinary incontinence: epidemiology and clinical research update. J Urol. 2009;182(6 Suppl):S2–7. [PubMed: 19846133]
3. Daneshgari F, Liu G, Hanna-Mitchell AT. Path of translational discovery of urological complications of obesity and diabetes. Am J Physiol Renal Physiol 2017;312:F887–F896. [PubMed: 28052873]
4. Leiria LO, Silva FH, Davel AP, Alexandre EC, Calixto MC, De Nucci G, et al. The soluble guanylyl cyclase activator BAY 60–2770 ameliorates overactive bladder in obese mice. J Urol 2014;191:539–547. [PubMed: 24050894]
5. Calmasini FB, de Oliveira MG, Alexandre EC, da Silva FH, da Silva CP, Candido TZ, et al. Long-term treatment with the beta-3 adrenoceptor agonist, mirabegron ameliorates detrusor overactivity and restores cyclic adenosine monophosphate (cAMP) levels in obese mice. Neurourol Urodynam 2017;36:1511–1518.
6. He Q, Babcook MA, Shukla S, Shankar E, Wang Z, Liu G, et al. Obesity-initiated metabolic syndrome promotes urinary voiding dysfunction in a mouse model. Prostate 2016;76:964–976. [PubMed: 27040645]
7. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860–867. [PubMed: 17167474]
8. Lee WC, Chien CT, Yu HJ, Lee SW. Bladder dysfunction in rats with metabolic syndrome induced by long-term fructose feeding. J Urol 2008;179:2470–2476. [PubMed: 18433789]
9. Lin WY, Wu SB, Lin YP, Chang PJ, Levin RM, Wei YH. Reversing bladder outlet obstruction attenuates systemic and tissue oxidative stress. BJU Int 2012;110:1208–1213. [PubMed: 22564765]

10. Oberbach A, Jehmlich N, Schlichting N, Heinrich M, Lehmann S, Wirth H, et al. Molecular fingerprint of high fat diet induced urinary bladder metabolic dysfunction in a rat model. PLoS One 2013;8:e66636.
11. Gharaee-Kermani M, Rodriguez-Nieves JA, Mehra R, Vezina CA, Sarma AV, Macoska JA. Obesity-induced diabetes and lower urinary tract fibrosis promote urinary voiding dysfunction in a mouse model. Prostate 2013;73:1123–1133. [PubMed: 23532836]
12. Bjorling DE, Wang Z, Vezina CM, Ricke WA, Keil KP, Yu W, et al. Evaluation of voiding assays in mice: impact of genetic strains and sex. Am J Physiol Renal Physiol 2015;308:F1369–F1378. [PubMed: 25904700]
13. Ryan TE, Schmidt CA, Green TD, Spangenburg EE, Neufer PD, McClung JM. Targeted Expression of Catalase to Mitochondria Protects Against Ischemic Myopathy in High-Fat Diet-Fed Mice. Diabetes 2016;65:2553–2568. [PubMed: 27284110]
14. Alexandre EC, Calmasini FB, de Oliveira MG, Silva FH, da Silva CPV, André DM, et al. Chronic treatment with resveratrol improves overactive bladder in obese mice via antioxidant activity. Eur J Pharmacol 2016;788:29–36. [PubMed: 27316789]
15. Fan EW, Chen LJ, Cheng JT, Tong YC. Changes of urinary bladder contractility in high-fat diet-fed mice: the role of tumor necrosis factor-α. Int J Urol 2014;21:831–835. [PubMed: 24661252]
16. Aizawa N, Homma Y, Igawa Y. Influence of High Fat Diet Feeding for 20 Weeks on Lower Urinary Tract Function in Mice. Low Urin Tract Symptoms 2013;5:101–108. [PubMed: 26663378]
17. Munoz A, Boone TB, Smith CP, Somogyi GT. Diabetic plasticity of non-adrenergic non-cholinergic and P2X-mediated rat bladder contractions. Brain Res Bull 2013 95:40–45. [PubMed: 23562604]
18. Lin CS, Wu TT, Chang CH, Cheng JT, Tong YC. Changes of Bladder M1,3 Muscarinic Receptor Expression in Rats Fed with Short-Term/Long-Term High-Fat Diets. Low Urin Tract Symptoms 2018 10:315–19. [PubMed: 28681521]
19. Peachey LD, Rasmussen H. Structure of the toad's urinary bladder as related to its physiology. J Biophys Biochem Cytol 1961 10:529–53. [PubMed: 13733732]
20. Wendt IR. Effects of substrate and hypoxia on smooth muscle metabolism and contraction. Am J Physiol 1989;256:C719–27. [PubMed: 2705512]
21. Arner A, Malmqvist U, Uvelius B. Metabolism and force in hypertrophic smooth muscle from rat urinary bladder. Am J Physiol 1990;258:C923–32. [PubMed: 2333984]
22. Hsu TH, Levin RM, Wein AJ, Haugaard N. Alterations of mitochondrial oxidative metabolism in rabbit urinary bladder after partial outlet obstruction. Mol Cell Biochem 1994;141:21–26. [PubMed: 7877605]
23. Damaser MS, Haugaard N, Uvelius B. Partial obstruction of the rat urinary bladder: effects on mitochondria and mitochondrial glucose metabolism in detrusor smooth muscle cells. Neurourol Urodyn 1997;16:601–607. [PubMed: 9353808]
24. Kwon W, Lee T. Learning from heart failure: How will we lead bladder failure into the future? Int Neurourol J 2016;20:1–2. [PubMed: 27032549]
25. Marquez J, Lee SR, Kim N, Han J. Rescue of heart failure by mitochondrial recovery. Int Neurourol J 2016;20:5–12. [PubMed: 27032551]
26. Brown DA, Perry JB, Allen ME, Sabbah HN, Stauffer BL, Shaikh SR, et al. Expert consensus document: Mitochondrial function as a therapeutic target in heart failure. Nat Rev Cardiol 2017;14:238–250. [PubMed: 28004807]
27. Kim JW, Jang HA, Bae JH, Lee JG. Effects of coenzyme Q10 on bladder dysfunction induced by chronic bladder ischemia in a rat model. J Urol 2013;189:2371–2376. [PubMed: 23306086]

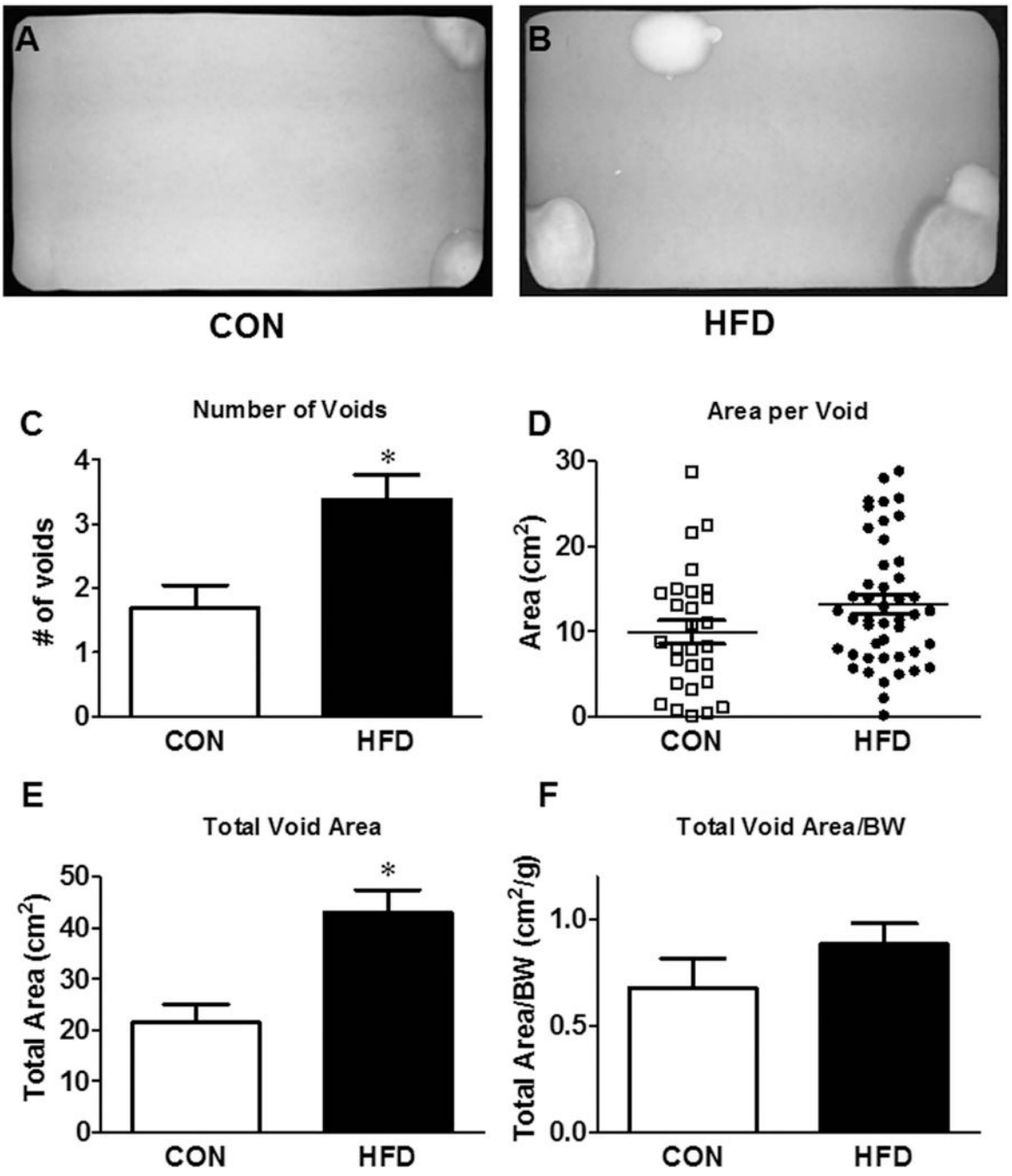


**Figure 1.**

Chronic high-fat feeding increased diuresis compared to controls. Representative images from 4 hour void spot assay in control (A) and HFD (B) mice. Data was quantified as the number of voids (C), area per void (D), the total void area (E) and the total void area normalized to body weight (BW; F). Data are mean ± SEM. n=12–14/group; *p<0.05 vs CON.

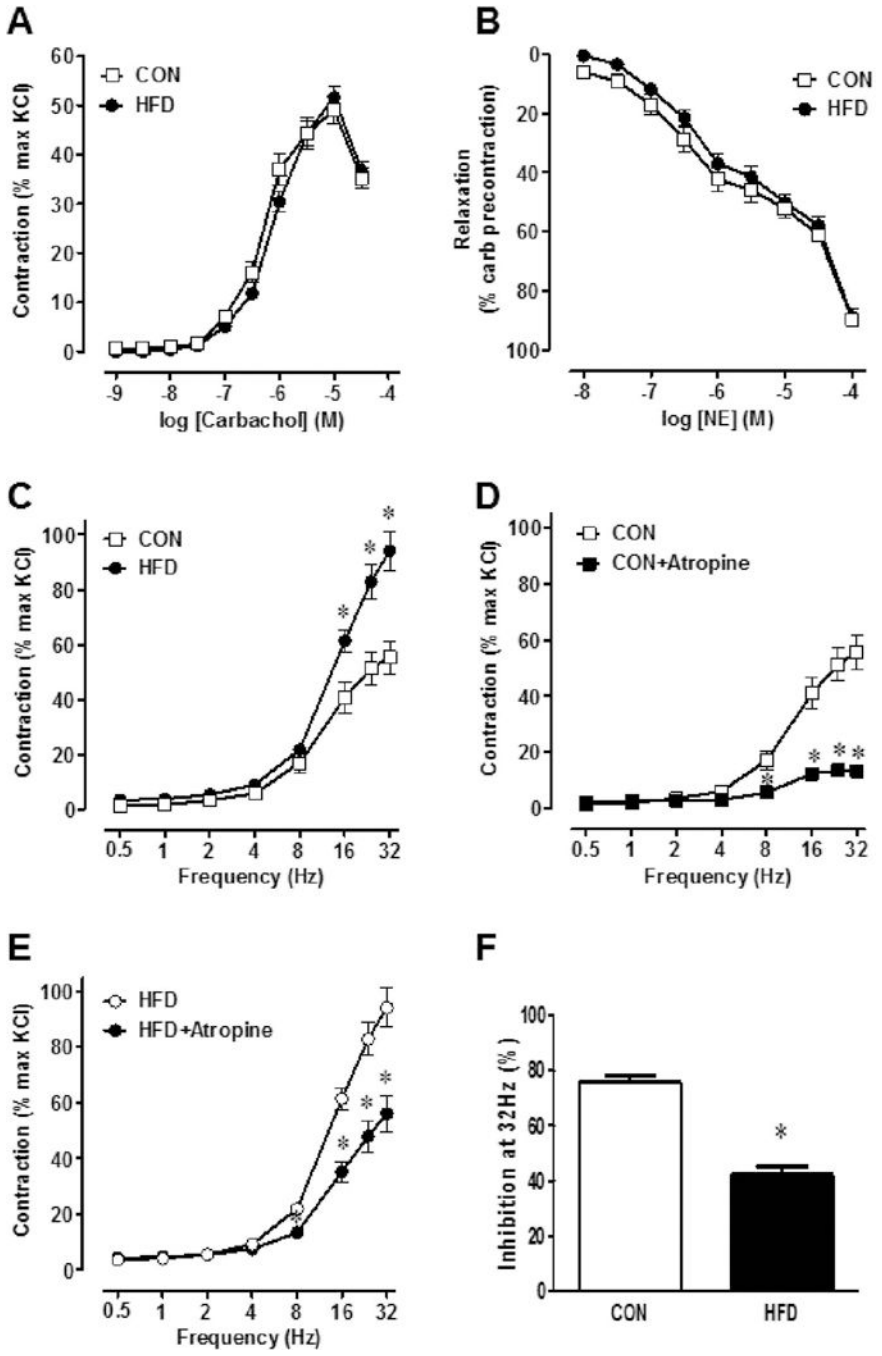


**Figure 2.**

Chronic high fat diet (HFD) did not impact high potassium carbachol induced contractile responses (A) or relaxation to norepinephrine (NE) in detrusor strips (B). Electrical field stimulated (EFS) contractile responses were increased in HFD detrusor (C). Inhibition of muscarinic receptors with atropine produced greater inhibition to carbachol in control detrusor (D) compared to HFD (E). The percent inhibition at 32 Hz is summarized in panel F. Data are mean ± SEM. n=8/group; *p<0.05 vs CON.

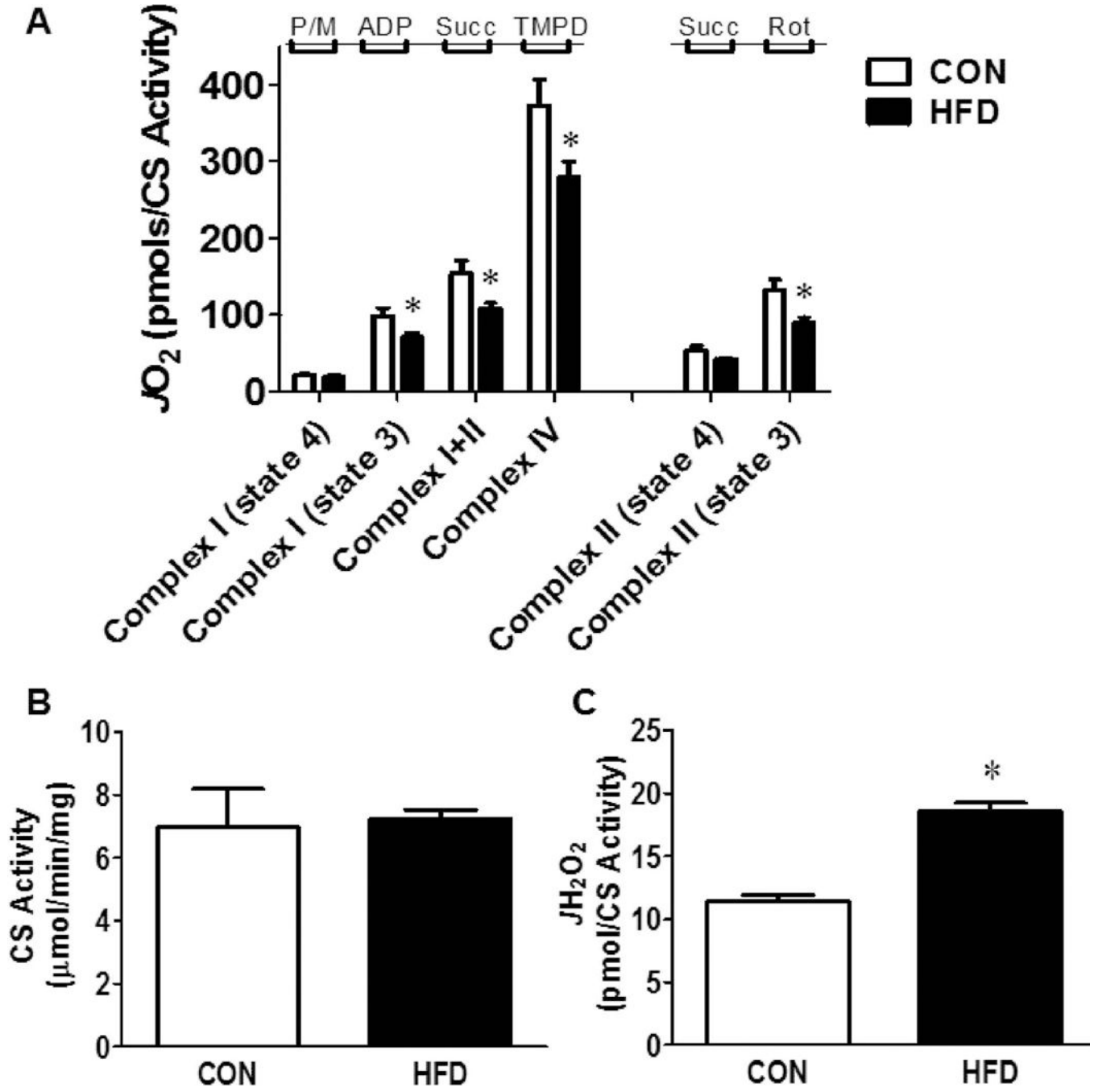


**Figure 3.**

Mitochondrial respiration was decreased in HFD detrusor smooth muscle. High-resolution respirometry of detrusor smooth muscle homogenates was decreased in HFD mice (A). Citrate synthase (CS) activity was unchanged between CON and HFD (B). The rate of mitochondrial hydrogen peroxide emission ($JH_2O_2$) was increased in HFD detrusor smooth muscle (C). Data are mean ± SEM. n=8/group; *p<0.05 vs CON. P/M: pyruvate/malate; ADP: adenosine diphosphate; Succ: succinate; TMPD: N,N,N',N'-Tetramethyl-p-Phenylenediamine; Rot: rotenone.

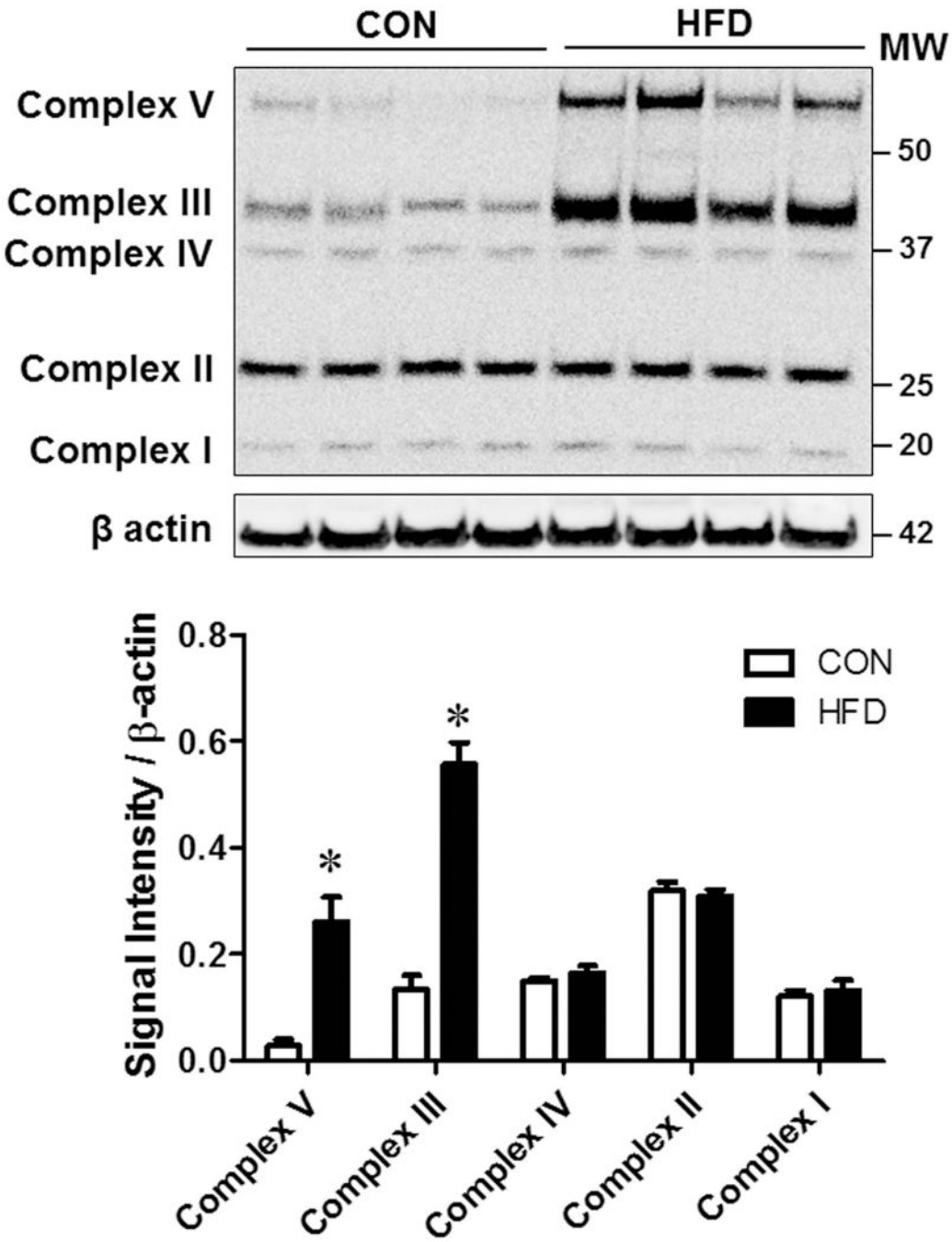


**Figure 4.**

Detrusor mitochondrial electron transport chain protein complexes I-V were assessed by Western blot. Analysis by densitometry showed that complex V and III were increased in HFD detrusor. Data are mean ± SEM. n=8/group; *p<0.05 vs CON.

**Table 1.**

Physiological variables

| | Body Weight (g) | Visceral Fat pads (g) | Bladder (mg) | Bladder (% body wt) | Blood glucose (mg/dL) |
|---|---|---|---|---|---|
| **CON** | 30.3 ± 2.15 | 0.73 ± 0.157 | 32.1 ± 4.63 | 0.11 ± 0.018 | 96 ± 28.6 |
| **HFD** | 48.7 ± 2.60 * | 4.76 ± 1.067 * | 32.1 ± 5.26 | 0.07 ± 0.009 * | 159 ± 17.4 * |

Data are mean ± SEM.

* $p<0.05$ vs CON. n=16/group