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Physiologic and metabolic safety of butyrylcholinesterase gene therapy in mice

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Abstract

In continuing efforts to develop gene transfer of human butyrylcholinesterase (BChE) as therapy for cocaine addiction, we conducted wide-ranging studies of physiological and metabolic safety. For that purpose, mice were given injections of adeno-associated virus (AAV) vector or helper-dependent adenoviral (hdAD) vector encoding human or mouse BChE mutated for optimal cocaine hydrolysis. Age-matched controls received saline or AAV-luciferase control vector. At times when transduced BChE was abundant, physiologic and metabolic parameters in conscious animals were evaluated by non-invasive Echo-MRI and an automated “Comprehensive Laboratory Animal Monitoring System” (CLAMS). Despite high vector doses (up to 10^{13} particles per mouse) and high levels of transgene protein in the plasma (~ 1500-fold above baseline), the CLAMS apparatus revealed no adverse physiologic or metabolic effects. Likewise, body composition determined by Echo-MRI, and glucose tolerance remained normal. A CLAMS study of vector-treated mice given 40 mg/kg cocaine showed none of the physiologic and metabolic fluctuations exhibited in controls. We conclude that neither the tested vectors nor great excesses of circulating BChE affect general physiology directly, while they protect mice from disturbance by cocaine. Hence, viral gene transfer of BChE appears benign and worth exploring as a therapy for cocaine abuse and possibly other disorders as well.

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CONFLICT OF INTEREST None

Keywords

Butyrylcholinesterase; Organophosphate toxicity; cocaine addiction; gene therapy; adeno-associated viral vector; helper-dependent adenoviral vector

INTRODUCTION

Cocaine addiction is a leading cause of sudden death and is highly treatment-resistant (Devlin and Henry, 2008), lacking any effective, proven therapy. However treatment with plasma butyrylcholinesterase (BChE) is emerging as a promising new approach. This widely expressed enzyme has no well-identified function (Giacobini, 2003; Lockridge, 2003), but slowly cleaves cocaine into ecgonine methyl ester and benzoic acid, which lack stimulant properties (Davies *et al.*, 1960; Masson *et al.*, 2007). Over the past decade, guided by molecular dynamics simulations, BChE mutations have yielded a “true” cocaine hydrolase (CocH) with ~ 1500-fold improved catalytic efficiency over wild-type BChE (Sun *et al.*, 2002; Xie *et al.*, 1999; Yang *et al.*, 2010; Zheng *et al.*, 2010). CocH reduces brain cocaine access and drug reward. It now seems likely that sustained CocH expression might reduce relapse rates in users trying to remain abstinent (Brimijoin and Gao, 2012; Brimijoin *et al.*, 2013a).

Exogenous enzymes disappear from the circulation within days, necessitating frequent and costly injections even with slow-release formulations. Viral gene transfer provides a possible solution. Our rodent gene transfers achieve durable levels of plasma CocH that suppress responses to cocaine (Carroll *et al.*, 2012; Gao and Brimijoin, 2004). We found that CocH transduced by helper-dependent adenoviral vector (hdAD) blocked reinstatement of cocaine-evoked operant responding in rats for at least six-months (Anker *et al.*, 2012), obliterated mouse reactions to lethal cocaine doses (Brimijoin *et al.*, 2013b; Gao *et al.*, 2013; Geng *et al.*, 2013), and fully suppressed ongoing operant responding for i.v. drug (Zlebnik, 2014).

To prepare for a human trial, safety studies of BChE and vector are crucial. We and others saw no toxicity from large amounts of native or mutant human BChE protein (Brimijoin *et al.*, 2008; Gao and Brimijoin, 2004; Saxena *et al.*, 2011; Saxena *et al.*, 2005; Weber *et al.*, 2011). Nor have we seen adverse effects from viral gene transfer in mice and rats (Geng, *et al.*, 2013; Zlebnik, 2014) or rhesus monkeys (Carroll and Brimijoin, unpublished data). Therefore we hypothesized that a sustained hundred-fold increase in plasma BChE does not disturb physiological function. However, anticipating FDA requirements for an investigational new drug permit we examined physiologic and metabolic parameters with a sophisticated “Comprehensive Animal Monitoring System” (CLAMS), and Echo MRI. These studies investigated O₂ consumption, CO₂ production, food intake, 24-hr activity levels, and glucose homeostasis in Balb/c mice after viral gene transfer of CocH, native mouse BChE, and human BChE.

MATERIALS AND METHODS

Drug

Cocaine HCl obtained from the National Institute of Drug Abuse (Research Triangle Institute, Research Triangle Park, NC USA) was freshly dissolved in 0.9% NaCl in each experiment for i.p. delivery, 40 mg/kg (~ 200 µl volume).

Animals

Balb/c male mice (6-7 weeks old) from Harlan (Madison, WI) were housed in plastic cages with access to water and food (Purina Laboratory Chow, Purina Mills, Minneapolis, MN, USA) in rooms controlled for temperature (24 °C), humidity (40–50%), and illumination (light/dark, 12h/12-h, lights on at 6:00 a.m.). Experiments were conducted in accordance with the Principles of Laboratory Animal Care in an AAALAC-accredited facility on protocol A20812, approved by Mayo Clinic's Institutional Animal Care and Use Committee.

Viral gene transfer

Two viral vectors and three enzymes were tested. Certain experiments used AAV-8 vectors incorporating cDNA for CocH (human or mouse BChE, mutated respectively for improved cocaine hydrolysis (A199S/F227A/S287G/A328W/Y332G or A199S/S227A/S287G/A328W/Y332G, respectively). As described (Geng, *et al.*, 2013), modified hBChE sequence was ligated into pAAV-VIP vector (Balazs *et al.*, 2012) between Not I and BamH I restriction sites. An AAV-VIP-mBChE mutant plasmid was co-transfected into HEK293T cells with pHelper and pAAV2/8, using FuGene HD Transfection Reagent (Roche). AAV virus was purified from cell lysates by ultracentrifugation against Optiprep Density Gradient Medium-Iodixanol (Sigma-Aldrich, St Louis, MO). Other experiments used helper-dependent adenoviral vector (hdAD) with cDNA for mouse CocH regulated by a human ApoE hepatic control region (Kim *et al.*, 2001), and a bovine growth hormone polyadenylation sequence cloned into a derivative of the p28lacZ hdAD-backbone plasmid. Vector was propagated with AdNG163 helper virus (Parks, *et al.*, 1996). Helper virus contamination was ~ 0.2% for both loaded and empty vectors. Vector (200 µl, 10^{11} - 10^{13} viral particles) was delivered to 6-7 week old mice via tail-vein.

Blood collection and enzyme assay

Blood (< 0.1 ml) was taken from the cheek with a 21-gauge mouse-lancet and bleeding was stopped with sterile gauze. Samples were centrifuged 15 min (8000g) and plasma was stored at -20° C. Cocaine hydrolase in 50 µl aliquots was assayed in a solvent-partitioning assay with ³H-cocaine (50 nCi, 18 µM) as described (Brimijoin *et al.*, 2002). Native mouse BChE activity was assayed spectrophotometrically with butyrylthiocholine (Ellman *et al.*, 1961).

Whole body composition analysis

Whole body composition was evaluated in conscious mice with an EchoMRI-100 (Echo Medical Systems LLC, Houston TX, USA) as described (LeBrasseur *et al.*, 2009). After calibration, individual mice were placed into a polymethyl-methacrylate tube for insertion

into the EchoMRI slot for a ~ 2 minute scan to measure fat, lean tissue, free water and total water masses.

Metabolic and behavioral parameters

Metabolic rates were measured at ambient temperature by indirect calorimetry of single animals in open-circuit “oxymax” CLAMS chambers (Columbus Instruments, Columbus, OH) as described (Akasaki *et al.*, 2013). Indirect calorimetry was performed during light and dark cycles to determine the extent to which treatment affected metabolic parameters during conditions of rest (light cycle) and activity (dark cycle). In addition, the effects of treatment on metabolic flexibility were assessed by measuring metabolic parameters under fed conditions (provision of a predominantly carbohydrate-based fuel source) and then fasted conditions (reliance on endogenous lipid-based energy stores). On the day of the experiment, mice were weighed and acclimated overnight. Over the next 48 hrs, locomotor activity, oxygen consumption (VO_2) and CO_2 production (VCO_2) were monitored in real time. Mice had food and water *ad libitum* during the first 24 hrs and were fasted during the subsequent 24. Respiratory exchange ratio ($\text{RER} = \text{VCO}_2/\text{VO}_2$) and metabolic rate ($\text{MR} = (3.815 + 1.232 \times \text{RER}) \times \text{VO}_2$) were calculated. This standard metabolic equation defines the calorific value (kcal) associated with the oxidation of mixtures of carbohydrate and fat (Izumiyama *et al.*, 2008; Bernardo *et al.*, 2010).

Glucose tolerance tests

Mice fasted for 6 hr received glucose i.p. at a dose of 1.0 g/kg. Blood samples were obtained from the tail tip at indicated times. A glucometer (Breeze2, Bayer Healthcare LLC, Mishawaka, IN, USA) measured glucose levels before glucose injection (0 min) and at 15, 30, 60, 90, and 120 min afterwards.

Statistics

Data were analyzed using single-factor (group) analysis of variance (ANOVA) or two-way analysis of variance (ANOVA) with Holm-Sidak post hoc testing (GraphPad Prism Statistical Software Version 6.0, San Diego, CA, USA).

RESULTS

Experimental Design

After acclimation on arrival, mice were subjected to viral gene transfer. When stable enzyme expression was confirmed (~ 4 weeks later) they sequentially underwent EchoMRI, CLAMS and Glucose tolerance tests (Flowchart, Fig. 1A).

Cocaine hydrolysis in vivo

This study addressed the safety of an experimental BChE gene-transfer therapy for cocaine addiction. As illustrated (Fig. 1B), transduction of murine or human CocH by AAV and hdAD vectors increased cocaine hydrolysis activity in plasma 500,000-fold (from 0.00004 U/ml to 20 U/ml). Calculated BChE protein increased 1,500-fold, but acetylcholine-hydrolysis activity rose only ~1000-fold (not shown) as CocH is roughly 30% less active

with acetylcholine than wild type BChE (Geng, *et al.*, 2013). Enzyme levels were sustained for eight to sixteen months.

Body composition

Echo-MRI analysis of vector-treated and control mice was performed 3-4 months after initial treatment. Body weights, water content, lean mass and fat mass of mice receiving CocH vectors remained similar to age-matched luciferase- and saline-controls (Table 1).

Activity and metabolism

Two-way ANOVA with CLAMS data on ambulation and rearing across a 48-hr window indicated no main effect of vector (Fig. 2A and 2B). VO_2 monitoring also revealed no difference between vector-treated and control groups (Fig. 2C). These measures were unaffected by feeding conditions. Post-hoc testing (Holm-Sidak) suggested slightly higher O_2 consumption by fasted AAV-hCocH mice at night, (Fig. 2C) but there was no main effect. As with motor activity and VO_2 , overall metabolic rate (Fig. 2D) remained similar to controls under both fed and fasted conditions.

Food intake tracked for 48 hr in all animals showed no group differences (Fig. 2F) but mice given AAV human CocH vector weighed less than saline- and luciferase vector controls ($p < 0.01$ and $p < 0.001$, respectively, Fig. 2E), possibly reflecting chronic low-level immune responses to foreign gene product (see DISCUSSION).

Glucose tolerance

Looking for dysregulation of whole-body metabolism, we examined glucose tolerance, i.e., rate of return to normoglycemia after acute i.v. glucose injection (Fig. 3). Again mice expressing BChE (~ 8 months after vector treatment) showed no difference from age-matched luciferase and saline controls.

Positive and negative controls

The lack of metabolic effects after CocH gene transfer necessitated a positive control. For that purpose mice were monitored 6 hr in CLAMS, then given cocaine (40 mg/kg, i.p.) and returned to the chamber for another 3 hr. The aim was to determine if post-cocaine data would show stimulation compared with the preceding period, each mouse as its own control. This design (Fig. 4A) involved 2-day observations under fed and fasting conditions with two 12-hr light/dark cycles per day. Since drug effects on locomotor activity and metabolic parameters were closely comparable under both conditions, we present fed state data only.

For adequate statistical power without excessive animal use, we examined mice previously studied, of the same strain and ages, but with varying treatments: 1) controls given saline ($n = 18$) or AAV luciferase ($n = 4$); and 2) "CocH vector," given AAV human CocH ($n = 8$), AAV mouse CocH ($n = 7$) or hdAD mouse CocH ($n = 8$). In line with prior experience, acute cocaine treatment induced large and statistically significant increases in motor activity in pooled controls (saline + AAV Luciferase) but not in CocH-vector mice. Thus, *for ambulation* (Fig. 4B), 2-way ANOVA indicated a significant main effect between control and CocH-Vector ($F(1,84) = 31.24$; $p < 0.0001$). A within-groups comparison (before-cocaine

and after-cocaine) showed significant interaction between controls and CocH-vector treatment ($F(1,84)=26.46$; $p<0.0001$) as well. Similarly, for *rearing* (Fig. 4C), 2-way ANOVA indicated a difference between control and CocH-vector mice ($F(1,84)=11.46$; $p=0.001$), and interaction between control and Coc-vector treatment ($F(1,84)=7.417$; $p=0.008$). However no significant effect was detected in CocH-vector mice before and after cocaine ($F(1,84)=3.451$; $p=0.067$). Multiple-comparison testing showed hyper-ambulation in non-CocH control mice after cocaine exposure ($p<0.0001$). But in mice given CocH vector, cocaine caused *no* increase in ambulation or rearing. In fact, post-cocaine values of both measures were non-significantly lower than pre-cocaine values (i.e., not changed) and dramatically below those in the “unprotected” controls ($p<0.0001$).

Acute cocaine treatment weakly affected metabolic parameters including VO_2 (Fig. 4D) and metabolic rate (Fig. 4E), but again, only in controls. For VO_2 , there was an overall significant effect between control and CocH vector-treatment ($F(1,84)=6.510$; $p<0.001$) and in within-group comparisons, before and after cocaine ($F(1,84)=9.114$; $p<0.0034$). There was also a significant interaction between control and CocH vector-treatment ($F(1,84)=6.552$; $p<0.0001$).

With metabolic rate, there was a significant effect between non-CocH control and CocH vector-treated mice ($F(1,84)=6.646$; $p=0.01$) and in within-group comparisons before- and after-cocaine ($F(1,84)=5.039$; $p=0.02$). The interaction between control and vector treatment was significant as well ($F(1,84)=5.074$; $p=0.02$). As with VO_2 , metabolic rate in CocH vector mice after cocaine was unchanged from before.

Multiple-comparison testing for VO_2 and metabolic rate gave a similar outcome: a significant effect in non-CocH controls before and after cocaine, with $p=0.001$ (VO_2) and 0.01 (metabolic rate). Consistent with expectations, in CocH vector-treated groups neither measure differed from before-cocaine to after-cocaine, when values were again below those in cocaine-treated controls ($p<0.003$).

A final CLAMS experiment confirmed that our procedures would have revealed depressant effects like decreased VO_2 if they had occurred. Groups of untreated young mice (4 months) were compared with older mice (12 and 24 months of age). Observations under four conditions (fed, fasted, light cycle, dark cycle) found no differences between the 4- and 12-month mice (Fig. 5), but 24-month mice showed less spontaneous ambulation and lower 24-hr VO_2 (2-way ANOVA). Post-hoc tests indicated significant effects on ambulation ($p<0.05$) during the most active condition (fasted, dark cycle), whereas VO_2 was reduced during both the light and the dark cycle, but only during the fed condition ($p<0.05$).

DISCUSSION

To our knowledge this study represents the first attempt at testing fundamental aspects of metabolism in mice receiving high-dose BChE gene transfer that, as we reported elsewhere (Geng, *et al.*, 2013), generated extraordinarily high circulating enzyme levels. Our essentially negative findings add to a consistent pattern of BChE safety reported recently by ourselves and others (Brimijoin *et al.*, 2008; Gao and Brimijoin, 2004; Saxena *et al.*, 2011;

Saxena *et al.*, 2005; Weber *et al.*, 2011). The results justify optimism that similar procedures in humans would prove benign.

Key outcomes from our earlier work are as follows. First, active-site mutants of BChE do not appear immunogenic in same-species recipients. Gene transfer of native or mutant human BChE to mice or rats typically elicits anti-BChE antibodies that curtail enzyme expression (Gao *et al.*, 2005), but no such antibodies have been detected in mice expressing low or high levels of mouse BChE with equivalent mutations (Geng, *et al.*, 2013). This outcome allowed probing for long-term adverse effects. Of concern was possible damage to the liver, the major vector target and primary site of BChE production. However, post mortem histology at multiple time points revealed no liver pathology, and assays for alanine amino transferase (ALT) failed to detect any elevation of this sentinel biomarker (Geng, *et al.*, 2013). The same study found no evidence of damage to skeletal or cardiac muscle such as increased plasma levels of cardio- and muscle-specific troponin-I. However, some of the data, e.g., the lower body weight in mice with human BChE, may reflect low levels of inflammatory cytokines generated in immune responses to foreign enzyme or, transiently, to vector coat proteins (Muruve, 2004).

Besides tissue toxicity, there are concerns that high-level enzyme might disturb physiology by decreasing acetylcholine at cholinergic synapses. Such concerns are theoretically valid since CocH hydrolyzes acetylcholine nearly as well as native BChE (Geng and Brimijoin, unpublished), which in turn is about 5% as efficient as AChE itself (Moralev, 2007). Focusing on that issue, we conducted extensive neurobehavioral studies on mice given high-dose hdAD or AAV vectors encoding mutant mouse CocH. Although such treatments caused more than 1000-fold increases in plasma BChE activity (butyrylthiocholine and acetylcholine hydrolysis), they did not affect grip strength, forced maximal treadmill endurance, or maze learning and retention in the structured “Stone T-water maze” (Murthy *et al.*, 2013). In hindsight, these results were predictable given the protected nature of the neuromuscular synapse with its high AChE content (Anglister *et al.*, 1998), and the efficiency of the blood brain barrier against enzyme entry (Vogler *et al.*, 2005). Because autonomic synapses are more open to plasma proteins they could be more vulnerable. However, we have preliminary evidence that extreme increases in plasma BChE do not affect heart rate and EKG in vector treated mice (Murthy *et al.*, unpublished). Cage-side observations have also failed to reveal signs of altered intestinal motility (e.g., diarrhea or constipation) that would accompany disturbed autonomic function. The results are in line with a preclinical animal study involving direct injection of BChE in large amounts (Weber, *et al.*, 2011) and one on high dose adenoviral gene transfer of unmodified murine BChE in mice, which also found no dysfunction (Parikh *et al.*, 2011). Thus, much evidence has emerged to indicate that, as with direct dosing of BChE protein, high-level BChE gene transfer is not harmful.

In the present study, we tested the safety of cocaine hydrolase vectors on metabolic and whole-body physiology in saline and cocaine-treated mice. A systematic examination of such phenomena requires positive controls for validation. Hence, CLAMS testing included one-time treatment with cocaine in both control and CocH-vector mice, along with a separate experiment comparing naïve mice in three groups with large differences in mean

age. The cocaine dose of 40 mg/kg was sufficient to produce a robust increase in locomotor activity in control mice, which was completely blocked in mice that received CocH vector beforehand, in line with our previous reports (Geng, *et al.*, 2013; Murthy, *et al.*, 2013). Results confirmed that CLAMS detects small changes in energy utilization and metabolism from acute stimulation or aging (Shigenaga *et al.*, 1994). The lack of such effects in the main study suggests that high BChE expression in host liver does not impair glucose tolerance, energy metabolism or oxidative phosphorylation and ATP generation, indicating preservation of pancreatic insulin release and mitochondrial function. The whole body measures investigated by Echo-MRI indicated no change in body composition such as shift in lean to fat ratio, or water content, which this instrumentation is designed to detect (Ronn *et al.*, 2013). Altogether, present findings agree with our previous observations indicating that BChE gene transfer is unlikely to present serious risk in humans, apart from the acknowledged hazards of any viral gene transfer (Brunetti-Pierri and Ng, 2011).

Chief among gene transfer hazards are innate immune responses to viral coat proteins (Muruve, 2004), which can be deadly, as in the tragic “Gelsinger incident” (Raper *et al.*, 2003). However, there are means of minimizing this danger, specifically by administration of immunosuppressant such as dexamethasone, used in organ transplant surgery and demonstrated to reduce the “cytokine storm” that can be evoked by viral vectors (Seregin *et al.*, 2009) and vaccinations (De Rosa *et al.*, 2004). We utilized it successfully in rhesus macaques to suppress interleukin 6 after doses of AAV vector exceeding 10^{13} viral particles per kg (Carroll and Brimijoin, unpublished results). Further study is required, but we are cautiously optimistic that a proper balance of risk and effectiveness can be established for treating cocaine abuse.

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A.

Experimental Flow

B.

Figure 1.

A) Experiment flow chart.

B). Cocaine hydrolase activity across time after vector transduction.

Mice received viral vector (AAV human CocH, n=14, closed circles; AAV mouse CocH, n=8, open circles; hdAD mouse CocH, n=10, closed invert triangles) at approximately 8 weeks of age and were followed for over one year. CocH activity in plasma samples is expressed in units (U) of micromoles cocaine hydrolysis per min. Baseline activity (pretreatment levels) was typically less than 0.001 U. General testing was performed on mice between the ages of 3 months and 5 months old. Positive control experiments involved mice between 9 and 15 months of age.

Figure 2.

Comprehensive Laboratory Animal Monitoring System (CLAMS). Multiple metabolic parameters were monitored in mice expressing AAV human CocH (n=12), AAV mouse CocH (n=9), and hdAD mouse CocH (n=8) and control groups, saline (n=18) and AAV LucZ vector (n=8). A) Ambulation; B) Rearing; (C) oxygen consumption; (D) metabolic rate; (E) body weight; (F) food consumption in grams per kg body weight. Mice were individually housed in metabolic chambers, with ad lib access to food for 24 hr (12 hr Fed state-light cycle; 12 hr Fed state-dark cycle); followed by 24 hr under fasting conditions (12 hr Fasted state-light cycle; 12 hr Fasted state-dark cycle). There were no significant changes observed across any treatments in activity or metabolic rate in both fed and fasted states. AAV hCocH consumed more oxygen compared to saline control in the fasted dark cycle (* $p < 0.05$). AAV hCocH showed lower body weight compared to saline control (** $p < 0.01$) and luciferase vector control (***) $p < 0.001$). No significant changes were seen in food intake relative to body weight across any treatments.

Figure 3. Glucose tolerance. Mice expressing AAV human CocH (n=9), AAV mouse CocH (n=9), and control groups, AAV LucZ vector (n=8) and saline (n = 8) were tested for glucose tolerance. Data are presented as mean $\pm$ standard error. There was no statistical difference across treatments.

Figure 4.

Scheme of Cocaine-CLAMS experiments. (A) After chamber acclimation overnight, mice were monitored for 48 hr while metabolic and locomotor data were recorded, with brief timeouts (~ 1 min) for cocaine injections (40 mg/kg, i.p.) at noon (arrows). With each mouse, data from the 6-hr control period (light gray bars) were compared with data from the first 3 hr after cocaine (dark gray bars). Motor activity, oxygen consumption and metabolic rate were measured in pooled control mice [Saline (n=18) and AAV Luciferase vector treated (n=4)] and pooled vector-treated mice [(AAV hCocH (n=8) + AAV mCocH (n=7) + hdAD mCocH (n=8)]. The mice were individually housed in CLAMS chambers for 12 hr with free access to food, while motor activity was continually quantitated as ambulation (B) and rearing (C). From 6 AM to 12 noon (day cycle), control data were recorded (light bars). At noon, 40 mg/kg cocaine was injected i.p. and data were recorded for the next 3 hr (black bars). After normalization for session length, the data were analyzed by two-way ANOVA and post hoc testing. Statistical significance is indicated: * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

Figure 5.

Age-related changes. Young, “mature,” and older mice [4 (n=5), 12 (n=4) and 24 (n=5) months of age] were compared in CLAMS for motor and metabolic parameters. Ambulatory and rearing activity (A & B) as well as VO_2 (C), were monitored for periods of 12 hr under each of four separate conditions: Fed state-light cycle; Fed state-dark cycle; Fasted state-light cycle; Fasted state-dark cycle. Two-way ANOVA revealed a significant main effect on both ambulation and VO_2). Post hoc testing showed significant reductions in ambulation and VO_2 in the oldest mice. Statistical significance is indicated: * $p < 0.05$.

Table 1

Body Composition.

	Weight (g)	Free Water (g)	Total Water (g)	% Fat	% Lean	Fat/Lean
Control (n=24)	26.6 ± 0.6	0.11 ± 0.01	17.5 ± 0.4	10.9 ± 0.3	79.1 ± 0.3	0.14 ± 0.01
AAV LucZ (n= 10)	28.9 ± 0.7	0.10 ± 0.01	18.8 ± 0.5	11.3 ± 0.3	78.0 ± 0.4	0.15 ± 0.01
AAV mCocH (n=10)	27.2 ± 0.3	0.11 ± 0.01	17.8 ± 0.2	11.2 ± 0.4	77.4 ± 0.4	0.14 ± 0.01
hdAD mCocH (n=8)	25.9 ± 0.9	0.11 ± 0.02	16.6 ± 0.7	12.9 ± 0.9	77.5 ± 0.9	0.17 ± 0.01
AAV hCocH (n=12)	23.5 ± 0.7	0.08 ± 0.01	15.4 ± 0.5	12.4 ± 0.7	78.0 ± 0.8	0.16 ± 0.01

Mice given AAV human CocH, AAV mouse CocH, or hdAD mouse CocH vector, were analyzed non-invasively for fat and water content along with control mice given AAV LucZ vector or saline. Data are presented as mean ± standard error. There were no statistical differences between treated mice and controls.