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# How vanadium and manganese compounds impact cardiac mitochondrial function

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**Introduction:** Vanadium and manganese are two biologically relevant redox-active first row transition metals. Both metals have been associated with protective and deleterious effects in the cardiovascular system depending on the biological context, chemical species and metal oxidation state investigated. Many studies have indicated that these metals elicit their effects in part by influencing mitochondrial function, with potential variations due to their redox properties and complexation.

**Methods:** To better understand these relationships, we investigated the effects of vanadium and manganese salts ( $V^{IV}OSO_4$ ,  $NaV^{VO}_3$ ,  $Mn^{II}Cl_2$ ) and acetoacetate (Hacac) complexes ( $V^{IV}O(acac)_2$  and  $Mn^{II}(acac)_2$ ) on murine cardiac mitochondrial function. Metal speciation calculations were performed to predict the chemical species present under biological assay conditions.

**Results and Discussion:** Both vanadium and manganese salts decreased rates of mitochondrial respiration in a concentration dependent manner, which was attenuated when the metals were complexed to an organic ligand. In contrast, only  $V^{IV}OSO_4$  and  $V^{IV}O(acac)_2$  induced significant mitochondrial swelling, with greater sensitivity over  $NaV^{VO}_3$ ,  $Mn^{II}Cl_2$ ,  $Mn^{II}(acac)_2$  and free Hacac ligand. Swelling induced by both vanadium(IV) species was fully abolished by inhibition of the mitochondrial calcium uniporter and was partially dependent upon the voltage-dependent anion channel. In addition to the simple monomeric form ( $V^{IV}O(H_2O)_5^{2+}$ ), a second active vanadium species is the dimer ( $(V^{IV}O)_2(OH)_5^-$ ), while for manganese the main active species is  $Mn^{2+}$ . In summary, these studies demonstrate distinct effects of vanadium and manganese on cardiac mitochondrial function that vary in part with the chemical speciation and metal oxidation state.

## KEYWORDS

cardiac mitochondrial function, vanadium, manganese, salts, coordination complexes, mitochondrial calcium uniporter, mitochondrial swelling, speciation

## 1 Introduction

Mitochondrial function is imperative for cell survival, particularly in organs with high metabolic demands such as the heart. Consequently, mitochondrial dysfunction has been implicated in the pathogenesis of cardiac injury and disease (Zhou and Tian, 2018; Kuznetsov et al., 2019; Bauer and Murphy, 2020; Ramachandra et al., 2020), as well as cancer, diabetes and neurodegenerative diseases (Diaz-Vegas et al., 2020; Wang et al., 2019). Transition metals have the potential to accumulate within cells and impact mitochondrial function to exert protective or pathologic effects by mechanisms that are not fully understood (Gunter et al., 2006; Gavin et al., 1999; Liu et al., 2013; Crans and Kostenkova, 2020). Manganese is an essential element and a co-factor for superoxide dismutase (Hamanaka and Chandel, 2010) and vanadium may have multiple effects on signal transduction as a phosphatase inhibitor (Treviño et al., 2019; Imhoff et al., 2024), but how these effects may impact mitochondrial function is unclear. Vanadium has been shown to have deleterious effects on liver mitochondria (Zhao et al., 2010; Lei et al., 2007), and manganese on cardiac mitochondria (Jiang and Zheng, 2005), but both have also been associated with protective effects against cancer and diabetes (Sun et al., 2025; Huang et al., 2025; Paolillo et al., 2025; Koziel et al., 2024), as well as cardiac injury and disease (Bhuiyan and Fukunga, 2009; Xiao et al., 2021). Since most studies reported have been on liver mitochondria, we investigated the cardiac mitochondrial function to obtain a better understanding of how the chemical state and speciation of transition metals impacts their effects on mitochondrial function in tissues of various sources.

Metal ions, particularly first-row transition metals, can serve as cofactors in several enzymes necessary for proper biological functioning (Kostenkova et al., 2022). Transition metals are also used in diagnostics and therapeutics, such as for cancer treatment (Wang et al., 2023; Phillips and Pombiero, 2020; Ma et al., 2020; Karges et al., 2021; Kostova, 2009). The example of a particularly successful transition metal therapeutic is cis-platin and other platinum-containing agents to treat several cancers. A significant problem with transition metals is that they can show toxic effects linked to disease pathogenesis (Crans and Kostenkova, 2020; Wang et al., 2023; Phillips and Pombiero, 2020; Ma et al., 2020; Karges et al., 2021; Kostova, 2009; Jaishankar, et al., 2014). Understanding the underlying disease mechanisms induced by transition metals is important due to their widespread use and exploration for potential therapeutic use against cancer,

Parkinson's, Alzheimer's, and diabetes (Lei et al., 2024; Wang et al., 2024; Souza-Coelho et al., 2024; Kostova, 2009; Prihantono and Raya, 2020; Kowalski et al., 2020; Murakami et al., 2022; Kumar et al., 2024).

Transition metals show different activity based on their specific complexes and speciation which can potentially allow for fine-tuning beneficial or toxic effects. It is well known that different forms (Dinda et al., 2025), oxidation (Buglyó et al., 2005), and protonation states (Buglyó et al., 2005), nuclearity (Samart et al., 2018), coordination to potential metabolites and ligands can dramatically affect their activity (Crans et al., 2013; Kostenkova et al., 2022). For example, organic ligands coordinating to the transition metals forming coordination complexes can alter activity compared against their biological activity of their salts (Willsky et al., 2011). Recent vanadium and manganese complexes have shown activity against cancer cell lines (Prihantono and Raya, 2020; Kowalski et al., 2020; Murakami et al., 2022; Lima et al., 2021); however, little is known about the mechanism of action, but prevailing data indicates trends (Kowalski et al., 2020). Significant amounts of literature indicate mitochondria as potential targets for transition metals and given the redox properties of these materials reported reactive oxygen species (ROS) formation or consumption depending on the specific metal species (Korotkov, 2023; Aureliano et al., 2023; Xiong et al., 2021). Mitochondrial oxidative stress apoptosis induced by a number of metal ions including  $\text{Hg}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cr}^{6+}$ ,  $\text{U}^{6+}$  and other toxic metals may display opening of the mitochondrial permeability transition pore (MPTP), mitochondrial swelling, an increase in the production of ROS and  $\text{H}_2\text{O}_2$ , lipid peroxidation, or reduced glutathione and oxygen consumption (Korotkov, 2023). Several studies have shown that vanadium complexes have differential impact on mitochondria (Zhao and Zhao, 2013) and some literature shows that oxidation state is critical because  $\text{Mn}^{2+}$  is more likely to have a biological effect than  $\text{Mn}^{3+}$  (Gunter, 2017). However, little has been done to characterize the effects of Mn salts and complexes that undergo speciation under physiological conditions to understand the role that the manganese ion may play on biological activity (Gunter et al., 2006; Gavin et al., 1999; Liu et al., 2013). Reports show that stable hydrophobic  $\text{Mn}^{\text{II}}$  coordination complexes can behave as efficient  $\text{O}_2^-$  scavengers and reduce oxidative cell injury (Nistri et al., 2015) and has a direct protective action on H9c2 rat cardiac muscle cells subjected to hypoxia and reoxygenation (Becatti et al., 2019). However, these studies preclude observation of the effects of the free Mn-ions since the hydrophobic complexes were designed for high stability under physiological conditions. Vanadium and manganese have been reported to have similar biological outcomes including the loss of mitochondrial membrane potential, effects on mitochondrial protein, promotion of chronic diseases and cell death (Schell et al., 2025; Avila et al., 2013; Aureliano and Crans, 2009; Bhat et al., 2015; Pajarillo et al., 2021; Ohiomokhare et al., 2020; Ścibior et al., 2023; Kwakye et al., 2015; Guilarte, 2010; He et al., 2020). However, vanadium has also been reported to be potentially protective in some disease contexts (He et al., 2020; Aureliano et al., 2023). In these studies we seek to identify the potential similarities and differences on cardiac

**Abbreviations:** acac, Acetylacetonate (Hacac – protonated acetylacetonate); CsA, Cyclosporin A; DS16570511, (3-[3-[[[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolyl]methyl]amino]-benzoyl]-1H-indole-1-butanoic acid; ETS, Electron Transfer System; MCU, Mitochondrial calcium uniporter; Mn, Manganese;  $\text{MnCl}_2$ , Manganese(II) Chloride;  $\text{Mn}(\text{acac})_2$ , Manganese(II) acetylacetonate; MnSOD, Manganese superoxide dismutase; MPTP, Mitochondrial permeability transition pore;  $\text{NaVO}_3$ , Sodium metavanadate; ROS, Reactive oxygen species; SOD, Superoxide dismutase; V, Vanadium;  $\text{V}^{4+}$ , Vanadyl;  $\text{V}^{5+}$ , Vanadate; VBIT-12, N-[[1-(1-naphthalenylmethyl)-4-(phenylamino)-4-piperidinyl]carbonyl]-glycine; VDAC, Voltage dependent anionic channel;  $\text{VOSO}_4$ , Vanadyl sulfate;  $\text{VO}(\text{acac})_2$ , Bis(acetylacetonato) oxovanadium(IV).

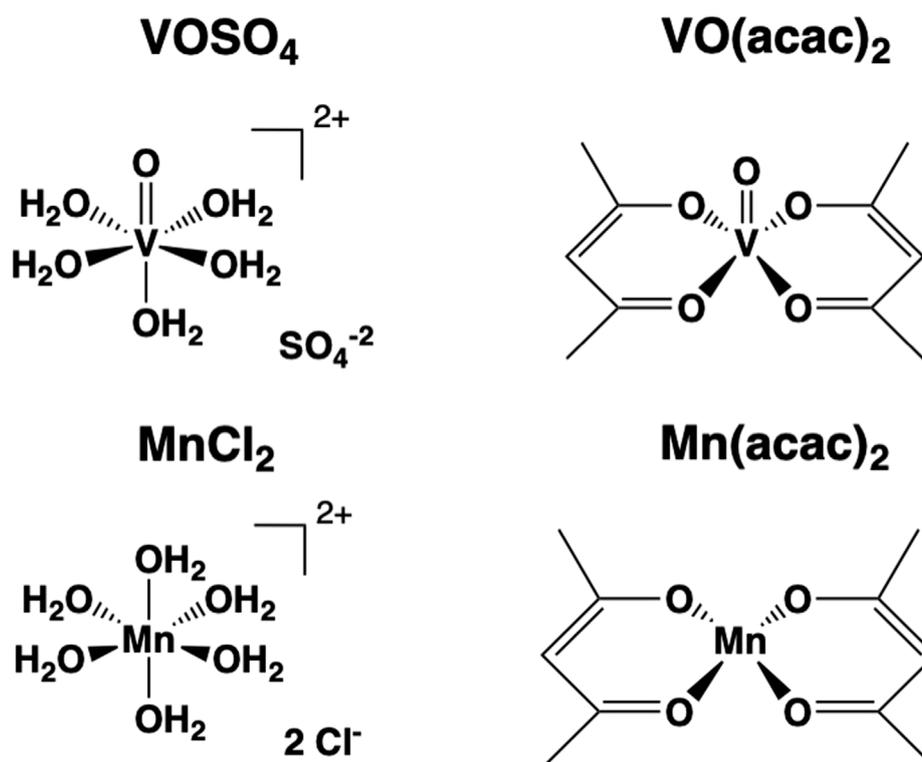


FIGURE 1

The structures of the compounds formed in solution from the solid compounds used in this study: Two vanadium(IV) compounds ( $V^{IV}OSO_4$  and  $V^{IV}O(acac)_2$ ), one vanadium(V) salt  $NaV^{V}O_3$  and two manganese(II) compounds ( $Mn^{II}Cl_2$ , and  $Mn^{II}(acac)_2$ ).

mitochondrial function of vanadium(IV) and manganese (II) salts and their respective coordination complexes.

In this study, we observed the effects of different geometries and oxidation states of vanadium and manganese salts and coordination complexes have on cardiac mitochondrial function. We chose to investigate the effects on cardiac mitochondrial respiration and swelling by vanadium species that are structurally analogous to the Mn(II)-salt known to have potent effects on Parkinsons disease and for comparison to the simple salt a known Mn(II)-coordination complex. In Figure 1 we show the structures for the salts vanadyl sulfate ( $V^{IV}OSO_4$ ) and manganese chloride ( $Mn^{II}Cl_2$ ) as well as the coordination complexes vanadyl acetylacetonate ( $V^{IV}O(acac)_2$ ), and manganese acetylacetonate ( $Mn^{II}(acac)_2$ ). The two coordination complexes were chosen because the two metal complexes were structurally similar and  $V^{IV}O(acac)_2$  is known to have a greater effect than the simple salt (Reul et al., 1999). Since vanadyl sulfate readily oxidize near neutral pH one would anticipate that some of the vanadium(IV) would oxidize to vanadium(V) under the assay conditions (Crans et al., 1995) so we also investigated the effects of sodium metavanadate ( $NaV^{V}O_3$ ). However, since manganate ( $K_2Mn^{VI}O_4$ ) and permanganate ( $KMn^{VII}O_4$ ) are strongly oxidative and do not form under the assay conditions the effects of these anions were not included in this study. The studies included investigation of the roles that different mitochondrial pores and channels have in vanadium and manganese uptake. We supported these results with speciation studies to better understand the role that speciation and oxidation state of these metals may have in affecting mitochondrial function.

## 2 Materials and methods

### 2.1 Materials

Amplex™ UltraRed Reagent, manganese(II) chloride ( $Mn^{II}Cl_2$ ) (both anhydrous and  $Mn^{II}Cl_2 \cdot 4H_2O$  form  $Mn^{2+}$  in aqueous solution), and Vanadyl sulfate ( $V^{IV}OSO_4$ ) was purchased from Thermo Fisher Scientific (Waltham, MA). Manganese(II) acetylacetonate ( $Mn^{II}(acac)_2$ ) was purchased from Aldrich Chemical Company (now Sigma Aldrich (Milwaukee, WI)). Acetylacetonone was purchased from Oakwood Chemical (Estill, SC). Bis(acetylacetonato) oxovanadium ( $V^{IV}O(acac)_2$ ), sodium metavanadate ( $NaV^{V}O_3$ ), L-(–)-Malic Acid sodium salt, sodium pyruvate, L-Glutamic Acid, sodium succinate dibasic hexahydrate, adenosine 5'-diphosphate monopotassium salt dihydrate (ADP), and horseradish peroxidase (HRP) were purchased from Sigma Aldrich (St. Louis, MO). (3-[3-[[[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolyl]methyl]amino]benzoyl]-1H-indole-1-butanoic acid (DS16570511) and N-[[1-(1-naphthalenylmethyl)-4-(phenylamino)-4-piperidiny]carbonyl]-glycine (VBIT-12) were purchased from Cayman Chemicals (Ann Arbor, Michigan) and prepared in DMSO.

### 2.2 Isolation of cardiac mitochondria

All procedures were approved by Colorado State University Animal Care and Use Committee in accordance with

recommendations from the Declaration of Helsinki and the Guiding Principles on the Care and Use of Animals. C57 BL/6 mice (10–28 months old) were housed in a climate-controlled facility on a traditional 12-h light/dark cycle with *ad libitum* food and water access. Mice were sacrificed by lethal carbon dioxide inhalation followed by midline thoracotomy and removal of the heart immediately after confirming the absence of a tail-pinch reflex. Cardiac mitochondria were isolated from heart tissue as previously described (He et al., 2020). All procedures were performed on ice or controlled at 4°C. In brief, hearts were harvested immediately after sacrifice and rinsed with ice-cold Chappell-Perry (CP1) buffer consisting of (in mM) 1 ATP, 100 KCl, 50 MOPS, 1 EDTA, 5 EGTA, and 5 MgSO<sub>4</sub>·7H<sub>2</sub>O at pH 7.4 with KOH. Minced tissue was homogenized for 15 s using a polytron, incubated for 7 min in CP1 containing trypsin (~5 mg/g tissue), quenched with CP2 (CP1+2 mg/mL Bovine Serum Albumin), then subjected to 12 passes with a glass Teflon Potter Elvehjem homogenizer before centrifugation at 600 g. The mitochondria-rich supernatant was collected and centrifuged at 7,000 g. Supernatant was discarded and the pellet resuspended in CP2, before being followed by three 7,000 g clarifying spins in CP2 and then one spin in KME buffer (100 mM KCl, 50 mM MOPS, and 0.5 mM EGTA). Final mitochondrial pellets were suspended in 350 µL of KME. A Bicinchoninic acid protein assay (BCA; Thermo Fisher Scientific, San Jose, CA) was utilized to determine specific protein concentration of mitochondrial isolates before 30 µg protein was added to each respiratory chamber (15 µg/mL).

### 2.3 Monitoring mitochondrial function by high-resolution respirometry

Mitochondrial respiration was investigated using two Oxygraph O2k high-resolution respirometer (Oroboros Instruments, Innsbruck, AT), which consists of two sealed temperature-controlled chambers that allow for real-time measurement of total O<sub>2</sub> flux in the chamber. Samples were run in MiRO5 respiration medium containing 0.5 mM EGTA, 3 mM MgCl<sub>2</sub> hexahydrate, 60 mM lactobionic acid, 20 mM taurine, 10 mM KH<sub>2</sub>PO<sub>4</sub>, 20 mM HEPES, 110 mM sucrose, and 0.1% bovine serum albumin (BSA), pH 7.1 with KOH. The instrument was calibrated using the Datlab software (Oroboros Instruments, Innsbruck, Austria) to accurately measure O<sub>2</sub> concentration in the chamber and the rate of oxygen consumption (JO<sub>2</sub>) which is calibrated prior to sample addition. Each chamber was allowed to equilibrate to room O<sub>2</sub> concentration and kept at a temperature of 37°C. Following O<sub>2</sub> stabilization, 30 µg of isolated mitochondria were added to the chamber and were treated with substrates (1 mM malate, 5 mM pyruvate, 10 mM glutamate, 10 mM succinate and 10 mM ADP). Each sample was treated separately with titrations of V<sup>IV</sup>OSO<sub>4</sub>, Mn<sup>II</sup>Cl<sub>2</sub>, V<sup>IV</sup>O(acac)<sub>2</sub> and Mn<sup>II</sup>(acac)<sub>2</sub> from 1 µM to 275 µM.

### 2.4 Evaluation of mitochondrial swelling induced by V<sup>IV</sup>OSO<sub>4</sub>, V<sup>IV</sup>O(acac)<sub>2</sub>, NaV<sup>VO</sup>O<sub>3</sub>, Mn<sup>II</sup>Cl<sub>2</sub>, Mn<sup>II</sup>(acac)<sub>2</sub>, and Hacac

Changes in the volume of mitochondria induced by shifts in osmotic pressure from metal uptake were measured by ultraviolet

absorbance at 540 nm (A<sub>540</sub>). Mitochondria isolated from the heart of 10–12-month-old mice were diluted in 2RX respiration buffer (500 mM sucrose, 20 mM Tris-MOPS, 0.02 mM EGTA, 10 mM pyruvate, 2 mM malate, adjusted to pH 7.4 with KOH) to a concentration of 60 µg/100 µL RX buffer. In a clear 96 well plate, 100 µL of the mitochondria/RX buffer sample were added V<sup>IV</sup>OSO<sub>4</sub>, V<sup>IV</sup>O(acac)<sub>2</sub>, NaV<sup>VO</sup>O<sub>3</sub>, Mn<sup>II</sup>Cl<sub>2</sub>, and Mn<sup>II</sup>(acac)<sub>2</sub>, at concentrations of 3.2 µM, 20 µM, 200 µM, and 400 µM were prepared fresh in distilled water for each experiment. Acetylacetonate was tested as a control with concentrations of 6.4 µM, 40 µM, 400 µM, and 800 µM. Each well was brought to a final volume of 200 µL with the addition of 100 µL of each metal sample so that the final concentrations of metals were 1.6 µM, 10 µM, 100 µM, 200 µM. Absorbance at 540 nm was measured every 2 min for 60 min with a microplate spectrophotometer (VersaMax, Molecular Devices).

### 2.5 Effect of inhibiting mitochondrial pores and channels on vanadium and manganese induced swelling

In a 96 well plate 100 µL of master mix of 60 µg/100 µL mitochondria and 2RX buffer (500 mM sucrose, 20 mM Tris-MOPS, 0.02 mM EGTA, 10 mM pyruvate, 2 mM malate, adjusted to pH 7.4 with KOH). Three inhibitors (cyclosporin A, VBIT-12, DS16570511) were tested. Stocks solutions of 4 mM VBIT-12 and DS16570511 as well as 2 mM of CsA were prepared in DMSO. At the bottom of separate wells 1 µL of the 2 mM CsA stock, 5 µL of a 4 mM VBIT-12 stock, and 5 µL of a 4 mM DS16570511 were added followed by the addition of 100 µL of the master mix. The assay was started by the addition of metal compounds (V<sup>IV</sup>OSO<sub>4</sub>, NaV<sup>VO</sup>O<sub>3</sub>, V<sup>IV</sup>O(acac)<sub>2</sub>, Mn<sup>II</sup>Cl<sub>2</sub>, Mn<sup>II</sup>(acac)<sub>2</sub>) to bring the well to a final volume of 200 µL and a concentration of 200 µM metal compound. Each inhibitor was run with an N of 6–9. As a control 400 µM acetylacetonate(acac)-ligand was tested. Absorbance at 450 nm was measured every 2 min for 60 min using a microplate spectrophotometer (VersaMax, Molecular Devices).

### 2.6 Speciation calculations of V<sup>IV</sup>OSO<sub>4</sub>, V<sup>IV</sup>O(acac)<sub>2</sub>, NaV<sup>VO</sup>O<sub>3</sub>, Mn<sup>II</sup>Cl<sub>2</sub>, and Mn<sup>II</sup>(acac)<sub>2</sub>

Transition metal ions undergo hydrolytic, protonation and redox reactions, and differences have been reported regarding which species interact with proteins, with interphases or with intact cells. In this work the effects of these coordination complexes and their respective salts on the cardiac mitochondria was investigated. However, since most of these studies were carried out at µM concentrations, it is not possible to measure the different species observed because the concentrations of the metal species are present below detection limits by most experimental methods. Thus, we resorted to the estimation of the speciation based on the known reported speciation constants. In such case the speciation will not be exact, but since the comparisons done with speciation constants in different biological systems yielded

concentrations within a factor of two (Crans, 1994), the evaluations determined by use of known speciation constants will be representative and provide some information on the systems we have been investigating. In the following how the speciation profiles for each of the two salts and the two coordination complexes were obtained will briefly be described. Some of the considerations, such as the speciation observed in the stock solutions (10  $\mu\text{M}$ ) used as well as under the conditions of the study ( $\mu\text{M}$ ) are provided in the [Supplementary Material](#).

### 2.6.1 VOSO<sub>4</sub> speciation

The speciation diagrams were calculated using the HYSS program and the IUPAC Stability Constants Database Software (Version 5.81) [Data version 4.62] (2000). Speciation profiles were constructed using the following formation constants (Crans et al., 2004):  $[\text{V}^{\text{IV}}\text{O}(\text{OH})]^+$  ( $\log\beta_{-1,1} = -5.94$ ),  $[(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_2]^{2+}$  ( $\log\beta_{-2,2} = -5.94$ ),  $[\text{V}^{\text{IV}}\text{O}(\text{OH})_3]^-$  ( $\log\beta_{-3,1} = -18.0$ ),  $[(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_5]^-$  ( $\log\beta_{-5,2} = -22.5$ ),  $\{\text{V}^{\text{IV}}\text{O}(\text{OH})_2\}_n$  (s) solubility product value of  $K_{\text{sp}} = 6.6 \times 10^{-23} \text{ M}^3$ ,  $[\text{V}^{\text{IV}}\text{O}(\text{HSO}_4)]^+$  ( $\log\beta_{1,1,1} = 1.74$ ),  $\text{V}^{\text{IV}}\text{OSO}_4$  ( $\log\beta_{0,1,1} = -2.51$ ).

### 2.6.2 VO(acac)<sub>2</sub> speciation

The speciation diagrams were calculated using the HYSS program and the IUPAC Stability Constants Database Software (Version 5.81) [Data version 4.62] (2000). The speciation profiles illustrates the concentration of VO(acac)<sub>2</sub> and hydrolytic species using the following formation constants (Crans et al., 2004; Martell and Smith, 1977):  $[\text{V}^{\text{IV}}\text{O}(\text{OH})]^+$  ( $\log\beta_{-1,1} = -5.94$ ),  $[(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_2]^{2+}$  ( $\log\beta_{-2,2} = -5.94$ ),  $[\text{V}^{\text{IV}}\text{O}(\text{OH})_3]^-$  ( $\log\beta_{-3,1} = -18.0$ ),  $[(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_5]^-$  ( $\log\beta_{-5,2} = -22.5$ ) [35],  $\{\text{V}^{\text{IV}}\text{O}(\text{OH})_2\}_n$  (s) solubility product value of  $K_{\text{sp}} = 6.6 \times 10^{-23} \text{ M}^3$ ,  $[\text{V}^{\text{IV}}\text{O}(\text{acac})]^+$  ( $\log\beta_{0,1,1} = 17.67$ ),  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$  ( $\log\beta_{0,1,2} = 33.62$ ).

### 2.6.3 MnCl<sub>2</sub> speciation

The speciation diagrams were calculated using the HYSS program. The diagrams illustrate the concentration of MnCl<sub>2</sub> and hydrolytic species. Diagrams have been constructed based on the most abundant species at physiological pH (pH = 7.4) using the following formation constants (Crans et al., 2004; Martell and Smith, 1977):  $[\text{Mn}^{\text{II}}(\text{OH})]^+$  ( $\log\beta_{-1,1} = -10.58$ ),  $\text{Mn}^{\text{II}}(\text{OH})_2$  ( $\log\beta_{-2,1} = -22.18$ ),  $[\text{Mn}^{\text{II}}(\text{OH})_3]^-$  ( $\log\beta_{-1,1,1} = -34.34$ ),  $[\text{Mn}^{\text{II}}(\text{OH})_4]^{2-}$  ( $\log\beta_{-4,1} = -48.28$ ) [37],  $\text{Mn}^{\text{II}}(\text{OH})_{2(s)}$  and  $\text{Mn}^{\text{II}}\text{O}_{(s)}$  solubility product value of  $\log\beta = 15.19$  and  $\log\beta = 17.94$  respectively,  $[\text{Mn}^{\text{II}}\text{Cl}]^+$  ( $\log\beta_{0,1,1} = 3.69$ ),  $\text{Mn}^{\text{II}}\text{Cl}_2$  ( $\log\beta_{0,1,2} = 6.09$ ),  $[\text{Mn}^{\text{II}}\text{Cl}_3]^-$  ( $\log\beta_{0,1,3} = 10.02$ ),  $[\text{Mn}^{\text{II}}\text{Cl}_4]^{2-}$  ( $\log\beta_{0,1,4} = 12.63$ ).

### 2.6.4 Mn<sup>II</sup>(acac)<sub>2</sub> speciation

The speciation diagrams were calculated using the HYSS program. The diagrams illustrate the concentration of Mn<sup>II</sup>(acac)<sub>2</sub> and hydrolytic species using the following formation constants (Crans et al., 2004; Martell and Smith, 1977):  $[\text{Mn}^{\text{II}}(\text{OH})]^+$  ( $\log\beta_{-1,1} = -10.58$ ),  $\text{Mn}^{\text{II}}(\text{OH})_2$  ( $\log\beta_{-2,1} = -22.18$ ),  $[\text{Mn}^{\text{II}}(\text{OH})_3]^-$  ( $\log\beta_{-1,1,1} = -34.34$ ),  $[\text{Mn}^{\text{II}}(\text{OH})_4]^{2-}$  ( $\log\beta_{-4,1} = -48.28$ ),  $\text{Mn}^{\text{II}}(\text{OH})_{2(s)}$  and  $\text{Mn}^{\text{II}}\text{O}_{(s)}$  solubility product value of  $\log\beta = 15.19$  and  $\log\beta = 17.94$  respectively,  $[\text{Mn}^{\text{II}}(\text{acac})]^+$  ( $\log\beta_{0,1,1} = 13.20$ ),  $\text{Mn}^{\text{II}}(\text{acac})_2$  ( $\log\beta_{0,1,2} = 25.28$ ).

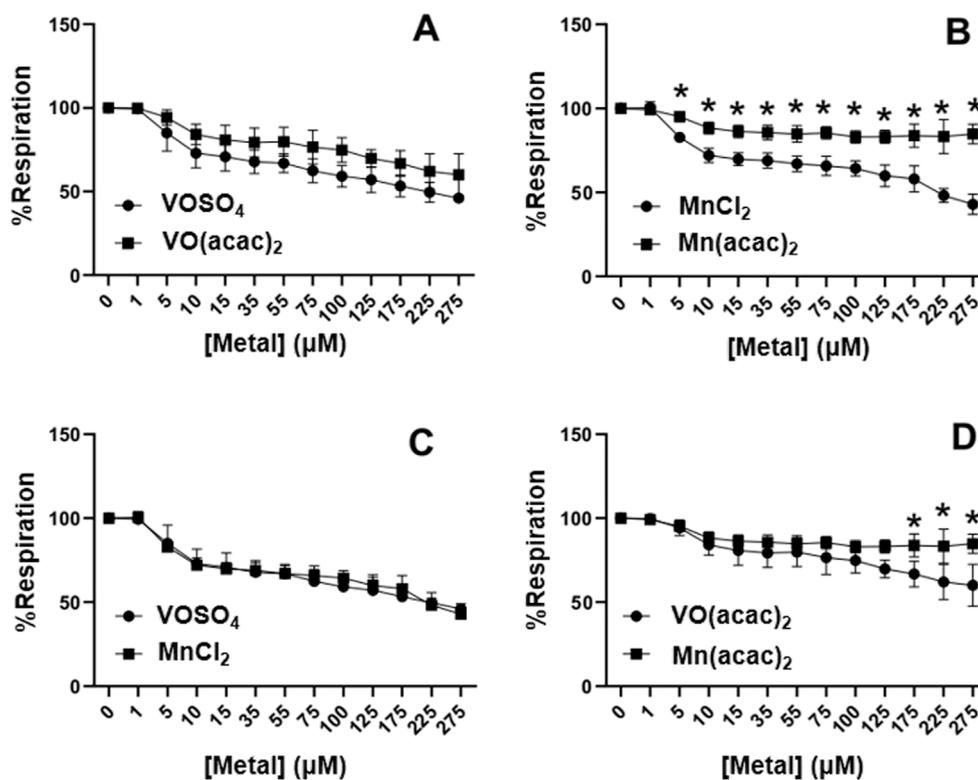
## 3 Results

### 3.1 Mn<sup>II</sup>Cl<sub>2</sub> and V<sup>IV</sup>OSO<sub>4</sub> inhibit mitochondrial respiration while Hacac-complexes show mitigated inhibition of respiration

The effects of manganese and vanadium on cardiac mitochondrial respiration varied depending on metal, ligand and oxidation state (Figure 2). To account for individual variations and limitations caused by depleting O<sub>2</sub> concentrations in the chamber, the data shown is expressed as a % respiration from the max respiration followed by the addition of ADP. Titrations were stopped after 275  $\mu\text{M}$  addition due to volume displacement effecting mitochondrial concentration in the chamber. V<sup>IV</sup>OSO<sub>4</sub> and Mn<sup>II</sup>Cl<sub>2</sub> inhibited mitochondrial respiration by nearly 28% at 15  $\mu\text{M}$  concentration but had no significant differences between vanadium or manganese (Figure 2C). Both V<sup>IV</sup>O(acac)<sub>2</sub> and Mn<sup>II</sup>(acac)<sub>2</sub> inhibit respiration only 15–20% and begin to differ significantly above 175  $\mu\text{M}$  ( $p < 0.05$ ) with Mn<sup>II</sup>(acac)<sub>2</sub> showing significantly less inhibition. There was a metal dependent effect on Hacac-complex respiration inhibition ( $p < 0.0001$ ) (Figure 2D). Both manganese compounds inhibited respiration, however, there was a species dependent affect ( $p < 0.0001$ ) with Mn<sup>II</sup>(acac)<sub>2</sub> having greater inhibition of respiration than Mn<sup>II</sup>Cl<sub>2</sub> above 1  $\mu\text{M}$  concentration ( $p < 0.05$ ) (Figure 2B). Vanadium compounds showed no significant difference in inhibition of respiration at any concentration but there was a speciation dependent difference on respiration inhibition between V<sup>IV</sup>OSO<sub>4</sub> and V<sup>IV</sup>O(acac)<sub>2</sub> ( $p < 0.05$ ) (Figure 2A). The metal and type of compound play a significant role in controlling the inhibition of mitochondrial respiration.

### 3.2 V<sup>IV</sup>OSO<sub>4</sub> and V<sup>IV</sup>O(acac)<sub>2</sub> induce mitochondrial swelling, while Na<sup>V</sup>O<sub>3</sub>, Mn<sup>II</sup>Cl<sub>2</sub>, Mn<sup>II</sup>(acac)<sub>2</sub> and free Hacac ligand do not

Mitochondrial swelling refers to the process by which water enters the mitochondria matrix from the surrounding media or cytosol of intact cells due to an increase in osmotic pressure, which is typically observed following excessive uptake of cations capable of crossing the inner mitochondrial membrane. The canonical cation capable of causing mitochondrial swelling is Ca<sup>2+</sup> (Matuz-Mares et al., 2022) which accumulates within the mitochondrial matrix during cellular Ca<sup>2+</sup> overload associated with pathologic states such as cardiac ischemia (Bertero et al., 2024). Excessive swelling can ultimately cause mitochondria to rupture and lose functionality, and therefore might explain the reduction in mitochondrial respiration observed in response to vanadium and manganese compound titrations. The ability of vanadium salts and selected complexes to induce mitochondrial swelling has been previously reported in liver (Zhao et al., 2010); however, the tissue-specificity impacts of and chemical speciation on these effects are less clear. Though manganese(III) was shown to induce cellular swelling (Ramao Rao et al., 2007) and manganese(II) was shown to induce mitochondrial swelling in animal cells (Mousa and Shehab,



**FIGURE 2**  
Concentration dependent effects of  $V^{IV}OSO_4$ ,  $Mn^{II}Cl_2$ ,  $V^{IV}O(acac)_2$ , and  $Mn^{II}(acac)_2$  on cardiac mitochondrial respiration. (A)  $V^{IV}OSO_4$  and  $V^{IV}O(acac)_2$ . (B)  $Mn^{II}Cl_2$  and  $Mn^{II}(acac)_2$ . (C)  $Mn^{II}Cl_2$  and  $V^{IV}OSO_4$ . (D)  $V^{IV}O(acac)_2$  and  $Mn^{II}(acac)_2$ . Respiration was measured using two Oxygraphs using a Clark type electrode. Statistical analysis was performed using a 2-Way ANOVA using GraphPad Prism. Each experiment was completed with an  $N = 3$ . (\* $p < 0.05$ )

2015), the effect of speciation of manganese compounds was not investigated. Furthermore, the current literature lacks consideration of the different action of manganese salts and complexes on mitochondrial or cellular swelling (Ramao Rao et al., 2007; Mousa and Shehab, 2015).

To address this gap in knowledge, we tested the effect of three vanadium compounds ( $V^{IV}OSO_4$ ,  $NaVVO_3$ ,  $V^{IV}O(acac)_2$ ) and two manganese compounds ( $Mn^{II}Cl_2$ ,  $Mn^{II}(acac)_2$ ) on cardiac mitochondrial swelling (Figure 3). The effects were compared to a sample where an aqueous solution was administered in place of compounds and called “mito control.”  $V^{IV}OSO_4$  and  $V^{IV}O(acac)_2$  allow for direct comparison of  $V^{4+}$  in a salt and a complex while  $NaVVO_3$  offers a comparison with a  $V^{4+}$  in case some of the  $V^{4+}$  oxidized. Similarly,  $Mn^{II}Cl_2$  and  $Mn^{II}(acac)_2$  represent a salt and complex and allows for a comparison of  $Mn^{2+}$  that is comparable to  $V^{4+}$  as well as determine if specific ligands offer similar effects. To study speciation differences, we tested both  $V^{4+}$  and  $V^{5+}$  salts to look at differences in swelling. The  $V^{4+}$  compounds showed rapid and severe mitochondrial swelling starting at 100  $\mu M$  when compared to the control mitochondrial sample (Figures 3A,B), whereas sodium metavanadate (Figure 3C) and manganese(II) salt and coordination complex (Figures 3D,E) had no significant effects on mitochondrial swelling at 200  $\mu M$  concentration; the results of all five compounds is summarized in Figure 3F

at the 60 min timepoint for convenient comparison. The effect of vanadate and Hacac ligand on the mitochondrial swelling is minimal as is shown in (Supplementary Figures S1, S2) although the Hacac ligand will not prevent the cardiac mitochondrial swelling inhibition by  $Ca^{2+}$  as shown in Supplementary Figure S3.

### 3.3 Mitochondrial calcium uniporter and voltage-gated anion channel impact swelling induced by vanadium compounds in cardiac, but not liver, mitochondria

To investigate the mechanisms by which vanadium compounds mediate mitochondrial swelling, we employed selective inhibitors of two canonical mitochondrial ion transporters: the mitochondrial calcium uniporter (MCU) and the voltage-dependent anion channel (VDAC) (Marchi and Pinton, 2014; Shoshan-Barmatz and De, 2017). Inhibition of the mitochondrial calcium uniporter by 100  $\mu M$  DS16570511 completely inhibited cardiac mitochondrial swelling induced by 200  $\mu M$   $V^{IV}OSO_4$  (Figure 4A) and  $V^{IV}O(acac)_2$  (Figure 4B), while inhibition of VDAC with 100  $\mu M$  VBIT-12 partially attenuated swelling induced by both vanadium complexes (Figures 4A,B). Consistent with the

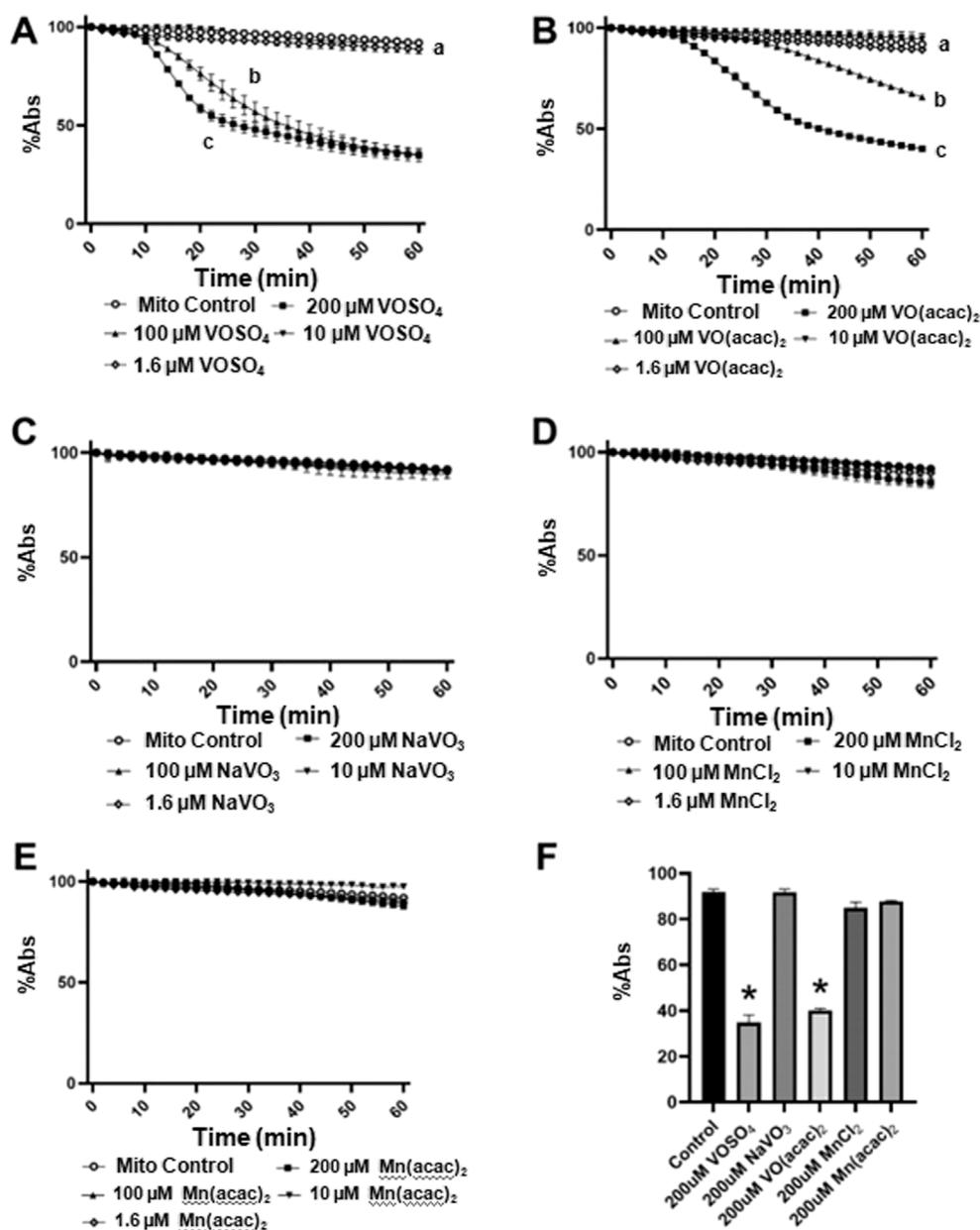


FIGURE 3

Concentration-dependent effects of vanadium and manganese compounds on cardiac mitochondrial swelling. Swelling was measured as a decrease in absorbance at 540 nm at 25°C in response to increasing concentration of  $V^{IV}OSO_4$  (A),  $V^{IV}O(acac)_2$  (B),  $NaVO_3$  (C),  $Mn^{II}Cl_2$  (D), or  $Mn^{II}(acac)_2$  (E). In the control sample ("mito control") an aqueous blank solution was added in place of compound. The final extents of swelling at 60 min under all conditions are summarized in panel (F). Rates of swelling were compared across concentrations for each compound by one-way ANOVA with repeated measures and Dunnett's Multiple Comparison Test. Different lowercase letters indicate significant variations in the rate of swelling for each experimental condition. Data represent mean  $\pm$  SEM of 3 separate experiments per group at each time point. \* $P < 0.001$  vs other groups by post-hoc comparisons at the 60 min time point.

findings from the  $V^{IV}OSO_4$  and  $V^{IV}O(acac)_2$  dose response curves (Figures 3A,B),  $V^{IV}OSO_4$  induced an earlier onset of swelling than  $V^{IV}O(acac)_2$  (arrows in Figures 4A,B) in cardiac mitochondria. Similar effects are not observed with  $NaVO_3$  as shown in Supplementary Figure S4. Given previous evidence for less robust impacts of MCU inhibition on vanadium-induced swelling in liver mitochondria, we repeated experiments to determine any tissue-specific responses and found that MCU inhibition with DS16570511 similarly abolish liver mitochondrial swelling induced by both 200  $\mu M$   $V^{IV}OSO_4$

(Figure 3C) and  $V^{IV}O(acac)_2$  (Figure 3D). However, no effect of VDAC inhibition with 100  $\mu M$  VBIT-12 was seen, nor any effect of vanadium species on the onset of swelling.

### 3.4 Speciation differences in vanadium and manganese salts and complexes

In the Supplementary Material we provide speciation diagrams for the salts and coordination complexes used in this work. These

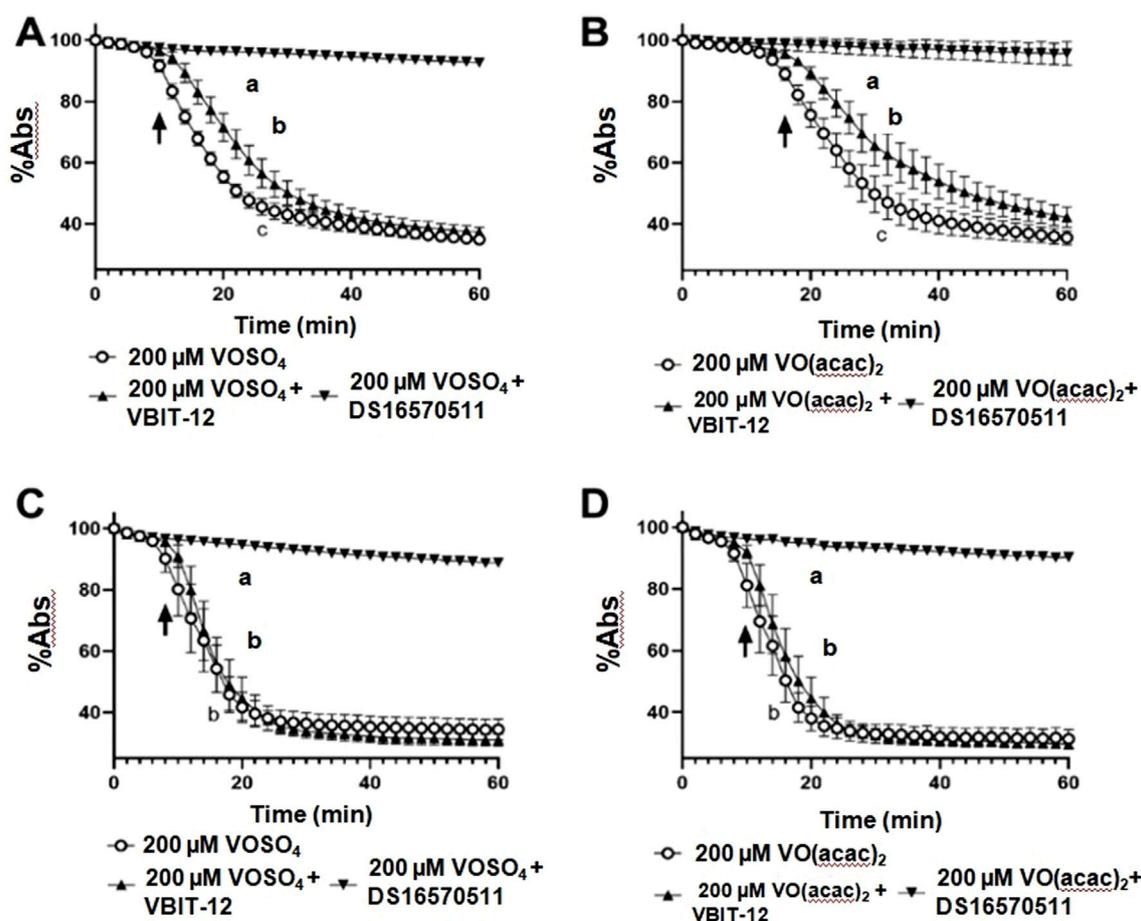


FIGURE 4

Impacts of MCU and VDAC on vanadium-induced swelling in cardiac and liver mitochondria. Mitochondrial swelling induced by 200  $\mu\text{M}$   $\text{V}^{\text{IV}}\text{OSO}_4$  or  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$  was monitored at 25°C as a decrease in absorbance at 540 nm of intact cardiac (A, B) and liver (C, D) mitochondria, respectively. Impacts of the mitochondrial calcium uniporter (MCU) and voltage-gated anion channel (VDAC) were investigated by co-incubation the MCU-inhibitor DS16570511 or VDAC inhibitor VBIT-12. Extents of mitochondrial swelling were compared by one-way ANOVA with repeated measures and Dunnett's Multiple Comparison Test. Different lowercase letter indicate significant variations in the rate of swelling for each experimental condition. Data represent mean  $\pm$  SEM of 6–9 separate experiments per group at each time point.

profiles illustrate the diversity of the systems, and the fact that several different species exist varying in protonation states and composition. Both  $\text{V}^{\text{IV}}\text{OSO}_4$  and  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$  and the  $\text{Mn}^{\text{II}}\text{Cl}_2$  and  $\text{Mn}^{\text{II}}(\text{acac})_2$  contain several species which vary dramatically with pH. Since the studies with the mitochondria are mainly done at pH 7.4, the speciation plots provided here are focusing on the changes as a function of added metal ion concentration in the swelling experiments. The speciation diagrams shown in Figures 5A–D illustrate the distribution of species as a function of concentration used in the swelling assays.

For  $\text{V}^{\text{IV}}\text{OSO}_4$  it is shown that two major species are present, namely, the monomer  $\text{V}^{\text{IV}}\text{O}(\text{OH})_3^-$  and a dimeric species  $(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_5^-$ . The species in solution is mainly a dimeric form of  $\text{V}^{\text{IV}}$  (Figure 5A). Since the dimer contains two vanadium atoms—we show both the concentration of molecule as well as the concentration of V-atoms in the dimeric species  $(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_5^-$ . Calculations from  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$  show that three major species are present, namely, the  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$ , the hydrolyzed monomer  $\text{V}^{\text{IV}}\text{O}(\text{OH})_3^-$ , and a dimeric species  $(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_5^-$

shown in Figure 5B. Since the dimer contains two vanadium atoms—we show both the concentration of molecule as well as the concentration of V-atoms in the dimeric species  $(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_5^-$ . Based on these speciation estimations it would be clear that most of the observed effects could be attributed to the hydrolyzed  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$ , and thus the monomeric and dimeric forms of  $\text{V}^{\text{IV}}$ .

For  $\text{Mn}^{\text{II}}\text{Cl}_2$  it is shown that two major species and two minor species are present at pH 7.4 in the concentration range up to 200  $\mu\text{M}$ . The two major species are the intact  $\text{Mn}^{\text{II}}\text{Cl}_2$  molecule and the partially hydrolyzed species  $\text{Mn}^{\text{II}}\text{Cl}^+$ . The two minor species present are  $\text{Mn}^{\text{II}}(\text{OH})^+$  and intact  $\text{Mn}^{\text{II}}\text{Cl}_2$  (Figure 5C). For  $\text{Mn}^{\text{II}}(\text{acac})_2$  three major species are present, namely, the  $\text{Mn}^{\text{II}}(\text{acac})_2$ , the hydrolyzed  $\text{Mn}^{\text{II}}(\text{acac})^+$ , and the plain  $\text{Mn}^{2+}$  cation (Figure 5D). Based on these speciation estimations it would be clear that most of the species present is the hydrolyzed  $\text{Mn}^{\text{II}}(\text{acac})^+$  cation, however the two other ions present are in sufficient amounts that any observed effects should be attributed to these forms of  $\text{Mn}^{2+}$ .

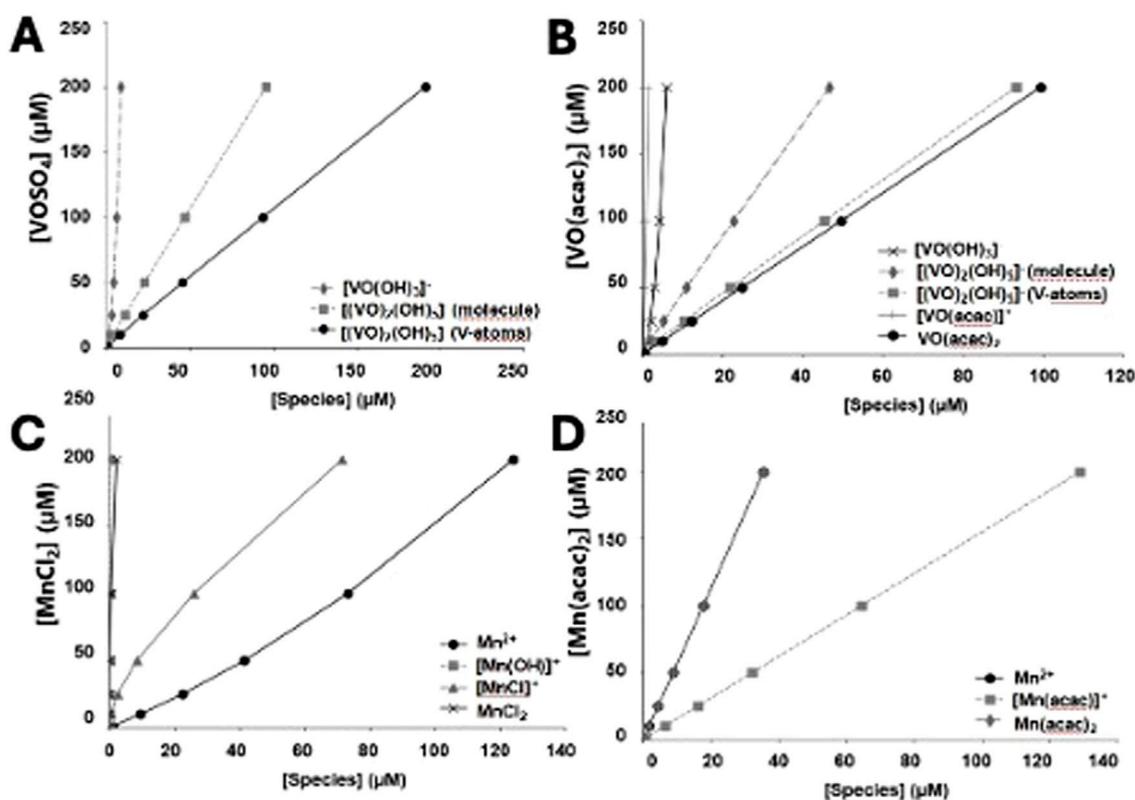


FIGURE 5

Speciation profiles illustrate the concentration of metal ion species as a function of the overall metal ion concentration. Diagrams have been constructed at physiological pH (pH = 7.4) in order to identify the species present under those conditions (A)  $\text{V}^{\text{IV}}\text{OSO}_4$ ; (B)  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$ ; (C)  $\text{Mn}^{\text{II}}\text{Cl}_2$ ; (D)  $\text{Mn}^{\text{II}}(\text{acac})_2$ .

## 4 Discussion

Transition metals and transition metal complexes play an important role in biological processes and medical applications. Redox-active metals are often used to achieve redox balance in cellular environments and play a key role in enzymatic processes. Previous studies observed that different vanadium compounds exerted varying effects on rat liver mitochondria; this study done in cardiac mitochondria tissue expanded the scope by estimating the speciation of both salts and coordination complexes and by comparing the effects of two redox-active first row transition metal ions. As a result, we assign speciation and suggest active forms (species) which causes mitochondrial swelling.

Speciation differences may account for mechanistic variation between vanadium and manganese and facilitating mitochondrial dysfunction. Therefore, the results from Figure 5 will be discussed first. Comparison of speciation calculation between metals indicates significant differences of species in solution. The primary species in solution for the vanadium compounds at 200  $\mu\text{M}$  is the dimer  $[(\text{V}^{\text{IV}}\text{O})_2(\text{OH})_5]^-$  as well as  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$  for the  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$  (Figures 5A,B). The similarities seen in mitochondrial swelling (Figure 4) may be attributable to the dimeric form of the vanadium in solution, but the existence of  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$  may limit the slightly lowered inhibition of respiration observed (Figure 2A). Below 50  $\mu\text{M}$ , where the most immediate inhibition of respiration took place, the same primary species exists in solution, lending credence to the hypothesis

that the inhibition of respiration can be attributed to a dinuclear species (Figures 5A,B). Similarly, the primary species in solution for the manganese compounds show some similarities while also having an outlier.  $\text{Mn}^{\text{II}}\text{Cl}_2$ 's primary species below 50  $\mu\text{M}$  and at 200  $\mu\text{M}$  is  $\text{Mn}^{2+}$  and  $\text{Mn}^{\text{II}}\text{Cl}_2$  (Figure 5C) while  $\text{Mn}^{\text{II}}(\text{acac})_2$ 's primary species are  $\text{Mn}^{2+}$  and  $\text{Mn}^{\text{II}}(\text{acac})_2$  (Figure 5D). The presence of  $\text{Mn}^{\text{II}}(\text{acac})_2$  may be the cause for the significant reduction in inhibition of respiration observed (Figure 2B). Therefore, the speciation of the metal compounds may play a role in what biological effects of these compounds are observed on cardiac mitochondria.

Concentration, speciation and oxidation state of metal ion can affect the role the metal ion play in biological systems, and even change the observed effects from therapeutic to toxic responses (Rojas-Lemus et al., 2021). To investigate these potential differences, we monitored mitochondrial function with increasing concentrations of  $\text{V}^{\text{IV}}\text{OSO}_4$ ,  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$ ,  $\text{Mn}^{\text{II}}\text{Cl}_2$ , and  $\text{Mn}^{\text{II}}(\text{acac})_2$ . Both salts showed similar effects on inhibiting mitochondrial respiration while  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$  and  $\text{Mn}^{\text{II}}(\text{acac})_2$  inhibited respiration significantly less when compared to the respective metal salts but not when compared to each other. Earlier reports have shown that cell culture with 100  $\mu\text{M}$   $\text{Mn}^{\text{II}}\text{Cl}_2$  (Sarkar et al., 2018) or 10–100  $\mu\text{M}$   $\text{Mn}(\text{II})$  acetate (Rao et al., 2004) will inhibit mitochondrial function over time. However acute additions of metals with levels under 15  $\mu\text{M}$   $\text{Mn}^{\text{II}}\text{Cl}_2$  have not previously been reported to inhibit mitochondrial function. In this work we observed a significant amount of the inhibition at

concentrations near physiologically relevant concentrations under 15  $\mu\text{M}$  metal ion. Previous reports have shown that to inhibit mitochondrial respiration excessive amounts of  $\text{Mn}^{2+}$  ion are needed (Warren et al., 2020), which is contrary to our findings and may be subject to the specific conditions and alteration in speciation; that is specifically complexation of the  $\text{Mn}^{2+}$  ion in the medium used in the study where the  $\text{Mn}^{2+}$  ion was found to be less active.

Mitochondrial swelling allows for further investigation into the mechanism of metal induced mitochondrial dysfunction in part because the mitochondrial swelling can be caused by rapid influx of calcium ions into the matrix. Calcium ions can enter mitochondria and cause shifts in osmotic pressure. These shifts can occur to such an extreme that mitochondria can burst, leading to a loss of function. This can also lead to the loss of the membrane potential limiting respiratory capacity of the ETS. Inhibition of vanadium and manganese induced swelling by the blocking of ion channels and pores help elucidate potential mechanisms of mitochondrial swelling by vanadium and manganese complexes. DS16570511 is a novel inhibitor of MCU which can block calcium transport into the mitochondria (Belosludtsev et al., 2021) and VBIT-12 acts as an inhibitor for VDAC (Wan et al., 2023), neither of which have been tested with vanadium or manganese. Previous literature has used a range of concentrations for these molecules and to ensure max inhibition we used 100  $\mu\text{M}$  of both agents. DS16570511 completely inhibited vanadium induced swelling while VBIT-12 delayed swelling. There was no significant effect of these inhibitors on swelling by manganese. These findings show that while these ions have many similarities, the mechanism of vanadium and manganese induced cardiac mitochondrial dysfunction are radically different, and we suggest that this can partially be attributed to speciation and the fundamentally different effects of the simple ions.

Heavy metals, as well as transition metals including vanadium and manganese, are identified by the Environmental Protection Agency as toxic byproducts of many industrial processes such as fossil fuel extraction and refinement and thus pose a risk to human health (Queiroz et al., 2021; Hanus-Fajerska et al., 2021; Imtiaz et al., 2015; Markiv et al., 2023). However, some vanadium and manganese species have been shown to have profound effects and potential for medical application when incorporated in therapeutic complexes. The present study emphasizes the importance of metal speciation when considering their biological mechanism and outcome. An important aspect for the therapeutic potential of these complexes is stability of the complex and the organic ligand which can affect uptake and biological activity. If manipulation of the ligand can alter biological effects this could lead toward controllable toxicity and targeted treatments. Further investigation of different vanadium and manganese complexes into their biological effects on cells and mitochondria will be necessary to test the differences in mitochondrial swelling, ROS release, membrane potential and lipid peroxidation damage. Manganese's inhibition of mitochondrial respiration without impacts on swelling may make it a promising alternative for transition metal-based therapeutics.

Both manganese and vanadium are first row transition metals and some similarities would be anticipated. Manganese is an essential element and functions as a cofactor in MnSOD in the conversion of superoxide into  $\text{H}_2\text{O}_2$  in mammals. Vanadium is a known cofactor in vanadium peroxidases and vanadium nitrogenase

in plants and prokaryotes, but in contrast to manganese is not essential in human beings. However, vanadium has been reported to have favorable biological activity throughout the biosphere and has shown strong biological activity that might be used therapeutically. Each of these metals has two physiologically common oxidation states, and in the case of vanadium can undergo redox cycling between oxidation state IV and V through Fenton chemistry (Avila et al., 2013; Rehder, 2013; Treviño et al., 2019). Manganese also changes redox state under physiological conditions, however, this occurs when it is bound to SOD (as MnSOD) and therefore likely causing a different phenotype change than vanadium compounds induce. A primary cellular location for such redox cycling is the mitochondria and would be consistent with the earlier reports showing a multifaceted effect on mitochondrial function. One of the primary mechanisms of toxicity by both manganese and vanadate are changes in mitochondrial membrane potential and inner membrane protein function (Zhao et al., 2010; Aureliano et al., 2023; Li and Yang, 2018; Hosseini et al., 2013; Galvani et al., 1995). Because of reports of similar biological effects (Sun et al., 2025; Huang et al., 2025; Paolillo et al., 2025; Koziel et al., 2024; Korotkov, 2023; Bhuiyan and Fukunga, 2009; Xiao et al., 2021; Becatti et al., 2019; Nistri et al., 2015), we were interested in comparing the speciation of manganese and vanadium while these materials were exerting effects on cardiac mitochondria. The comparison of both salts and complexes allowed us to investigate if a ligand could modify the biological effects through governing speciation under physiological conditions. However, since we found that similar responses were observed on respiration but not on mitochondrial swelling we wondered if the speciation of both Hacac complexes were similar. Indeed, both complexes were found to hydrolyze to form salts, although the species that form are different. Hence, we suggest that the observed effects on the cardiac mitochondria for the metal-acac complexes could be attributed to the formation of salt because limited effect was observed by the ligand Hacac. The major effect of the ligand may thus indicate that the Hacac ligand simply alters the delivery and uptake process of the metal ion. This conclusion is supported by the previous reports of  $\text{Mn}^{\text{II}}$ -complexes that were found to scavenge ROS and  $\text{H}_2\text{O}_2$  but that this complex was significantly more stable than the  $\text{Mn}^{\text{II}}$ (acac)<sub>2</sub> complex under physiological conditions ((Nistri et al., 2015; Becatti et al., 2019). Indeed, the report for a number of other metal complexes showing apoptosis which in part was attributed to ROS as well as other effects somewhat specific for each metal ion (Korotkov, 2023).

Further investigation into the differences between effects exerted by these metals should be carried out using multiple speciation states and complexes, lower concentrations and buffers that do not associate with the metal ion (Levina, et al., 2017). The mitochondrial swelling observed occurred above physiologically relevant concentrations and does not explain the inhibition of respiration observed in this work below 15  $\mu\text{M}$ . Cell culture studies tested with these metals could allow for more insight into the mechanistic differences between vanadium and manganese in biological systems, specifically considering oxidation states and speciation. Measuring mitochondrial ROS production and associated damage, intact cellular respiration, and specific enzyme activities and protein expression would provide more in-depth information of the effects that these metals may have on

function. This information could prove useful for future therapeutic design by offering a mode of action and a target for future research.

## 5 Conclusion

Vanadium and manganese both are redox active transition metals, but their redox properties and effects in biological systems can be very different, leading to potentially different capabilities and outcomes *in vivo*. Despite these differences, similar biological effects have been reported with these elements and in this work both salts and complexes were investigated for their effects on cardiac respiration and mitochondrial swelling. In the present study, both vanadium and manganese inhibited cardiac mitochondrial respiration at low concentrations, however the effect decreased when the metal ions were complexed with a ligand. In contrast, only high concentrations of vanadium induced mitochondrial swelling, which was attenuated by ligand Hacac complexing at 100  $\mu\text{M}$ , but not 200  $\mu\text{M}$ . We attribute this to the speciation chemistry and the fact hydrolysis of the complex occurs much faster at high micromolar concentrations. Further investigation at concentrations under 20  $\mu\text{M}$  using metal complexes that do not undergo rapid hydrolysis and in buffers/media that do not associate with the metal ion (Levina et al., 2017) are suggested to verify this interpretation. Finally, we have shown that vanadium-induced mitochondrial swelling is dependent on the mitochondrial calcium uniporter in both liver and cardiac mitochondria, suggesting possible mechanism of entry into the mitochondrial matrix and/or impacts on mitochondrial calcium handling, corroborating an earlier report of oxidative stress in the liver mitochondria and opening of the mitochondrial permeability transition pore (Zhao et al., 2010). In summary, these studies show that both vanadium and manganese can impair mitochondrial function, but by different mechanisms that depend on the concentration and speciation of metal under the conditions of the system.

## Data availability statement

Additional original data contributions obtained are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

## Ethics statement

All procedures were approved by Colorado State University Animal Care and Use Committee in accordance with recommendations from the Declaration of Helsinki and the Guiding Principles on the Care and Use of Animals.

## Author contributions

CD: Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review and

editing. LW: Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review and editing. ED: Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review and editing. LR: Investigation, Writing – original draft, Writing – review and editing. AC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Project administration, Supervision, Funding acquisition, Writing – original draft, Writing – review and editing. DC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Project administration, Supervision, Funding acquisition, Writing – original draft, Writing – review and editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that DC was an editorial board member of *Frontiers*, at the time of submission. This had no impact on the peer review process and the final decision.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fchbi.2025.1602602/full#supplementary-material>

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