

ORAL CREATINE HYDROCHLORIDE SUPPLEMENTATION: ACUTE EFFECTS
ON SUBMAXIMAL, INTERMITTENT BOUTS OF BENCH PRESS AND VERTICAL
JUMP EXERCISES

by

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DEDICATION

This is dedicated to my parents, Rebecca and David Holmes, and my uncle, Louie Medrano; wouldn't be here without you guys. And to my four brothers, Rich, Kamran, Jason, and J.D., who believed I could do this, but aren't here to see me finish it out. Rest in peace.

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ABSTRACT

The purpose of this study was to examine the acute effects of oral creatine hydrochloride (CrHCl) supplementation on three repeated bouts of bench press and vertical jumping exercises and body composition measures (body weight (BW), fat-free mass (FFM) and fat mass (FM)). *Methods:* Fifteen resistance trained males completed 3 sets of the barbell bench press (70% 1RM) and 3 sets of the repeated counter-movement vertical jump (CMJ; 85% maximal CMJ height), with 2 min rest between sets, before and after a 7 d CrHCl intervention (4 g·day⁻¹). A two-factor repeated measures ANOVA was used to determine significant main effects (time and set) with post-hoc Bonferroni analysis and interaction effects (time x set) for bench press and CMJ performances from pre- to post-intervention. A one-factor repeated measures ANOVA was used to assess pre- to post-intervention differences in body composition. *Results:* Significant main and interaction effects for time and set were found in the bench press from pre- to post-intervention ($p < 0.005$, for all) with Bonferroni analysis indicating increased performance on later sets (eg, Set 3 > Set 2 > Set 1, $p < 0.005$). Significant main effects for time and set were found in the CMJ test from pre- to post-intervention ($p < 0.005$) but no interaction effect was found ($p > 0.05$). Post hoc analysis indicated increased performance on later sets. BW was the only body measure to reach significance ($p < 0.005$). *Conclusion:* Supplementation with 4 g of CrHCl for 7 d in healthy, resistance trained men significantly increased the number of repetitions performed during intermittent bouts of submaximal bench press and CMJ exercises and body weight.

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LIST OF ABBREVIATIONS

BSU	Boise State University
Cr	Creatine
CrHCl	Creatine Hydrochloride
CrH ₂ O	Creatine Monohydrate
ATP	Adenosine Triphosphate
PCr	Phosphocreatine
PF	Physical Fitness
PA	Physical Activity
O ₂	Oxygen
H ₂ O	Water
CK	Creatine Kinase
TCr	Total Creatine
FCr	Free Creatine
ADP	Adenosine Diphosphate
P _i	Inorganic Phosphate
CHO	Carbohydrate
1RM	One repetition Maximum
g	Gram
kg	Kilogram
cm	Centimeter

CHAPTER ONE: INTRODUCTION

Physical Fitness and Physical Activity

Physical fitness (PF) is a set of skill- or health-related attributes which may be evaluated through a variety of physical tests (eg, the mile-run test).¹ One's level of PF is imperative to their health and overall quality of life, and according to the American College of Sports Medicine (ACSM), embodies five fundamental components: 1) cardiovascular endurance, 2) muscular endurance, 3) flexibility and mobility, 4) muscular strength, and 5) body-composition.^{2,3} A sixth component suggested by Clarke is muscular power.⁴ Physical activity (PA) is movement of the body produced by action of the muscle, causing an increase in energy expenditure and is critical to improving and maintaining PF.¹ PA creates the greatest demand for the transfer of energy in the body.¹ There are three distinct systems utilized to provide the energy, and the intensity and duration of a given physical task determines which predominates: 1) immediate-term adenosine triphosphate-phosphocreatine (ATP-PCr) system, 2) short-term glycolytic system, and 3) long-term aerobic system.⁵

Energy Transfer During PA

ATP is the body's energy currency because this energy-rich nucleotide-molecule provides power for all cells' energy-driven processes.⁶ Energy is released from ATP when it loses an inorganic phosphate (P_i) molecule to form adenosine diphosphate (ADP).⁷ ATP is restored by adding back the P_i to ADP.⁸ There is limited ATP storage to increase the sensitivity and responsiveness of energy supply.⁸ PCr is stored as the energy

reservoir because this high-energy, intracellular phosphate-compound undergoes anaerobic (without oxygen (O₂)) splitting of one of its P_i molecules for the rapid, short-term re-synthesis of ATP.⁹ Both molecules maintain similar characteristics as a vast amount of free-energy (~7.3 kcal·mol⁻¹) gets released when the P_i is released in a reversible reaction.¹⁰ Creatine kinase (CK, ~5% on the outer membrane of the mitochondria, ~4% on the sarcomere, ~90% in the cytosol) catalyzes the near-equilibrium equation: PCr + ADP + hydrogen (H⁺) ↔ Cr + ATP.¹⁰⁻¹² Because PCr's CK-driven hydrolysis has a much greater free energy than that of ATP, it rapidly drives the phosphorylation of ADP to resynthesize ATP.^{11,13} During periods of all-out muscle contractions and a high demand for ATP, this process is driven to the right which reduces PCr concentration while simultaneously increasing free Cr (FCr) and P_i ion concentrations and maintaining ATP concentration nearly unchanged.^{10,14} The process is reversed during recovery periods, as the free Cr and one P_i ions bind, favoring PCr synthesis.¹⁰ This energy system is engaged at the initiation of PA and in all-out PA as skeletal muscle has the unique ability to rapidly increase its rate of energy consumption. Under conditions where explosive, maximal contractions are required, the millisecond increase in the turnover of energy can be up to 300-fold from resting to the fully-activated state.^{10,15,16} Consequently, the availability of contributing sources to the ATP-PCr energy system within the muscle cells, such as Cr concentration, become crucial as short-duration, near-maximal exercise proceeds and muscular fatigue begins to set in, requiring further ATP resynthesis.¹⁷ Cr serves as a buffer to H⁺ and ADP which accumulate in the muscle fibers as a product of ATP hydrolysis, lowering the pH and decreasing muscular performance.¹⁸ Given that skeletal muscle cells, especially type II,

fast-twitch fibers, store approximately four-to six-times more PCr than ATP, it is advantageous to maintain high levels of intramuscular total Cr (TCr) content (FCr + PCr) for those who participate in short duration, maximal-effort exercise lasting 5-8 s, and up to 30 s for near-maximal PA.^{10,14,16,19-21} Because of the training protocols used, this applies too, to those who resistance train with the intent to increase muscular strength, muscular power, and improve fat-free mass (FFM; total amount of lean (non-fat) parts of one's body).²² However, the ability to resynthesize ATP via PCr hydrolysis declines approximately three-times faster than that of glycolysis (carbohydrate (CHO) catabolism).^{23,24} Thus, a greater quantity of Cr and PCr in skeletal muscle may translate to a greater quantity of ATP resynthesis via PCr hydrolysis and allow for more energy to be derived from high-energy phosphates before shifting to glycolytic pathways.²⁵

Creatine

Cr is an organic, nitrogen-containing compound that is derived from the nonessential amino acids arginine, glycine, and methionine, that is, the body can manufacture creatine.²⁶ Primarily the liver, but also the kidneys and pancreas, synthesize approximately 1 g·day⁻¹ of Cr, in addition to the approximately 1 g·day⁻¹ obtained through the typical, non-vegetarian diet.²⁶⁻²⁸ 95%-98% of the body's 120-140 g of Cr is found in skeletal muscle, approximately 40% as FCr and the remaining 60% combined with P_i to form PCr.^{12,29} FCr undergoes nonenzymatic cyclization and approximately 1.6% of this TCr pool gets degraded to creatinine (Cn; sole byproduct of Cr metabolism) per day, and undergoes renal excretion.^{12,26,28-33} Therefore, a sedentary, 70 kg man who maintains a 120 g TCr pool, loses approximately 2 g Cr·day⁻¹ and TCr levels are maintained. However, Cn production and loss increases with PA levels.^{12,30} As the intensity and time

of PA increases, individuals who wish to maintain maximal effort may seek alternative methods of maximizing intramuscular TCr levels.^{12,30} Thus, the exogenous supplementation of Cr is a common practice among those who resistance train as a means of maintaining elevated intramuscular TCr levels and enhancing total work output.¹²

Creatine as a Supplement

The average daily intake of Cr is approximately $1 \text{ g}\cdot\text{day}^{-1}$ (0.2 kg of uncooked meat contains approximately 1 g of Cr) and may be much lower for vegetarians who may consume minimal amounts of animal-food sources.^{12,29,34-37} Cr content in meat (eg, beef and lamb) has been shown to decrease with cooking (90 min, 70°C), as evidenced by an increase in Cr levels.³⁸ Thus, exogenous ingestion of Cr through dietary supplements (commercially available products consumed in addition to one's usual diet) has long been practiced as an effective means for maintaining elevated intramuscular TCr levels.^{12,39} When ingested, Cr progresses through the intestinal mucosa of the digestive tract, arguably unaltered, where it then gets absorbed into the blood stream and ultimately, stored in skeletal muscle for the maintenance of energy homeostasis.⁴⁰ Muscle TCr stores may be increased via supplementation and reach their typical limits ($150\text{-}155 \text{ mmol}\cdot\text{kg}^{-1}$ (dry muscle; DM)), resulting in excess Cr not being taken up by the muscle and voided in the urine.⁴⁰

CrH₂O Supplementation

One of the most researched and consumed form of Cr supplements is Cr monohydrate (CrH₂O).⁴¹ Harris et al. were among the first to demonstrate that oral supplementation of $20\text{-}30 \text{ g}\cdot\text{day}^{-1}$ of CrH₂O for several days could increase intramuscular TCr content between 30-50%, with approximately 30% of the Cr being retained by the

muscle in the first 2 d.⁴² The majority of studies that supplemented Cr at the recommended doses (eg, 4-5 g·day⁻¹, 10 d or 2-4 g·day⁻¹, 30 d) reported performance enhancement on exercises where energy transfer is mostly from the phosphagen energy system, and the brief, between-bout rest periods are sufficient to restore intramuscular PCr in participants.

All-out PA with CrH₂O supplementation is enhanced because the elevation in intramuscular TCr content allows for a prolonged use of PCr and replenishment of ATP, especially during intermittent exercise.^{23,43-48} Thus, better performances on maximal-effort tasks that go beyond relying solely on readily available ATP (eg, 1RM bench press) and rely on PCr hydrolysis to maintain maximal exertion.⁴⁴⁻⁴⁶ Moreover, because elevated intramuscular PCr may promote buffering of H⁺ ions and ADP during maximal muscular contractions and reduce neuromuscular fatigue, this may allow for an increase in the number of muscular contractions possible during a given short-bout high intensity activity, such as resistance training.^{18,19,25,26,49-51} This ability for prolonged muscle contractions may ultimately lead to hypertrophy of the targeted skeletal muscle tissue and increased lean body mass due to the increased resistance training volume and elevated muscle protein synthesis.^{23,47,52-55} Phillips and colleagues propose that because one of the most crucial stimuli for elevating skeletal muscle protein synthesis is muscle contraction, the greater number of contractions in a given training period may promote an elevated cumulative stimulus for protein synthesis and greater net muscle protein retention (skeletal muscle hypertrophy).^{52,56}

Body Mass and Lean Mass Effects of CrH₂O Supplementation

Though most of the literature has found acute CrH₂O interventions to positively influence body composition, few studies have found no or insignificant (~0.5 kg) changes in FFM (healthy, older males) after 10 d of supplementing with CrH₂O (20 g·day⁻¹). Further, men tend to accrue greater changes than women, likely due to their larger muscle mass.^{26,57-59} Independent of changes in hormonal (cortisol or testosterone) concentrations, CrH₂O supplementation often results in increased body mass, ranging from 0.5-3 kg in periods between 1-3 wk.^{23,26,30,60-63} It remains unclear, however, the mechanism(s) by which this occurs, whether it be elevated intramuscular water retention, the direct anabolic effect of Cr on skeletal muscle tissue synthesis, or other indirect factors.⁶⁴ It was further found that CrH₂O supplementation, in addition to the resistance training protocol, promoted greater cross-sectional area of the muscle fibers: type I: 35% vs 11%; type IIA: 36% vs 15%; type IIAB: 35% vs 6%, with vs. without supplementation, respectively.⁶¹ Participants in this study who were given the CrH₂O treatment were able to train at higher volumes during wk 5-8, which suggests that increased quality in training sessions led to more favorable adaptations in their FFM and overall muscle morphologies.⁶¹

Issues and Non-Severe Adverse Effects with CrH₂O Supplementation

It is important to note that chronic Cr supplementation lasting up to 4 y has not yielded any serious adverse health effects and up to 5 y had no effect on renal functioning among healthy athletes.⁶⁵ Moreover, some anecdotes report hypertensive effects upon supplementing with Cr, though acute (5 d, 20 g·day⁻¹) and longer-duration (6 wk, 20 g·day⁻¹ (3 d), 10 g·day⁻¹ maintenance) interventions have been shown to have no effects on blood pressure (systolic, diastolic, and mean).^{66,67}

More commonly anecdotally reported non-severe adverse effects accompany CrH₂O supplementation, such as subcutaneous water retention, gastrointestinal distress, nausea, and vomiting. These may be attributed to factors, such as co-ingestion with other nutrients (eg, large doses of CHO), timing of ingestion (in the periworkout period), or a lack of ability for all of it to pass through the intestinal epithelial cells and make it into circulation.^{26,68,69} CrH₂O supplementation often accompanies weight gain, not just in FFM, however, but also by water retention in the muscle fiber and subcutaneous space.^{70,71} Moreover, supplementing with CrH₂O has caused gastrointestinal distress, with reports of abdominal aches and cramps.^{26,72,73} There is a possible correlation between mega-doses of CrH₂O in one serving, otherwise known as a “loading phase,” where 10 g or more is ingested, and increased risk of diarrhea.^{74,75} This effect is likely due to the fact that CrH₂O is poorly soluble in liquid and consequently, poorly absorbed by the intestines where the substance then sits and draws water in to the intestinal tract.^{26,69,72,74-76} Another study showed using an in-vitro model that a range of only 0.2-3% of ingested CrH₂O was able to pass through the intestinal cells for upwards of a 90 min time period with the remainder being excreted by the body.⁷⁷

A potential enhancement of CrH₂O uptake is that of insulin augmenting Cr uptake by the skeletal muscles.^{25,40,64,78-81} Only approximately 50% of participants who supplemented with CrH₂O alone, positively responded (significantly enhanced their intramuscular TCr), but 100% positively responded with the presence of a large bolus of simple carbohydrates (CHO), such as dextrose.^{42,81,82} It takes approximately 95 g of simple CHO to provide a sufficient insulin spike to positively affect the intramuscular

uptake of TCr upon supplementation with CrH_2O .^{40,78-80} This insulin effect on Cr retention is likely due to insulin-stimulating, Na^+ -dependent, muscle-Cr transport.^{26,40,83,84}

Cr retention in the body after supplementing can be tested via urinalysis, as Cr undergoes excretion by the kidneys, and increases gradually after the first few days as supplementation continues.^{12,26,28-33,85} Chanutin et al. reported that approximately 100% of ingested Cr passes through the intestines and makes it into the bloodstream, though most of the body's Cr retention takes place within the first couple of days and approximately 90% of ingested Cr is excreted thereafter, with continued supplementation.⁸⁵ Thus, as myocytes no longer maintain the capacity to extract circulating Cr from the blood after the first few days of supplementation ($10\text{-}20\text{ g}\cdot\text{day}^{-1}$), bodily retention will decline, and the excess will be excreted as Cr in the urine.⁸⁵ However, elevated TCr levels may be maintained if one continues to supplement with Cr while following a resistance training program, as trained skeletal muscle tissue better takes up circulating Cr, especially as intramuscular Cr levels decrease from training and natural metabolic processes.⁴²

Given the popularity of this dietary supplement and these potential adverse effects associated with it, manufacturers have attempted to produce different forms of Cr with the same performance benefits, minus the ill-effects of the monohydrate form. Cr hydrochloride (CrHCl) was introduced to the market around 2009 as new form of Cr and is claimed to reduce the adverse effects of CrH_2O while providing the same performance and FFM benefits as the CrH_2O form.⁷⁶

Creatine Hydrochloride

CrHCl is created using CrH_2O and acetyl chloride (CH_3COCl) as reactants with ethanol ($\text{C}_2\text{H}_6\text{O}$) as the solvent, which then produces a granular precipitate consisting of

CrHCl salt, both in a high purity and yield.⁷⁶ CrHCl products are typically much more expensive than CrH₂O products (eg, NutraBio Creatine HCl, 135g, \$29.99; NutraBio Creatine Monohydrate Powder, 500g, \$14.99; NutraBio Labs, Middlesex, NJ).⁸⁶

Enhanced Solubility and Intestinal-Uptake of CrHCl

CrHCl was created as a form of Cr that yields higher aqueous solubility with the intention of being better mixed in liquid and better absorbed and retained with the same intended applications as CrH₂O.⁷⁶ The CrHCl patent reported the aqueous solubility of CrHCl to be in excess of approximately 150 mg·mL⁻¹, while that of CrH₂O and Cr citrate salt was only approximately 10-15 mg·mL⁻¹.⁷⁶ Subsequent research has confirmed their claims, finding CrHCl to be 38-times more soluble in solution than its CrH₂O counterpart.⁸⁷ Following, Gufford and colleagues reported that when Cr ethyl ester was placed in a highly acidic state (HCl), with the pH stabilized at pH 1.0, the amount of Cr absorbed by the intestines consequently increased 60%, due to the enhanced solubility and greater permeability characteristics under these acidic conditions, in comparison to CrH₂O, which is more basic in nature.⁶⁹ Gufford et al. propose that because the added HCl group is highly stable at the pH level of the gastrointestinal tract, its greater permeability and absorption are superior to that of Cr, alone.⁶⁹ This contradicts the aforesaid findings in the Chanutin et al. study, as it was found that nearly all of the Cr ingested passed through the intestines and made it into circulation.⁸⁵ Nevertheless, the implication is that CrHCl can be supplemented at a much smaller dose than CrH₂O, while still producing similar performance benefits with a reduced potential for the reported side effects. Therefore, with greater solubility in liquid, more efficient absorption by the intestines, and a smaller required dose than CrH₂O, CrHCl *may* minimize the stomach

and water retention issues reported by those who supplement with CrH_2O . However, a study examining the body's retention of Cr upon supplementing with CrHCl would be needed to tell whether these retention-effects are truly prevalent; currently, no such literature exists.

Need for the study

The study at hand will be among the first to investigate the acute resistance training responses after 7 d of supplementing with CrHCl . This study will bring value to the literature as it will add to the sparse literature pertaining to CrHCl as a dietary supplement and will provide data that can be compared with previous findings in regards to CrH_2O 's ability to acutely affect submaximal, intermittent exercise performances.^{23,40,42,78} These findings will allow for researchers and consumers to decide which form of Cr is superior for consumption based on dosing requirements and performance benefits.

Hypotheses

It was hypothesized that:

- 1) 4 g of $\text{CrHCl}\cdot\text{day}^{-1}$ for 7 d will significantly increase repetitions completed on three sets of the repeated barbell bench press and CMJ exercises at 70% of participants' 1RMs.
- 2) 4 g of $\text{CrHCl}\cdot\text{day}^{-1}$ for 7 d will significantly increase BW, FFM and decrease FM.

Delimitations

The study at hand will require participants to be currently resistance trained (minimum of 3 d $\cdot\text{wk}^{-1}$) with a minimum of 1 y experience. Moreover, participants will be

between the ages of 18 and 34 y, will not use anabolic steroids, be vegans or vegetarian, and/or must cease the use of their current nutritional supplement regimens.

Limitations

Limitations of the current study include untested participants who are:

- Untrained and low-trained.
- Youth, not 18 y of age and adults and elderly, greater than 34 y of age.
- Anabolic steroid users.
- Vegans and vegetarians.
- Diabetics.
- Unwilling to stop taking dietary supplements.

CHAPTER TWO: LITERATURE REVIEW

Selection Criteria

Selection criteria for this review gave preference to studies investigating CrH₂O's retention, and training studies pertaining to both CrHCl and CrH₂O whose participants were: 1) non-vegetarian or vegan, 2) male and female, 3) currently well trained (minimum 1 y of current and consistent resistance training experience), 4) non-steroid users, 5) not taking any other supplements at the time of the respective study, and 6) non-diabetics. Studies investigating the retention of TCr after supplementing with CrH₂O will be restricted to 1992, and beyond, as this was the year of the seminal study by Harris et al. that first investigated this.⁴² Any study examining CrHCl supplementation will be restricted to 2009 and beyond, as this was when it was first brought to the market for commercial use.^{42,76} No such literature exists relative to TCr pool enhancement and retention upon supplementing with CrHCl and thus, will be disregarded from this review. Studies detailing the intramuscular ATP-PCr energy system and/or skeletal muscle structure and function will have no date restrictions.

Review Format

The review starts with a brief section detailing the ATP-PCr energy system. Following is a section reviewing CrH₂O retention studies. Subsequent is a section reviewing the ergogenic benefits of Cr supplementation across different forms of PA, followed by a section reviewing anecdotal adverse effects associated with CrH₂O

supplementation. Following will be a section on CrHCl and any literature available pertaining to it, which currently is very limited.

Key Terms and Phrases

Key terms and phrases used in this search were “ATP-PCr,” “anaerobic energy,” “anaerobic metabolism,” “creatine,” “creatine monohydrate,” “creatine hydrochloride,” “creatine HCl,” “creatine retention,” “creatine carbohydrate,” “creatine insulin,” “creatine solubility,” “muscle fiber,” and “type II muscle fiber.”

Search engines utilized for this search included Web of Science, Google Scholar, Medline, PubMed, Boise State University Albertson Library Worldcat, Science Direct, and ASC Publications.

ATP-PCr Energy System

Enzymes: The Biologic Catalysts

Enzymes are large protein catalysts that are highly specific in function, catalyzing the forwards and backwards rates of chemical reactions without being altered or consumed during the respective reactions.³⁴ They reduce the required activation energy (initial energy to start a reaction), changing the rate of the reaction without changing equilibrium constants and free energy.³⁴ Their globular, three-dimensional protein structure determines the interaction with a specific substrate.²² This interaction takes place, described as a “lock-and-key mechanism” or “induced fit,” when the active site on the outer surface of the protein interlocks perfectly with the active site of the respective substrate.^{34,88,89} Subsequent to the formation of the enzyme-substrate complex, a new product with the new bonds is created after the initial splitting of the chemical bond.³⁴

For all-out PA, associated energy system enzyme activity increases to about 100-times their resting level, especially during each step of the hydrolysis process.²²

Hydrolysis Reactions

The hydrolysis, or “water splitting,” process catabolizes complex, organic molecules, with the splitting of chemical bonds initiating the decomposition process by adding the components of water (H₂O; hydrogen atom (H⁺) and a hydroxyl group (OH⁻)) to the byproducts of the reaction, as shown in the basic hydrolysis reaction equation: (AB + HOH → A-H + B-OH).⁹⁰ Each step of the decomposition process is accelerated by specific enzymes.⁹¹ During maximal-effort PA, the enzyme-mediated hydrolysis of both PCr and ATP are critical for maintaining adequate ATP levels and overall intramuscular energy homeostasis.⁴⁰

ATP: The Energy Currency

ATP is a naturally occurring nucleotide that is found in every cell of the human body.⁸ Approximately 10% more ATP is found in type II muscle fibers than in oxidative, type I fibers (eg, the vastus lateralis versus the soleus muscle).⁹² Its base is purine (adenine) and ribose (adenosine) and is linked to three negatively-charged P_i groups.^{8,90} The fact that these negatively charged P_i groups are so closely packed and repel each other makes ATP highly unstable.^{8,90} Chemically speaking, the triphosphate tail is similar to a tightly-bounded spring that is ready to uncoil with great energy.^{8,90} Thus, ATP is the ideal agent of energy-transfer, as it stores potential energy from ingested food molecules within its phosphate bonds and readily transfers this energy to other low-energy compounds to raise them to a greater activation level.⁹⁰

When ATP undergoes hydrolysis and its terminal, energy-packed P_i group is anaerobically cleaved off, the chemical “spring” becomes relaxed, making the entire molecule more stable.⁹⁰ Upon the release of this P_i from ATP, an abundance of free energy gets released ($\sim 7.3 \text{ kcal}\cdot\text{mol}^{-1}$), $ATP + H_2O \xrightarrow{ATPase} ADP + P_i + 7.3 \text{ kcal}$.^{34,93} This energy from the hydrolysis of ATP (mediated by the ATPase enzyme) powers all bodily functions, such as nerve transmission, tissue synthesis, and muscular action.¹³ In muscle cells, molecular motors are powered and muscle shortening is energized thanks to this release of free energy.⁶⁰ ADP is then anaerobically converted back to ATP – primarily in the muscle cells’ cytosol, as PCr undergoes hydrolysis in order to maintain a continuous supply of ATP.²² Hence, PCr, an energy reservoir, is a critical component of the phosphate energy system.

PCr: The Energy Reservoir

ATP’s storage is limited due to its heavy weight and to increase sensitivity of the energy production response.²⁹ This makes maximal-effort PA possible for only a few muscle-twitches, or 4-10 s, if relying primarily on ATP as an energy source.^{3,7} To maintain energy homeostasis and keep intramuscular levels of ATP stable (at approximately resting levels), PCr is hydrolyzed and phosphorylates the direct byproduct of ATP hydrolysis, ADP, within a fraction of a second to resynthesize ATP.^{28,78,90,94} Phosphocreatine, also referred to as creatine phosphate, is a phosphorylated Cr molecule ($Cr + P_i$).^{3,7} The enzyme-mediated (CK) hydrolysis of PCr yields a much higher free energy ($10.3 \text{ kcal mol}^{-1}$) than the ATPase-mediated hydrolysis of ATP, which is enough energy to power the phosphorylation of ADP to ATP.³ PCr, thereby, serves as the high-energy P-bond reservoir.³

Immediate Energy via the ATP-PCr Energy System

During maximal exercise, the rate of ATP turnover in skeletal muscle may increase several hundredfold.¹⁷ Despite this vast increase in ATP turnover, intramuscular ATP content remains relatively unchanged.^{17,34} This ATP hydrolysis/resynthesis balance is possible because ATP is resynthesized via PCr.¹⁷ The ATP-PCr energy system maintains four significant roles within skeletal muscle: 1) temporally buffers muscle ATP (to maintain ATP stores), 2) spatially buffers ATP (decrease cellular gradients of both ATP and ADP), 3) controls the oxidative phosphorylation process, and 4) acts as the primary buffer of H⁺ in the skeletal muscle cell.¹⁷

Muscular fatigue has been associated with decreased PCr levels and intramuscular pH, resulting in the inability to continue muscular contractions.^{6,95-98} Muscular energy deficiency may not be the product of ATP shortage but may rather be due to increases in ADP and P_i (products of ATP hydrolysis), as it has been shown that muscle force, velocity, shortening, and rate of relaxation may be impaired by the increased presence of both ADP and P_i.¹⁷ This suggests that muscular fatigue is more so a result of product inhibition than a lack of substrate availability.¹⁷ Increasing available intramuscular substrates through Cr supplementation has been shown to delay muscular fatigue and increase muscular performance by better utilizing the ADP and P_i for resynthesis of the high energy phosphates.²⁶

As ATP is used, the increase in byproducts of the high-energy phosphate hydrolysis, adenosine monophosphate (AMP; result of ATP utilization exceeding ATP regeneration) and P_i, initiate the breakdown of glucose through glycolysis without the need for O₂.^{14,17,20,99,100} Glycolysis breaks down the six-carbon glucose into two three-

carbon pyruvates as well as liberating electrons and H^+ .¹⁴ Pyruvate can either continue to be broken down via the Krebs's cycle if O_2 is present or, if energy demand is high, be cleared by forming lactate.¹⁴ Ensuing PCr degradation, anaerobic glycolysis predominates and provides approximately 70% of the entire energy demand for near-maximal PA lasting approximately 30-180 s.^{14,16,20,99} Anaerobic glycolysis (lactate end-product) is a rapid process that yields only limited amounts of ATP.¹⁰¹ Considerable amounts of lactate can accumulate in the blood and increase more than 30-fold above resting levels during maximal movements utilizing large muscle groups.^{14,16,20,99} One's muscular endurance heavily relies on this energy system and has implications for PA, such as maximal sprints and resistance training that lasts for approximately 30 s.^{14,16,20,99} Further, this applies for activities lasting slightly longer, such as during the last few hundred yards of a mile run or maximal effort on a 440 m run or 100 m swim.²²

When the pyruvate end-product of glycolysis enters the Krebs's cycle, it is a slow process that requires O_2 and yields substantial amounts of ATP to provide almost all the necessary energy for PA lasting for several minutes and beyond.^{102,103} Taking the pyruvate formed in glycolysis, the Krebs's cycle transfers electrons and H^+ atoms to the electron transport chain (ETC).¹⁰³ It is within the ETC that aerobic metabolism relies on O_2 , as it is the final electron acceptor within the respiratory chain, where it bonds with H^+ to form water (H_2O) as ATP is formed.¹⁰³ Energy substrate availability determines one's cardiovascular endurance and the ability of the aerobic system to replenish ATP, which has implications for endurance activities, such as long-distance walking, jogging, running, and swimming.²² The amount of ATP turnover during aerobic activities is very

high, yet this is a slow process when compared to fast glycolysis and the phosphagen system.¹⁷

Creatine Supplementation: Ergogenic Benefits

Cr is purchased over-the-counter as a dietary supplement and comes in numerous forms: powder, capsules, tablets, and stabilized liquid, though purity is not guaranteed as dietary supplements are unregulated by the Food and Drug Administration.^{26,104,105} In 1994, a regulatory framework pertaining to dietary supplements in the United States was established by the Dietary Supplement Health and Education Act (DSHEA, 1994), which eliminated regulation of the supplement industry. Consequently, sales of dietary supplements in the US increased from \$4 billion (1994) to \$35 billion (2015), more than an eight-fold increase over a 21 y time span.^{39,104-107} Around the year 2000, alone, an estimated 2.5-thousand metric tons of Cr was consumed, which coincides with 2001 sales of Cr supplements (\$400 million), and one can assume that as US dietary supplement sales have continued to increase over time, Cr sales have, too.^{26,41} More than 48% of male collegiate athletes use, or have used Cr as a supplement in preparation for their respective competitions; such numbers for females in this population have not been reported.⁴¹ Among strength and power athletes (eg, Olympic lifters, powerlifters, strongman competitors, and recreational weightlifters), the prevalence of Cr is much greater, exceeding 80%.⁴¹ Further, upwards of 71% of youth and adolescent athletes report using dietary supplements, and 90% of those in high school who reported using dietary supplements reported supplementing with Cr.^{41,108}

CrH₂O supplementation during training has been found to enhance performances in running (improved final, total, and best times for 300 and 1,000 m and 4 x 1,000 m

runs), cycle ergometry (better maintenance of pedal frequency at 4-6 s (all three bouts) and at the end of a 10 s bout, and increased peak-power, mean power, and total work on bouts 1 and 2), bench press (increased 1RM and amount of repetitions completed at 70%-repetition maximum), isokinetic unilateral knee-extension (greater peak-torque production on bouts 2, 3, and 4), and the jump squat (increased peak-power production on all five trials), to list a few.^{23,43-48}

A myriad of studies, though not all, show that Cr supplementation can significantly enhance healthy young adults' abilities to produce greater muscular force and/or power output for maximal, short-duration exercise, especially those with brief intervals of rest between exercise bouts, and less so for enhancing single-effort performances (eg, 1RM testing).²⁶ Participants in these studies vary in athletic ability, ranging from untrained to high-level collegiate athletes, and the improved activities include short-duration, maximal cycling, repeated jumping, sprinting, swimming, and resistance training, to list a few.²⁶

Acute Cr supplementation has been found to enhance resistance training in multiple instances.^{23,47} In one study, Volek et al. split fourteen resistance trained men into two groups (placebo (PI)) or Cr group), and both groups executed a jump squat protocol (5 sets, 10 repetitions, 30% of participants' 1RM) and a bench press protocol (5 sets, failure, 10-repetition maximum). This took place on three occasions, each trial separated by 6 d while discontinuing resistance training. Prior to trial 1, neither group was supplemented, from trial 1 to trial 2 both groups ingested the PI treatment, and between trial 2 and trial 3, the Cr group consumed 25 g CrH₂O·d⁻¹ while the PI group consumed the same amount of the PI treatment. The PI group exhibited no significant increases in

bench press repetitions completed from trial 2 to trial 3, while the Cr group saw significant increases on all five sets ($p \leq 0.05$).²³ The authors concluded that 1 wk of 25 g·d⁻¹ Cr supplementation increases muscular performances during repeated sets of jump squat and bench press exercises, likely due to the greater TCr pools from supplementation.²³

Moreover, Earnest et al. reported similar findings in their investigation of CrH₂O supplementation among ten highly weight-trained male participants and its effects on anaerobic power indices, muscular strength, and body composition.⁴⁷ Participants underwent three consecutive 30 s Wingate ergometer tests with 5 min off in between each Wingate trial. Peak anaerobic power was defined as the highest power achieved within a given 5 s work interval, while anaerobic work was denoted as the total amount of performed work within the 30 s work period.⁴⁷ Further, the free weight bench press was used to assess 1RM as maximal muscular strength, followed by assessment of the number of repetitions achieved on the free weight bench press (70% 1RM) until fatigue, cadenced by a metronome (60 beats·min⁻¹).⁴⁷ Lastly, body composition was assessed using hydrostatic weighing techniques. Participants were either supplemented with (double-blind) Pl (n = 4) or CrH₂O (n = 4, 20 g·d⁻¹) and after 14 d they re-tested their Wingate performances and after 28 d re-tested their bench-pressing exercises and anthropometric measures. It was unspecified if participants resistance trained during their supplementation protocols. For all post-test Wingate re-trials, the Cr group saw significant increases in anaerobic work (13%, 18%, 18%, respectively, ($p < 0.05$), while the Pl group saw no significant increases.⁴⁷ Further, several 5 s intervals over all three re-trial Wingate periods showed significant ($p < 0.05$) increases (specific data not

reported).⁴⁷ 1RM bench press performance increased by 6% in the Cr group.⁴⁷ Total lifting volume was significantly greater in the Cr group (absolute: 26%, $p < 0.01$; relative: 29%, $p < 0.001$). No changes in body weight or fat free mass (FFM) were noted for the Pl group, though the Cr group saw significant increases in body weight but not FFM (body weight: 86.5 ± 13.7 vs. 88.2 ± 14.1 kg, $p < 0.05$; FFM: 77.6 ± 10.8 vs. 79.2 ± 11.6 kg, $p = 0.054$).⁴⁷ The authors concluded that CrH₂O could be utilized as an ergogenic aid to boost athletic performance as it increased submaximal resistance training volume.⁴⁷

Balsom and colleagues found CrH₂O supplementation to increase short-term, maximal cycling performance.⁴⁴ Participants ($n = 16$; resistance trained males) underwent two high-intensity, intermittent exercise protocols, once before and once after the supplementation of either CrH₂O (5 g CrH₂O + 1 g glucose·d⁻¹; five times, daily, 14 d) or Pl (6 g·d⁻¹ glucose; five times, daily). Participants were instructed to refrain from PA 24 h before each testing session but otherwise were instructed to maintain their typical daily PA. Each cycling protocol consisted of ten 6 s bouts of high-effort cycling at two different exercise intensities (130 revolutions·min⁻¹, ~820 W; 140 revolutions·min⁻¹ ~880 W).⁴⁴ The latter of the two intensities were meant to induce fatigue during the 10 s exercise bouts while the former was meant to allow for participants to execute the same amount of work pre and post trials. The Pl group exhibited no significant improvements in any of the assessed physiological measures.⁴⁴ In the 140-revolution trial, there was significantly increased performance (+10 revolutions·min⁻¹) towards the end (4-6 s, $p < 0.05$) of each bout of exercise when the participants were supplemented with CrH₂O.⁴⁴ The authors therefore concluded that CrH₂O ingestion allowed prolonged muscular performance at higher intensities.⁴⁴ They attributed this to an enhanced PCr pool in the

resting state before exercise, in addition to an accelerated resynthesis of PCr during the recovery periods.⁴⁴ Thus, Cr supplementation postpones the onset of muscular fatigue during repeated bouts of near-maximal exercise.⁴⁴

Sprinting performance has been shown to be enhanced after CrH₂O supplementation by Earnest and colleagues.¹⁰⁹ Their investigation was to determine if supplementation of CrH₂O would enhance anaerobic, intermediate-length, treadmill running to exhaustion. Investigators sought to see if anaerobic capacity, and time to exhaustion per trial (TTE_{trial}), and total time to exhaustion (TTE_{total}; cumulative, across all trials) could be enhanced with CrH₂O supplementation for bouts of work lasting up to 90 s. Eleven male participants ($n = 11$) received either a glucose PI or CrH₂O treatment (20 g·d⁻¹, 4 d and 10 g·d⁻¹, 6 d) while maintaining their normal PA routines during the treatment period. They performed two treadmill runs to exhaustion at individually prescribed grades with 8 min of rest between runs.¹⁰⁹ Participants executed the two exhaustive treadmill runs at 214 m·min⁻¹ at either a 10, 15, or 20% grade.¹⁰⁹ TTE_{trial} and TTE_{total} were measured as a sum of both run trials. Results showed that there was a significant improvement for the Cr group for TTE_{total} with a 5.7 s increase ($p = 0.05$) and a decrease of 2 s in the PI condition (exhausted faster).¹⁰⁹ TTE_{total} was significantly enhanced in the posttest trial for the Cr group (pre-test: 176.5 ± 11.4 s; posttest: 182.2 ± 14.6 s).¹⁰⁹ Researchers also measured blood lactate levels, and as expected, as TTE increased, so too did the bioenergetic dependency on anaerobic glycolysis, and thus, the increased levels of blood lactate.²⁴ They concluded that the increased TTE_{total} was attributed to greater TTE capability for each bout and thus, Cr supplementation may

contribute to longer, intermediate-duration, near-maximal performances that are interval in nature.¹⁰⁹

Swimming performance has also been found by Grindstaff et al. to be enhanced by CrH₂O supplementation.¹¹⁰ The authors had eighteen competitive swimmers, male and female, supplement with either CrH₂O (21 g·d⁻¹) or Pl (maltodextrin, 21 g·d⁻¹) for 9 d during their training sessions. Both before and after the supplementation protocol, participants executed three 100-m (long-course) freestyle sprint-swims, and three 20 s maximal-effort, arm ergometer sprint tests (prone), both with 60 s of rest/recovery between exercise bouts.¹¹⁰ Following CrH₂O supplementation, participants in this group swam significantly faster than the Pl group in the 100 m swim (CrH₂O: Heat 1: -0.27 ± 0.3 s; Heat-2: -0.93 ± 0.4 s; Heat-3: -0.36 ± 0.3 s; Pl: Heat-1: 0.86 ± 0.3 s, Heat-2: -0.40 ± 0.5 s, Heat-3: 0.02 ± 0.3 s), significantly improved heat-2 swim time, and showed a tendency to better their cumulative swim times for all three 100 m swims ($p = 0.057$).¹¹⁰ As a whole, CrH₂O supplemented participants decreased their swim times by -1.57 ± 0.8 s in comparison to the Pl group which increased their swim times by 0.48 ± 0.6 s ($p = 0.057$).¹¹⁰ As for the ergometer for the upper extremity, sprint performance was significantly increased for the first of the three trials and in comparison to the Pl group, they showed 7.8%, 5.3%, and 0.5% increases in performance, respectively, across all three trials.¹¹⁰ The authors concluded that CrH₂O supplementation may enhance repetitive sprint-swim performance among competitive junior swimmers.¹¹⁰

Again, it is important to note that not all studies have found CrH₂O supplementation to instigate ergogenic benefits.^{82,111-117} It remains uncertain as to why there has been some discrepancies in the literature, though it has been suggested the

disparities may perhaps be due to: 1) placebo effects, 2) differing performance tasks used across studies, 3) small magnitudes of treatment effects, 4) relatively vast test-retest reliability error for the measurement of the given performance tasks, and 5) most likely because Cr supplementation is meant to increase intramuscular TCr stores, and as will be reviewed below, not everyone positively responds to Cr supplementation when consumed by itself.²⁶

As for the above studies that found positive performance effects associated with the oral supplementation with CrH₂O, they shared many common variables in terms of methodologies. For instance, nearly all the studies mentioned had only male participants. This alone disregards any discrepancies that may have been prevalent had female participants been included in the data sets. Indeed, one study noted that women showed less absolute increases in lean body mass after CrH₂O supplementation versus their male counterparts, which was attributed to their smaller muscle mass, by nature.¹¹⁸ This may also indicate that women have altered Cr uptake abilities in comparison to men, and thus, it is important that women be included in future Cr studies.

Moreover, all the studies investigated near-maximal PA that was interval in nature. This calls for brief periods of rest/recovery between bouts of exercise, which allows for the ATP-PCr energy system to recover ATP levels by way of PCr hydrolysis.^{29,42,48} As the number of exercise trials increased per a given study, so too did the effects of the Cr treatments. While some acute performance increases occurred as well (eg, trial-1 on upper-extremity ergometer testing and 1RM bench press performance), the majority of the ergogenic benefits came from repeated bouts of executing a single performance task.¹¹⁰

As for the supplemental protocols, participants who were in the experimental Cr groups were typically supplemented with about 20-30 g CrH₂O·d⁻¹. This dose was shown by Harris and colleagues to adequately increase intramuscular TCr content among most participants, and thus, has been used in the hundreds of Cr studies since.⁴² Supplemental protocols in the above literature ranged from acute to somewhat long-term, and all were associated with positive performance outcomes. This is likely because, as will be discussed in the following section, most Cr uptake by the body occurs within the first couple of supplementation days and decreases thereafter.^{42,85,119}

In all, overwhelmingly more studies than not have found Cr supplementation to have positive performance outcomes when appropriately dosed and when the selected exercise protocol reflects the function of the ATP-PCr energy system than not. To best achieve these ergogenic benefits, it is imperative that Cr uptake by the body and skeletal musculature be maximized, which will be reviewed in the next section.

Creatine Monohydrate: Uptake and Retention Assessed via Urinalysis

Inulin increases caused by the intake of simple CHO has been shown to augment Cr retention in skeletal muscle.⁸¹ Thus, this section is dedicated to studies not supplementing Cr with simple CHO. This section is dedicated to studies that investigated CrH₂O supplementation and its ability to be absorbed by the intestines and retained by the body upon ingestion.

Among the first researchers to investigate oral Cr supplementation and its ability to be taken up by the body by way of tracing the Cr-creatinine (Cn; sole end product of Cr breakdown) balance via the urine, were Chanutin and Colleagues, in 1925.⁸⁵ For this experiment, the authors of the study also served as the participants ($n = 2$, males). 2 wk

prior to the start of the study, participants followed a Cr-free diet (no meat or fish). Further, participants engaged in daily, non-vigorous PA throughout the course of the study, with activities being varied and unspecified. This 2 wk period served as their control period from which they took their basal urine tests (participant 1 average daily outputs: Cn = 1.51 g; Cr retained = N/A; extra Cn eliminated = N/A).⁸⁵ The experimental periods lasted for 29 (participant 1) and 44 d (participant 2), and the administered CrH₂O doses were either 10 or 20 g·d⁻¹. Participant 1 first supplemented with 10 g·d⁻¹ for the duration of 10 d (average daily outputs (g): Cn = 6.19; Cr retained = 4.54; extra Cn eliminated=0.60).⁸⁵ Following, this participant ingested 20 g·d⁻¹ which lasted for 8 d (average daily outputs (g): Cn = 16.45; Cr retained = 4.23; extra Cn eliminated = 1.93).⁸⁵ Following, participant 1 underwent a 2 d period where was consumed 10 g·d⁻¹ again (average daily outputs (g): Cn = 11.43; Cr retained = -0.21; extra Cn eliminated = 1.16), then a final, 2 d period where no Cr was administered (average daily outputs (g): Cn = 3.71; Cr retained = N/A; extra Cn eliminated = 0.65).⁸⁵ He followed a different protocol, followed a control period (average daily outputs (g): Cn = 1.61; Cr retained = N/A; extra Cn eliminated = N/A), followed by three, 10 d (10 g·d⁻¹), supplemental periods (average daily outputs (g): Cn = 6.37; Cr retained = 4.48; extra Cn eliminated = 0.62: Period 2 average daily outputs (g): Cn = 10.10; Cr retained = 1.35; extra Cn eliminated = 1.22: Period 3 average daily outputs (g): Cn=10.44; Cr retained = 0.816; extra Cn eliminated = 1.03), a 5 d (10 g·d⁻¹), supplemental period (average daily outputs (g): Cn = 10.06; Cr retained = 1.09; extra Cn eliminated = 0.93), and concluded with 2 d of no Cr supplementation (average daily outputs (g): Cn = 2.82; Cr retained = N/A; extra Cn eliminated = N/A).⁸⁵ The first few d of Cr feeding allowed for very high Cr retention by

the body (Participant 1: d 1, 5.91 g, d 2, 5.32 g; Participant 2: d 1, 6.80 g, d 2, 6.15 g).⁸⁵ However, from these points forth, gradual decreases in the retention of Cr in the body are noted with coincidental increases in urinary Cr levels.⁸⁵ Both participants had approximately 115 g of baseline intramuscular Cr content and at the end of their respective experimental periods, Participant 1 retained 58 g of the 270 g (assumed in the skeletal muscle tissue) total Cr consumed over the 29 d period, while Participant 2 retained 38 g of the 340 g (assumed in the skeletal muscle tissue) consumed over the 44 d period.⁸⁵ This would equate to an elevation in intramuscular Cr storage of 33% (Participant 1) and 50% (Participant 2).⁸⁵ From these findings, the authors concluded that 1) intestinal absorption is approximately 100% (via urinalysis), 2) Cr excretion gradually increases as Cr supplementation continues, 3) most of the Cr retained by the body takes place within 1-2 d of supplementing, and 4) approximately 90% of the ingested Cr is excreted in the urine with prolonged supplementation, beyond the first few days.⁸⁵

A study by Harris et al. is much more recent and sought to see if doses of orally supplemented CrH₂O were 1) retained by the body and 2) increased intramuscular Cr content.¹ There were 17 participants (10 males, 7 females) between the ages of 20 and 62, two of which were vegetarian, and all with varied fitness levels (untested). Participants were not instructed to change their diets nor change their daily PA. 20 g·d⁻¹ (5 g, four times, daily) CrH₂O was consumed by Participants 1 and 2 (4.5 d), Participants 3 and 4 (7 d), and Participant 5 (10 d). 5 g·d⁻¹ (6 times, daily) CrH₂O was supplemented by Participants 6-8 (7 d) and Participants 9-12 (alternate d, 21 d). The rest of the participants (*n* = 5) underwent similar doses but were tested under exercised conditions to note any training effects associated with the Cr consumption and thus, will be disregarded by this

section of the review as it is solely focused on Cr retention by the body. Muscle biopsies, blood samples, and 24 h urine samples were taken from all participants, though only four participants had complete urine data collected. A 5 g dose of CrH₂O (about that found in 1.1 kg of raw steak) was selected based on blood sampling of participants as 7 h of sampling at this dose provided a high mean peak concentration of approximately 795 (\pm 104) $\mu\text{mol}\cdot\text{l}^{-1}$ at 1 hour with a stable Cn concentration of only 70-100 $\mu\text{mol}\cdot\text{l}^{-1}$, as compared to a 1 g administered dose (plasma Cr concentration only approximately 1 $\mu\text{mol}\cdot\text{l}^{-1}$).¹ Though there were only four complete urine collection analyses, consistent even with the incomplete analyses, it revealed that the greatest uptake of Cr occurred during the first 2 d, regardless of dose (20-30 g Cr).¹ Over the first 3 d for participants 13-15 (5 g, 6 times a day), renal excretion of the total 30 g dose was 40% \pm 14%, 61% \pm 3%, and 68% \pm 15%, respectively.¹ Among these participants during these first 2d, the estimated mean Cr uptake by the muscle was approximately 17.7 \pm 3.4 g which equates to approximately 32% of the administered Cr dose, 20% as PCr.¹ From d 2 to d 4, Cr uptake gradually increased another 9.1 \pm 1.6 g, approximately 50% of what was taken up the first 2 d.¹ This differed from the previous study in that 100% of the Cr was reported to be digested. In all, an intramuscular Cr concentration increased by approximately 25 $\text{mmol}\cdot\text{kg}^{-1}$ (dry muscle; DM), however, with between-subject variation rather vast (2-40 $\text{mmol}\cdot\text{kg}^{-1}$ (DM)).¹ This variation should be expected given the varied supplementation protocols participants were assigned to.

Hultman and colleagues followed up the Harris et al. study in 1996 with their investigation of Cr retention among men.¹¹⁹ Thirty-one men participated in this study which had four different experiments (each participant assigned to only one) and lasted

for 2 y. No participant was considered highly trained, though all reported being at least somewhat physically active. Further, participants were to maintain their usual diets and to refrain from strenuous PA throughout each experiment. Of the four experimental groups, Group-4 was the only group to test for Cr excretion via the urine. Group-4 consisted of seven participants (age = 23 ± 1.8 y, body mass = 74.3 ± 5.4 kg).¹¹⁹ Each participant consumed 20 g of the PI (Maxijul glucose polymer) for 5 d and 24 h urine samples were collected 1 and 6 d prior to the start of PI ingestion.¹¹⁹ Urine collection also took place on PI ingestion d 1, 3, and 5, and then on d 8, 11, 15, 22, and 25 for a 20 d period that followed the placebo (PI) ingestion period. Following this period, participants then repeated the previously mentioned procedures, but with $20 \text{ g}\cdot\text{d}^{-1}$ of Cr rather than the PI. These supplemental periods were originally planned to last 6 d each, but as previously found in Groups-1 and -2, intramuscular Cr retention was peaked after d-5 and started to decline after (d 5: $142.6 \pm 2.3 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$; d 6: $147.2 \pm 4.7 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$).¹¹⁹ The authors reported that there was a vast between-subject variation for Cr excretion among participants for the first 20 d of this experimental trial, though on the final day 20 they could obtain a post-Cr consumption average for Cr excretion ($2.8 \text{ mmol}\cdot\text{d}^{-1} > \text{PI ingestion } (p < 0.05)$).¹¹⁹ The majority of the Cr retained by the muscle tissue took place during the first 2 d of Cr supplementation (~30%) as compared to only 15% on d 2-4.¹¹⁹ The mean increase in intramuscular TCr across all experimental groups who ingested $20 \text{ g}\cdot\text{d}^{-1}$ over the 6 d period (high-dose, fast Cr load) was $20 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$. Other groups (not mentioned earlier, no urinalysis) supplemented with only $3 \text{ g}\cdot\text{d}^{-1}$ over a 28 d period (a low-dose, long-duration Cr load), and while accumulation of Cr was slower, the 28 d result proved to show no difference in intramuscular TCr retention.¹¹⁹ It is important to

note, however, that the sample size in this study was small ($n = 13$) and additional studies are needed to draw more conclusive results.

Enhanced uptake of Cr may help negate typical anecdotes of associated gastrointestinal discomfort or others, which though are uncommon, are reviewed below.

Creatine Monohydrate: Adverse Effects

There have been numerous reported adverse effects associated with CrH_2O supplementation, ranging from gastrointestinal distress to muscle and cardiovascular issues.²⁶ However, Terjung and colleagues state that these findings are not definitive are not enough to deem Cr supplementation as a health risk.²⁶ At the same time, they state, it cannot be assumed that Cr supplementation is free from health risks; more research is needed.²⁶

One study by Vandebuerie and colleagues demonstrated that timing of Cr supplementation may be responsible for the reported associated stomach issues.⁶⁸ Twelve male, non-vegetarian participants underwent a 2 h and 30 min endurance cycling protocol on their own bicycles mounted to an electromechanically-braked system. Once the participants cycled and achieved exhaustion at their 4 mmol lactate thresholds, they immediately followed with five maximal sprints for 10 s, with 2 min intervals of recovery. Prior to the exercise protocol, all participants were “Cr-loaded,” as they consumed $25 \text{ g}\cdot\text{d}^{-1}$ (5 d), and some participants were given Cr ($5 \text{ g}\cdot\text{h}^{-1}$) or Pl *during* the workouts.⁶⁸ Four out of six participants who consumed the Cr during the exercise protocol reported post-exercise “distress,” while the Pl treatment during exercise yielded two reports of distress.⁶⁸

Juhn et al. and Schnirring's respective studies reported muscle cramping, stiffness, and strains among some of their athlete-participants.¹²⁰ Similarly, Tarnopolsky et al. found that among 81 participants who had neuromuscular disease, just one participant reported muscular cramping subsequent to supplementing with 10 g·d⁻¹ (5 d) followed by 5 g·d⁻¹ (5 d).¹²⁰ When they put this participant under the same experimental Cr conditions, however, they did not complain of any muscle cramps the following session, suggesting that Cr may not have been responsible.¹²⁰

There have also been anecdotes of hypertension among those who supplement with Cr.²⁶ However, Mihic and colleagues found via double-blind procedures that short-term Cr supplementation (20 g·d⁻¹, 5 d) among young male and female participants had no effects on mean, systolic, or diastolic blood pressures.⁶⁶ Peeters et al. found in a similar study that the same supplemental Cr protocol yielded no blood pressure effects over a longer duration of time (6 wk, 20 g·d⁻¹ (3 d), 10 g·d⁻¹ maintenance).⁶⁷ Two more studies found that blood pressure was not affected among participants with congestive heart failure who supplemented with Cr (20 g·d⁻¹, 5 d and 20 g·d⁻¹, 10 d).^{121,122}

Lastly, Cr supplementation has been scrutinized by claims that it unfavorably alters renal functioning because its consumption increases urinary output of Cr and Cn and would therefore be expected to increase Cn concentrations in the plasma.²⁶ Short-duration (20 g·d⁻¹, 5 d) supplementation of Cr has been found to have no effects on glomerular filtration rate.^{65,123} Moreover, athletes supplementing with Cr for up to 5 y had no effect on renal functioning.⁵⁰

There have been numerous anecdotes associated with the consumption of large quantities of CrH₂O. As discussed in the next session, this has been attributed to the lack

of intestinal absorption of CrH_2O through the intestinal cells.^{69,76,87} Nevertheless, these anecdotes are typically single reports, and when in some cases, the affected participant is re-tested under the same Cr conditions, the report of distress does not reoccur. However, because of these perceived issues, companies have strived to come up with alternative forms of commercially available Cr that can be better taken up by the body, reduce these anecdotal reports of ill-effects, and still provide the ergogenic benefits found with the supplementation of CrH_2O . CrHCl is among the latest of these new forms, and shows promise in solubility and intestinal uptake abilities.

Creatine HCl: Solubility and Intestinal Uptake

Nutrients can make it through the epithelia of the intestinal tract through numerous mechanisms, including active transport, facilitated transport, diffusion, or paracellular pathways.¹²⁴ Because Cr shares similar structural characteristics to simple amino acids it may enter systemic circulation through peptide transporters, amino acid transporters, or specialized transporters (eg, taurine), or possibly even through the paracellular route.^{124,125} Karlsson and colleagues reported that Cr has a molecular weight of $131 \text{ g}\cdot\text{mol}^{-1}$, a net positive charge, and an approximate partition coefficient of -2.7 , and thus, should bypass the intestinal epithelia through paracellular pathways.¹²⁶ However, Dash et al. found CrH_2O to have very poor ability to bypass the intestinal epithelia.¹²⁴ This is likely because the aqueous solubility of CrH_2O has been found to be only approximately $16.6 \pm 0.3 \text{ mg}\cdot\text{mL}^{-1}$.¹²⁷ Aqueous solubility of CrHCl was found to be 38-times greater than that of CrH_2O .⁸⁷

CrHCl was brought to the market as a dietary supplement around 2009 as an alternative form of supplemental Cr, more acidic than CrH_2O , that is supposed to be

much more soluble in liquid and consequently, better absorbed by the intestines.⁷⁶ To make CrHCl, the manufacturer mixes CrH₂O with acetyl chloride and ethanol is then added as a solvent.⁷⁶ This solution precipitates a granular form of HCl salt of Cr after the solution is heated between approximately 24°C and 50°C.⁷⁶ Lastly, the solution is reduced to ambient atmospheric temperature and pressure where the CrHCl granules are collected and packaged for commercial use and consumption by humans or livestock as a dietary supplement, in a solid, oral-dosage form, such as capsules or tablets.²

Gufford and colleagues investigated the aqueous solubility ability of CrHCl and other commercially available Cr salts in comparison to that of CrH₂O.⁸⁷ The purpose of the study was to identify a water soluble Cr salt that would have increased solubility and bioavailability, a lower needed oral dose, and a decrease in side effects in comparison to CrH₂O. Each of the six salts underwent nuclear magnetic resonance, elemental analysis, and melting-point determination, followed by high performance liquid chromatography (HPLC). From there, determination of saturation solubility of each salt took place in deionized water by slowly adding it to 5 mL of solvent in glass bottles which were settled in a shaking water-glass (25° C).⁸⁷ Following, 2 mL aliquots were extracted and centrifuged (11,000 rpm) for 5 min.⁸⁷ HPLC was then used to determine Cr concentrations. All salts were found to be significantly more soluble than CrH₂O, with CrHCl producing the most significant increase (CrHCl: 709 ± 7 ; CrH₂O: 17.1 ± 0.4).⁸⁷ The authors proposed that a contributing factor is likely due to the fact all of the saturated salt solutions were in highly acidic states (unbuffered water; pH 0.3-3.1) as compared to the CrH₂O solution (pH 8.6).⁸⁷ In biological fluid or neutral pH, Cr is in its least-soluble, electrically-neutral state due to its 7.4 isoelectric point.⁸⁷

In another study, Gufford and colleagues investigated the permeability of CrHCl across the human Caco-2 epithelial cell monolayers.⁶⁹ Permeability of both CrH₂O and CrHCl were investigated using the cell-line (epithelium of human intestine) from a human carcinoma of the colon (Caco-2).⁶⁹ The epithelial cells were seeded into specialized, 24 mm diameter, polycarbonate membrane inserts at 60,000 cells·cm².⁶⁹ These cells were kept in a 5% carbon dioxide environment for approximately 21 d and supplemented with 10% fetal bovine serum, from which point, merged monolayers were reached.⁶⁹ 1.5 mL of simulated intestinal fluid in addition to 10 mM of CrHCl replaced the apical compartment of the monolayers after a 30 min period of incubation.⁶⁹ The highest permeability in the Caco-2 monolayers was noted for CrHCl, as it was approximately 15-times greater than that of CrH₂O (CrH₂O: 0.12 ± 0.004 cm/s; CrHCl: 4.3 ± 0.4 cm·s⁻¹ (*p* < 0.05)).⁶⁹

Per these studies, it is apparent that CrHCl is indeed a much more soluble form of Cr in aqueous solution in comparison to CrH₂O. Further, while CrHCl showed promise bypassing intestinal cells in vitro, TCr uptake by the body upon supplementing with it has yet to be quantified in a human model. Thus, the study at hand will be among the first to do so.

CrHCl and Resistance Training

Research investigating CrHCl supplementation and its effects on resistance training is lacking. To the author's knowledge, only three studies to date have considered CrHCl as an ergogenic aid.

The first study compared strength and body composition effects of a high CrHCl (5 g·d⁻¹) group, a low CrHCl group (1.5 g·d⁻¹), a CrH₂O group (5 g·d⁻¹), and a Pl group

over a 4 wk period.⁷⁵ Participants ($n = 30$; both sexes) were randomly divided into one of the four groups and underwent a given treatment while adhering to a prescribed 4 wk resistance training protocol. 1RM bench press and leg press were assessed both pre- and post-experiment as well as body-composition (skin fold). 1RM leg press was significantly increased ($p < 0.05$) in all groups while 1RM bench press was only significantly increased in the low CrHCl group ($p = 0.003$).⁷⁵ Between group differences were not found. Both CrHCl groups resulted in significantly decreased FM (high: $p = 0.034$; low: $p = 0.005$) while FFM was significantly increased only in the high CrHCl group.⁷⁵ It was concluded that both CrHCl and CrH₂O promote gains in strength but only CrHCl promotes significant changes in body composition with differences in doses, which contradicts other research showing that CrH₂O can positively influence body composition (eg, increase fat free mass, decrease fat mass).⁷⁵ However, it is important to note that participants in this study were asked to refrain from any form of resistance training 4 wk prior to the start of the study, which may have had an impact on strength and body composition results.

Another study by Reuland investigated training effects associated with a single dose of CrHCl supplementation ($0.033 \text{ g} \cdot \text{kg}^{-1}$ bodyweight, 30 min before testing) and differences across genders.¹²⁸ This study investigated pre- and post-total training volume (TTV) on five repeated sets of the barbell bench press and the incline lying leg press exercises with 75 s rest between each set. Lab visits were separated by 48 h (paperwork, 1RM testing, control trial, and experimental trial) and participants were asked to refrain from exercise during these periods. Results for the leg press showed that women in the Cr and Pl groups all significantly increased TTV from the control to the experimental trial (p

= 0.004, $p = 0.02$, respectively).¹²⁸ Men in the Cr and Pl groups also significantly increased leg press TTV ($p = 0.03$, $p = 0.003$, respectively).¹²⁸ However, women in the Cr group had significantly higher score differences from the control to the experimental trials than their male counterparts ($p = 0.01$).¹²⁸ Women's TTV for the bench press showed no significant differences from pre- to post-intervention in the experimental or control group ($p = 0.2$, $p = 0.21$) and the same held true for men ($p = 0.12$, $p = 0.68$).¹²⁸ The authors concluded that the efficacy of CrHCl on resistance training remains unclear.

Lastly, a study by Packer and colleagues investigated the acute effects of CrHCl supplementation over a 6 d period on 1RM performance on the barbell bench press, barbell deadlift, and CMJ among trained powerlifters.¹²⁹ Participants ($n = 9$) were spread unevenly into three groups (CrHCl ($n = 4$): two doses at $0.750 \text{ mg} \cdot \text{d}^{-1}$; CrH₂O ($n = 3$): two doses at $5 \text{ g} \cdot \text{d}^{-1}$; Pl ($n = 2$)).¹²⁹ Participants were instructed not to resistance train or perform any anaerobic-style PA during the 6 d intervention period. No statistically significant changes were found in 1RM on any of the three performance measures from pre- to post-treatment ($p > 0.05$).¹²⁹ Percent-changes for all three performance tasks for the CrHCl, CrH₂O and Pl groups, respectively, were calculated (barbell back squat: 2.44%, 1.54%, and 10.33%; deadlift: 1.4%, 2.21%, and 6.67%; CMJ: 2.04%, 3.61%, and -2.15%) and resulted in the conclusion that neither form of Cr as a dietary supplement is effective at augmenting 1RM on these three tasks over a 6 d supplemental period.¹²⁹ Results contrary to the previous studies were likely produced due to a very small sample size and because participants were instructed not to exercise during the 6 d Cr intervention period, which is counterintuitive due to the fact that exercised skeletal muscle tissue better retains Cr as shown by Harris and colleagues and once a muscle's Cr

stores are full supplemented Cr is simply excreted in the urine.¹²⁹ Moreover, 1RM performance does not truly mimic the ATP-PCr energy system where an influx of intramuscular Cr and PCr would be useful for ATP and PCr resynthesis during longer periods of near-maximal effort PA; likely, the ATP stores in the muscle prior to the 1RM attempts were being used as the sole energy source. Thus, Cr supplementation should not have been expected to increase performances on the respective 1RMs.

Closing

It has been established that the oral supplementation of CrH₂O can enhance whole body and intramuscular levels of TCr, even more so when taken in conjunction with an insulin-stimulating companion. Because of this increase of intramuscular TCr, namely PCr, ATP can be resynthesized for longer durations of short-duration, maximal-intensity PA, especially when interval in nature with recovery-time in between exercise bouts to allow for further synthesis of ATP.

However, there have been many anecdotal reports of study participants who supplemented with high doses of CrH₂O (20-30 g) who complained about issues, such as gastrointestinal distress and muscle cramps, with the former of the two being attributed to CrH₂O's lack of ability to bypass the intestinal cells due to its low solubility in aqueous solution. Thus, another form of Cr (CrHCl) was introduced to the market in 2009 as a form of Cr that is more soluble in aqueous solution and bypasses the intestinal epithelial cells at a much higher rate with the purpose of providing consumers with the ergogenic benefits of CrH₂O without the reported ill-effects reported by those who supplement with it. However, there are only three studies to date looking at the ergogenic benefits

associated with its consumption. Thus, the study at hand will be one of the first to investigate this.

CHAPTER THREE: METHODS

Participants

Sixteen healthy, resistance trained males between the ages of 18 and 34 y were recruited to participate in this study. Inclusion in the study required participants to have at least 1 y of current, consistent (minimum of 3 d·wk⁻¹) resistance training experience, which was verbally verified by the PI prior to the start of the study. Exclusion criteria, which was also verbally assessed prior to the start of the study, excluded participants who were not between the ages of 18 and 34 y, had less than 1 y of current and consistent resistance training experience, used anabolic steroids, were vegans or vegetarians, diabetic, and/or were unwilling to cease the use of their current dietary supplement regimens.

Participation in this study was completely voluntary and required a signed, written informed consent (APPENDIX A) approved by the BSU Institutional Review Board (IRB #103-MED17-003). The revised, 2017 version of the Physical Activity Readiness Questionnaire (PAR-Q; APPENDIX B) was distributed to all participants to ensure that they had no previous or current health conditions that would prevent them from being able to participate in this in the study. All screening information and collected data was handled with complete confidentiality. Only the PI had access to the participants' screening documents locked in a designated cabinet within BSU's Human Performance Laboratory (HPL). Collected data was accessed only by the PI. All participant information was kept electronically on a secured, BSU password-protected computer.

Further, each participants' information was kept private and was stored using assigned IDs created by the PI of this study. The participant ID codes were kept in a separate location from the participant data.

Procedures

A pre-post experimental design was followed, as the same group of participants were tested twice on the same variables before and after the 1 wk treatment period.

The Cr hydrochloride (CrHCl) supplement (NutraBio Creatine HCl; NutraBio Labs, Middlesex, NJ) was administered in a 1 wk supply (28 g) to each participant, in 2 g doses (powdered form). Participants were instructed to take two, 2 g doses daily, 30 min pre-, and immediately following their respective daily resistance training workouts. On days when participants did not resistance train, they were instructed to consume a 4 g dose in the morning upon waking. To minimize any potential differences in rates of intestinal Cr absorption, all participants were instructed to mix the powder in identical volumes (8 oz) of cold water and were asked to consume the mixture within a 5 min period from the end of mixing, starting from their first sip.⁷⁸

Two wks prior to the start of the treatment period, participants agreed to cease their current use of dietary supplements, which served both as a Cr washout (allowing intramuscular TCr levels to return to pre-supplemented levels) and control period.⁴⁰ Furthermore, participants agreed to refrain from consuming alcohol and engaging in any form of strenuous exercise 24 h before each testing protocol.⁷⁸

During visit 1, participants completed all paperwork and completed their maximal countermovement vertical jump (CMJ) height and 1 repetition maximum (1RM) on the bench press. Participants were then asked to recall their typical daily dietary intakes and

were asked to maintain similar caloric intakes throughout the course of the study (eg, make no major dietary changes, such as vast amounts of fast foods).

Participants were also asked to maintain their current resistance training splits and continue to resistance train a minimum of 3 d·wk⁻¹. All participants reported resistance training during the treatment period (per their usual routines) at least 3 d·wk⁻¹, per study requirements. Of the fifteen participants, about half ($n = 8$) reported resistance training 4 d·wk⁻¹ while the remaining participants ($n = 7$) reported resistance training 5 d·wk⁻¹ during the 7 d treatment period. All participants reported engaging in some form of a periodized, split-body training routine where each muscle major muscle group was only trained 1 d·wk⁻¹. No participant reported engaging in PA that was outside of their normal training routines.

Pre- and post-intervention body measures were taken on visits 2 and 3 in the BSU HPL (height (cm): standard anthropometry stadiometer; body weight (BW; kg) and body composition: whole body densitometry via air displacement (Bod Pod; COSMED USA, Concord, CA) for fat free mass (FFM; kg) and fat mass (FM; kg), followed by the respective upper and lower body muscular performance assessments. Completion of visit 2 took approximately 45 min to complete and initiated the 1 wk treatment period, as participants were distributed their 1 wk portions of the CrHCl treatment. Visit 3 was identical to visit 2 as participants repeated the performance and body composition assessments described above.

The study protocol is displayed below (Table 3.1):

Table 3.1 Study Protocol by Week

Time (wk)	Activity	Data
0	Recruitment	None
0-2	No supplements (Cr wash-out) Visit 1 (1 h): Pre-screening/Introduction 1RM bench press testing Maximal CMJ testing Protocol familiarization Assess “typical” daily diet intake	1RM bench press Maximal vertical jump height Typical daily diet intake
2	Visit 2 (45 min): Body measures (height, weight, body composition) Pre-treatment bench press test Pre-treatment lower body jump test Distribute CrHCl supplement	Major dietary changes (if any)? Other supplement consumption? Body measures Bench press repetitions Number of jumps completed
2-3	Daily supplement intake (pre-and post-workout)	None
3	Visit 3 (45 min): Body measures (height, weight, body composition) Post-treatment bench press test Post-treatment lower body jump test	Major dietary changes (if any)? Other supplement consumption? Body measures Bench press repetitions Number of jumps completed

Measures

Purity of the CrHCl Product

CrHCl product samples were tested and analyzed for purity using an Agilent 1100 Series High-Performance Liquid Chromatography (HPLC) Value System machine (Agilent Technologies, Waldbronn, Germany).¹³⁰ The Agilent 1100 HPLC machine allows for the separation, identification, and quantification of all components of a given mixture, with efficient mixing and pulse-free delivery of solvents in order to provide fast and accurate analytical results of a given sample.¹³⁰ 10 mg of CrHCl was placed in 10 mL

of Nanopure Water, which yielded a concentration of 6.7 mM. Solvents used in the HPLC included ammonium formate (aqueous) and acetonitrile (organic). Sample run time in the HPLC machine lasted 10 m, and progressed in this fashion:

- 0-3 min: Solution is 70% ammonium formate/30% acetonitrile;
- 3-8 min: solution is 30% ammonium formate/70% acetonitrile;
- 8-10 min: solution is 70% ammonium formate/30% acetonitrile.

1RM Bench Press

1RM on the bench press was initially estimated using the Epley Formula:

$$1RM = [1 + (0.0333 \times \text{repetitions completed})] \times \text{weight lifted.}^{131}$$

Participants estimated a weight for which they could perform ten repetitions on the barbell bench press. This weight was multiplied by 1.33 (per the Epley Formula), which yielded an approximate 1RM and a starting point for testing.¹³¹ Testing began with a progressive barbell bench press warmup, starting with 3-5 repetitions at a very light weight (50% of established, estimated 1RM), resting for 2 min, completing 2-4 repetitions at 70% of the estimated 1RM, resting for 4 min, completing 1-2 repetitions at 80-85% of the estimated 1RM, resting for 5 min, followed by testing the determined estimated 1RM weight. If the participant successfully completed the first 1RM attempt, they were instructed to rest for 4 min, then make another attempt after adding either 5-10 or 10-20 lbs, depending on the perceived difficulty of the previously completed 1RM, as has been found to be effective by Hoeger and colleagues for testing a true 1RM.¹³² This was continued until a true 1RM was established. The PI served as a spotter on all lifts to ensure participant safety while performing the barbell bench press, where proper form entailed feet maintaining contact with the floor and hips and head kept in contact with the

bench. If participants broke proper form at any point during a 1RM attempt, the PI racked the weight and ceased any further testing, and used the previous successfully completed 1RM attempt in the data set.

Maximal CMJ Height

Maximal CMJ height was established using a commercially-available, vertical jump measuring device (Vertec; Sports Import Inc., Columbus, OH). The participants' maximum standing reach heights (cm) were first be measured by the PI. Following, participants were guided through a warmup by performing 5 min of low-intensity cycling (60 revolutions·min⁻¹; 1.5 kp) on a stationary cycle ergometer (Monark Ergomedic 828 E; Monark Exercise AB, Vansbro, Sweden). Following, a brief dynamic warmup took place which consisted of ten BW squats and five CMJ performed at 50% maximal intensity. Then, three trials of the maximal CMJ were completed, with 1 min of rest between each trial. Participants initiated the jump with both feet on the ground and descended into a countermovement before jumping vertically and swiping the Vertec measurement device at the highest possible point with the middle finger of the dominant hand, and then landed safely with flat feet and their knees slightly bent. Each jump height was recorded to the nearest 0.5". Participants were allowed three maximal CMJ attempts. If a participant showed progression in jump height on each consecutive attempt, they were allowed more attempts until they failed to bypass the previous attempt. Maximal CMJ heights were calculated by subtracting the respective jump heights by the participants' maximum standing reach heights (cm).

Anthropometric Measures

To measure body height and weight, participants removed their shoes and only wore their shorts or under garments. Body heights were recorded using a stadiometer (Seca 213 Portable Stadiometer; Seca, Chino, CA). BW, FFM, and FM were measured using a Bod Pod as described by Antonio and colleagues in a similar study.¹³³ Both pre- and post-treatment body measures were assessed in this fashion.

Repeated Bench Press

Repetitions completed per set and total training volume (TTV; sets x repetitions) on three sets the submaximal (70% of participants' 1RM) barbell bench press were assessed before and after the 1 wk treatment period. Participants performed the barbell bench press exercise (three sets, maximal repetitions) at 70% of their previously determined 1RM, with 2 min of rest and recovery between each set. The bench press warmups for visits 2 and 3 were identical to that of visit 1 with the progressing percentages adjusted for the 70% submaximal weight. A metronome was utilized ($60 \text{ beats} \cdot \text{min}^{-1}$) to control repetition tempo and to ensure all participants performed the exercise in the same fashion on all trials. Participants performed the exercise until complete muscle failure or until proper form (previously described) was broken, as determined by the PI. This was repeated until all three sets were complete. The PI served as the spotter on all lifts to ensure participant safety while performing the barbell bench press.

Repeated CMJ

Repetitions completed per set and TTV were assessed on three sets of the repeated, submaximal (85% of participants' maximal vertical jump height) CMJ using the Vertec device before and after the 1 wk treatment period. Using previously determined maximal

CMJ height, participants completed as many consecutive submaximal CMJs as possible at 85% of their maximal CMJ height. The warmup for this test was identical to that of the maximal CMJ test during visit 1, described above. The Vertec device was set to a height where the top horizontal measuring-stick was at 85% of the participants' maximal CMJ heights. The participants were instructed to only slightly touch the Vertec horizontal measuring sticks on each successful submaximal jump attempt. Each jump was interspersed by 3 s (recorded by the PI) upon landing from the previous jump to allow proper reset of participants' original jumping position and for the PI to quickly reset the moved horizontal measuring stick to its set position, if necessary.¹³⁴ This was timed using a standard hand held digital stopwatch (Ultrak 495 Digital Stopwatch; CEI; Gardena, Ca). The PI was stationed on a latter placed next to the Vertec device to ensure participants successfully completed each submaximal CMJ and to allow for easy reset of the horizontal measuring stick after each attempt, if necessary. This continued until the participant could no longer jump at the desired height; the test was terminated after one unsuccessful repeated CMJ attempt. The number of successfully completed jumps at this height was recorded. Each set was separated by 2 min of rest/recovery. This took place for three trials before and after the 1 wk treatment period.

Data Analysis

Statistical analyses utilized the IBM Statistical Package for the Social Sciences (SPSS; IBM, Armonk, New York) software. Descriptive statistics (means and standard deviations) were calculated for age, height, BW, FFM, FM, 1RM bench press and maximal CMJ height. A series of one-way, repeated-measures ANOVAs were used to find any significant changes in body composition, bench press repetitions and number of

submaximal CMJs from pre- to post-treatment on a per-set and TTV basis. A significance level was set at $p < 0.05$.

CHAPTER FOUR: RESULTS

Sixteen participants were initially recruited for this investigation. One participant dropped out due to personal health issues. Fifteen healthy, resistance trained males completed the study, whose baseline characteristics are listed in Table 4.1.

Table 4.1 Participant Baseline Characteristics (n = 15).

	Minimum	Maximum	Mean	SD
Age (yr)	20	27	22.8	2.0
Height (cm)	168.0	191.0	178.5	5.8
Body Weight (kg)	70.1	105.4	81.6	9.9
1RM ^a Bench Press (kg)	65.9	255.0	120.2	42.4
Max. ^b Vertical Jump Height (cm)	51.2	87.6	70.1	9.8

^a One-repetition maximum

^b Maximum vertical jump height

Dietary Supplement and Nutrition Intake

At the end of visits 2 and 3 participants were asked to recall their dietary intakes for any significant caloric changes and other dietary supplement intakes (if any). No participant reported changes in dietary intake at any point during the study. Every participant reported consuming meat daily, and all reported consuming a balanced diet with most of their caloric intakes coming from CHO, followed by protein, then dietary fats. All participants reported adhering to the request to cease the consumption of alcohol 24 h prior to all testing sessions.

All participants adhered to the study guidelines and refrained from using any dietary supplements other than the CrHCl treatment during the study (eg, whey protein and caffeine). Of the fifteen participants, all but one reported no use of any dietary supplements prior to the start of the study. These participants bypassed the 2 wk Cr washout period and began their visit 1s upon their earliest convenience. The one participant who reported using a pre-workout dietary supplement (with Cr and other nutrients) reported adhering to the 2 wk Cr washout phase before reporting to the laboratory for visit 1. All fifteen participants reported adhering to the mixing and consumption protocols to ensure consistent absorption across participants. Further, all participants reported using the entirety of the distributed 1 wk supply of the CrHCl treatment and maintained proper consumption of the treatment during the periworkout period on training days and during the morning on rest-days, per study requirements. No participant reported stomach issues upon ingestion of the treatment.

CrHCl Purity

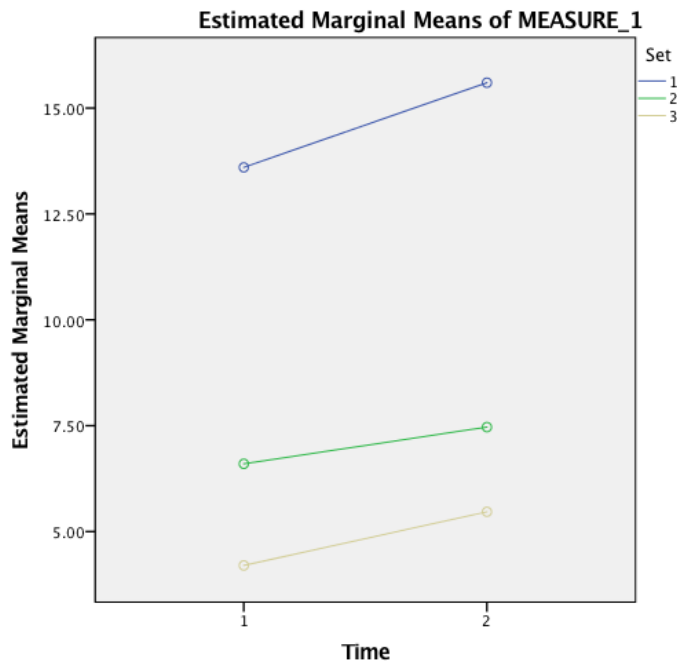
HPLC analysis revealed the CrHCl product to be 100% pure. Over a 10 min sample run time, there was a retention time of approximately 2 min and the absorption of the analyte was approximately 320 milli absorbance units (mAu) (Figure 4.1). Product purity of 100% assured that 1) participants were consuming a safe and pure product without any unknown substances and 2) any reported treatment effects, or lack of, could attributed to the CrHCl treatment with greater confidence.

Repeated Bench Press

There was a significant main effect of time on bench press performance score, indicating that from pre- to post-intervention, participants' bench press performances

improved across all three sets (Figure 4.1: $F(1, 14) = 106.4, p < 0.005, \eta^2 = 0.88$).

Further, a significant main effect for set on bench press performance was found ($F(2, 28) = 268.3, p < 0.005, \eta^2 = 0.95$). Post-hoc Bonferroni analysis indicated that participants performed significantly better at later sets compared to earlier sets (eg, Set 3 > Set 2 > Set 1, $p < 0.005$). A significant interaction between time and set on bench press was found ($F(2, 28) = 4.15, p = 0.026, \eta^2 = 0.23$), which indicates relatively larger improvement in bench press from pre- to post-intervention in Set 1 (Cohen's $d = 0.71$) and Set 3 ($d = 0.65$), but smaller improvement for Set 2 ($d = 0.39$). This suggests that from pre- to post-intervention, fatigue was better overcome when participants' upper-body muscles were unfatigued (Set 1) and at the end of the assessment, when their muscles were highly fatigued (Set 3). Bench press data are presented in Table 4.2.



Note: $p < 0.005$ for all sets.

Figure 4.1 Effect for Time: Repeated Bench Press (Repetitions)

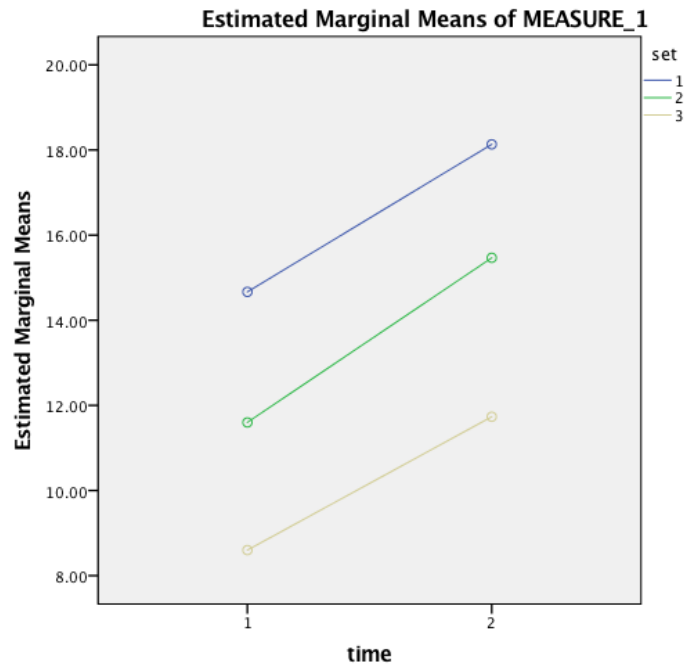
Table 4.2 Participant Repeated Bench Press Characteristics (repetitions).

	Pre-Intervention		Post-Intervention	
	Mean	SD	Mean	SD
Set 1	13.6	2.6	15.6*	3.0
Set 2	6.6	2.3	7.5*	2.2
Set 3	4.2	1.8	5.5*	2.2

* $p < 0.005$

Repeated CMJ

There was a significant effect for time on CMJ performance score, such that from pre- to post-intervention, participants' CMJ performances increased on all three sets (Figure 4.2: $F(1, 14) = 55.61, p < 0.005, \eta^2 = 0.80$). Additionally, a significant main effect for set was found ($F(2, 28) = 27.5, p < 0.005, \eta^2 = 0.66$). Post-hoc Bonferroni results signified that participants performed significantly better at later sets compared to earlier sets (eg, Set 3 > Set 2 > Set 1, $p < 0.005$). No significant interaction effect ($F(2, 28) = 0.96, p > 0.05, \eta^2 = 0.06$) was observed between time and set on the CMJ test, indicating that there were no significant improvements in CMJ from pre- to post-intervention on any set (Set 1, $d = 0.41$; Set 2, $d = 0.59$; Set 3, $d = 0.63$). Data for the CMJ test are presented in Table 4.3.



Note: $p < 0.005$ for all sets.

Figure 4.2 Effect for Time: Repeated CMJ (Repetitions)

Table 4.3 Participant Repeated CMJ Characteristics (repetitions).

	Pre-Intervention		Post-Intervention	
	Mean	SD	Mean	SD
Set 1	14.7	7.9	18.1*	8.3
Set 2	11.6	5.8	15.5*	7.2
Set 3	8.6	4.7	11.7*	5.0

* $p < 0.005$

Body Measures

There were no statistically significant changes in FFM ($F(1, 14) = 3.06, p > 0.05, \eta^2 = 0.179$) or FM ($F(1, 14) = 0.423, p > 0.05, \eta^2 = 0.029$) from pre- to post-treatment.

Notably, however, there was a significant increase in BW from pre- to post-treatment ($F(1, 14) = 6.00, p < 0.05, \eta^2 = 0.30$). Data for all body composition outcomes are presented in Table 4.4.

Table 4.4 Participant Body Composition Characteristics (kg).

	Pre-Intervention		Post-Intervention	
	Mean	SD	Mean	SD
BW ^a	81.6	9.9	82.2*	9.6
FFM ^b	70.4	8.0	71.3	7.9
FM ^c	11.3	3.1	10.9	3.8

* $p < 0.05$

^a Body weight

^b Fat-free mass

^c Fat mass

CHAPTER FIVE: DISCUSSION

Consuming CrH₂O as a dietary supplement with the intent of enhancing anaerobic muscular performance and body composition (eg, increasing FFM, decreasing FM) has shown consistent efficacy in the literature. Companies are producing alternative forms of Cr, such as CrHCl, to offset the various anecdotal reports of biological distress associated with consuming the relatively large amounts of CrH₂O required to reap the ergogenic benefits. However, literature examining CrHCl in a human model is lacking and its efficacy remains unclear in augmenting muscular performance and favorable adaptations in body composition. Identifying the effect of oral CrHCl supplementation on submaximal, intermittent bouts of resistance exercise will help establish its effectiveness as a dietary supplement and will allow consumers to make educated decisions when purchasing Cr products.

In this study, measurements of completed repetitions on three sets of the barbell bench press and CMJ exercises were taken pre- and post-intervention in human participants. The results from the present study suggest that consuming 4 g CrHCl·d⁻¹ in the periworkout period for 7 d is beneficial for increasing repetitions on repeated bouts of submaximal bench press and CMJ exercises when taken to complete muscle failure. This supports the hypothesis that 7 d of CrHCl supplementation (4 g·d⁻¹) taken in the periworkout period will increase repetitions on three repeated sets of the barbell bench press and CMJ exercises. Though participants were all previously well-versed in both performance assessments prior to the start of the study and practiced both tests during

their Visit 1, it is important to acknowledge that learning effects or self-competition (trying to improve upon their baseline performances) may have contributed to these results. However, previous research examining repeated bouts of high intensity resistance exercise noted that learning effects from pre- to post-intervention are unlikely due to participants' previous resistance training experience (≥ 1 y).¹³⁵ Thus, the lack of a control group in the present study likely was not an issue. Moreover, similar findings have been reported in the literature examining CrH₂O supplementation and its effects on repeated bouts of submaximal exercise.^{23,136}

A similar experiment investigated CrH₂O supplementation for 7 d (25 g·d⁻¹) and its effects on five intermittent bouts of submaximal bench press (10 RM, failure) and jump squat exercises (30% 1RM barbell back squat, ten repetitions), with each trial separated by 2 min rest.²³ Peak power output (W) on all five sets of the jump squats and total training volume (TTV) on all five sets of the bench press were significantly enhanced ($p < 0.05$), with the highest numbers recorded on the first sets and progressively decreasing on the four subsequent sets.²³ Though the experimental design (maximal repetitions at 85% maximal CMJ height) and performance measure (maximal repetitions) for the repeated jumps test was different in the present study, similar results were produced. The significant effect for time on repeated CMJ performance suggested a significant increase in completed repetitions across all three sets from pre- to post-intervention. Thus, participants in the current study were also able to prolong the production of lower body peak power output and maintain their submaximal CMJ performances during post-intervention testing, as performance increased concurrently with increases in body mass. Bench press results in the present study were also similar, in

that repetitions across sets were significantly enhanced from pre- to post-intervention. These results are likely attributed to more energy being derived from the enhanced phosphagen pool from the CrHCl treatment and a delayed transition into anaerobic glycolysis.^{44,46}

An investigation of acute CrH₂O supplementation (20 g·d⁻¹, 5 d) on maximal repeated vertical jumping performance (45 s) reported significant ($p < 0.05$) enhancement in average jump height during the first 15 s (+ 7%) and 30 s (+ 12%), but not the final 15 s, when the energy-shift was likely being made to anaerobic glycolysis and aerobic metabolism.¹³⁶ These results are similar to those in the present study where participants were likely able to produce greater muscular power as time and fatigue increased and continue reaching their 85% maximal CMJ heights, as evidenced in the post-intervention CMJ performance results, described above. In slightly longer CrH₂O interventions (14 d), three pre-/post-trials of the 30 s maximal Wingate test were significantly enhanced (+13%, +18%, +18%, respectively, $p < 0.05$) as quantified by total anaerobic work and for submaximal bench press testing (28 d; 70% 1RM, one trial) for maximal repetitions (+26%, $p < 0.05$).⁴⁷ Similarly, the present study saw upper and lower body increases in total anaerobic work capacity as seen by the increases in repetitions for the submaximal repeated CMJ and bench press exercises following the Cr treatment. However, the study at hand shows that similar performance results can be achieved in only 7 d.

Results associating CrH₂O supplementation with prolonged muscular power production during submaximal, intermittent bouts of exercise may be attributed to an increased quantity of PCr resynthesis due to increases in intramuscular Cr during the prescribed rest/recovery periods, and ultimately, an elevated PCr pool at the initiation of

each successive bout of exercise.²⁵ To maintain elevated power output during repeated maximal jumps, for example, requires constant ATP recycling via PCr hydrolysis at the myosin cross-bridge sites to maintain ideal myosin-head cycling with active sites located on the actin filament and elevated intramuscular PCr would enhance this.^{23,60} Brief periods of rest/recovery (eg, 2-5 min) allow for rapid PCr resynthesis which can be approximately 95% restored in 3-4 min.⁹ Thus, repeated, intermittent performances on the submaximal bench press and CMJ exercises can be expected to be improved by Cr supplementation as demonstrated in this study.

Total intramuscular TCr seems to play a role in performance.^{19,25} Individuals with lower basal TCr concentrations (eg, non-supplemented individuals or vegetarians) experience more benefit as they tend to have a greater relative and absolute increase in TCr pools upon supplementation (+ ~20%) and the extent to which intramuscular TCr pools increase is related to the enhancement in muscular performance.³⁰ With TCr retention so important for reaping ergogenic benefits from Cr supplementation, those consuming Cr would ideally increase their intramuscular TCr pools from the average 120-140 mmol·kg⁻¹ (DM) to the known upper limit of 150-155 mmol·kg⁻¹ (DM).⁶⁰

However, publications examining CrHCl supplementation and retention have yet to be conducted in a human model, making it unclear as to the appropriate dose and timing needed to properly increase intramuscular TCr levels. This may explain why there have been disparate results in the literature across the few available CrHCl training studies. Possible discrepancies in the literature may be attributed to factors, such as varied dosing of the CrHCl treatments, length of the treatment periods, or testing protocols not fully utilizing the phosphagen energy system.^{26,30,60} One study that

examined the 4 wk effects of a high dose ($5 \text{ g}\cdot\text{d}^{-1}$) or low dose ($1.5 \text{ g}\cdot\text{d}^{-1}$) of CrHCl versus $5 \text{ g}\cdot\text{d}^{-1}$ CrH₂O and a PI group reported increases in 1RM leg press in all groups but only 1RM bench press in the low CrHCl group.⁷⁵ Participants in this study were asked to refrain from all PA 4 wk prior to the start of the study which may have played a role in the reported strength increases. Another study had participants consume a single dose ($0.033 \text{ g}\cdot\text{kg}^{-1}$) of CrHCl, 30 min prior to pre- and post-testing on five repeated sets of the bench press and leg press with 75 s rest between each set.¹²⁸ It was found that TTV for men and women in the leg press significantly improved (men: + 12.9%, $p = 0.03$; women; + 32.7%, $p = 0.004$) for all groups including the PI while TTV on the bench press showed no significant improvements in any group.¹²⁸ This is likely due to only a single dose of CrHCl not being enough to maximize TCr stores in the muscle cells. In addition, participants were asked to refrain from exercise between the control and experimental trials although exercised muscle tissue better takes up and stores circulating Cr than unexercised muscle tissue. It is also possible that the 75 s rest periods were not sufficient enough for PCr resynthesis between exercise bouts.⁶⁰ A third investigation looked at 6 d CrHCl supplementation ($750 \text{ mg}\cdot\text{d}^{-1}$, twice daily) on 1RM performances on the bench press, back squat, and deadlift exercises and found no significant changes post-treatment.¹²⁹ Because 1RM attempts do not rely on phosphate sources other than already available ATP, increased intramuscular TCr through supplementation without resistance-training for only 6 d would not likely instigate the necessary training effects necessary to augment significant gains in maximal muscular strength.⁶⁰

In an attempt to build on the aforementioned literature and eliminate discrepancies in results, the present study took each potential flaw in the aforesaid

research designs and addressed them accordingly. First, participants were asked to maintain their usual resistance training regimens (minimum 3 d·wk⁻¹) before and throughout the Cr intervention period. This would help eliminate performance improvements being attributed to unexercised muscle tissue undergoing a resistance training *and* Cr intervention, as opposed to previously resistance trained muscle tissue continuing to resistance train while undergoing a Cr intervention. Further, a single dose of Cr taken 30 min prior to a maximal, weight-bearing performance assessment would not likely elevate intramuscular TCr levels enough to provide a difference in performance, especially when asked to refrain from resistance training leading up to the assessment. Thus, the present study had participants resistance train while supplementing with 4 g of CrHCl in the periworkout period around their respective resistance training workouts, over a 7 d span, which would likely better increase their skeletal muscle TCr stores and improve their intermittent, submaximal resistance training performances. Additionally, rest periods under 2 min, as discussed previously, are not sufficient enough to allow sufficient PCr resynthesis following a trial of maximal-effort PA.⁹ Therefore, the study at hand utilized 2 min rest periods between testing trials which would allow sufficient PCr resynthesis by utilizing ATP from oxidative metabolism and the anaerobic metabolism of hexose monophosphates (accumulated during intense exercise) to pyruvate before the successive testing trials.⁹ Lastly, studies testing pre- and post-1RM after an acute Cr treatment period should not expect to see significant improvements, as such lifts tend to use energy from readily available phosphates, namely ATP.³⁰ Thus, the current study designed a performance protocol that utilized exercises that rely primarily on the ATP-PCr energy system and would more likely benefit from Cr supplementation.

Regardless, there are still some limitations in the design of this study, which will be discussed at the end of this section.

In this investigation, FFM and FM saw no statistically significant changes from pre- to post-intervention ($p > 0.05$ for both). However, BW was significantly increased from pre- to post-intervention ($F(1, 14) = 6.00, p < 0.05, \eta^2 = 0.30$). Mean bodyweight in the present study increased approximately 0.6 kg. Many CrH₂O studies with acute treatment periods (6-14 d) have reported similar increases in body mass, ranging from 0.5-2.0 kg.^{23,25,44,47,61,80,81,114,137} As indicated by decreases in urinary volume upon Cr supplementation, this may be mostly attributed to increased retention of body water.¹¹⁹ This may be due to the fact that increased Cr content in the muscle cell increases osmotic pressure, which moves H₂O into the cell and retains it, leading to weight gain.⁷⁰ It is important to note that no participants in the present study reported any biologic distress during the intervention period. Further, consuming CrH₂O in the periworkout period has been found to increase FFM while decreasing FM, which may help further influence changes in body composition.¹³³ Thus, the study at hand had participants split their daily CrHCl doses pre- and post-workout on days where they resistance trained.

These results suggest that supplementing with 4 g of CrHCl over a 7 d period may benefit those who seek to improve muscular performances during intermittent, submaximal PAs and avoid possible biologic distress associated with CrH₂O supplementation at such high doses. Thus, supplementing with CrH₂O is approximately \$0.29 cheaper per day to supplement with compared to CrHCl. This may have more specific implications for those other than just healthy men who resistance train. For instance, the National Football League Scouting Combine utilizes a 225 lb (~102.3 kg)

bench press test (maximal repetitions to fatigue) to assess upper body muscular strength and endurance. A potential recruit may be 1 wk out from the Scouting Combine and still need to improve on the number of repetitions he can perform during the submaximal bench press test. With this knowledge, his trainer may have him supplement with 4 g of $\text{CrHCl} \cdot \text{d}^{-1}$ around his workouts, for 7 d, with the intent of increasing his intramuscular TCr stores, and thus utilizing more phosphate energy sources before transitioning to anaerobic glycolysis. This would allow for more completed repetitions on the submaximal bench press test.

Additionally, players who participate in sports that require repeated bouts of maximal vertical jumping, such as volleyball, may benefit from these findings. The ability to sustain near-maximal CMJ height may allow players to compete at higher levels through the duration a given match. Coaches can therefore utilize these results and have their players practice and resistance train their lower extremities while supplementing with 4 g of $\text{CrHCl} \cdot \text{d}^{-1}$ during the week before a match. With an increased TCr pool in the lower extremities, the athletes should expect to maintain submaximal CMJ performances for longer periods of time during a given match.

Regardless of the sport, to maximize the benefits of CrHCl supplementation, the PA one wishes to improve upon should stress the phosphagen energy system and should have brief periods of rest/recovery between bouts, ranging from 2-5 min. This has proven to be an effective alternative to supplementing with high doses of CrH_2O .

However, a 500 g bottle of CrH_2O has a retail cost of \$14.99 (USD; $20 \text{ g} \cdot \text{d}^{-1} = \$0.60 \cdot \text{d}^{-1}$) while a 135 g bottle of CrHCl sells at retail for approximately \$30.00 (USD; $4 \text{ g} \cdot \text{d}^{-1} = \$0.89 \cdot \text{d}^{-1}$).⁸⁶

Limitations and Future Research

There were some limitations in the present study. One major limitation of the current study was the small sample size. Data from 15 participants is not enough to generalize the results to larger populations outside of a cohort of healthy, resistance trained men. Additionally, the brief, 7 d treatment period may not have been sufficient for some individuals as they may respond slower to Cr supplementation (delayed uptake of intramuscular TCr) or may not respond at all (Cr treatment has no influence on intramuscular TCr content) and this was not assessed in this study.²⁶ Further, muscle biopsy techniques or tracer methodologies were not used to quantify intramuscular TCr content pre- and post-intervention, so it cannot be concluded that the improvements in performance were a direct result of the Cr treatment. Moreover, although all participants reported consuming their normal diets throughout the intervention, only general diet information was collected and specific caloric intakes, especially from meat, were not tracked throughout the intervention. Differences in energy intake, especially from Cr-dense meat, could have further increased participants' intramuscular TCr stores and influenced performance results. Lastly, this was an open trial as participants were not blinded to the CrHCl treatment, which may be a cause for bias.

Follow up studies should longitudinally investigate CrHCl supplementation in resistance trained men and women with a greater sample size and investigate the long-term effects on performance and body composition. Further, CrHCl supplementation among older populations of both sexes should be examined for TCr retention, body composition and anaerobic performance outcomes to further assess its effects on other populations. Moreover, basal and post-intervention intramuscular TCr content should be

measured via biopsy techniques or tracer methodologies to measure the retention effects of CrHCl in comparison to CrH₂O, and assess the correlation between retention level and performance changes. Caloric intake, especially meat intake, should be controlled to ensure the consumption of meat products remains consistent throughout the treatment period. Lastly, participants should be blinded to the treatment and the CrHCl group should be compared against a CrH₂O group and a Pl group to avoid potential bias.

Conclusion

In conclusion, supplementation with 4 g of CrHCl for 7 d in healthy, resistance-trained men significantly increases the number of repetitions performed during intermittent bouts of submaximal bench press and CMJ exercises and body weight.

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APPENDIX A

Informed Consent Form

Study Title: Oral Creatine Hydrochloride Supplementation: Acute Effects on Submaximal, Intermittent Bouts of Bench Press and Vertical Jump Exercises

Principal Investigator:

DJ McDonough

Co-Investigator:

Dr. Shawn Simonson

Sponsor: N/A

This consent form will give you the information you will need to understand why this research study is being done and why you are being invited to participate. It will also describe what you will need to do to participate as well as any known risks, inconveniences or discomforts that you may have while participating. We encourage you to ask questions at any time. If you decide to participate, you will be asked to sign this form and it will be a record of your agreement to participate. You will be given a copy of this form to keep.

➤ **PURPOSE AND BACKGROUND**

The purpose of this research is to examine the effects of 1-week of oral creatine hydrochloride supplementation on three sets of the free weight barbell bench press exercise at 70% of your 1 repetition maximum and repeated submaximal countermovement vertical jump exercise at 85% of your maximal vertical jump height. This will provide us with further information about the potential athletic-enhancing effects of supplementing with a newer form of creatine for 1-week. To participate in this study, you must be a non-vegetarian male between the ages of 18 and 34 years, in good physical health (no diagnosed cardiovascular, pulmonary, metabolic, joint, or chronic disease), and have a minimum of 1-year of resistance-training experience and currently resistance-train a minimum of 3-times per week. Further, you must agree to cease the use of all dietary supplements for 3-weeks, during the study.

➤ **PROCEDURES**

You will be asked to come to the Boise State University Kinesiology Annex Building on 3 occasions.

Visit 1 – Paperwork, body composition measurement, and familiarization trial (2 hrs)

During your first visit, you will complete all paperwork, have your body height and composition (% body fat, fat free mass, and fat mass) measured, and complete a practice trial of the protocol procedures that will take place during visits 2 and 3. Body composition will be measured with a system that measures the amount of air your body takes up within a chamber. This is an egg-shaped structure that uses changes in air pressure and your body size to determine your total body composition breakdown.

You will be asked to wear a tight-fitting bathing suit or lycra/spandex shorts with a swim cap covering the hair to reduce air blockage. You will enter the system and sit for

approximately one minute. You will breathe regularly and remain motionless during the testing procedure. A large window is centered in the front of the system so you may see out into the laboratory and may communicate with an investigator if necessary. An emergency release button is located inside the system should you need to terminate the test for any reason.

Next, you will be asked to provide a dietary recall of your typical daily food consumption. This will be used to assess any major dietary changes that may take place during the study. Following, you will be asked to complete practice trials on the free weight bench press and repeated countermovement vertical jumping exercises. This will be identical in nature to the exercise sessions completed during visits 2 and 3. Following, you will be asked to complete testing for your 1 repetition maximum on the free weight bench press exercise, followed by maximal countermovement vertical jump height testing using the Vertec, which is a vertically adjustable height-measuring device. Before leaving, you will be distributed a 1-week (28 grams (fourteen 2 gram packets): 7-days, 4 grams per day) supply of the creatine hydrochloride treatment. You will be asked to take one 1-serving packet (2 grams) of the creatine supplement 30 minutes before your resistance training workout and one 1-serving packet (2 grams) immediately after your resistance training workout. On days where you do not resistance train (rest-days), you will be asked to take two 2-serving packets (4 grams) in the morning at the same time on every rest-day. Each time you consume the creatine supplement, you will be asked to take it in 300mL of semi-cold water, and consume within 5-minutes after mixing.

Visit 2 and 3 – Experimental trials (1.5 hrs)

During visits 2 and 3 you will assess all body measures in the same fashion as visit 1. Following, you will complete a progressive bench press warmup until working up to your 70% 1 repetition maximum bench press, established during visit 1. Following, you will complete three trials of the bench press at this weight identical to the practice trials during visit 1. Following, you will complete a low-intensity, 5-minute warmup on the stationary cycle ergometer before completing 3 trials of the repeated vertical jumping test, identical to the practice trial during visit 1.

➤ RISKS

The potential risks that may occur with participating in this study include those associated with any exercise. These include muscle/joint soreness, lightheadedness, nausea, and in rare instances, fainting, and heart attack. However, the possibility of serious events happening in people who have no previous history of heart disease is low. The Human Performance Laboratory has a planned response to any emergency procedure and all testing personnel are CPR certified. Consuming high doses of creatine has been associated with some anecdotes of gastrointestinal distress. However, the creatine hydrochloride supplement you will be asked to take will be consumed at a fraction of the dose as typically used in research studies.

BENEFITS

There will be no direct benefit to you from participating in this study. However, the results of this study may provide information as to which form of commercially available creatine, sold as a dietary supplement, is superior for acute ergogenic benefits.

EXTENT OF CONFIDENTIALITY

Reasonable efforts will be made to keep the personal information in your research record private and confidential. Any identifiable information obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by law. The members of the research team and the Boise State University Office of Research Compliance (ORC) may access the data. The ORC monitors research studies to protect the rights and welfare of research participants.

Your name will not be used in any written reports or publications which result from this research. Data will be kept for three years (per federal regulations) after the study is complete and then destroyed.

➤ **PAYMENT**

You will not be paid for your participation in this study. You will receive 1 free, unopened bottle of creatine upon full completion of the study.

➤ **PARTICIPATION IS VOLUNTARY**

You do not have to be in this study if you do not want to. You may also refuse to answer any questions you do not want to answer. If you volunteer to be in this study, you may withdraw from it at any time without consequences of any kind or loss of benefits to which you are otherwise entitled.

➤ **QUESTIONS**

If you have any questions or concerns about your participation in this study, you may contact the Principal Investigator, Daniel McDonough: 650-580-6253 or djmcdonough@u.boisestate.edu.

If you have questions about your rights as a research participant, you may contact the Boise State University Institutional Review Board (IRB), which is concerned with the protection of volunteers in research projects. You may reach the board office between 8:00 AM and 5:00 PM, Monday through Friday, by calling (208) 426-5401 or by writing: Institutional Review Board, Office of Research Compliance, Boise State University, 1910 University Dr., Boise, ID 83725-1138.

DOCUMENTATION OF CONSENT

I have read this form and decided that I will participate in the project described above. Its general purposes, the particulars of involvement and possible risks have been explained to my satisfaction. I understand I can withdraw at any time.

Printed Name of Study
Participant

Signature of Study
Participant

Date

Signature of Person Obtaining Consent

Date

APPENDIX B

Physical Activity Readiness Questionnaire (PAR-Q)

2017 PAR-Q+






The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS




Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition <input type="checkbox"/> OR high blood pressure <input type="checkbox"/> ?	<input type="checkbox"/>	<input type="checkbox"/>
2) Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	<input type="checkbox"/>	<input type="checkbox"/>
3) Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	<input type="checkbox"/>	<input type="checkbox"/>
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? PLEASE LIST CONDITION(S) HERE: _____	<input type="checkbox"/>	<input type="checkbox"/>
5) Are you currently taking prescribed medications for a chronic medical condition? PLEASE LIST CONDITION(S) AND MEDICATIONS HERE: _____	<input type="checkbox"/>	<input type="checkbox"/>
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer NO if you had a problem in the past, but it <i>does not limit your current ability</i> to be physically active. PLEASE LIST CONDITION(S) HERE: _____	<input type="checkbox"/>	<input type="checkbox"/>
7) Has your doctor ever said that you should only do medically supervised physical activity?	<input type="checkbox"/>	<input type="checkbox"/>

 **If you answered NO to all of the questions above, you are cleared for physical activity. Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.**

-  Start becoming much more physically active – start slowly and build up gradually.
-  Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
-  You may take part in a health and fitness appraisal.
-  If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
-  If you have any further questions, contact a qualified exercise professional.

 **If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.**

 **Delay becoming more active if:**

-  You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
-  You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
-  Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.



2017 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

- 1. Do you have Arthritis, Osteoporosis, or Back Problems?**
If the above condition(s) is/are present, answer questions 1a-1c If **NO** go to question 2
- 1a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 1b. Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)? YES NO
- 1c. Have you had steroid injections or taken steroid tablets regularly for more than 3 months? YES NO
-
- 2. Do you currently have Cancer of any kind?**
If the above condition(s) is/are present, answer questions 2a-2b If **NO** go to question 3
- 2a. Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck? YES NO
- 2b. Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)? YES NO
-
- 3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm**
If the above condition(s) is/are present, answer questions 3a-3d If **NO** go to question 4
- 3a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 3b. Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction) YES NO
- 3c. Do you have chronic heart failure? YES NO
- 3d. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months? YES NO
-
- 4. Do you have High Blood Pressure?**
If the above condition(s) is/are present, answer questions 4a-4b If **NO** go to question 5
- 4a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 4b. Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure) YES NO
-
- 5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes**
If the above condition(s) is/are present, answer questions 5a-5e If **NO** go to question 6
- 5a. Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies? YES NO
- 5b. Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness. YES NO
- 5c. Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet? YES NO
- 5d. Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)? YES NO
- 5e. Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future? YES NO

2017 PAR-Q+






6. **Do you have any Mental Health Problems or Learning Difficulties?** *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*
If the above condition(s) is/are present, answer questions 6a-6b If **NO** go to question 7
- 6a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 6b. Do you have Down Syndrome **AND** back problems affecting nerves or muscles? YES NO
-
7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*
If the above condition(s) is/are present, answer questions 7a-7d If **NO** go to question 8
- 7a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 7b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy? YES NO
- 7c. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week? YES NO
- 7d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs? YES NO
-
8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*
If the above condition(s) is/are present, answer questions 8a-8c If **NO** go to question 9
- 8a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 8b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting? YES NO
- 8c. Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)? YES NO
-
9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*
If the above condition(s) is/are present, answer questions 9a-9c If **NO** go to question 10
- 9a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES NO
- 9b. Do you have any impairment in walking or mobility? YES NO
- 9c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months? YES NO
-
10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**
If you have other medical conditions, answer questions 10a-10c If **NO** read the Page 4 recommendations
- 10a. Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months? YES NO
- 10b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)? YES NO
- 10c. Do you currently live with two or more medical conditions? YES NO


PLEASE LIST YOUR MEDICAL CONDITION(S)
AND ANY RELATED MEDICATIONS HERE: _____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.



2017 PAR-Q+

-  **If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:**
-  It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
 -  You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
 -  As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
 -  If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

-  **If you answered YES to one or more of the follow-up questions about your medical condition:**
You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the ePARmed-X+ at www.eparmedx.com and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

-  **Delay becoming more active if:**
-  You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
 -  You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
 -  Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

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The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity
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The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.



APPENDIX C

Institutional Review Board Protocol

This research was conducted under approval from the Institutional Review Board at Boise State University, protocol #103-MED-003.