# A Nano-Scale Device (NSD) approach to Mitochondrial Uncoupling

A platform technology for the treatment of human disease including: Obesity, Diabetes, Stoke, Traumatic Brain Injury

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# Increasing Metabolism by Short-Circuiting the Mitochondrial ETS-OxPhos Circuit

- Increasing proton conductance across the inner mitochondrial membrane using chemical uncouplers has been demonstrated and results in significant weight loss in humans

# Downside of chemical uncouplers

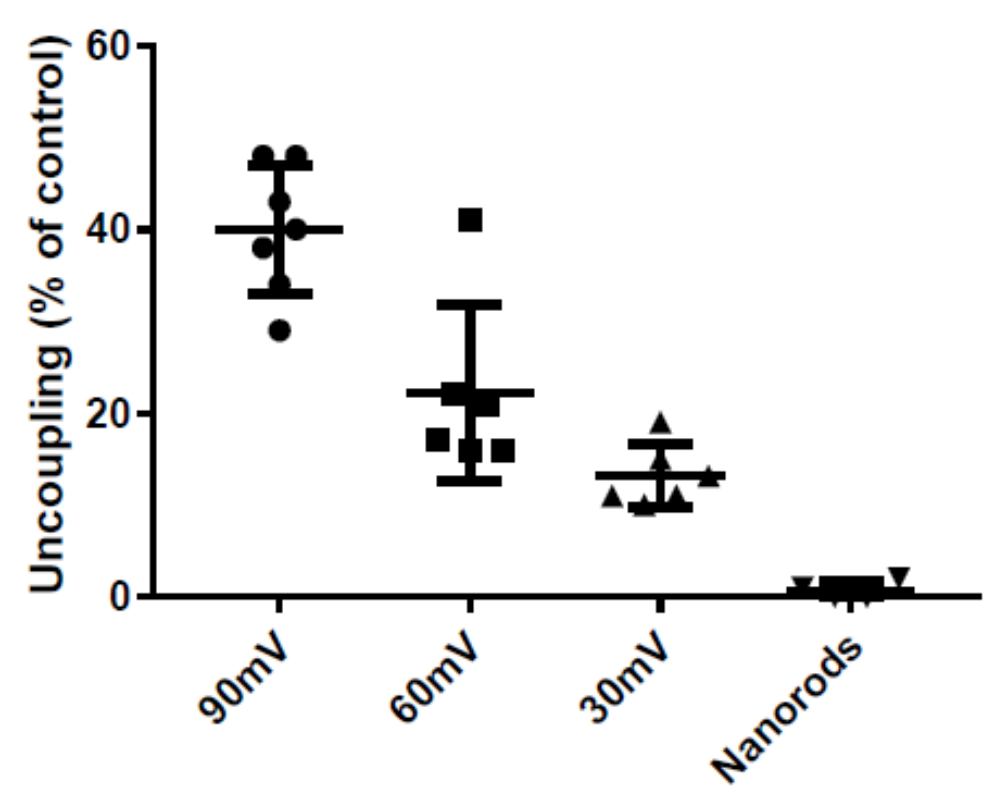
- Uncontrolled drop in membrane potential: completely dissipates $\Delta\Psi$.
- FDA pulled the chemical uncoupler 2,4-DNP from the shelves due to multiple severe side effects, including death.

# What we are shooting for:

- **Nano-scale devices that increases respiration (proton conductance) without comprising ATP production: Speed up mitochondrial ETS (substrate burn) by lowering membrane potential.**

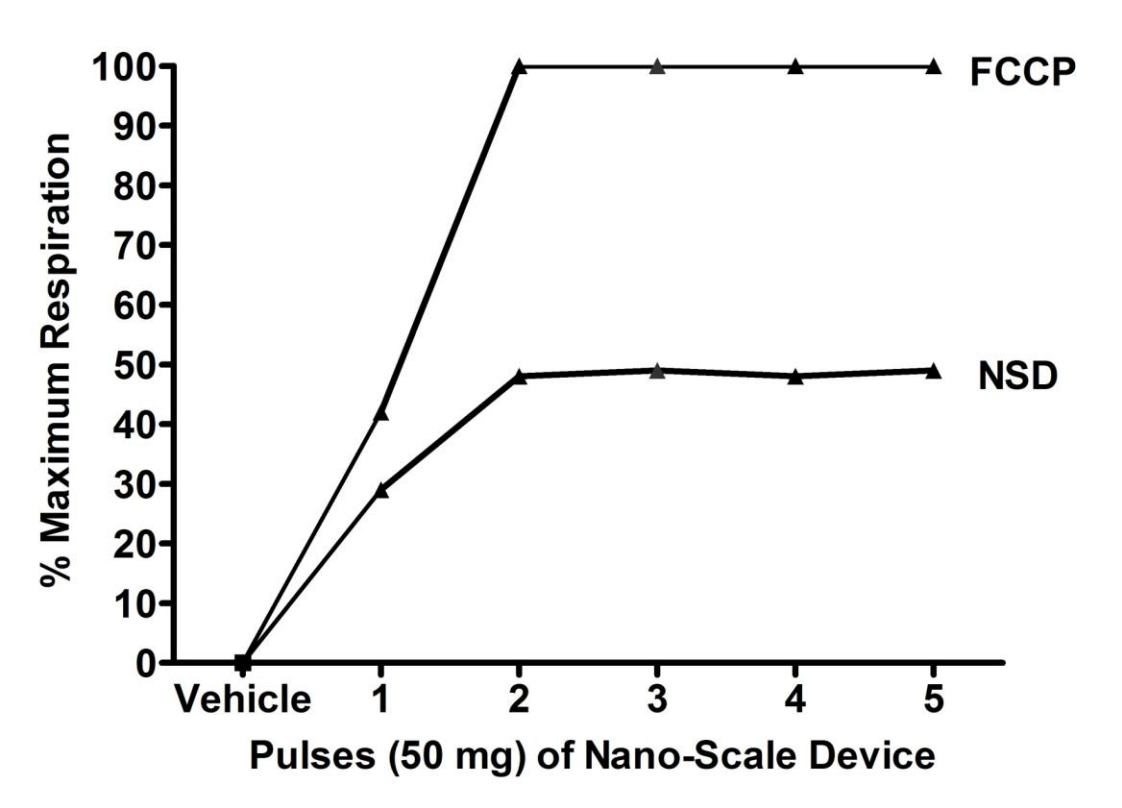
- **Our nanotubes are the device and control the flow of protons across inner mitochondrial membrane.**

# NSDs are tunable



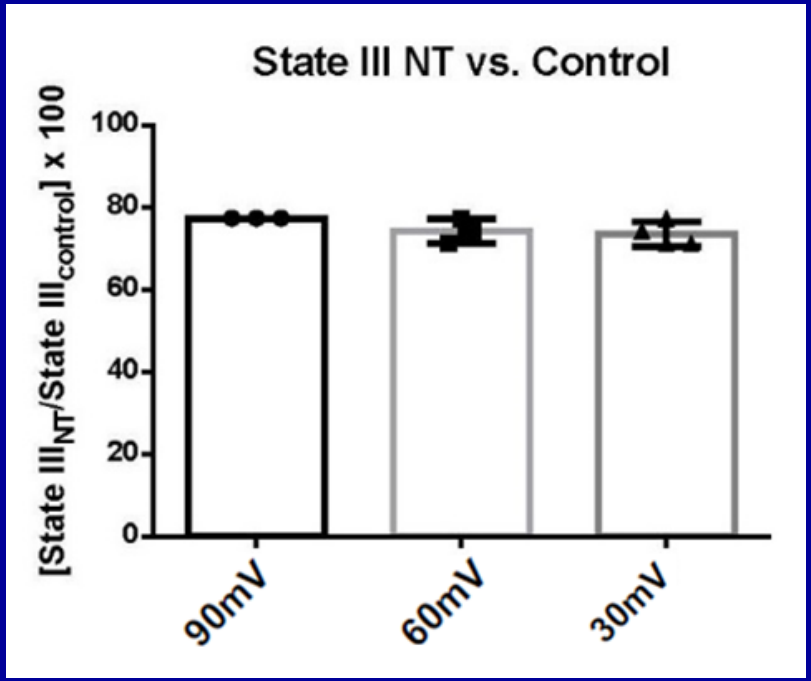

NSDs were manufactured to increase mitochondrial uncoupling in a step-wise manner without compromising mitochondrial bioenergetics, translating to safe uncoupling

# NSDs will not exceed their tuned uncoupling capacity regardless of the concentration



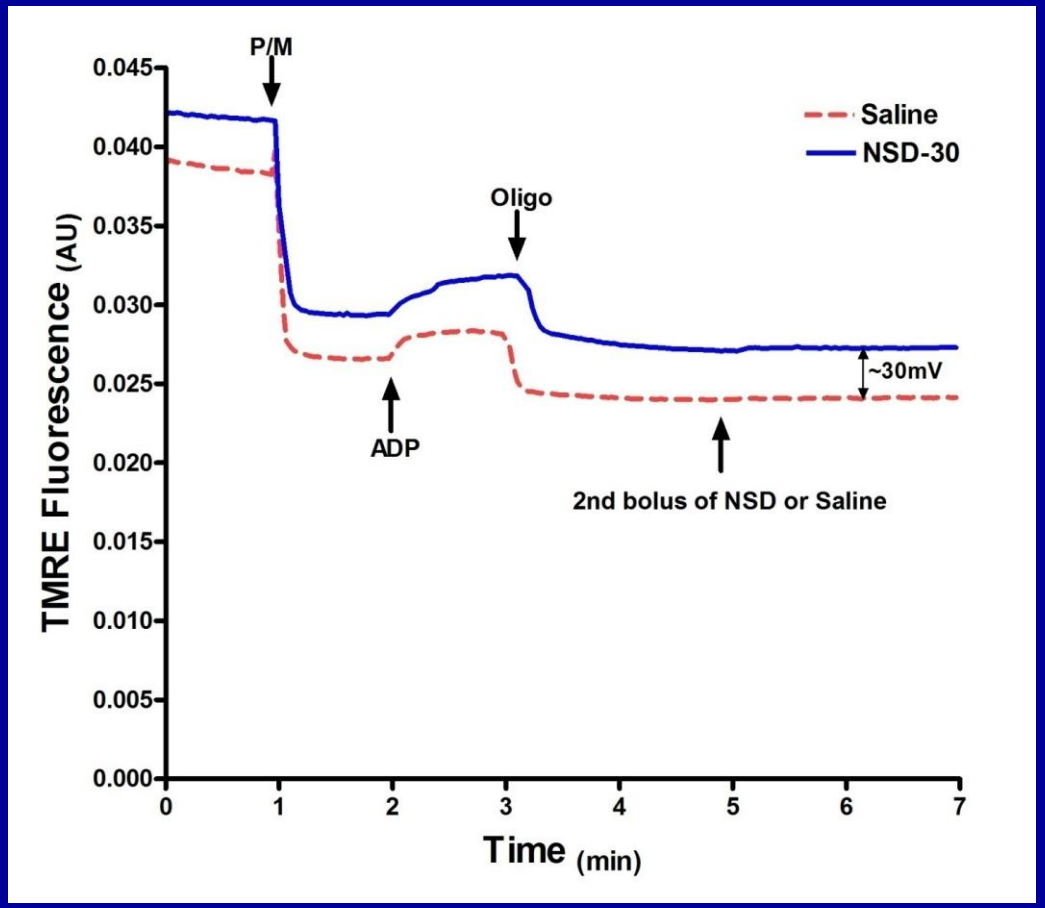

**NSDs self-rectify and will not completely uncouple mitochondria regardless of the concentration.** Cortical mitochondria were isolated from adult Sprague-Dawley rats. Oxygen consumption was assessed in a sealed, thermostated and stirred chamber equipped with a Clark-type electrode. Pyruvate (10 mM) and malate (5 mM) were added to the chamber as substrates. Oligomycin (1 μM) was then added to inhibit ATP synthase and lock the mitochondria in state IV to directly assess proton conductance across the inner mitochondrial membrane. Following the addition of vehicle (saline or EtOH), the chemical uncoupler FCCP (250 nM) or NSD manufactured to reduce ΔΨm ~30 mV (NSD-30) were injected into the chamber (50 mg/g mitochondrial protein) and the percent increase over state IV oxygen consumption measured. FCCP completely uncoupled the mitochondria at a concentration of 500 nM (2 additions) indicating a complete collapse of ΔΨm. In contrast, the NSD-30 only increased oxygen consumption by ~48% even after 5 additions (250 mg/g mitochondrial protein) indicating that **membrane potential was only being dissipated partially and that the NSDs were in fact shutting off once the membrane potential was reduced by ~ 30 mV.**

# NSDs do not compromise mitochondrial bioenergetics-ATP production




NSDs were manufactured to reduce ΔΨm ~ 30, 60, or 90mV. They were incubated (10 mg/g mitochondrial protein) with naïve isolated cortical mitochondria from mice and oxygen consumption was measured. State III respiration was induced by the addition of ADP and oxygen consumption measured in the absence and presence of various NSD with different uncoupling rates. **State III respiration was maintained at 80% for all NSD tested, indicating that we can alter uncoupling without significantly reducing ATP production.**

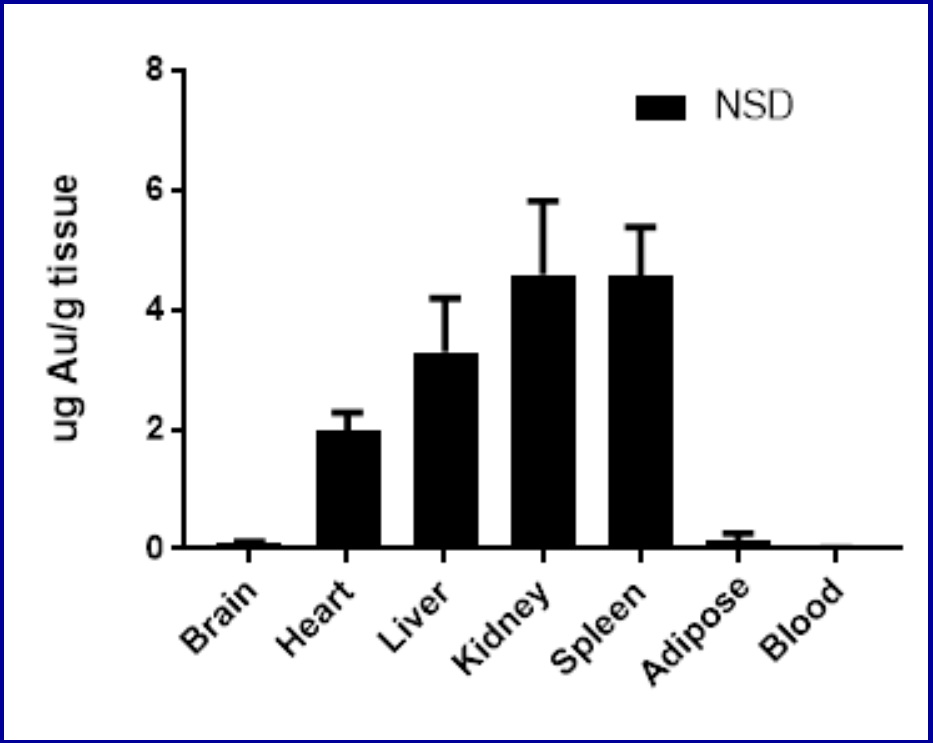
# Nano-scale Device reduces Mitochondrial Membrane Potential




**Self-rectifying nano-scale devices reduce ΔΨm**. Cortical mitochondria were isolated and placed in a sealed, thermostated and constantly stirred chamber and TMRE fluorescence measured in the presence of vehicle (saline) or NSD (100 mg) designed to reduce ΔΨm ~30 mV. The substrates pyruvate and malate (P/M; 10 and 5 mM) were added and due to electron transport system activity, a membrane potential was generated, indicated by a drop in fluorescence as the TMRE was sequestered and the indicator stacked in the mitochondrial matrix. ADP (150 nM) was then added to ensure the mitochondria were coupled. ΔΨm was utilized to phosphorylate ADP and this is demonstrated by the increase in TMRE fluorescence. Oligomycin was then added to inhibit proton flux through the ATP synthase which yields maximum membrane potential. **NSDs reduced ΔΨm ~30 mV which is illustrated by the higher TMRE fluorescence** (lower ΔΨm, less indicator quenched or stacked in the matrix**). A second bolus of NSDs was then added to the chamber to assess the self-rectifying properties of the NSDs and illustrated by the absence of any change in ΔΨm which would have resulted in an increase in TMRE fluorescence.**

***Increase in potential = Decrease in fluorescence**

# Biodistribution of NSDs

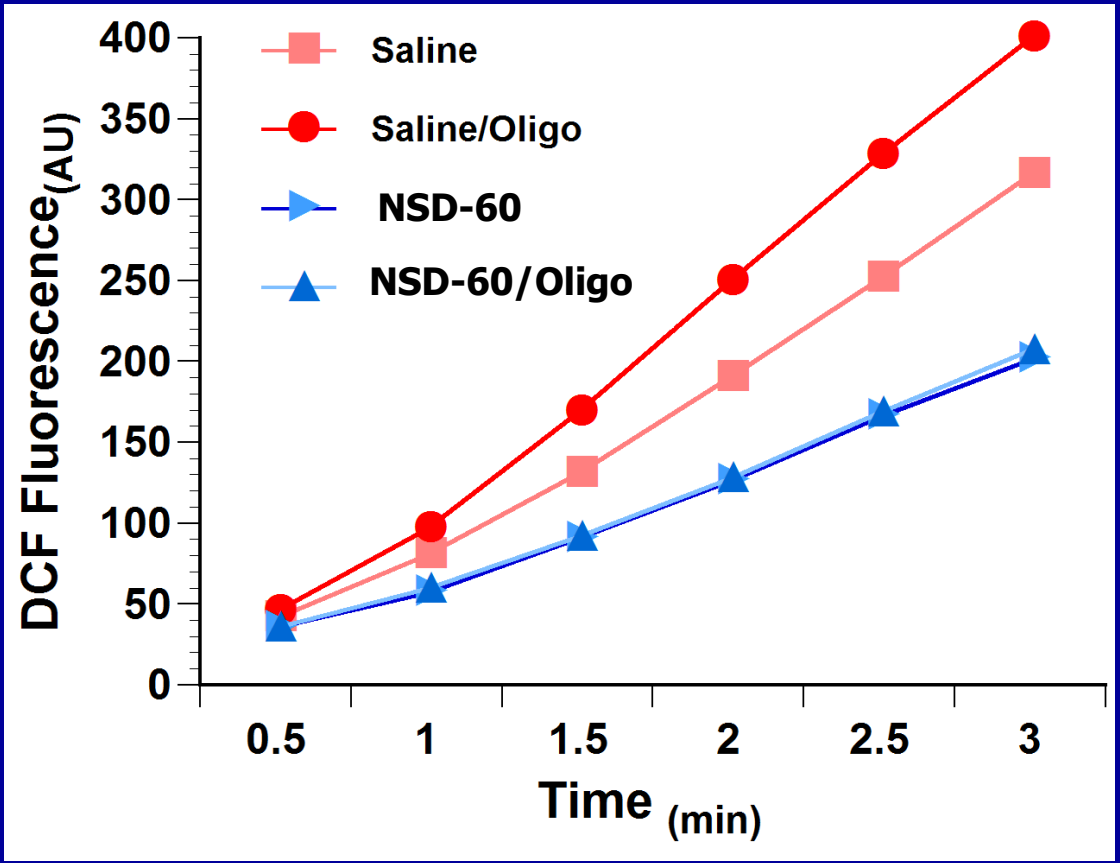



Mice were dosed with NSDs(n=4/group; 50 mg/kg every other day for 16 days following by 2 day washout period). Lyophilized tissue samples were digested using 5 mL of concentrated trace-metal grade HNO3 acid ramping to 110°C and holding under reflux for 10 min. This was followed by adding 5 mL of concentrated trace-metal grade HCl and heating under identical conditions. Reagent blanks were included with each digestion set. The samples were then diluted and analyzed using ICP-MS.

NSDs in brain was nearly undetectable

Nano-Mite has preliminary data demonstrating NSDs can be targeted to the adipose

# Nano-scale Device Reduces Mitochondrial ROS Production

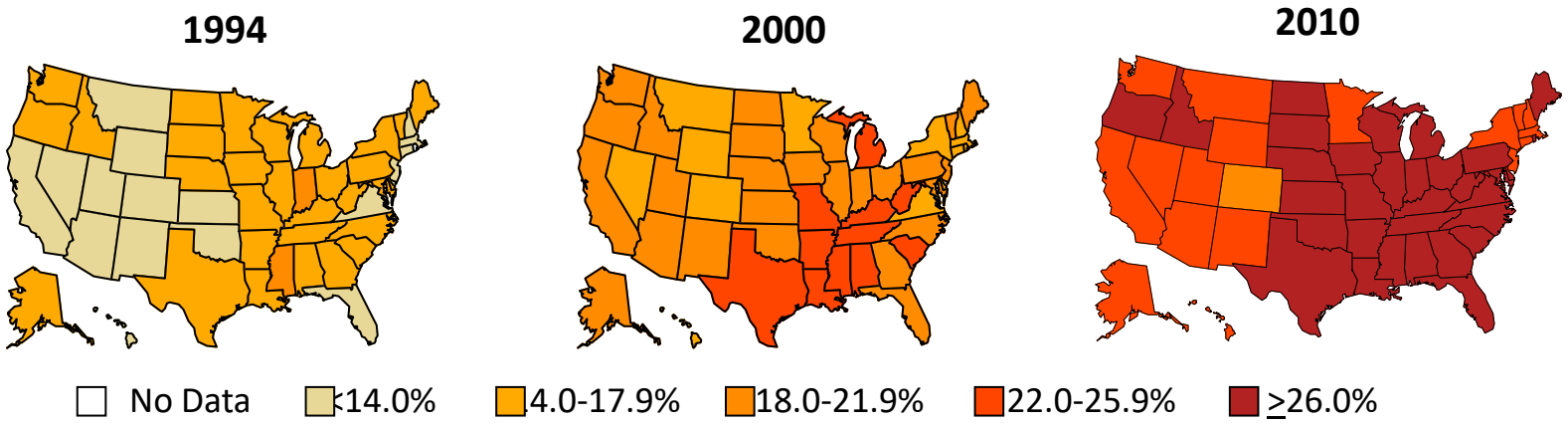



Mitochondrial ROS production is intimately linked to ΔΨm such that hyperpolarization (high ΔΨm) increases and promotes ROS production, **decreasing metabolism resulting in weight gain.** The underlying mechanism is the altered redox potential of electron transport chain carriers (reduced) and an increase in semiquinone anion half-life (high ΔΨ prevents bh oxidation of cytochrome b1 in the Q cycle). In other words, at a high ΔΨm, protons can no longer be pumped out of the matrix (against the electrochemical proton gradient) so electron transport slows/stalls resulting in intermediates staying reduced longer and increasing the chance that the electrons escape from these intermediates, reduce O2 and increase ROS production. Since the magnitude of ROS production is largely dependent on, and correlates with, ΔΨm even a modest reduction via increased proton conductance (decreased ΔΨ, the electrochemical proton gradient) across the mitochondrial inner membrane (uncoupling) **reduces ROS formation, increasing metabolism and promoting weight loss. As illustrated, NSDs can reduce both basal and maximal reactive oxygen species produced by mitochondria.**

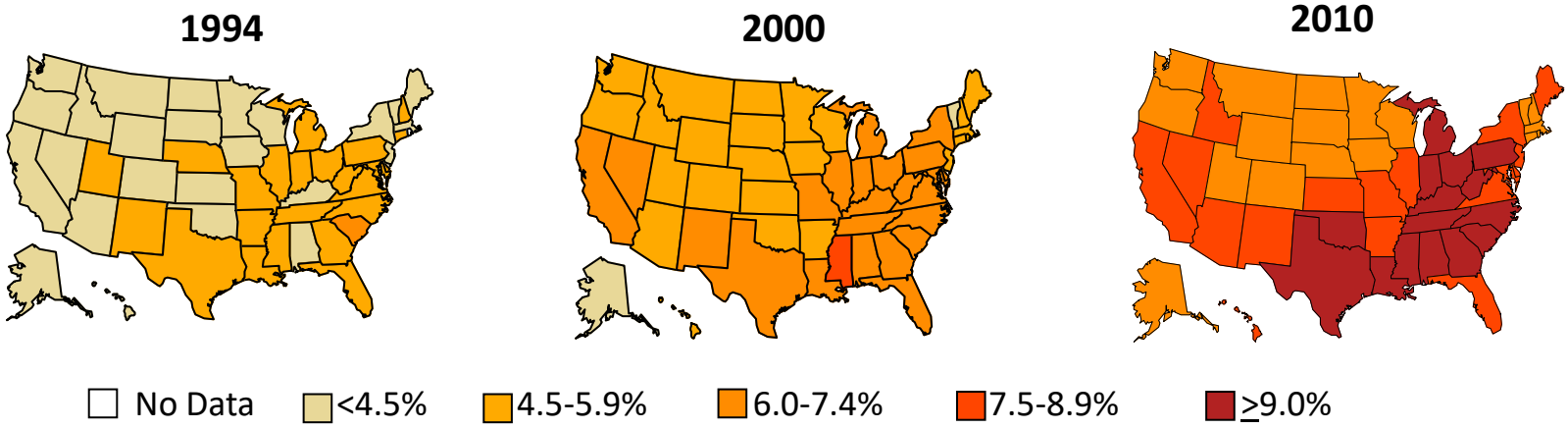
**Self-rectifying NSDs reduce mitochondrial ROS production**. Cortical mitochondria were isolated and ROS production measured using the fluorescent indicator dihydrodichlorofluorescein diacetate (DCF), which fluoresces when oxidized leading to an increase in signal. Mitochondrial reactive oxygen species were assessed under both basal conditions (substrates pyruvate and malate (P/M; 10 and 5 mM only) and in the presence of oligomycin (substrates pyruvate/malate and oligomycin (P/M; 10 and 5, oligo 1 μM) to maximize ΔΨm and induce maximum, ΔΨm-dependent ROS production. NSD-60 (60 mV reduction in ΔΨm) reduced both basal and maximal reactive oxygen species produced by mitochondria.

# Prevalence of obesity and diagnosed diabetes among U.S. adults

## Obesity (BMI ≥30 kg/m²)

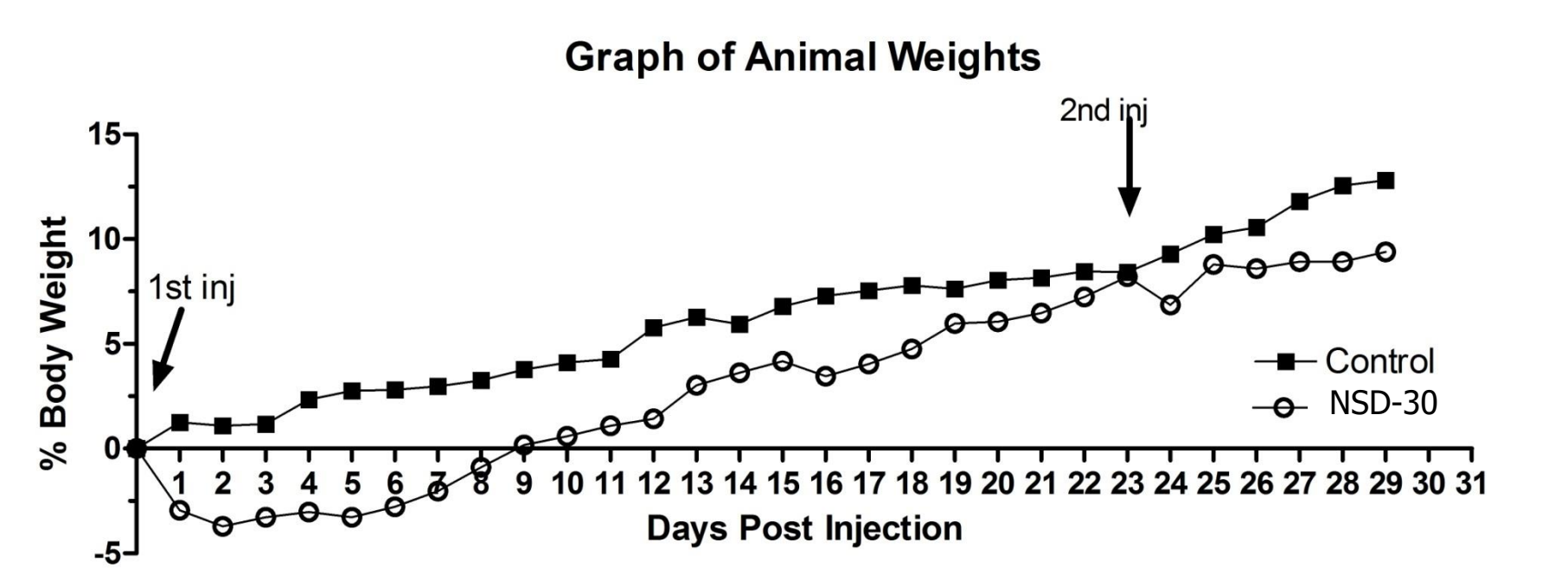



## Diabetes



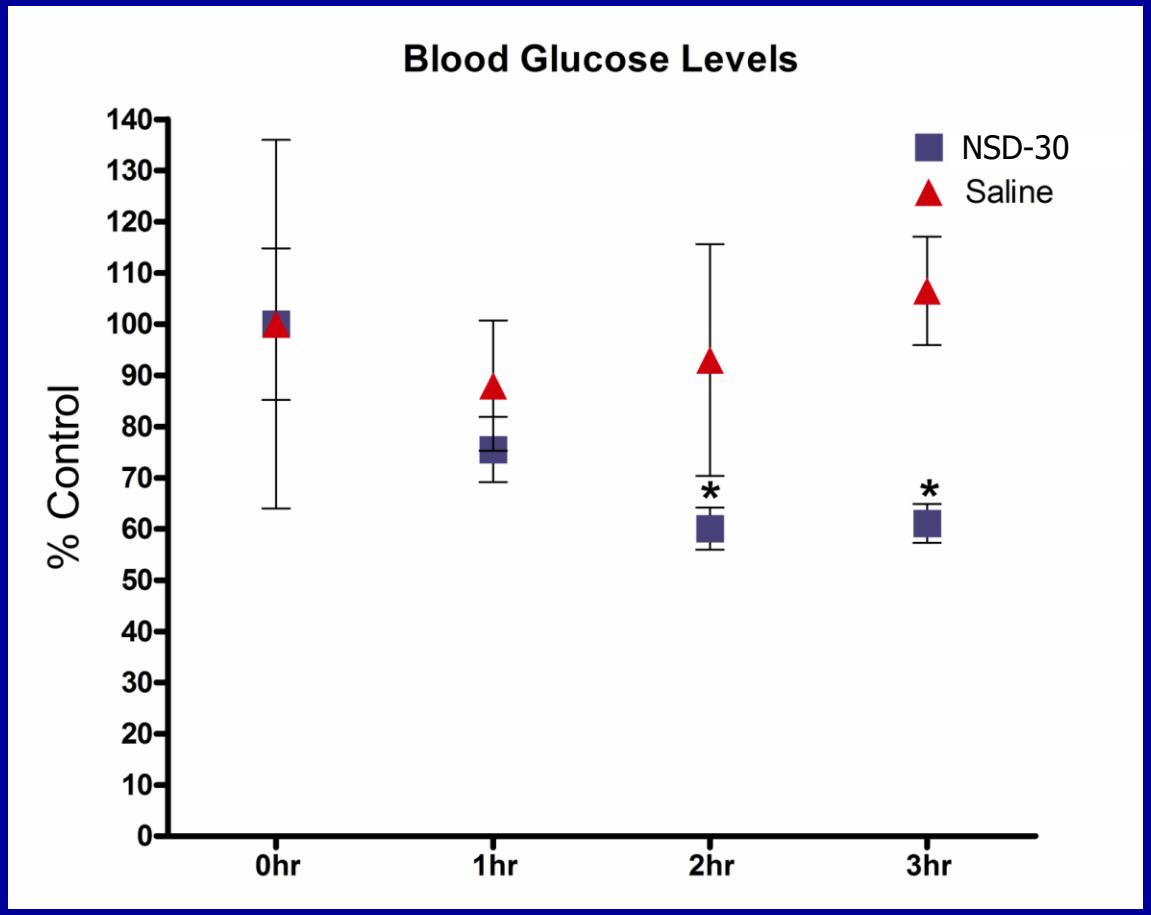

CDC's Division of Diabetes Translation. National Diabetes Surveillance System available at http://www.cdc.gov/diabetes/statistics

# NSDs cause weight reduction and limit gain in adult rats




Adult Male Sprague-Dawley Rats were administered 25 mg/kg of NSD-30 and weights monitored over 31 days. Following injection of NSD-30, a **rapid loss of weight (~4%) was observed that was maintained over 6 days post-injection.** A second injection of NSD-30 (25 mg/kg) was administered on day 23 which was **again followed by a rapid weight loss over the next 5 days.** Note food intake was available ad lib. Data are group average (n=4/group).

# NSDs Reduces Blood Glucose Levels Possible Diabetes Applications

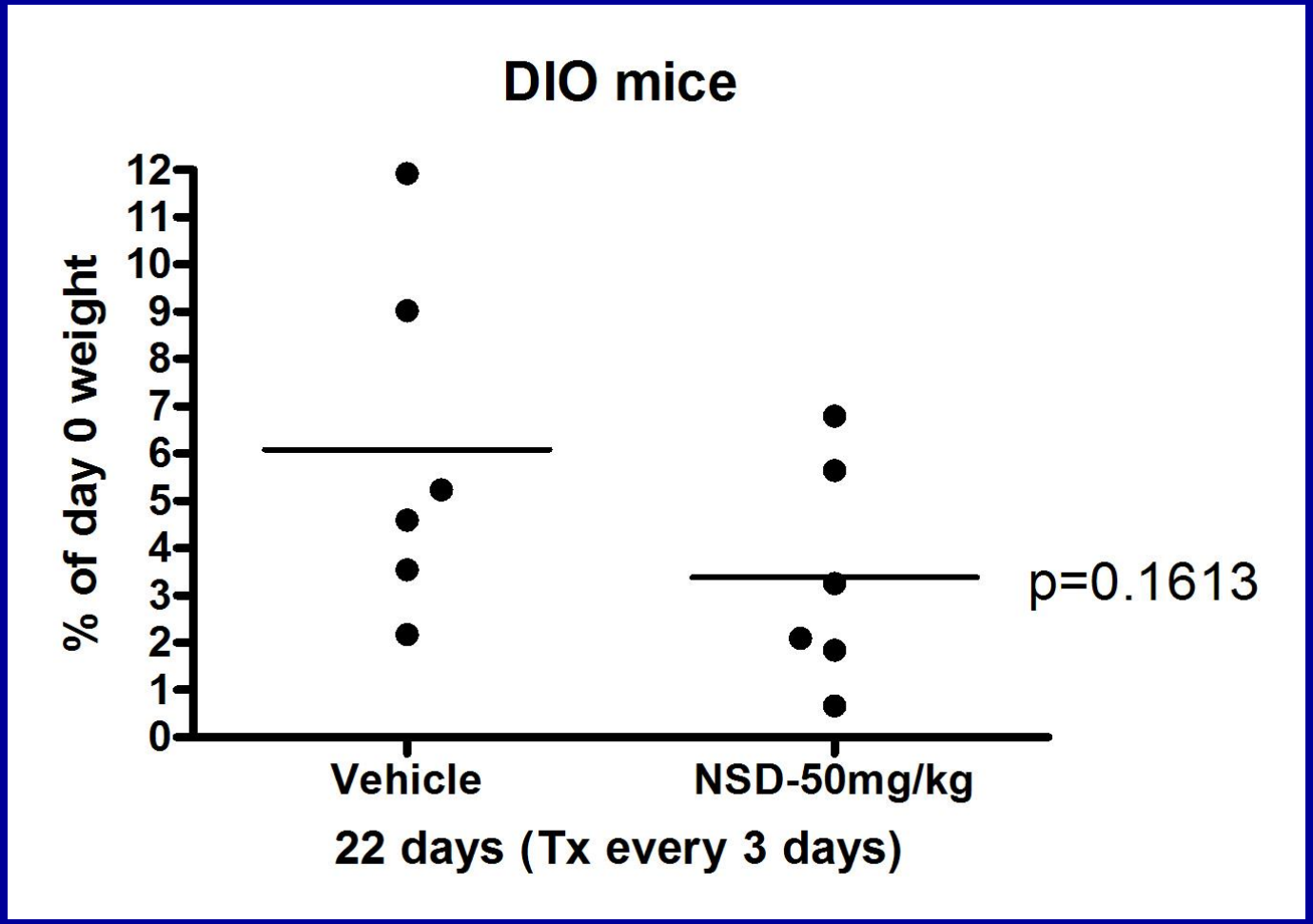



NSD-30 (25 mg/kg) or saline were administrated to Adult Male Sprague-Dawley Rats and blood glucose levels measuring at various times post administration. **The results demonstrate NSD can increase metabolism and decrease blood glucose levels.**

# Dose response paradigms in diet-induced obesity (DIO) mice experiments

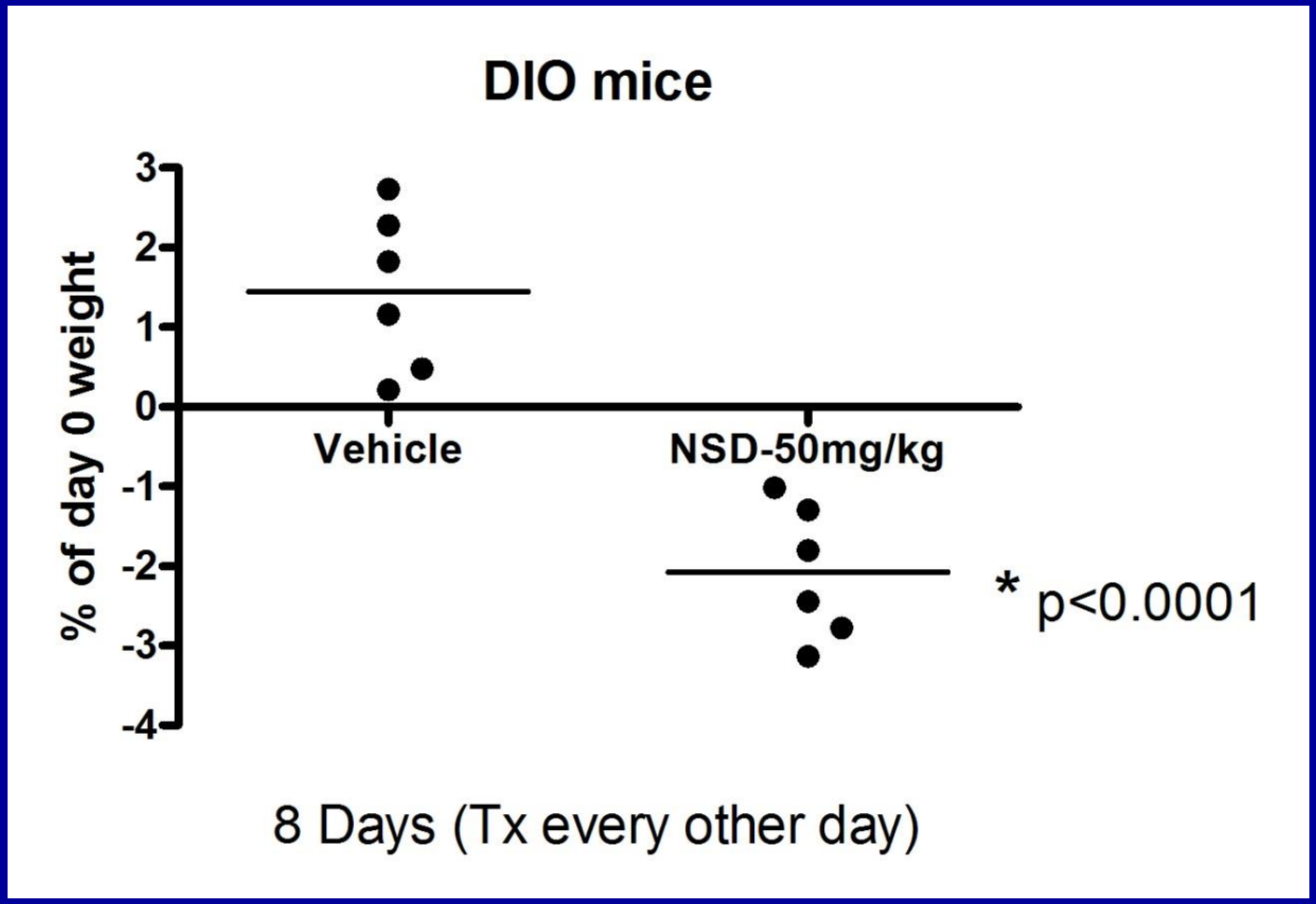
- Female DIO mice ordered from Taconic
- Randomly assigned to groups; Vehicle or Nano-Scale Device (NSD), weights obtained every other day
- Pilot study indicated very rapid turnover of NSD in mice compared to canines or rats so dosing at every 1, 2 or 3 day intervals
- No toxicity observed in any group at any dosage

# Paradigm 1



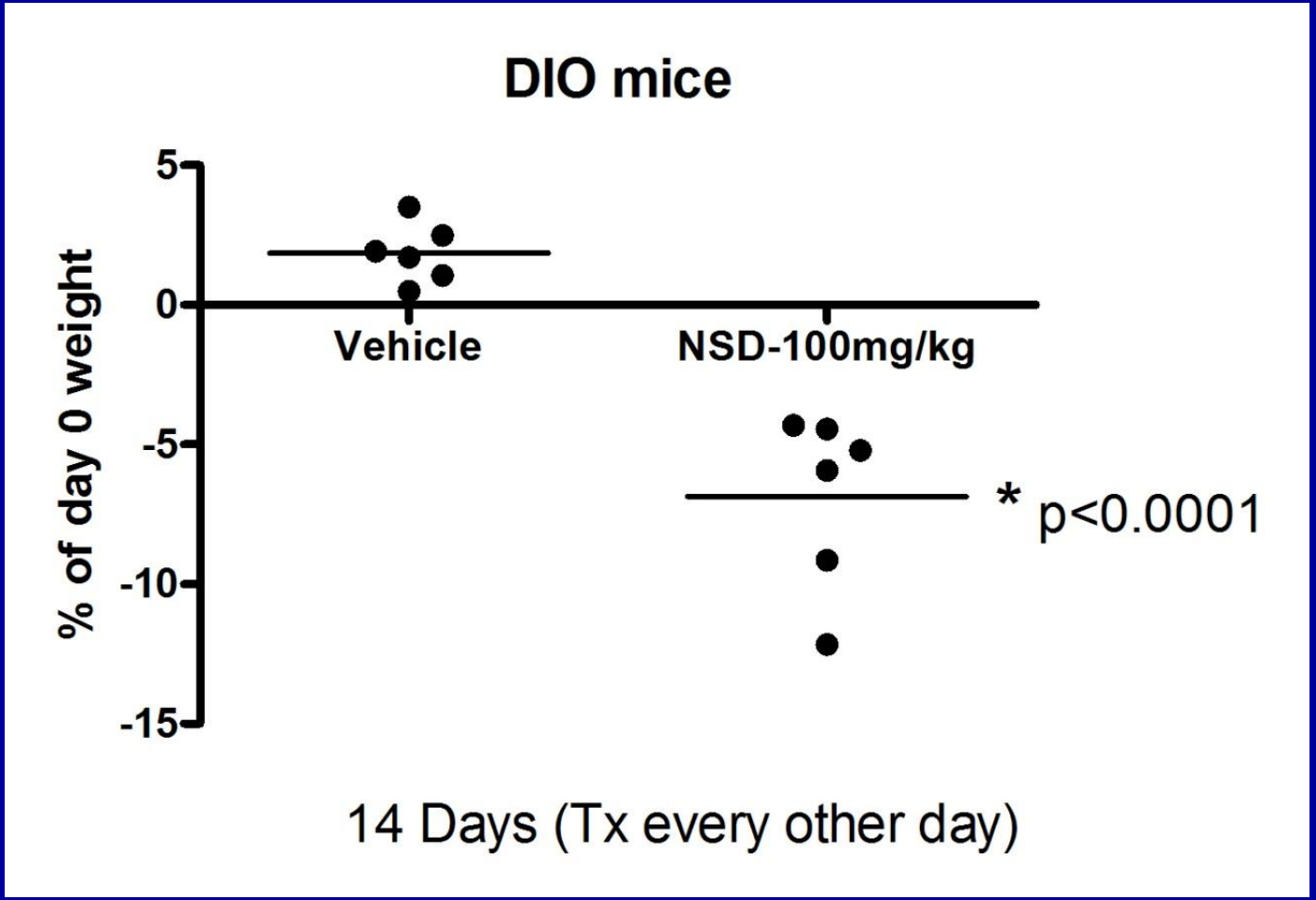

NSD (50 mg/kg) administered every 3 days for 22 days

# Paradigm 2



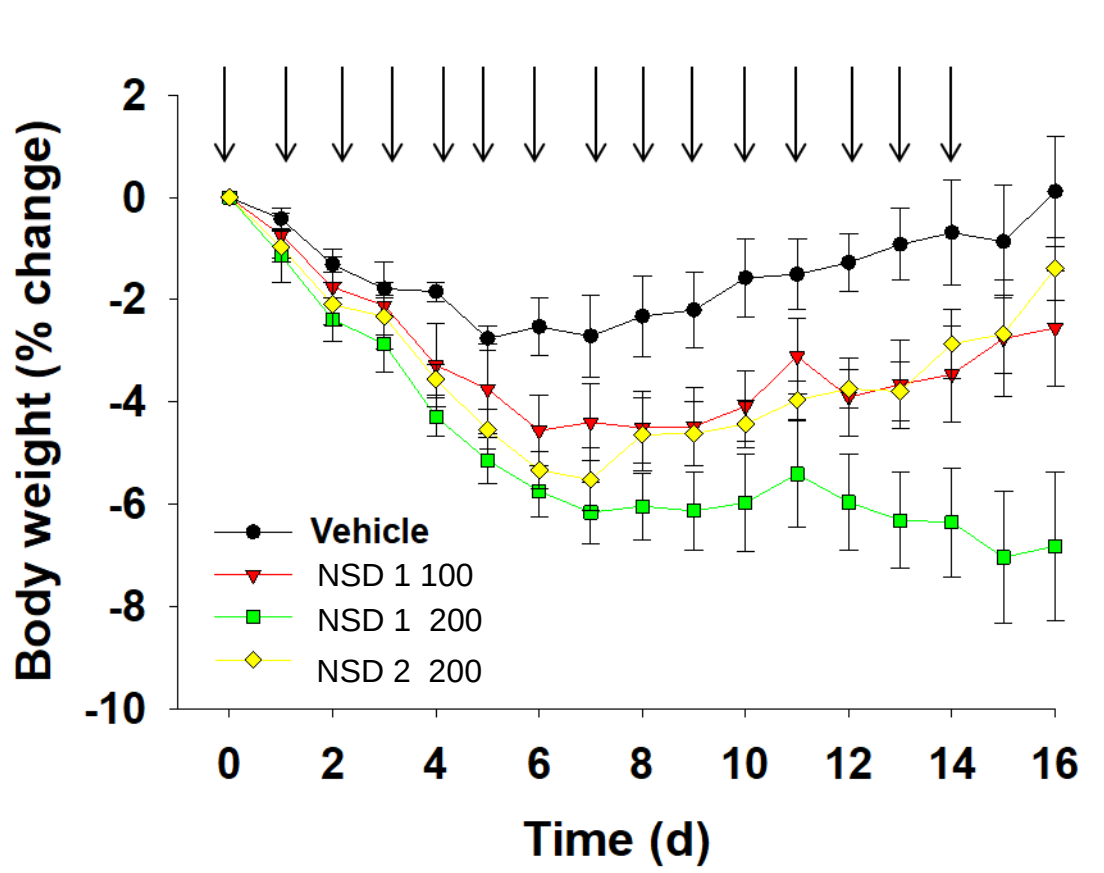

**NSD (50 mg/kg) administered every other day for 8 days**

# Paradigm 3



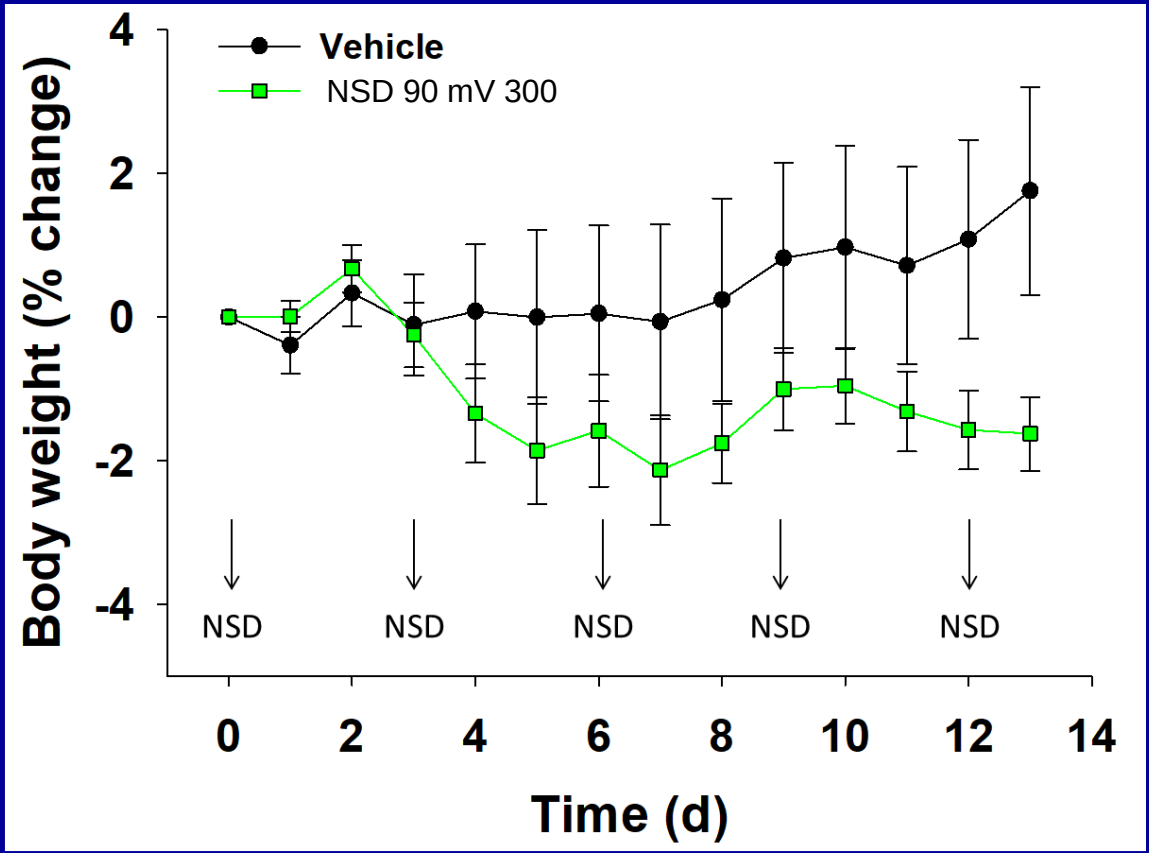

**NSD (100 mg/kg) administered every other day for 14 days**

# Paradigm 4




**DIO mice (n=20/group, means +SD) were administered were NSDs manufactured to reduce ΔΨm at varying levels ~60 and 90mV at 100 mg/kg or 200 mg/kg daily for 16 days and weights measured. A dose response was demonstrated where the highest uncoupling NSD (NSD1) at the highest dose caused significant weight loss (~7% loss of body weight at day 16) compared to any other NSD or NSD dose.**

# Paradigm 5




DIO mice (n=8/group, means +SD) were administered NSD manufactured to reduce ΔΨm ~90mV at 300 mg/kg every 3 days for 13 days and weights measured. The results demonstrate that lower dosing at higher frequency may affect body weight loss more robustly than dosage based on these results. Nonetheless, body weights on days 12 and 14 were significantly different between the experimental groups.

# Independent (big pharma*) Verification of Effect of Nano-Scale Devices: Toxicity and Weight Loss in Obese Canines

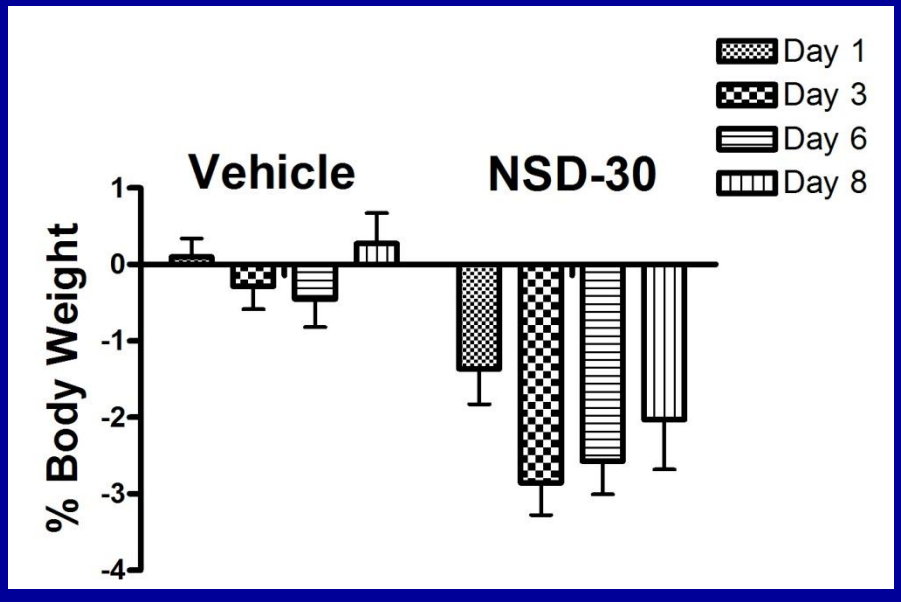
- **Trial # 1: Toxicity studies using 0.3-3 mg/kg/day repeated dosing over 10 days (IP and IV routes used).**
  - **No changes in > 20 clinical pathology endpoints but dose-dependent weight loss of ~1 to 4% over 7 days (raw data available).**

- **Trial # 2: 14 animals (n=7/group), single dose of 3 mg/kg IP, all animals that received Nano-scale Device lost weight (range 1-2.5% of body weight over 7 days).**

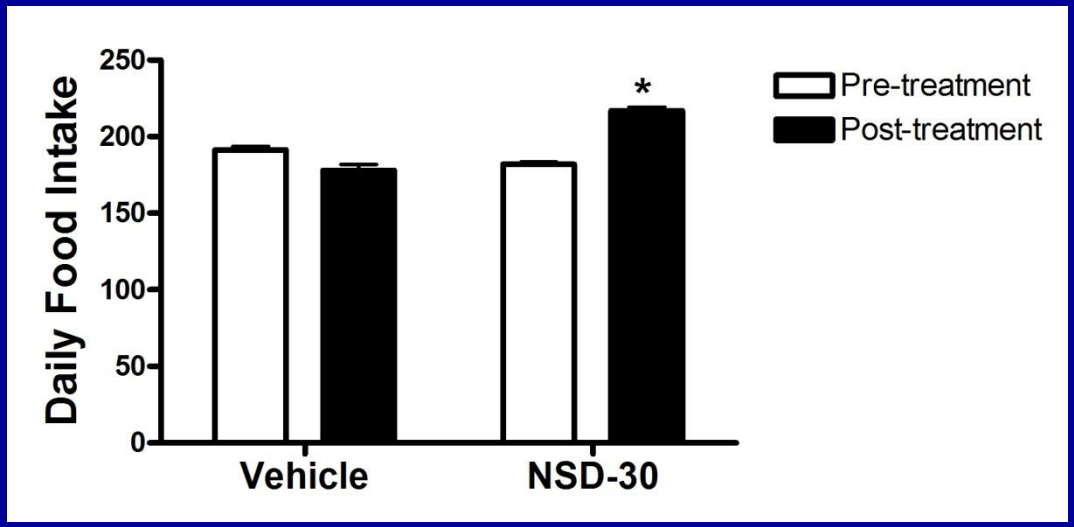
- **Trial # 3: 18 animals (n=6/group), single dose of 50 mg/kg IP, all animals except 1 that received Nano-scale Devices lost weight (~1-4.5% of body weight over 7 days).**
  - **All animals that received Nano-scale Devices had significant increase in food Intake (> 15% increase!).**

*Big pharma partner was deep in negotiations to license NSDs but deprioritized and shut down all obesity/metabolism programs. Providing Nano-Mite with excellent validation data for new partnership prospects.

# Nano-Scale Devices reduce Body Weight in Obese Canines




Young adult overweight beagles were administered NSD-30 (50 mg/kg IP) and all animals that received NSD-30 had weight loss (~1-4.5% of body weight) over 7 days.




Animals that received NSDs had significant increases in food intake (> 15% increase) over the duration of the short-term experiment, indicating increased metabolism and that hyperphagia did not negate NSD effect.

# NSDs: a therapeutic platform

**Additional benefits of this technology include treatments for acute neurological disorders such as:**

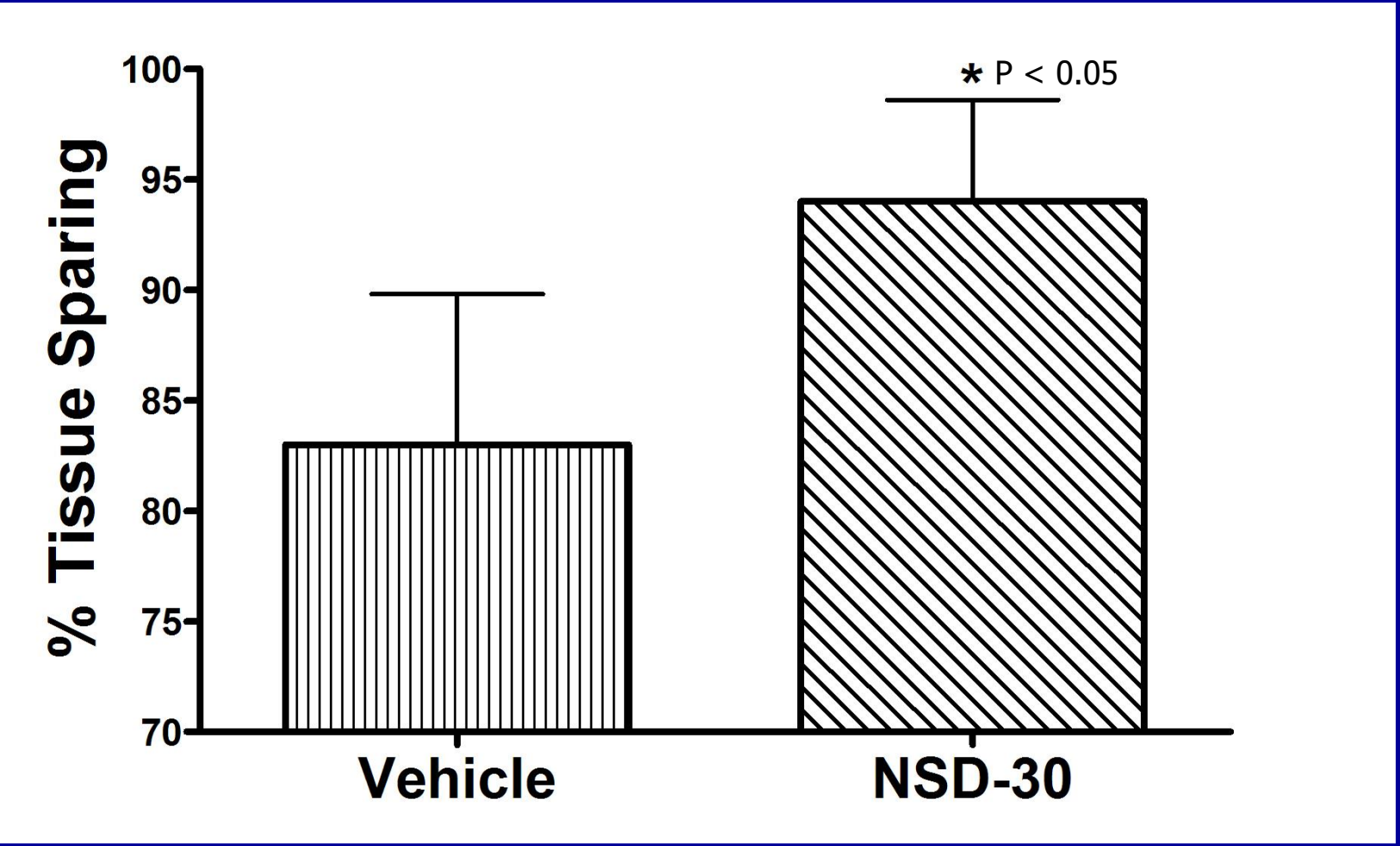
- **Traumatic brain and spinal cord injury**
- **Ischemia**

**Other applications are treatments for chronic neuropathological conditions such as:**

- **Alzheimer's Disease**
- **Parkinson's Disease**
- **ALS**
- **Pathological Aging**

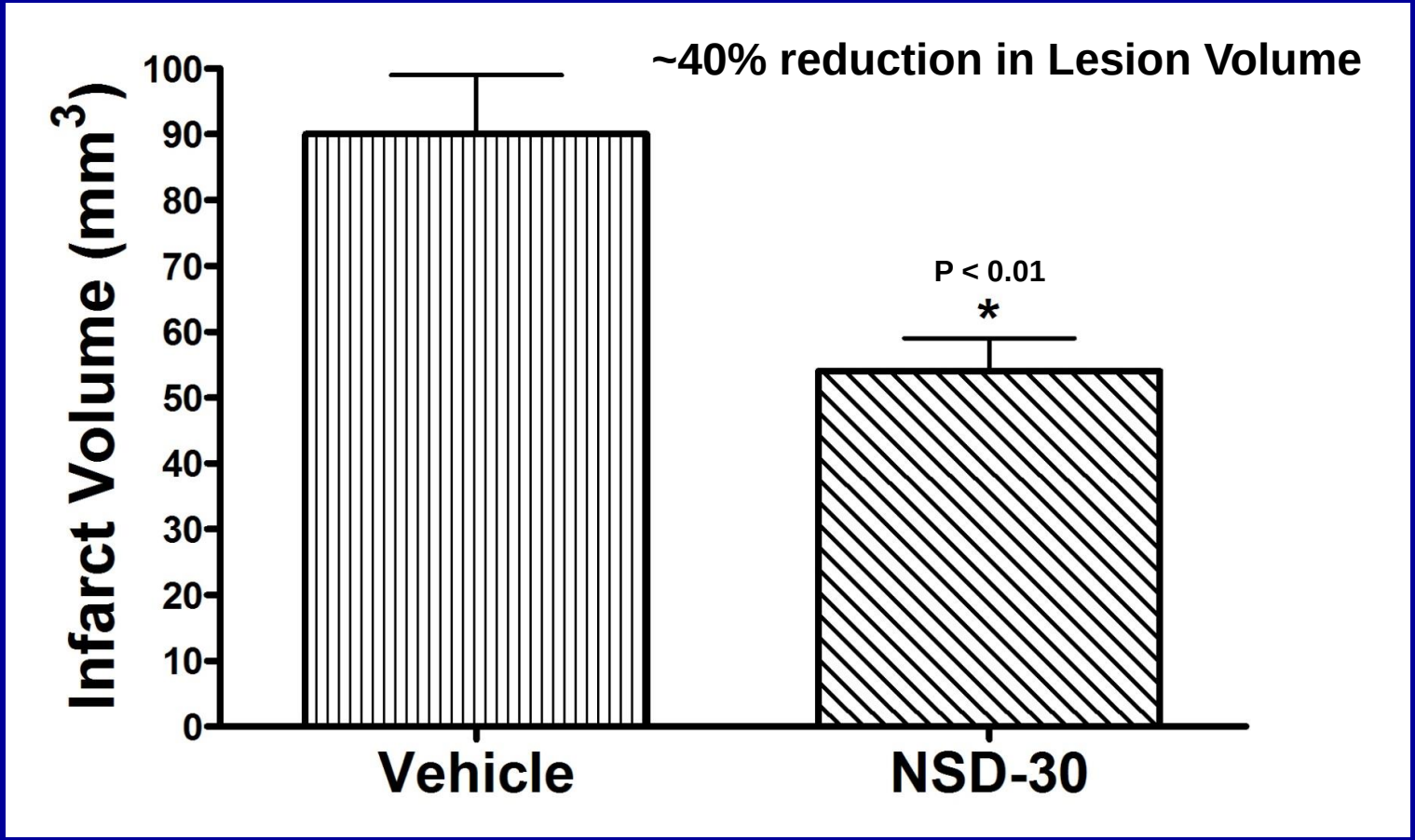
**Other: hypertension, atherosclerosis, aneurysm, cancer**

# Nano-Scale Devices increase Tissue Sparing following Traumatic Brain Injury




Animals were injured using a controlled cortical impact model of TBI and tissue sparing assessed 15 days post-injury. NSD-30 were administered 15 mins post-injury ip.

# Self-rectifying NSDs are Neuroprotective following Ischemia/Reperfusion




Animals underwent 2 hrs of ischemia. One hour following reperfusion, animals were injected with 30 mg/kg of a nano-scale device that depolarizes mitochondria 30mV (NSD-30).

# Our Licensing Process

- **Nano-Mite management has elected to license the NSD platform (or sell the company)**