Effect of oral creatine supplementation on upper extremity anaerobic response in females
by Karyn Hamilton Ward

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Health and Human Development
Montana State University

Abstract:
Most oral creatine monohydrate (CrH2O) research has been focused on lower body strength and power in males. The purpose of this study was to investigate the influence of standard doses of oral CrH2O on muscular performance during elbow flexion (EF) and shoulder internal rotation (IR) in females. Following written informed consent, 24 females involved in overhand sports were pair-matched on lean body mass, % body fat, height, weight (Wt), and age, and assigned to either placebo (P) (n=13) or CrH2O (n=11) groups in a randomized, double-blind fashion. Peak concentric (CON) and eccentric (ECC) isokinetic (IK) torque, isotonic (IT) one repetition maximum (1RM), and muscular fatigue (FAT) during EF, and IT 1RM, FAT, and peak velocity (V) during IR were evaluated. Following habituation, subjects consumed either P or 25 g CrH2O/day for a seven day dietary phase. All subjects were weighed and tested before and after the dietary phase. Repeated measures MANOVAs revealed a significant interaction between treatment and trial for EF (F 419=5.30, p=0.005) but not for IR (F319=0.06, p=0.98) or Wt (F123=0.66, p=0.43). Post hoc univariate analysis indi-cated a significantly (p=0.006) greater change in EF Fat following CrH2O than following P. Results suggest oral CrH2O did not influence peak EF or IR strength, IR work to fatigue, or peak IR velocity in strength-trained, female athletes. Therefore, CrH2O supplementation may be of benefit for enhancing strength conditioning but not performance of overhand sports.

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Karyn Hamilton Ward

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Michael C. Meyers, PhD, MS, FACSM Chairperson, Graduate Committee	M C. Meyers (Signature)	11-30-96 Date
William A., Skelly, PhD Co-chair, Graduate Committee	W A Skelly (Signature)	12-2-96 Date
Robert J. Marley, PhD Graduate Committee Member	Robert J. Marley (Signature)	12-2-96 Date

Approved for the Department of Health and Human Development

Ellen Kreighbaum, PhD Department Head	Ellen Kreighbaum (Signature)	12/2/96 Date

Approved for the College of Graduate Studies

Robert Brown, PhD Interim Graduate Dean	R L Brown (Signature)	12/10/96 Date

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ABSTRACT

Most oral creatine monohydrate (CrH_2O) research has been focused on lower body strength and power in males. The purpose of this study was to investigate the influence of standard doses of oral CrH_2O on muscular performance during elbow flexion (EF) and shoulder internal rotation (IR) in females. Following written informed consent, 24 females involved in overhand sports were pair-matched on lean body mass, % body fat, height, weight (Wt), and age, and assigned to either placebo (P) (n=13) or CrH_2O (n=11) groups in a randomized, double-blind fashion. Peak concentric (CON) and eccentric (ECC) isokinetic (IK) torque, isotonic (IT) one repetition maximum (1RM), and muscular fatigue (FAT) during EF, and IT 1RM, FAT, and peak velocity (V) during IR were evaluated. Following habituation, subjects consumed either P or 25 g CrH_2O/day for a seven day dietary phase. All subjects were weighed and tested before and after the dietary phase. Repeated measures MANOVAs revealed a significant interaction between treatment and trial for EF ($\underline{F}_{4,19}$=5.30, $\underline{p}$=0.005) but not for IR ($\underline{F}_{3,19}$=0.06, $\underline{p}$=0.98) or Wt ($\underline{F}_{1,23}$=0.66, $\underline{p}$=0.43). Post hoc univariate analysis indi-cated a significantly ($\underline{p}$=0.006) greater change in EF_{FAT} following CrH_2O than following P. Results suggest oral CrH_2O did not influence peak EF or IR strength, IR work to fatigue, or peak IR velocity in strength-trained, female athletes. Therefore, CrH_2O supplementation may be of benefit for enhancing strength conditioning but not performance of overhand sports.

CHAPTER I

INTRODUCTION

The use of an ergogenic substance refers to the application of a pharmacological, nutritional, physiological, psychological, or mechanical aid which is perceived to enhance athletic performance by improving strength, speed, endurance, or recovery of an athlete (McArdle, Katch, & Katch, 1991). Ergogenic aids may exert their effects by directly acting on muscle tissue, supplying fuel necessary for muscle contraction, or minimizing effects of metabolic by-products. Enhancing cardiovascular and circulatory response and stimulating the central nervous system are two additional routes through which ergogenics may act (Berglund & Hemmingsson, 1982; Chandler & Blair, 1980; Fox, Bowers, & Foss, 1993).

Enhancement of athletic performance with the use of ergogenic aids is a topic of international controversy. The use of anabolic steroids, amphetamines, and blood doping, for example, is prohibited by national and international sports medicine groups and governing bodies including the American College of Sports Medicine, the International Olympic Committee, the United States Olympic Committee, and the American Medical Association (American College of Sports Medicine, 1987a; American College of Sports Medicine, 1987b; United States Olympic Committee, 1989). However, the application of other ergogenics, such as training, dietary manipulation, nutritional supplements, and high performance equipment, is deemed acceptable at this time.

Athletes striving for a "competitive edge" constitute a vulnerable population typically targeted for ergogenic sales (Karpovich, 1971; McArdle et al., 1991). With a growing number of people participating in recreational and amateur sports, production and sales of ergogenic aids has become a lucrative industry with a steady increase in the number and variety of new products marketed annually. One such product is creatine monohydrate (CrH_2O). When taken orally during training, CrH_2O is purported to enhance fuel supply for working muscles, ultimately resulting in improved strength, endurance, and fat-free mass (Balsom, Ekblom, Soderlund, Sjodin, & Hultman, 1993a; Bleue & Goodman, 1995; Greenhaff et al., 1993b; Lemon et al., 1995).

The majority of research conducted on the efficacy of CrH_2O, though somewhat equivocal, includes compelling findings with regard to improved lower extremity strength and anaerobic capacity primarily in males of varying fitness classifications (Balsom et al., 1993a; Bleue & Goodman, 1995; Greenhaff et al., 1993b; Lemon et al., 1995). However, information on the effects of CrH_2O on upper body strength and anaerobic power is limited. In addition, since the majority of research has included primarily male subjects, little is known concerning the physiological response of females following CrH_2O supplementation. Therefore, the purpose of this study was to quantify the effects of CrH_2O supplementation on the upper extremity anaerobic response of females. The results of this study should be of value to female athletes who are using or considering the use of oral creatine supplements for the purpose of enhancing upper body strength. Exercise scientists, coaches, and athletic trainers should also benefit through a greater knowledge of nutritional supplementation when counseling athletes.

Statement of the Problem

The purpose of this study was to quantify the effects of oral creatine monohydrate on upper extremity isokinetic and isotonic strength and velocity in females.

Research Objectives

The investigation attempted to fulfill the following objectives:

1. Quantify the efficacy of oral creatine supplementation on upper extremity isotonic peak torque and velocity in females.

2. Quantify the efficacy of oral creatine supplementation on upper extremity isokinetic peak torque in females.

Hypotheses

The following null hypotheses were tested:

1. Oral creatine supplementation will have no effect on upper extremity isotonic peak torque and velocity in females.
2. Oral creatine supplementation will have no effect on upper extremity isokinetic peak torque in females.

Assumptions

1. Subjects were adequately informed and trained on all procedures and phases of the investigation to facilitate optimal completion of the study.
2. Subjects put forth maximal effort for optimal physiological response to achieve a maximal level of upper extremity strength, endurance, and biomechanical efficiency.

Limitations

1. Results were generalizable only to college-aged, strength-trained females participating in competitive or recreational overhand sports.
2. Results were derived within a laboratory setting rather than during performance in an actual sport-specific environment.

Delimitations

1. Inference was made only to college-aged, strength-trained females participating in overhand sports.
2. Subjects were tested using sport-specific movements to simulate a sport environment.

CHAPTER II

REVIEW OF THE LITERATURE

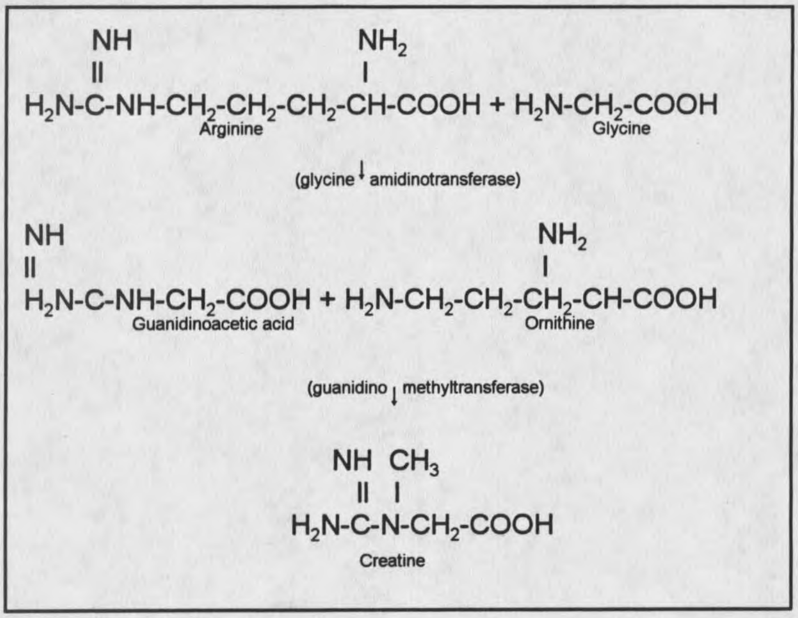
Anaerobic Energy Production

Muscular work is dependent on availability of energy derived from dephosphorylation of adenosine triphosphate (ATP) (diPrampero, 1976; Marechal, 1981; Rall, 1988). Work requiring efforts of maximal intensity, but of short duration, depends highly on anaerobic production of energy through only two processes: (1) the adenosine triphosphate-phosphocreatine (ATP-PC) system, and (2) glycolysis, i.e., the lactic acid system (diPrampero, 1976; Newsholme, 1981). The ATP-PC system is a metabolic process which provides immediate, high power, short-term energy for muscle contraction (Davies, 1965; Hultman, 1967; Karlsson, 1971). The lactic acid system provides energy for up to approximately two to three minutes resulting in lactate accumulation which can contribute to a decline in blood pH (diPrampero, 1976; Fox et al., 1993).

Phosphocreatine Availability, Storage, and Degradation

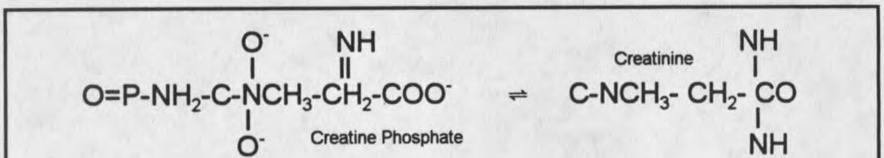
An understanding of the availability, storage, and breakdown of phosphocreatine (PC) is imperative for an appreciation of its physiological roles. Creatine (Cr), the unphosphorylated form of PC, is available both from dietary intake of meat and fish (which provide about 5 g Cr/kg or an estimated average daily Cr intake of 1 g/day) and via biosynthesis in the liver, pancreas, and kidneys from the non-essential amino acids arginine and glycine (see Figure 1; Balsom, Soderlund, & Ekblom, 1994; Devlin, 1982; Hoogwerf, Laine, & Greene, 1986; Lykken, Jacob, Munoz, & Sandstead, 1980; Walker, 1960). Biosynthesis, alone, appears to be capable of supplying adequate amounts of Cr in normal individuals as demonstrated by normal muscle Cr concentrations in vegetarian subjects (Harris, Soderlund, & Hultman, 1992). Regardless of its origin, Cr is transported by the blood

Figure 1. Biosynthesis of Cr From Arginine and Glycine



and either phosphorylated and stored directly in muscle cells, the fate of approximately 95% of Cr, or spontaneously converted to creatinine for excretion in the urine (approximately 2 g/day; see Figure 2) (Balsom et al., 1994; Delanghe et al., 1989; Hoberman, Sims, & Peters, 1948; Hunter, 1928; Zubay, 1988). Creatine gains entry into muscle cells via a sodium-dependent transporter (Guimbal & Kilimann, 1993). The body's total Cr pool is estimated to be approximately 120 g in a 70 kg human or approximately 124 mmol/kg dry muscle (Harris, Hultman, & Nordesjo, 1974; Walker, 1960).

Figure 2. Interconversion of PC and Creatinine



Phosphocreatine provides energy for muscular contraction via continual storage and degradation. Though ATP is the major energy source for muscular work, ATP storage in muscle is very limited with an estimated total energy availability of only 1.2-1.8 kcal/kg muscle used, assuming 10 kcal/mole of ATP and 30 kg of muscle in a 70 kg man (Hultman, 1967; Karlsson, 1971). Since the demand for ATP greatly exceeds its storage, working skeletal muscle must rely on other substrates for energy. As one of the first stored forms of high energy phosphate to be discovered, PC is an energy reservoir for the resynthesis of ATP (Fiske & Subbarow, 1929). Estimated PC availability is 4.5-5.1 kcal/kg muscle (Hultman, 1967; Karlsson, 1971; McArdle et al., 1991). When PC is dephosphorylated through enzymatic action of creatine kinase (CK), a large amount of energy is liberated and used to rephosphorylate adenosine diphosphate (ADP), a precursor to ATP (see Figure 3; Davies, 1965; McArdle et al., 1991).

Figure 3. Utilization of PC for Resynthesis of ATP

$$\boxed{\begin{array}{l} \text{PC} \underset{\text{CK}}{\rightleftharpoons} \text{Creatine + Phosphate + Energy} \\ \hspace{14em}\downarrow \\ \hspace{6em}\text{ADP + Phosphate} \rightleftharpoons \text{ATP} \end{array}}$$

Buffering and Phosphocreatine

In addition to its role of rapidly regenerating ATP, PC may also play a role in shuttling energy and buffering environmental changes at sites of high energy utilization during muscle contraction (Davies, 1965; McArdle et al., 1991; Wallimann, Wyss, Brdiczka, Nicolay, & Eppenberger, 1992). Phosphocreatine and Cr are capable of “shuttling” high energy phosphates between the mitochondria and the myofibrils (Bessman & Geiger, 1981; Jacobus & Lehninger, 1973; Meyer, Sweeney, & Kushmerick, 1984; Savabi, Carpenter, Mohan, &

Bessman, 1988; Wallimann et al., 1992). The "PC shuttle" is a communicator and energy carrier between locations of high energy production and locations of high energy utilization. Three sites at which the PC shuttle operates have been proposed: (1) at the myosin heads during muscle contraction, (2) in the mitochondria, and (3) between these two areas of energy production and utilization (Bessman & Carpenter, 1985; Wallimann et al., 1992).

At sites of accelerated energy utilization near the contractile proteins, PC buffers changes in concentrations of ATP, ADP, and protons via CK (Figure 3). Low pH resulting from H^+ accumulation is thought to contribute to muscle fatigue via direct action on skeletal muscle contractile proteins and by disturbing the equilibrium of key enzymes such as myosin phosphofructokinase (PFK), phosphorylase, ATP-ase, and CK (Fabiato & Fabiato, 1978). Because regeneration of ATP from ADP involves acceptance of a proton (H^+), the CK reaction may help minimize H^+ accumulation, thus, delaying a decrease in pH and the subsequent onset of fatigue (Iyengar, 1984; Iyengar, Fluellen, & Iyengar, 1982; Wallimann et al., 1992). Following regeneration of ATP, the free Cr can then be transported back to the mitochondria, undergo rephosphorylation by CK, and be shuttled back to areas of high energy utilization (Bessman & Geiger, 1981; Jacobus & Lehninger, 1973; Meyer et al., 1984; Wallimann et al., 1992).

The role of PC may be important from the onset of high-intensity exercise through early recovery. Muscle PC concentration rapidly declines within the first two minutes of intense physical work as PC is utilized for ATP regeneration, and then stabilizes unless work loads exceed maximal capacity for steady state (Greenhaff, Soderlund, Ren, & Hultman, 1993c; Harris, et al., 1976; Hultman, Bergstrom, & McLennan-Anderson, 1967). The "PC shuttle" carries essential energy from locations of synthesis and storage to areas of intense muscle contraction (Bessman & Geiger, 1981; Jacobus & Lehninger, 1973; Meyer et al., 1984; Wallimann et al., 1992). PC's buffering functions may be particularly important during high-intensity exercise when anaerobic glycolysis predominates, and increases in lactic acid, H^+, and fatigue are observed (Wallimann et al., 1992). With supramaximal workloads, muscle PC concentration can decrease rapidly to near zero with subsequent cessation of muscle contraction. Whether cessation of muscle contraction occurs voluntarily or as a result of depleted PC stores is still speculative.

Phosphocreatine and Fatigue

During high-intensity exercise, muscle fatigue may be related to PC depletion (Hultman et al., 1967). Decreased sprinting speed may be related to a decline in energy production resulting from depletion of high energy phosphate bonds (Hirvonen, Rehunen, Rusko, & Harkonen, 1987). Type II muscle fibers, the type used predominantly during high-intensity activities, exhibit higher PC concentrations, accelerated rates of utilization, and slower recovery than type I fibers (Edstrom, Hultman, Sahlin, & Sjoholm, 1982; Greenhaff et al., 1994b; Soderlund, Greenhaff, & Hultman, 1992). It has been hypothesized that higher PC utilization and slower repletion characteristic of type II fibers may promote a reduction in force and contribute to the onset of fatigue during high-intensity exercise (Hultman & Greenhaff, 1991).

Phosphofructokinase, an important enzyme in the glycolytic pathway, may be another factor that links PC concentrations and the onset of fatigue. Phosphofructokinase appears to be inhibited by normal concentrations of PC (Storey & Hochachka, 1974). While PC cleavage and anaerobic glycolysis occur simultaneously during high intensity-exercise, PC is depleted more rapidly than glycogen forcing the contracting muscles to rely predominantly on anaerobic glycolysis for continued energy supply (Hirvonen et al., 1987; Hultman et al., 1967; Spriet, Soderlund, Bergstrom, & Hultman, 1987). When PC concentrations decline during high-intensity exercise, PFK inhibition may be released allowing accelerated glycolytic flux and greater ATP availability for continued muscle contraction. It has, therefore, been proposed that supplemental Cr may spare glycolytic substrates during predominantly anaerobic activity. The resultant reduction in lactate accumulation and maintenance of a favorable pH could, theoretically, delay the onset of fatigue (Balsom et al., 1994; Storey & Hochachka, 1974).

Creatine Supplementation

Given the extensive roles and limited storage capacity of PC during exercise performance, supercompensation using oral Cr has become an area of interest to researchers, athletes, and manufacturers. Several companies currently market oral CrH_2O, a compound consisting of Cr stabilized with a water molecule. The product is accompanied by claims of increased peak strength, muscular endurance, body weight, and fat-free mass within a week of supplementation.

Biochemical Effects of Creatine Supplementation

Interest in the potential benefit of supercompensation with oral CrH_2O has led to investigation of tissue level effects. Oral CrH_2O supplementation has been shown to result in increases in the body's Cr pool (Chanutin, 1926; Crim, Calloway, & Margen, 1976; Hoberman et al., 1948). Increases in both plasma and total muscle Cr concentration, as determined by quadriceps femoris muscle biopsies, have been reported following CrH_2O ingestion (Harris et al., 1992). Muscle biopsies of subjects with varying, unquantified fitness levels, given CrH_2O in 5 g doses (each dose providing the Cr equivalent of approximately 1 kg uncooked beef) varying from 70 g over 3.5 days to 330 g over 21 days, revealed that Cr uptake was greatest within the first two to three days of supplementation. Increases in Cr were most substantial in subjects with the lowest initial total muscle Cr concentrations (Harris et al., 1992).

Similar oral doses of CrH_2O (20 g/day for five days) have also resulted in increased muscle PC concentration (Greenhaff et al., 1993a), and an increase in the rate of muscle PC resynthesis following recovery from electrically evoked isometric contraction (Greenhaff, Bodin, Soderlund, & Hultman, 1994a). Further investigation, using comparable doses of CrH_2O, revealed an increase in muscle Cr resynthesis as determined by magnetic resonance spectroscopy (MRS) of the anterior tibialis muscle and quadriceps femoris muscle biopsies following 20 intense, percutaneous electrically-evoked isometric contractions (Greenhaff et al., 1993a). Muscle energetics were similarly investigated using MRS during thirty-second isometric MVCs of the ankle extensors before and after CrH_2O supplementation (Lemon et al., 1995). Results indicated increases in pre-exercise Cr/ATP ratios, total and maximal force production, glycolytic rate, and rate of oxidative phosphorylation.

Effects of Creatine Supplementation on Exercise Performance

Over the last few years, the effects of CrH_2O on exercise performance has become an area of substantial research. Following CrH_2O supplementation in moderately trained subjects given four daily doses of 5 g CrH_2O for five days, muscle torque was sustained longer without a significant change in blood lactate accumulation during repeated bouts of maximal isokinetic contractions (Greenhaff et al, 1993b). The same group of investigators observed significantly improved running times in 300 and 1000 m track events following CrH_2O ingestion (Harris, Viru, Greenhaff, & Hultman, 1993). Further research, however, indicated no significant increase in running velocity through three testing zones using the 60 m sprint (20-30 m, 40-50 m, and 50-60 m) following ingestion of 25 g CrH_2O/day for seven days (Redondo et al., 1995). More recently, peak power output during repeated sets of jump squats was increased in active males following CrH_2O supplementation. No difference in post-exercise lactate concentration, however, was observed (Volek et al., 1996).

Studies of athletes performing high-intensity, intermittent cycle exercise indicated that subjects maintained higher pedal frequency following CrH_2O ingestion (Balsom et al., 1993a). Researchers postulated a change in energy sources and a decrease in adenine nucleotide degradation. Further investigation using the widely accepted Wingate anaerobic test (Ayalon, Inbar, & Bar-Or, 1974; Bar-Or, 1987), revealed an increase in total work output following 14 days of supplementation with 20 g of CrH_2O/day (Earnest, Snell, Rodriguez, Almada, & Mitchell, 1995b). In another study of athletes performing repeated bouts of high-intensity, intermittent cycling, investigators found that during the first two exercise bouts, peak power, mean power, and total work output increased following CrH_2O supplementation though no difference in peak lactate was observed (Birch, Noble, & Greenhaff, 1994). Similar increases in anaerobic work capacity were also attributed to CrH_2O supplementation following four bouts of cycling exercise at work rates designed to elicit fatigue within one to ten minutes (Hall, Smith, Stephens, Snell, & Earnest, 1995). However, peak power, mean power, and rate of power decline during maximal cycling exercise were not significantly altered by CrH_2O ingestion despite significantly increased total muscle Cr concentration (Ruden et al., 1996). The outcome of this investigation may have been influenced by the cross-over design with a 14-day wash-out period which may have been inadequate for ensuring complete removal of supplemental Cr (Greenhaff, 1995).

During continuous exercise there are brief periods of high-intensity efforts during which time anaerobic systems are used such as at the start of a race or during a hill climb (Fox et al., 1993). Therefore, the potential ergogenic effect of CrH_2O on continuous exercise was investigated. In one study, no significant improvements in performance times, relative oxygen consumption (VO_2), or blood lactate were documented following a CrH_2O-supplemented exhaustive treadmill run or during a 6 km run on a forest track with variable terrain (Balsom, Harridge, Soderlund, Sjodin, & Ekblom, 1993b). Time to exhaustion and blood lactate accumulation during intermediate length treadmill runs, designed to fatigue subjects within 90 seconds, were also unaffected by CrH_2O supplementation (Earnest, Rash, Snell, Almada, & Mitchell, 1995a). Performance and energy metabolism during intermittent submaximal treadmill runs were, similarly, unaffected in subjects of varying fitness levels ingesting CrH_2O (Green et al., 1994).

While it appears that CrH_2O supplementation may improve performance of short-term anaerobic work that primarily utilizes large muscle groups of the lower body, the effects of CrH_2O on activities that rely primarily on upper body movement has gained only minimal attention. Only five investigations addressing oral CrH_2O supplementation and upper body anaerobic performance have been published. Two of these five studies addressed sports involving whole body anaerobic performance rather than predominantly upper extremity strength. Supplemental CrH_2O given to elite swimmers resulted in no improvement in velocity of 25, 50, and 100 m swim sprints (Burke, Pyne, & Telford, 1995). Similarly, in a study of competitive rowers, CrH_2O supplementation did not result in significantly improved rowing times (Rossiter, Cannel, & Jakeman, 1996). Weight-trained male subjects ingesting daily doses of a high carbohydrate, high protein supplement with 20 g CrH_2O/day, experienced no significant enhancement of average peak power, peak force, or mean force during maximal bench press repetitions (Grindstaff et al., 1995). Absolute peak power, however, was significantly increased following supplementation. Following three more weeks of the same regimen (28 days total), improvements in absolute and relative power and force and in total work output were evident in both placebo and supplemented groups suggesting a training effect rather than an ergogenic influence (Almada et al., 1995). In a recent investigation of normally-active males taking either a placebo or CrH_2O, a significant improvement in the total number of bench press repetitions was observed following CrH_2O (Volek et al., 1996).

To date, no research has been published regarding the effect of oral CrH_2O on explosive, upper body, sport-specific movements such as overhand throwing, swinging a bat or racquet, or spiking/serving a volleyball. In addition, the majority of existing studies regarding CrH_2O have focused primarily on exercise response of males with limited comparison between gender (Brees et al., 1994; Ruden et al., 1996). While research on gender-specific effects of Cr supplementation on athletic performance is lacking, there is evidence of higher total muscle Cr/PC content in female versus male non-athletes (Forsberg, Nilsson, Werneman, Bergstrom, & Hultman, 1991). It is unknown, however, whether the relative quantity of PC stored in muscle relates to efficacy of CrH_2O supercompensation. Because of inherent gender differences in lean body mass (McArdle et al., 1991) and the possible gender difference in Cr/PC muscle content relative to tissue weight, the efficacy of Cr supplementation on the exercise response of females should be quantified.

In summary, the effects of Cr supercompensation on exercise performance remain equivocal. Supplementation with CrH_2O appears to alter muscle energetics possibly resulting in more efficient use of metabolic fuels and a delay in muscle fatigue (Greenhaff, et al., 1993a, 1993b; Lemon et al., 1995). It appears that supplementation with 25-30 g of CrH_2O daily for five to seven days may improve performance of high-intensity, short-term, anaerobic work utilizing large muscle groups (Balsom et al., 1993a; Earnest et al., 1995b; Hall et al., 1995; Harris et al., 1993). There is little evidence, however, that supplementation with CrH_2O has any buffering effect during anaerobic activities (Birch et al., 1994; Greenhaff et al., 1993b; Volek et al., 1996). It also remains unclear whether oral CrH_2O can improve high-intensity performance of the upper extremities or whether similar effects are observed in females. Furthermore, there remains a strong reliance on published abstracts with limited peer-reviewed, exercise performance-related publications in existence (Balsom et al., 1993a, 1993b, 1994; Earnest et al., 1995a, 1995b; Greenhaff, 1995; Greenhaff et al., 1993b; Maughan, 1995; Volek & Kraemer, 1996). Therefore, investigation on the effects of CrH_2O supplementation on upper body anaerobic response in females is warranted.

CHAPTER III

EXPERIMENTAL PROCEDURES

Description of Subjects and Movements Tested

Following written informed consent and approval from the Montana State University-Bozeman Human Subjects Committee, 24 college-aged females, who met the inclusion criteria of participation in upper extremity strength training and either recreational or competitive overhand sports, took part in this study. Inclusion criteria were based on prior research indicating overall strength and biomechanical differences between throwing athletes and non-throwing athletes (Brown, Niehues, & Harrah, 1988; Cook, Gray, & Savinar-Nogue, 1987; Ellenbecker, Davies, & Rowinski, 1988; Ng & Kramer, 1991; Perrin, Robertson, & Ray, 1987; Wooden, Greenfield, & Johanson, 1992). Subjects met criteria for involvement in overhand sport based on reported frequency of participation in such sports and on observed throwing accuracy. All testing was conducted at the Montana State University-Bozeman Human Performance and Ergonomics Laboratory.

Testing included two movements, elbow flexion (EF) and shoulder internal rotation (IR). Elbow flexion is commonly used to evaluate concentric (CON) and eccentric (ECC) strength of the biceps brachii, brachioradialis, and brachialis muscles (Adams, 1994; Rutherford & Corbin, 1994). The movement is also commonly used as a component of strength conditioning programs (Adams, 1994; DeLorme & Watkins, 1951; Fox et al., 1993; Heywood, 1991; Wilmore & Costill, 1988; Withers, 1970). Therefore, EF was selected to evaluate the efficacy of CrH_2O supplementation in strength conditioning of the upper extremities.

In contrast to EF, which incorporates a finite number of muscles, an overhand throwing motion utilizes numerous muscle groups of the upper body (Bartlett, Storey, & Simons, 1989). The muscles responsible for rotating the shoulder internally, a movement dominant in the overhand throw, are active during the upper extremity projectile motions used in a variety of sport movements such as the baseball pitch, tennis serve, volleyball spike and serve, water polo throw, and javelin throw (Cohen, Mont, Campbell, Vogelstein, & Loewy, 1994). The acceleration of such movements is dependent upon explosive contraction of the internal rotators (coracobrachialis, biceps-short head, subscapularis, teres major, latissimus dorsi, anterior deltoid, and pectoralis major) which may either provide the power for the movement, stabilize the glenohumoral joint during the movement, or both (Atwater, 1979; Cohen et al., 1994; Perry, 1983; Toyoshima & Hoshikawa, 1974). Additionally, upper extremity peak torque has been shown to be positively correlated with throwing speed in competitive athletes (Bartlett et al., 1989; Pedegana, Elsner, & Roberts, 1982). Thus, shoulder IR was selected to evaluate the potential use of CrH_2O supplementation in a variety of overhand throwing skills.

Pre-testing Procedures

Prior to initiation of testing, supine, hyperventilation, and standing modified Mason-Likar twelve-lead electrocardiogram (ECG) recordings (Dubin, 1989; Marquette Max-1, Marquette Electronics Inc., Milwaukee, WI), heart rates, and blood pressures were obtained from each subject to identify any contraindications to participation (American College of Sports Medicine, 1995). Height (Ht; cm) and weight (Wt; kg) were determined using a calibrated Detecto physician's beam scale (Cardinal Scale Manufacturing Co., Webb City, MO). Body composition was evaluated via the seven-site skinfold technique (Brozek, Grande, Anderson, & Keys, 1963; Jackson & Pollock, 1977; Pollock, Schmidt, & Jackson, 1980) using a Lange skinfold caliper (Cambridge Scientific Industries, Inc., Cambridge, MD). After obtaining three measurements at each site using appropriate landmarks on the right side of the body, the mean measurement at each site was used to calculate percent body fat (BF; %) and lean body mass (LBM; kg) via the following equations:

Eq. 1 D_b = 1.0979 - 0.0004697(sum of 7 sites) + 0.00000056(sum of 7 sites)^2 - 0.00012828(age)

Where:

D_b	= body density
1.0979	= regression coefficient for variance in body circumference
0.0004697	= regression coefficient for variance in sum of skinfolds
0.00000056	= regression coefficient for variance in squared sum of skinfolds
0.00012828	= regression coefficient for variance in age

Eq. 2 $\%BF = \underline{4.570} - 4.142 \times 100$ over D_b

$$\%BF = \frac{4.570}{D_b} - 4.142 \times 100$$

Where:

4.57	= constant for density of fat tissue
4.142	= constant for density of fat-free tissue

Eq. 3 LBM = (100 - %BF) x Wt (kg)

Testing

The study included five testing sessions (Figure 4). The first three sessions were conducted for the purpose of habituation; the last two sessions constituted data collection.

Figure 4. Time Line for Completion of the Five Testing Sessions

Day 1: Test 1	Day 3: Test 2	Day 6: Test 3	Day 13: Test 4	Day 20: Test 5
Neither CrH_2O nor P was delivered	*Neither CrH_2O nor P was delivered*	*7-day dietary habituation began following testing*	*CrH_2O or P began following testing*	*CrH_2O or P was completed prior to testing*

At each session, peak strength during EF and IR was evaluated using the one repetition maximum (1RM), one of the most operational definitions for strength (Eq. 4; DeLorme & Watkins, 1951). One repetition maximum was expressed as the isotonic resistance (Nm) at which each subject could only complete one repetition. Strength was also expressed as the total number of repetitions that could be completed at 70% of 1RM, a workload selected to

Eq. 4 1RM (Nm) = Nm at RM between 2-20 repetitions/[100% - (RM x 2)]

facilitate sport-specific testing, with nine to twelve repetitions per set typically employed in resistance programs designed to achieve both strength and endurance gains (Dons, Bollerup, Bonde-Petersen, & Hancke, 1979; Withers, 1970). All testing was conducted using a LIDO WorkSET™ dynamometer (Loredan Biomedical, Inc., West Sacramento, CA). The LIDO WorkSET™ facilitates isokinetic (IK), isotonic (IT), and isometric evaluation throughout concentric (CON), eccentric (ECC), and continuous passive motion modes. Modes utilized in this study included IK (CON/ECC) and IT (CON). The LIDO WorkSET™ dynamometer has been found to have test-retest reliabilities of 0.82-0.96 for peak torque and average peak torque and a high degree of internal validity ($R^2=1$) with regard to torque, power, and work (Matheson et al., 1992). Test-retest reliability coefficients for this study were determined via intraclass correlation which employs ANOVA for calculation of R (see Appendix C, Table 1). The LIDO was interfaced with a personal computer via a digital to analog board and Labtech Notebook (version 6) software (Laboratory Technologies Corporation, Wilmington, MA) to facilitate collection of position and torque data at a sampling rate of 150 Hz. Isotonic strength testing was performed to facilitate measurement not only of EF and IR strength, but also of velocity throughout the execution of shoulder IR (Adams, 1994). Inclusion of IK strength testing enabled measurement of torque throughout the active EF range of motion (ROM) while the dynamometer maintained a constant angular velocity (Hislop & Perrine, 1967).

Torque "overshoot" or impact torque, evidenced by prominent initial and terminal spikes in torque output curves, was disregarded in the determination of peak IK torque (Perrine & Edgerton, 1978; Sapega, Nicholas, Sokolow, & Saraniti, 1982). It is thought that these spikes do not represent intermittent surges of muscular contractile force but, rather, forces associated with the initial accelerations, final decelerations, and subsequent velocity fluctuations of an initially overspeeding lever system (Sapega et al., 1982). Compensation for the effect of gravity on the LIDO lever arms was accomplished internally by the dynamometer except during IK testing. Compensation for gravity during IK testing was made by hand calculation based on torque caused by gravity on the lever arm in the position approximating that for peak torque during EF.

Habituation Phase

The first three of the five total testing sessions were designed to obtain proper equipment settings, familiarize subjects with the procedures for testing and supplementation, and promote biomechanical efficiency during execution of both movements tested. Appropriate settings for EF on the LIDO were obtained and recorded after instructing subjects on proper positioning. Positioning for EF testing required standing with the feet shoulder width apart, stabilizing the elbow against the iliac crest, and keeping the shoulders back and the head facing straight forward. Range of motion for EF testing was measured from the point of maximal flexion to that of maximal extension. The lateral epicondyle of the dominant limb was located and aligned with the center of rotation on the lever arm of the LIDO. Proper lower body positioning for testing shoulder IR was determined by measuring the distance between the leading and trailing foot at the point of ball release during a typical overhand throw. This measured distance was marked on the floor parallel to the LIDO to facilitate lower body positioning during testing of IR that duplicated that during the subject's overhand throw. With the dominant arm in 90° of elbow flexion, 90° of shoulder abduction, and approximately 20° of forward flexion to facilitate testing in the scapular plane (Ellenbecker, Feiring, & DeHart, 1992), the olecranon process of the dominant arm was located and aligned with the center of rotation on the lever arm of the LIDO. Internal rotation was evaluated from each subject's point of maximal external rotation, with the arm in 90° of shoulder abduction and 90° of elbow flexion, through horizontal forward (forearm parallel with the ground; Hinton, 1988). Shoulder IR was evaluated without immobilizing the lower extremity or the torso. Aggressive verbal cues were provided to minimize involvement of muscles other than those responsible for IR.

Following determination of appropriate settings, habituation testing sessions were conducted. On the day of the third habituation testing session, all subjects began consumption of 6 oz herbal tea, five times daily for one week to become familiar with supplementation procedures. Following habituation testing sessions, subjects were pair-matched based on Ht, Wt, BF, LBM, and age (years), and randomly assigned to receive either CrH_2O (n=11) treatment or P (n=13).

Testing Sessions

Subjects began each of the five testing sessions by warming-up with three submaximal EF IK CON/ECC contractions (approximately 50% of maximal voluntary contraction, MVC) on the dominant side at 90°/s in a standing position (Adams, 1994; Morris, Lussier, Bell, & Dooley, 1983; Osternig, 1986; Sale, 1991; Stafford & Grana, 1984). Following a standard two-minute rest period, subjects completed one MVC and the peak CON and ECC torques were recorded as EF IK_{CON} (Nm) and EF IK_{ECC} (Nm), respectively.

Evaluation of EF_{1RM} began with a warm-up of three submaximal CON contractions using approximately 50% of estimated EF_{1RM} (or 50% of previously determined 1RM) on the dominant side. Approximation of EF_{1RM} was made based on the linear relationship between the intensity and the number of repetitions performed (see Eq. 4; DeLorme & Watkins, 1951; Hoeger, Barette, Hale, & Hopkins, 1987; Landers, 1985). Approximated EF_{1RM} was then verified by counting the number of repetitions that could be completed at the calculated load. Load adjustments were made as necessary, until EF_{1RM} (Nm) was determined. Subjects then completed successive EF repetitions in the IT mode at 70% of 1RM until volitional fatigue (FAT; Osternig, 1986; Perrine & Edgerton, 1978). The number of repetitions was recorded as EF_{FAT}. All EF tests were separated by standard two-minute rest periods.

Evaluation of shoulder IR began with a warm-up of 3 submaximal IT repetitions using 50% of estimated IR_{1RM} (or 50% of previously determined IR_{1RM}). Approximated IR_{1RM} was verified by counting the number of repetitions that could be completed by the subject at the calculated load. Load adjustments were made as necessary to determine IR_{1RM}. Subjects then performed successive IR repetitions at 70% of 1RM to the point of volitional fatigue. The number of repetitions was recorded as IR_{FAT}. Finally, velocity during IR (IRV; °/sec) was determined as each subject completed one repetition as rapidly as possible at 25% of 1RM. All IR tests were separated by two-minute rest periods.

Testing sessions for each subject were conducted at similar times of the day (± 1 hour). Subjects were required to exert maximal effort. Verbal encouragement was provided using a standardized script and every effort was made to maintain a consistent testing environment with all subjects. Throughout data collection, subjects were instructed to maintain upper extremity strength conditioning without progressing the resistance to facilitate accurate evaluation of treatment effects without the positive influence of progressive resistance exercise (PRE; Hellebrandt & Houtz, 1956; Pearson & Costill, 1988). Each subject's physical activity was quantified by the investigator (average estimated METS per day) using the 7-day physical activity recall method (Blair, 1984) on days one and seven of the dietary phase (see Appendix A).

Dietary Phase

Following pair-matching and subsequent randomization into treatment groups, subjects began consuming either CrH_2O or P for seven days (Table 1). Treatment doses were consumed in the presence of the investigator in the Human Performance and Ergonomics Laboratory. All subjects were blind to the treatment received.

Table 1

Composition of Dietary Supplements

Group	Composition	Cr Content	CHO Content	Frequency
CrH_2O	5 g CrH_2O[a] + 2 g sucrose	30 g	2 g	5 x/day
P	2.12 g Polycose®	0 g	2 g	5 x/day

[a] Harris et al., 1992

Each supplement dosage was dissolved in approximately 6 oz of warm-to-hot caffeine-free herbal tea to facilitate dissolution without formation of creatinine (Harris et al., 1992). Herbal tea was chosen as a pleasant-tasting medium for delivery of CrH_2O that did not appreciably increase caffeine or simple carbohydrate intake and was not likely to have interfered with CrH_2O stability (Harris et al., 1992). The dosage was then consumed by the subjects within ten minutes of mixing. Subjects were not permitted to see the supplement being prepared. The dietary doses were administered a minimum of one hour apart and one hour before/after meals to maximize supplement absorption. Pre-treatment testing sessions were conducted within an hour prior to beginning supplementation. Post-treatment testing was conducted approximately 12 hours following completion of the dietary phase.

Though it is unlikely that small differences in the amount of Cr derived from the average non-vegetarian diet would influence the utilization or exercise performance effects of supplemental CrH_2O (Brees et al., 1994), all subjects were instructed to eat a non-vegetarian diet. Subjects were asked to eat three balanced meals daily, consisting of approximately six ounces of meat, six to eight servings of complex carbohydrates, two servings of fruit or fruit juice, one serving of milk or equivalent, and non-starchy vegetables and non-caloric beverages ad lib. Vegetarians were excluded from the study due to reports of increased Cr uptake by skeletal muscle in subjects with lower initial muscular Cr concentration (Harris et al., 1992), and lower normal serum Cr reference values reported for vegetarians (Delanghe et al., 1989). Food frequency questionnaires (see Appendix A), completed during pre- and post-treatment testing, were analyzed using a nutritional analysis software package (Food and Diet Analyzer, Fitness Technologies Press, Ann Arbor, MI) to quantify meat, fish, and poultry consumption (kg) for estimation of dietary Cr intake (g).

Statistical Analyses

Data were grouped by movement (EF or IR) and subjected to test-retest reliability procedures. Multivariate analysis was performed on pair-matching variables to verify efficacy of matching procedures. Separate multivariate analyses were performed on the EF and IR groups of variables using the General Linear Model (GLM) procedure of the SAS^R Proprietary Software Version 6.08 (SAS Institute, Carey, NC) running on a VAX/VMS platform. Variables were assessed with regard to unequal sample size, multivariate normality, linearity, outliers, homogeneity of variance, multicolinearity, and singularity (Tabachnick & Fidell, 1989). An experiment-wise type I error rate of 0.05 was established a priori and the Bonferroni method of multiple comparisons was used where appropriate (Tabachnick & Fidell, 1989). Subjects were stratified according to anthropometric variables and age, and treated statistically as a random effect. The model used was

$$Y_{ijkl} = \mu + \beta_k + \pi_{l(k)} + \alpha_j + \alpha\beta_{jk} + \xi_{ijkl}$$

where μ represents a mean, β_k represents the treatment effect, $\pi_{l(k)}$ represents subjects nested within treatment, α_j represents the trial effect, $\alpha\beta_{jk}$ represents the trial by treatment interaction, and ξ_{ijkl} represents the residual term. Wilks' criterion was used to identify significant effects and, when significant effects were observed, univariate F statistics were examined to identify the importance of individual variables.

CHAPTER IV

RESULTS

Initial Wt, LBM, BF, Ht, age, estimated weekly meat intake, and estimated weekly physical activity are shown in Table 2. Using Wilks' criterion, no significant differences existed between groups with respect to anthropometric parameters and age. Mean Wt, Ht, and BF for the sample were consistent with United States averages for females in this age group. No significant differences ($p>0.05$) existed between the groups with respect to estimated average Cr intake from meat (kg/wk; 1 kg providing approximately 5 g Cr; ($F_{1,22}=1.254$, $p=0.29$), or estimated average physical activity (METS/wk; $F_{1,22}=0.06$, $p=0.81$) during the dietary phase.

Table 2

Comparison of Subjects by Treatment Group

	Placebo		CrH_2O	
Variable	Mean ± SEM	Range	Mean ± SEM	Range
Initial Wt (kg)	65.4 ± 3.5	50.0-95.9	65.1 ± 2.2	54.1-79.1
LBM (kg)	49.7 ± 1.6	40.3-58.0	51.4 ± 1.3	44.3-60.3
BF (%)	22.2 ± 2.6	11.7-48.0	20.1 ± 1.6	12.9-31.0
Ht (cm)	166.7 ± 1.9	152.4-177.8	165.8 ± 1.2	160.0-172.7
Age (yrs)	23.9 ± 0.9	21.0-33.0	22.5 ± 0.8	19.0-29.0
METS/wk	66.9 ± 9.9	40.0-101.3	62.5 ± 13.9	24.9-113.5
Meat (kg/wk)	0.8 ± 0.1	0.4-1.1	1.0 ± 0.2	0.6-1.9

Pre- and post-treatment Wt and muscle response are summarized in Table 3. With respect to Wt, CrH_2O supplementation resulted in no significant treatment effect ($F_{1,22}=0.72$,

p=0.41), a significant trial effect ($F_{1,22}$=5.75, p=0.03), and no significant treatment by trial interaction ($F_{1,22}$=0.66, p=0.43). These findings indicate that while body weight did change over time, the change did not differ significantly between treatment groups. Weight increased by 0.6% and 1.2% from pre- to post-treatment in P and CrH_2O groups respectively.

Table 3

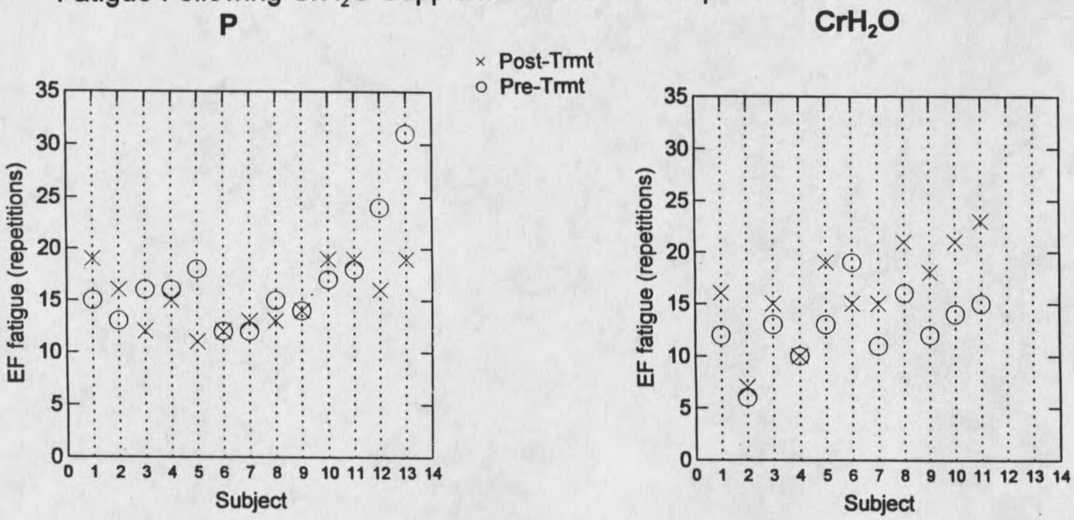
Body Weight and Muscle Response By Trial and Treatment Group

	Placebo[a]		CrH_2O[a]	
Variable	Pre	Post	Pre	Post
Wt (kg)	65.3 ± 3.5	65.7 ± 3.5	64.9 ± 2.2	65.7 ± 2.2
EF IK_{CON} (Nm)	42.9 ± 2.9	42.1 ± 2.5	44.2 ± 2.0	46.6 ± 1.6
EF IK_{ECC} (Nm)	55.2 ± 3.5	56.3 ± 4.2	62.0 ± 2.7	64.8 ± 5.1
EF_{1RM} (Nm)	34.4 ± 2.5	35.0 ± 2.6	36.2 ± 1.8	37.8 ± 1.7
EF_{FAT} (reps)[b]	17.0 ± 1.5	15.0 ± 0.8	12.8 ± 1.0	16.4 ± 1.5
IR_{1RM} (Nm)	35.4 ± 2.1	36.7 ± 2.1	34.1 ± 1.6	35.6 ± 1.5
IR_{FAT} (reps)	38.3 ± 8.0	47.5 ± 9.7	36.5 ± 6.3	47.3 ± 9.3
IRV (°/sec)	292.1 ± 8.0	287.9 ± 7.4	291.1 ± 4.3	286.5 ± 8.1

[a] Mean ± SEM
[b] The difference from pre- to post-treatment was significantly different (p<0.05) between treatment groups.

Analysis of EF using Wilks' criterion revealed a significant treatment by trial interaction ($F_{4,19}$=5.30, p=0.005) and a significant treatment effect ($F_{4,19}$ =6.71, p=0.0015). In the absence of interaction, significant univariate treatment effects for EF IK_{CON} ($F_{1,22}$ =13.39, p=0.001), EF IK_{ECC} ($F_{1,22}$=16.76, p=0.0005), and EF_{1RM} ($F_{1,22}$=18.08, p=0.0003) are not indicative of significant differences between treatment group responses. Treatment effects may be reflective of pre-existing strength differences and/or similar changes in strength between the groups following treatment. However, a significant treatment by trial interaction for EF_{FAT} ($F_{1,22}$=9.42, p=0.006), depicted in Figure 5, indicates that the increase in the number of repetitions completed prior to volitional fatigue following CrH_2O supplementation was significantly greater than that following P ingestion. Analysis of EF data revealed no significant trial effects. Internal rotation analysis revealed no significant interaction, a significant treatment effect ($F_{3,19}$=10.12, p=0.0003), and a significant trial effect using Wilks' criterion ($F_{3,19}$=9.93, p=0.0004). The significant univariate treatment effect for IR_{1RM} ($F_{1,21}$= 10.12, p=0.0003), without significant treatment by trial interaction, cannot be interpreted as a significantly different response between treatment groups. Significant univariate trial effects for IR_{1RM} ($F_{1,21}$=11.13, p=0.003) and IR_{FAT} ($F_{1,21}$=7.65, p=0.01) indicate that peak IR strength and IR repetitions to fatigue improved over time but with no significant differences between treatment groups.

Figure 5. Scatter Plots Depicting a Significantly Greater Increase in EF Repetitions to Fatigue Following CrH_2O Supplementation as Compared to P



Nonsignificant trends in muscle response are compared in Figure 6. Peak IT EF strength remained essentially unchanged or decreased following P in 70% of subjects, whereas 45% of subjects in the CrH_2O group experienced improvement in EF_{1RM}. Recalling that subjects were asked to discontinue progression of their resistance training during the study and assuming subject compliance was adequate, one should consider the possibility that detraining occurred in the P group but was prevented in the CrH_2O group. Peak EF IK_{CON} improved in 23% and 54% of subjects following P and CrH_2O, respectively; EF IK_{ECC} improved in 30% and 45% of subjects following P and CrH_2O, respectively. Following P, 30% experienced increased peak IR torques while 45% of those supplemented with CrH_2O increased IR_{1RM}. The majority of subjects, regardless of treatment group, experienced decreased velocity during IR. Improvements in IR repetitions to fatigue, experienced by 54% of subjects, occurred equally in both treatment groups.

Figure 6. Scatter Plots Comparing Trends of Nonsignificant Changes in Muscle Response Pre- and Post-Dietary Phase Across Treatment Groups

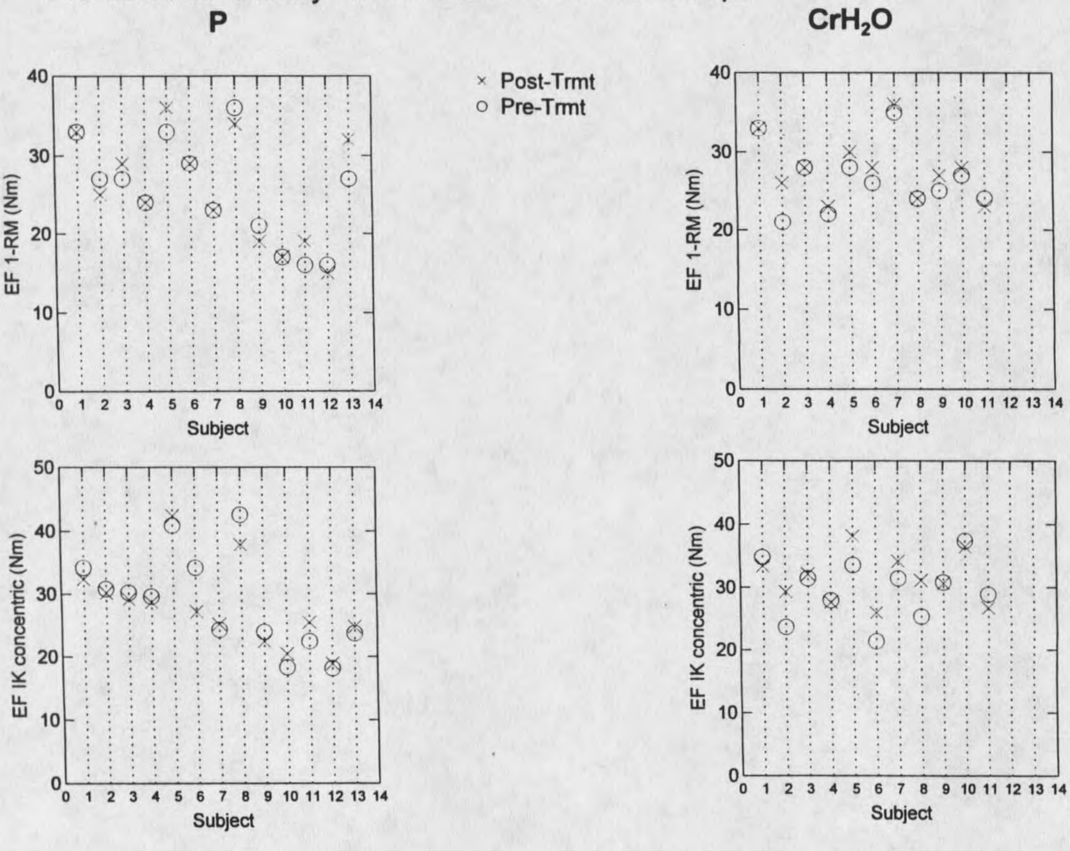
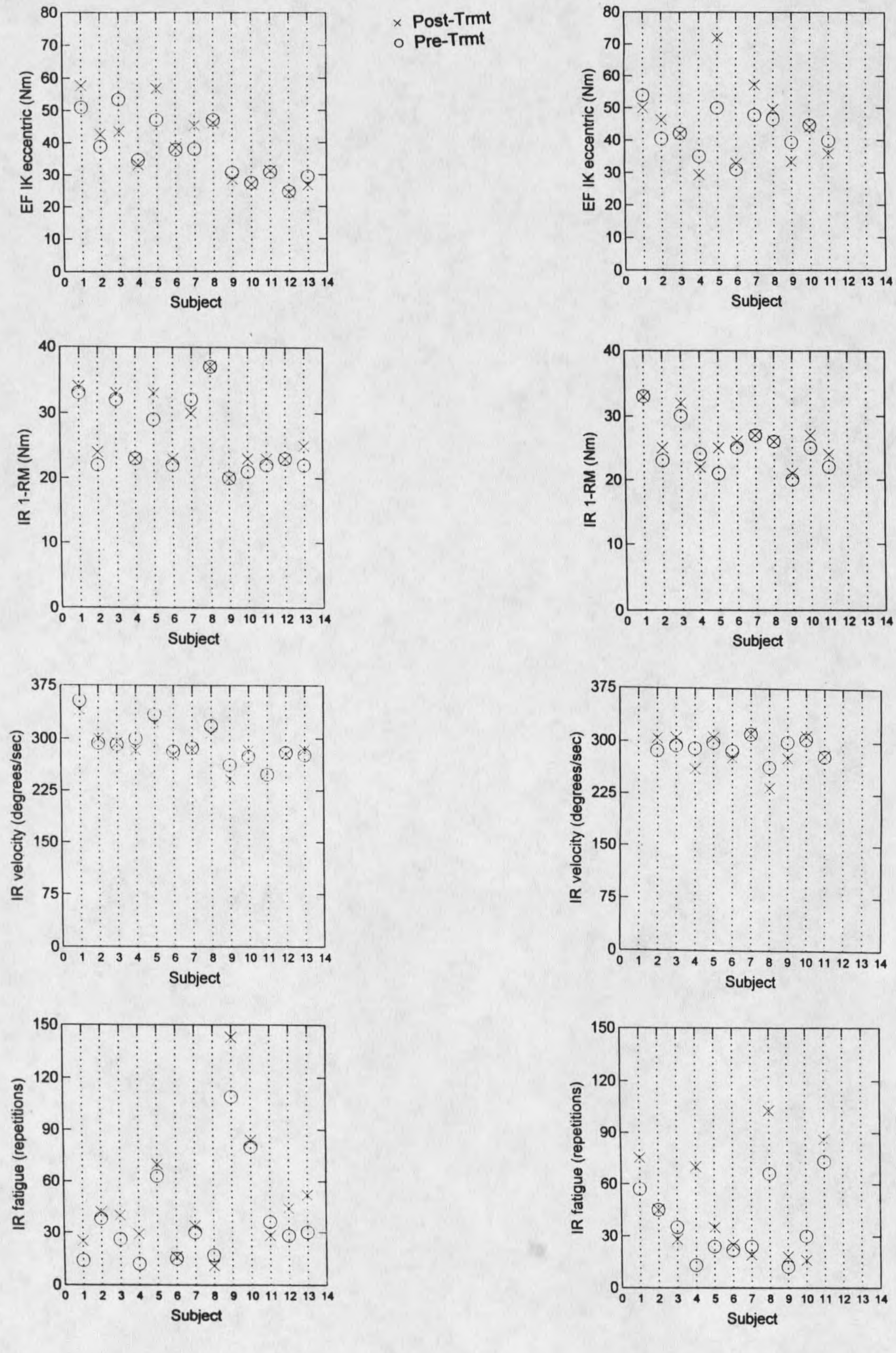


Figure 6. (cont'd) P CrH$_2$O



CHAPTER V

GENERAL DISCUSSION AND SUMMARY

The purpose of this study was to quantify the effects of oral Cr supplementation on upper extremity anaerobic response in females. An examination of four EF and three shoulder IR variables revealed a significant gain only in IT work to fatigue during EF. Overall tolerance to CrH_2O was adequate with only two isolated reports of gastrointestinal discomfort for which termination of supplementation did not become necessary. Supplement preparation in warm herbal tea facilitated delivery of CrH_2O in a pleasant-tasting medium that did not increase simple carbohydrate or caffeine intake.

Elbow Flexion

Average peak IK and IT EF torque observed in this investigation exceeded published values (Rutherford & Corbin, 1994). The results of this study suggest a role for CrH_2O supplementation in enhancing IT work to fatigue in female athletes during upper extremity conditioning movements such as the biceps curl. This finding of increased IT work to fatigue following CrH_2O ingestion is in agreement with previous findings applicable to lower body anaerobic work efforts (Balsom et al., 1993a; Birch et al., 1994; Earnest et al., 1995b; Hall et al., 1995) and upper body strength training (Volek et al., 1996).

Both EF IK_{CON} and EF IK_{ECC} increased to a greater extent in the CrH_2O group than in the P group (EF IK_{CON}: decreased 1.9% and increased 5.4% in P and CrH_2O groups respectively; EF IK_{ECC}: increased 1.1% and 4.5% in P and CrH_2O groups respectively). Therefore, while no significant interaction existed for IK peak CON and ECC torque during EF, the significant treatment effects for these variables may lend credence to an effect of Cr supplementation in increasing peak IK strength. Limited information in the literature exists concerning the effect of CrH_2O on IK performance. A previous investigation of Cr supplementation and IK lower body work revealed improvement in work capacity but did not include an evaluation of peak IK strength (Greenhaff et al., 1993b).

Shoulder Internal Rotation

Average peak IR torque equaled or exceeded published IR normal values for the same age and gender (Ivey, Calhoun, Rusche, & Bierschenk, 1985). No significant improvements were seen in any of the shoulder IR parameters suggesting that CrH_2O does not enhance performance of sports involving explosive overhand movement in female athletes. While this is the first study to examine the potential effects of CrH_2O on performance of overhand sports, previous investigations of upper extremity sports such as swimming and rowing have revealed no significant performance enhancement following CrH_2O ingestion (Burke et al., 1995; Rossiter et al., 1996).

Body Mass

In the present study, there was no significant change in body weight following CrH_2O ingestion. This contradicts the majority of existing literature which reports increases in body mass related to CrH_2O use (Balsom et al., 1993a, 1993b; Chanutin, 1926; Greenhaff et al., 1994a; Soderlund et al., 1994; Volek et al., 1996). Because treatment duration for CrH_2O studies is typically five to seven days, a time period during which changes in body composition are generally undetectable, the nature of the CrH_2O-related weight gain is unknown. The mechanisms by which CrH_2O may lead to weight gain are also unknown. It is possible that cessation of progressive resistance exercise may have counteracted any possibility of Cr-induced LBM gains in this investigation.

Physical Activity

No significant difference in estimated amount of physical activity existed between dietary groups. The subjects in this study were instructed to continue their current physical training but without progressive resistance. The majority of past research, however, does not include an indication of whether subjects were instructed to continue versus change their normal training. If no restrictions are placed on physical training during the supplementation period, it becomes difficult to ascertain whether improvements in performance are due to an ergogenic action of CrH_2O or merely to the effects of progressive resistance training. In a study comparing changes in skeletal muscle Cr concentration following CrH_2O supplementation in subjects engaged in normal physical activity versus those performing maximal one-legged exercise, investigators reported a significantly greater increase in muscle Cr concentration following exercise (Harris et al., 1992). Given the evidence that Cr uptake is accelerated when supplemented during participation in high-intensity exercise, the most beneficial method for evaluating the efficacy of CrH_2O becomes difficult to ascertain.

Dietary Creatine

Estimated average daily Cr intake from meat was slightly less than normal, regardless of treatment group. Based on renal creatinine excretion, an average 70 kg male requires approximately 2 g Cr/day, 50% of which is normally provided by the diet. The portion of the requirement not provided by the diet is met via biosynthesis which likely operates via a feedback system. That is, when dietary Cr decreases, biosynthesis increases. Thus, it is unlikely that fluctuations in dietary Cr necessarily deem an individual Cr deficient at the tissue level (Harris et al., 1992). While this, theoretically, may have influenced supplemental Cr metabolism, one should also be aware of the tendency to underreport food intake on food frequency questionnaires (Pekkarin, 1970; Young, Hagan, Tucker, & Foster, 1952).

Direction for Future Research

The influence of CrH_2O on repeated intermittent bouts of EF, particularly in the IK mode comparing CON and ECC work, should be investigated. As this study did not address the potential value of CrH_2O for enhancing intermittent bouts of shoulder IR in female or male athletes, further investigation is warranted. Such an investigation should be designed to be as sport specific as possible, for example, having tennis athletes do the same number of bouts of shoulder exercise as would occur during the average rally in a tennis match. Biomechanical techniques, such as measuring ball velocity and employing motion analysis for evaluation of throwing kinematics during overhand sports, may be another valuable avenue to pursue for the assessment of possible ergogenic-related changes.

The mechanisms by which CrH_2O may contribute to increased body mass should be investigated. Balsom et al. (1993a) have proposed that weight gain results from an accelerated uptake of water by skeletal muscle cells due to osmotic action of increased intracellular Cr. A study of patients with choroid and retinal gyrate atrophy given Cr supplements lends some support to the theory of cellular swelling resulting in weight gain (Sipila, Rapola, Simell, & Vannas, 1981). These patients, whose disease is attributed to a defect in Cr biosynthesis, experienced an increase in the diameter of type II muscle fibers in the vastus lateralis. Another theory attempting to account for weight gain associated with CrH_2O supplementation is based on Cr functioning as a signal for increased contractile protein synthesis (Ingwall, Weiner, Morales, Davis, & Stockdale, 1974). A recently published investigation was the first to employ CrH_2O dosing based on kg of body mass (Rossiter et al., 1996). Utilizing 0.25 g CrH_2O/kg body mass dosing, investigators effectively increased whole-body Cr stores as estimated by measurement of urinary creatinine and Cr concentrations. This information may be valuable for evaluating the influence of LBM on the efficacy of CrH_2O. Research efforts should be aimed at clarifying the mechanisms of action by which supplemental Cr interacts with and influences skeletal muscle cells and whether this interaction causes a change in body mass.

Further clarification of the effects of training volume on CrH_2O efficacy is needed. Few efforts have been made to control or manipulate physical activity during experimental Cr supplementation. Well-designed, highly controlled training studies that explore the relationship between CrH_2O efficacy and training volume could provide valuable information regarding the practical application of this potential ergogen.

Future investigation should also be focused on determining the exact mechanism(s) by which supplemental Cr may increase PC resynthesis and/or delay fatigue. Type II fibers have been shown to have higher PC concentration and turnover than type I fibers, yet slower PC resynthesis following high-intensity exercise, owing to the apparent correlation of PC concentration with muscle fatiguability (Edstrom et al., 1982; Greenhaff et al., 1994a; Soderlund et al., 1992; Tesch, Thorsson, & Fujitsuka, 1989). It is also well documented that following high-intensity activity with short recovery periods, performance is impaired (Balsom, Seger, Sjodin, & Ekblom, 1992a, 1992b). Hypotheses follow that inadequate PC resynthesis may be a causal factor in this performance impairment. There is growing support for the ability of Cr supplementation to accelerate the rate of PC resynthesis following intermittent exercise, yet it is still not clear if this enhanced resynthesis results in delayed fatigue (Greenhaff et al., 1993a, 1994b).

Because the time course of Cr degradation following supplementation has yet to be quantified, a cross-over design is contraindicated. Investigation of Cr degradation following supplementation at doses similar to those in the current study is in progress (Greenhaff, 1995). Current research suggests that supplemental Cr may remain in skeletal muscle longer than one month following supplementation (Benedict & Osterberg, 1923; Chanutin, 1926; Lemon et al., 1995). While care was taken to ensure that treatment groups were anthropometrically similar, utilization of a cross-over design would have been optimal for counterbalancing the effects of increased testing experience, time of day, maturation effects, and the interaction of any of these effects with selection by enabling each subject to serve as her own control. Until more conclusive insight into the half-life of supplemental Cr is gained, experimental design will continue to be a concern in CrH_2O research.

As the mechanisms by which CrH_2O may exert ergogenic effects are still unclear, efforts to discover whether other substances may further enhance any ergogenic capabilities of CrH_2O are being pursued. Investigation into the effect of insulin and glucose on Cr uptake by myocytes, first investigated over 20 years ago, is re-emerging (Haugland & Chang, 1975; Koszalka, & Andrew, 1972). Vitamin E deficiency may inhibit Cr metabolism (Gerber, Gerber, Koszalka, & Emmel, 1962) as may the concurrent use of drugs such as digoxin (Bennet, Bevington, & Walls, 1994). Cr supplementation in conjunction with chromium or with vanadyl sulfate, a substance which binds phosphate compounds and freely enters and accumulates in cells, is also currently under investigation.

Other areas in need of research are with regard to the biochemical and performance effects of long-term CrH_2O supplementation. Few investigators have pursued measurement of the effects of CrH_2O ingestion beyond an average of seven days of supplementation (Almada et al., 1995; Sipila et al., 1981). As the mechanisms by which CrH_2O acts may be related to the extent of muscle uptake which may, in turn, be related to LBM and physical training, investigation into the appropriate dosage and timing of supplementation with respect to training may be of value. This value may extend beyond the sporting environment into the clinical setting as a treatment for atrophy-related disease.

In summary, Cr is important in regulation of skeletal muscle metabolism. While the roles of endogenous Cr are reasonably well defined, the influence of supplemental CrH_2O is not. It is well accepted that both muscle and plasma Cr concentrations can be elevated by providing exogenous Cr without any apparent ill effects. Beyond this point, however, both biochemical and performance effects resulting from exogenous Cr supercompensation become less definitive. The exact mechanisms by which exogenous Cr invades skeletal muscle, in addition to the course of action that ensues once intracellular Cr concentrations are elevated, remain unknown. The value of CrH_2O supplementation for the athlete is currently limited to possible enhancement of short-duration, high-intensity, intermittent physical activity including powerful activities of both the lower and upper extremities. The practical application of CrH_2O for athletes wishing to significantly enhance sport performance is yet ill-defined. The results of this study indicate that oral CrH_2O supplementation has a positive effect on isotonic, upper extremity work to fatigue. Results further indicate that CrH_2O has no effect on isotonic, upper extremity peak strength, isotonic velocity, or isokinetic peak CON and ECC strength.

GLOSSARY

Adenosine triphosphate (ATP): A nucleotide formed with the energy released from food and the energy stored in cells, particularly muscle cells.

Anaerobic capacity: The amount of energy accessible for work in the absence of oxygen.

Anaerobic power: The maximum amount of energy that can be produced during maximal exertion per unit of time.

Buffer: A substance that reduces the change in hydrogen ion (H^+) concentration by binding or releasing H^+ in solution (Ganong, 1993).

Carbohydrate (CHO): A group of chemical compounds (including sugars, starches, and cellulose) containing carbon, hydrogen, and oxygen (Fox et al., 1993).

Creatine (α-methylguanidinoacetate; Cr): An amino-acid derived compound, stored primarily in skeletal muscle, serving as a reservoir of high-energy phosphate groups.

Creatine kinase (CK): An enzyme responsible for catalyzing the reversible phosphorylation of creatine to phosphocreatine.

Glycogen: The storage form of glucose; primarily stored in the liver and muscle tissue.

Glycolysis: The catabolic conversion of carbohydrate (glycogen or glucose) to pyruvate.

Isokinetic (IK): A contraction in which constant maximal tension is developed by a muscle while moving at a constant speed or velocity through a joint's full range of motion.

Isometric (IM): A contraction during which there is no change in the length of the muscle.

Isotonic (IT): A contraction in which a resistance is moved without changing velocity.

Lactic acid or lactate: A metabolic by-product resulting from the catabolism of carbohydrate in the absence of oxygen.

Mitochondrion (singular); Mitochondria (plural): Membranous structures found in the cytoplasm of all cells in which ATP is manufactured (Tortora & Anagnostakos, 1987).

Myofibril: The part of a muscle fiber containing actin, myosin, troponin, and tropomyosin filaments (Ganong, 1993).

Polycose®: Powdered glucose polymers (Abbott Laboratories, Columbus, OH).

Work: A force exerted with displacement but without respect to time (Fox et al., 1993).

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APPENDICES

APPENDIX A

FORMS USED IN THIS STUDY

SUBJECT CONSENT FORM
Wellness & Sport Testing Program
Human Performance and Ergonomics Laboratory
Montana State University

1. The procedures to be followed, including those which are experimental:
You are being asked to participate in a study to investigate the physiologic response to a nutritional supplement. The supplement is not investigational and is available commercially. As a participant, you may receive the supplement itself or a placebo.

The subject for this study is ______________________________. This subject exhibits no known cardiovascular or pulmonary disease, pregnancy, metabolic disorders (i.e., diabetes), or contraindications to exercise.

Physiologic measurements will include an initial evaluation of electrical activity of the heart (ECG), anthropometric measurements (total body weight, height, percent body fat, and lean mass), and upper body isokinetic and isotonic strength.

2. Participant time requirements:
The amount of total time that the subject will be asked to participate in this study is 20 days (see Fig. 1). During the 20 day study, the subject must attend 5 testing sessions, in the Human Performance and Ergonomics Laboratory (HPEL), for approximately 1 hour each. Additionally, during 14 days of the 20 day study, the subject will be required to report to the HPEL 5 times daily (total of 5 minutes/day) to consume the supplement or placebo.

Figure 1: Experimental Time Line

Day 1: Testing	Day 3: Testing	Day 6: Testing	Day 13: Testing	Day 20: Testing

Cross-hatches indicate that no supplement /placebo will be consumed; shading indicates that oral supplement/placebo will be consumed 5 times daily.

3. The participant discomforts and risks:
a. Electrocardiographic (ECG) evaluation- The preparatory phase for the ECG will involve roughing the skin with a piece of gauze and alcohol to optimize adhesion and conduction of the electrodes. Cardiac responses will be monitored through an electrocardiograph device (ECG) and blood pressures will be taken during supine (lying down), hyperventilation (breathing rapidly and deeply in a sitting position), and standing. The ECG preparation may cause slight discomfort in the areas of electrode placement which may sting slightly, similar to a rug burn, but the discomfort should subside within two days. Some discomforts associated with the testing procedure may include occasional abnormal blood pressure response, syncope (light-headedness), dysrhythmia (disturbance in normal heart rhythm), severe dyspnea (shortness of breath), and in very rare instances, heart attack. Every effort will be made to minimize these inherent risks through preliminary examination and observations during testing by trained personnel according to the American College of Sports Medicine guidelines for testing procedures. The overall procedural risk in this study is very small and the associated discomforts and risks will be reviewed just prior to the evaluation.
b. Upper body muscular strength- Each testing session will involve evaluation of strength through two movements: elbow flexion/extension (biceps curl) and internal rotation of the shoulder (simulated overhand throw). Strength will be evaluated in 2 modes; isokinetic (speed is controlled but resistance varies) and isotonic (resistance is constant but speed can vary). The subject will be asked to exert maximal effort during each testing mode and movement. Tests will be separated with two minute rest periods. Associated risks during these procedures are minimal since these movements are experienced in competitive sporting events and routine strength conditioning; however,

tests will be performed in a controlled situation. Given the nature of the subject, and prior warm-up and stretching procedures, the risks are minimal. The most common discomfort incurred may be temporary muscular soreness due to the maximal effort exerted.

c. Consumption of nutritional supplement- Few side effects associated with consumption of the supplement have been reported. They include occasional transient gastrointestinal discomfort (i.e., mild nausea, diarrhea) and temporary fluid retention.

d. Determination of lean body mass- The skinfold procedure, which involves measuring the width of a surface skinfold at particular sites on the body using a caliper, does not present a risk to the subject.

4. **Psychological assessment**
The subject will be asked to complete a widely-accepted psychometric inventory called the *Profile of Mood States (POMS)* which evaluates typical mood states including tension, depression, anger, vigor, fatigue, and confusion. The POMS will be completed 3 times during the 20 day study.

5. **The benefits expected:**
Initial ECG evaluation and upper body strength values may serve as an index of general cardiac health status, athletic performance potential, and physical well-being. Results should specifically be of value to female athletes who are using or considering the use of this nutritional supplement for the purpose of enhancing upper body strength and/or power.

6. **Statement of understanding:**
I, ____________________________ (please print), should benefit from this study by receiving information related to my cardiac health. I understand that the procedures are those which will offer the minimal amount of risk to me while allowing the collection of significant and relevant data.

If I desire, I will be given an copy of the results of my ECG evaluation and muscular strength/power. I understand there will be no monetary compensation for my participation in this study.

I have been offered an answer to any inquiries concerning the procedures prior to signing voluntarily this consent form and I expect that future inquiries will be answered to my satisfaction. I understand that I am free to withdraw my consent and to discontinue my participation in this study at any time and for any reason. Should I choose to do so, this will be without prejudice and what results were obtained will still be reported to me upon request.

I understand that participation in this study is voluntary and have been assured that steps will be taken to ensure the confidentiality of my individual results which will not be shared with anyone or will not affect my status on any team. The only information available to individuals other than myself will be in average and statistical form. Neither my name nor any other personal descriptor will be associated with publication or presentation of the results of this project.

I have not been requested to waive or release the institution, its agents, or sponsors from liability for the negligence of its agents or employees.

I, the undersigned, have understood the above explanations and give my consent to my voluntary participation in this research project.

If I have additional questions about my rights as a subject or the manner in which this research is conducted I can contact Dr. Stephen Guggenheim, Chair Human Subjects Committee at 406-994-4411.

I □ do □ do not (please check one) consent to being videotaped or photographed at some time during my testing sessions.

Participant Signature: ____________________ Witness Signature: ______________

Date:____________Please contact the following individuals if you have further questions.

Michael C. Meyers, PhD, MS, FACSM	Karyn H. Ward, RD
Assistant Professor, Exercise Physiology	Lab Technician; Graduate student
(406) 994-6324	(406) 994-6325

Montana State University

Human Performance and Ergonomics Laboratory

Medical History Form--All Information Is Private And Confidential

General Information

___Mr. ___Ms. ___Miss ___Mrs. ___Dr. Sex ____M ____F Date___/___/___

Name (Last, First, MI):____

Address ____
Number and Street City, State Zip

Phone (___)_______ SSN ___-___-___ DOB ___/___/___

Physician

Dr. ____ Phone (___)________

Address ____
Number and Street City, State Zip

Marital Status___Single ___Married ___Divorced ___Widowed ___Separated

Education (Check highest level attained)

___Grade School___ Jr. High___High School___2-yr college (or 4-yr not completed)

___College Graduate___Postgraduate School

Occupation________________________________ **Employer**____________________

____ (___)

Employer Address City, State Zip Phone

Women Only check all that apply:

___ Currently pregnant (if yes:______weeks) No. of pregnancies ______________

___ Taking oral contraceptives No. of children ______________

___ Menstrual problems (comment below if yes) 1st day of last menstruation__________

___ Recurrent itching/discharge Date last pelvic exam ____________

___ Childbirth complication (comment below if yes) results: ______nl._____abnl.

___ Breast discharge/lumps

___ Urinary incontinence

Comments: ____

____

Men and Women answer the following:

List any prescribed *and* any self-prescribed medications and dietary supplements you are taking:

____

____

____

Date of last complete physical examination: ____________________

results: ______nl. _______abnl. (please comment:________________________)

Date of last chest x-ray (if ever): ____________________

results: ______nl. _______abnl. (please comment: ________________________)

Date of last electrocardiogram (if ever): ____________________

results: ______nl. _______abnl. (please comment: ________________________)

Date of last dental examination: ____________________

results: ______nl. _______abnl. (please comment: ________________________)

List any other medical or diagnostic tests you have had in the past 5 yrs:

____

List hospitalizations (include dates and purpose for hospitalization):

____

List any drug or food allergies:

____

Please explain the purpose for your visit to this laboratory:
___ To participate in a research study
___ To determine current level of fitness/receive recommendations for an exercise program
___ Other (please explain): ______________________

Present Health

Check only those conditions that apply to you. Makes comments below if further explanation needed.

___ High blood pressure
___Low blood pressure
___ Chest pain
___ "Thumping" or "racing" of the heart
___ Heart skips a beat or seems to have extra beats
___ Ankles that tend to swell
___ Hands and feet that are often cold even in warm weather
___ Leg cramps
___ Difficulty breathing
___ Shortness of breath
___ Out of breath while sitting or laying down
___ High cholesterol
___ Chronic or recurrent morning cough
___ One or more episodes of coughing up blood
___ Anxiety or depression
___ Chronic fatigue, difficulty sleeping, and/or increased irritability
___ Migraine or recurrent headaches
___ My joints are swollen, stiff, or painful
___ Leg pain even after walking only a short distance
___ Back pain
___ Men only: Prostate trouble
___ Stomach or intestinal problems (heartburn, ulcers, constipation, diarrhea, etc.)
___ Vision or hearing problems
___ Recent change in a wart or mole

Comments:______________________

Please indicate if you've had any history of the following conditions:

___ Heart attack
How long ago? ________
___ Heart murmur
___ Diseases of the arteries
___Other heart problems
___ Varicose veins
___ Arthritis in legs or arms
___ Diabetes or abnl. blood sugar test
___ Phlebitis
___ Dizziness or fainting spells
___ Thyroid problems
___ Pneumonia
___ Bronchitis
___ Asthma
___ Abnl. chest x-ray
___ Other lung diseases
____ Injuries to back, arms, legs, joints

___ Broken bones
___ Jaundice or gallbladder problems
___ Kidney or urinary problems
___ Polio
___ Epilepsy or seizures
___ Stroke
___ Diphtheria
___ Scarlet Fever
___ Infectious Mononucleosis
___ Anemia
___ Nervous or emotional problems

Comments:______________________
____
____
____

Family Medical History

Father: __ Alive __current age: __yrs general health:__ excellent __ good __fair __ poor
__ Deceased at age:__yrs cause of death:______________________________

Mother:__ Alive__ current age:__yrs general health: __excellent __ good__ fair __ poor
___ Deceased at age:_____yrs cause of death:_____________________________

Siblings: No. of brothers:_____ No. of sisters:______ Age range:_________
Health problems:____
____

If your parents, siblings, grandparents, aunts, or uncles have had any of the following, please indicate with a check mark and comment below as needed:

___ Heart attacks under age 50
___ Strokes under age 50
___ High blood pressure
___ Elevated cholesterol
___ Diabetes
___ Asthma or hay fever
___ Congenital heart disease
___ Heart operations
___ Glaucoma
___ Obesity (20 or more lbs. overweight)
___ Leukemia or cancer under age 60

Comments:____
____

Additional Cardiac Risk Factor Assessment

SMOKING

Have you ever smoked (cigarettes, cigars, or a pipe)?___yes___no (go to WEIGHT)
Do you currently smoke? ___yes___no
If you do/did smoke, how many per day? ___ cigarettes cigars pipe Age started:___
If you have quit smoking, when was your last (month/year)? _________________

WEIGHT

What do you consider a good weight for yourself? _____lbs.
Most you've ever weighed (indicate if it is pregnancy weight)?_____lbs.@age_____
Current weight:______lbs.; Weight 1 yr. ago:______lbs.; Weight @ age 21:______lbs.

NUTRITIONAL INTAKE

The following is a food frequency list. Please indicate the average number of times you consume each food item per week.

______ Eggs ______ Beef ______ Pork ______ Fowl
______ Fish ______ Fried foods ______ Desserts ______ Coffee/Tea
______ Whole milk ______ Low fat milk ______ Skim milk

What is your usual intake of the following alcoholic beverages?
Beer ___ none ___ occ ___ often (#/wk:__) Wine ___ none ___ occ ___ often (#/week:____)
Hard liquor ___none ___ occ ___ often (#/week:_)
Has your intake of alcoholic beverages changed recently?______________________

EXERCISE

Are you currently involved in a regular exercise program? ___ yes ___no
Do you regularly walk or run one or more miles continuously? ___yes ___no
If yes, avg no. of miles you walk/run per workout or day: ______ Avg time/mile: ________
Do you lift weights or do calisthenics? ___yes ___no
Are you involved in an aerobic exercise program (e.g. aerobics class)? ___yes ___no
Have you recently taken any "fitness tests?" ___yes ___no If yes, please describe (include results):____
Do you frequently participate in competitive sports? ___yes ___no If yes, please list (include frequency): ____
Please list any high school or collegiate sports in which you participated: ______________
____

ELECTROCARDIOGRAPHY REPORT

RESTING ECG:

Supine:

Rate_______ PR Interval_________ QRS Duration________
QT Interval______ Axis_______ Rhythm_______ ST Segment_________
Remarks____

Hypervent:

Rate_______ PR Interval_________ QRS Duration________
QT Interval______ Axis_______ Rhythm_______ ST Segment_________
Remarks____

Standing:

Rate_______ PR Interval_________ QRS Duration________
QT Interval______ Axis_______ Rhythm_______ ST Segment_________
Remarks____

IMPRESSION: Normal________ Equivocal__________ Abnormal__________

EXERCISE ECG:

Protocol___________ MAX BP_____/______ MAX HR____________
Duration of test____

IMPRESSION:

___________ **Normal**

___________ **Inconclusive test**

_____ Submaximal _____ Mechanical Failure
_____ Drug Effect (Name)_____________
_____ Other______________________
Remarks_____________________________________

___________ **Equivocal**

______ST Segment Depression/Elev(.5-1 mm Amp,≥.08 sec)
______PVCs (10-20%/min)
______Excessive Hypertension
______T Wave Changes
______Other: ______________________________

___________ **Abnormal**

________ST Changes ≥1 mm
________Depression ________Elevation
________Downslope ________Upslope
________Horizontal
________Angina_____________________________
________LBBB (induced)_____________________
________Hypotension________________________
________R on T PVCs: #/min_________________
________Exercise Induced 2 or 3° Block______
________Uniform PVCs (>20-30%)
________A Couplet (with known CHD)
________Multiform PVCs: #/min______________
________Ventricular Tachycardia_____________
________RBBB (Induced)_____________________
________Supraventricular Tachycardia________
________Other

REMARKS:____

______________________________ ______________________________
Supervisor's Signature Date Physician's Signature Date

SKINFOLD ASSESSMENT

Name_______________________________ Date___________

Age_______Gender______
Wt_______lbs_________kg
Ht________in_________cm

I. **Seven-Site Skinfold**

Trial:	1	2	3	Avg
Chest	___	___	___	___
Axilla	___	___	___	___
Tricep	___	___	___	___
Subscapular	___	___	___	___
Abdominal	___	___	___	___
Suprailium	___	___	___	___
Thigh	___	___	___	___

Sum of 7 sites______

II. **Generalized Regression Equations**

A. **Body Density (D_b)** (Jackson & Pollock, 1977; Jackson, Pollock, & Ward, 1980)

Male ($\sum 7$): D_b = 1.112 - 0.00043499[sum of 7-sites(____)] +
0.00000055[sum of 7-sites^2(____)^2] - 0.00028826[age(___)] = _____

Female ($\sum 7$): D_b = 1.0979 - 0.0004697[sum of 7-sites(____)] +
0.00000056[sum of 7-sites^2(____)^2] - 0.00012828[age(___)] = _____

B. **Body Fat Percentage** (Brozek et al., 1963)

% body fat = $\frac{4.570}{D_b}$ - 4.142 x 100 = _________%

III. **Body Composition**

A. Fat Mass = Wt. (lbs) x % Body Fat/100 = __________lbs
B. Lean Body Mass = Wt. (lbs) - Fat Mass (lbs) = __________lbs
C. % Lean Body Mass = Lean Body Mass/Body Wt. x 100 = __________%

FOOD FREQUENCY QUESTIONNAIRE

The following is a list of foods grouped into 22 categories which include Baby Foods, Beverages, Breads, Breakfast Cereals, Combination Foods, Dairy Products, Desserts, Eggs, Fast Foods, Fats & Oils, Fish, Frozen Dinners, Fruits, Grains, Meats, Miscellaneous, Nuts & Seeds, Poultry, Sauces & Dips, Soups, Sugars & Sweets, and Vegetables. Please read through the lists and do the following:

1. **Circle** the food items that you normally consume on a weekly basis: Crackers-Ritz
2. Indicate with a **number** to the side how many times each week you normally consume a serving of the food item: 2 Crackers-Ritz
3. On the last page, **list** any foods not appearing on the list that you consume weekly.

When completing this Food Frequency Questionnaire, be as accurate as you can when you estimate the frequency with which you consume each of the food items during an average week. Remember to include between-meal snacks in addition to regular meals. Thank you for your participation.

BABY FOODS

Baby Food-cereal-barley-milk
Baby Food-cereal-egg yolks
Baby Food-cereal-mixed-milk -USDA
Baby Food-cereal-oatmeal-milk
Baby Food-cereal-rice-milk
Baby Food-cottage cheese + pineapple
Baby Food-dessert-apple betty
Baby Food-dessert-arrowroot cookie
Baby Food-dessert-bananas & tapioca
Baby Food-dessert-cookies-enrich
Baby Food-dessert-custard choc-USDA
Baby Food-dessert-custard-vanilla
Baby Food-dessert-dutch apple
Baby Food-dessert-fruit-str
Baby Food-dessert-mango/tapioca
Baby Food-dessert-orange pudding
Baby Food-papaya/apple/tapioca
Baby Food-dessert-peach cobbler-str
Baby Food-dessert-plums & tapioca
Baby Food-dessert-prunes & tapioca
Baby Food-egg yolks
Baby Food-fruit juice-apple
Baby Food-fruit juice-apple bluberry
Baby Food-fruit juice-apple peach
Baby Food-fruit juice-apple prune
Baby Food-fruit juice-mixed fruit
Baby Food-fruit juice-orange
Baby Food-fruit juice-orange apple
Baby Food-fruit juice-orange apricot
Baby Food-fruit juice-orange prune
Baby Food-fruit-applesauce
Baby Food-fruit-peaches
Baby Food-fruit-pears
Baby Food-fruit-pears & pineapple
Baby Food-macaroni & cheese
Baby Food-meat-beef
Baby Food-meat-beef & egg noodles
Baby Food-meat-beef lasagna
Baby Food-meat-beef stew
Baby Food-meat-beef-macr+tomato
Baby Food-meat-chicken
Baby Food-meat-chicken & noodles
Baby Food-meat-chicken stew-str
Baby Food-meat-ham
Baby Food-meat-lamb
Baby Food-meat-liver
Baby Food-meat-pork
Baby Food-meat-turkey
Baby Food-meat-turkey & rice
Baby Food-meat-veal
Baby Food-meat-veal /vegetables
Baby Food-pretzels-enrich
Baby Food-soup-cream of chicken
Baby Food-teething biscuits
Baby Food-vegetable-beef
Baby Food-vegetable-beef-liver
Baby Food-vegetables-beans-green
Baby Food-vegetables-beans-green/bu
Baby Food-vegetables-beets-str
Baby Food-vegetables-carrots-str
Baby Food-vegetables-corn-crm-str
Baby Food-vegetables-garden
Baby Food-vegetables-mixed
Baby Food-vegetables-peas
Baby Food-vegetables-peas-crm-str
Baby Food-vegetables-spinach-crm
Baby Food-vegetables-squash
Baby Food-vegetables-sweet potato
Baby Food-zwieback cookies

BEVERAGES

Ale-mild-American
Beer-Budweiser
Beer-light
Beer-Michelob
Beer-Natural light
Beer-reg
Br+Y-california
Br+Y-cognac-pony
Carnation inst breakfast-choc
Carnation inst breakfast-eggnog
Carnation inst breakfast-vanilla
Champagne-domestic-glassful
Choc beverage powd-dry milk add
Choc beverage powd-no dry milk
Cider-fermented
Cocktail-daiquiri
Cocktail-eggnog
Cocktail-gin rickey
Cocktail-highball
Cocktail-manhattan-frozen
Cocktail-martini-frozen
Cocktail-mint julep
Cocktail-old fashioned
Cocktail-pina colada-home recipe
Cocktail-planters punch
Cocktail-rum sour
Cocktail-tom collins-foz
Coffee substitute-made
Coffee-brewed
Coffee-decaffeinated-Sanka-made
Coffee-inst-made
Cordials/liqueur-54 proof
Fruit punch drink-can
Fruit punch-powd-made + water

Gatorade-thirst quenching drink
Hot Cocoa-made + milk-home recipe
Liqueurs-anisette
Liqueurs-apricot br+y
Liqueurs-benedictine
Liqueurs-creme de menthe
Liqueurs-curacao
Ovaltine-choc flavor-made/milk
Ovaltine-malt flavor-made + milk
Postum-inst grain beverage-dry mix
Soda-club-carbonate
Soda-cola type-carbonate
Soda-cream flavored-carbonate
Soda-diet cola-nutraswt-carbonate
Soda-Dr. Pepper type cola-carbonate
Soda-ginger ale-carbonate
Soda-grape-carbonate
Soda-lemon lime/7Up-carbonate
Soda-root beer-carbonate
Soda-tab-lo calorie cola-carbonate
Soda-tonic water/quinine-carbonate
Tang-inst break drink-orange-dry
Tea-brewed
Tea-herbal-brewed
Tea-inst-made-sweetened
Tea-inst-made-unsweet
Water-mineral-Perrier
Water-municipal tap
Whiskey/gin/rum/vodka-100 proof
Whiskey/gin/rum/vodka-80 proof
Whiskey/gin/rum/vodka-86 proof
Whiskey/gin/rum/vodka-90 proof
Whiskey/gin/rum/vodka-94 proof
Wine cooler-white wine + 7up
Wine-california/red-glassful
Wine-dessert
Wine-madeira-glassful
Wine-muscatel/port-glassful
Wine-red-table
Wine-rose-table
Wine-sauterne-glassful
Wine-sherry-dry-glassful
Wine-vermouth-dry-glassful
Wine-vermouth-sweet-glassful
Wine-white-table

Breads

Bagel-egg-3.5"
Bagel-water-3 inch diameter
Biscuit-baking powd-home recipe
Biscuit-baking powd-made from mix
Bread stick-vienna type
Bread-boston brown-can
Bread-cracked wheat-enr
Bread-crisp-breakfast-wasa
Bread-crisp-whole grain-wasa
Bread-french
Bread-italian
Bread-melba toast-plain
Bread-melba toast-wheat
Bread-melba toast-wheat-unsalted
Bread-mixed grain
Bread-mixed grain-toasted
Bread-oat bran-no chol-monterey
Bread-oatmeal + bran-oatmeal
Bread-pita-white
Bread-pumpernickel
Bread-raisin
Bread-rye-american-light
Bread-vienna
Bread-wheat-firm-enrich-toasted
Bread-wheat-soft-enrich-toasted
Bread-wheat-toasted/home recipe
Bread-white-comm prep
Bread-white-firm-enrich
Bread-white-firm-enrich-toasted
Bread-white-soft-enrich-crumbs
Bread-white-soft-enrich-cubes
Bread-white-soft-toasted
Bread-whole wheat-firm-enrich
Bread-whole wheat-home rec
Bread-whole wheat-soft-enrich
Breadcrumbs-dry-grated-enrich
Cornbread-homerecipe/whole milk
Cracker-animal-Ralston
Cracker-cheddar snacks-Ralston
Cracker-cheese
Cracker-cheese + chives-Ralston
Cracker-cheese snacks-Ralston
Cracker-graham-plain
Cracker-graham-sugar-honey
Cracker-oyster-Ralston
Cracker-rich + crisp-Ralston
Cracker-Ritz
Cracker Rye Krisp-natural-Ralston
Cracker-Rye Krisp-seasoned-Ralston
Cracker-Rye Krisp-sesame-Ralston
Cracker-Rye Snacks-Ralston
Cracker-Rye wafers
Cracker-saltines
Cracker-sesame + wheat-Ralston
Cracker-snackers-Ralston
Cracker-Triscuits
Cracker-wheat snacks
Cracker-Wheat Thins
Cracker-whole wheat-lo Na
Cracker-whole wheat-Na free
French toast-from froz-Campbells
French toast-from home recipe
French toast + butter
Muffin-blueberry bran-health val
Muffin-blueberry-from home recipe
Muffin-bran-from home recipe
Muffin-corn-from home recipe
Muffin-corn-from mix
Muffin-english-plain-toasted
Muffin-english-plain-untoasted
Muffin-home recipe-plain-each
Muffin-raisin oat bran-health val
Muffin-soy
Pancakes-buckwheat-from mix
Pancakes-plain-from mix
Pancakes-plain-home recipe
Pancakes-soybean-25% soy flour
Roll-brown & serve-enrich
Roll-cinnamon
Roll-cloverleaf-from home recipe
Roll-cloverleaf/pan-enriched
Roll-croissant-sara lee
Roll-hamburger-plain
Roll-hard
Roll-submarine/hoagie-enrich
Roll-whole wheat-homemade
Waffles-enrich-from home recipe
Waffles-enrich-from mix-egg & milk
Waffles-froz-each
Waffles-oat bran-no chol-eggo

Breakfast Cereals

Cereal-100% bran
Cereal-100% natural-plain
Cereal-40% bran flakes-Kelloggs
Cereal-40% Bran flakes-post
Cereal-All Bran-USDA
Cereal-Alpha bits-USDA
Cereal-Apple Jacks-USDA
Cereal-Bran Buds-USDA
Cereal-Bran Chex
Cereal-Bran Flakes-Ralston
Cereal-C.W. Post-plain
Cereal-C.W. Post-+ raisins
Cereal-Cap'n Crunch-crunchberries
Cereal-cap'n crunch-USDA
Cereal-Cheerios-USDA
Cereal-Cocoa Krispies
Cereal-Cocoa Pebbles-USDA
Cereal-Corn Bran-USDA
Cereal-Corn Chex
Cereal-corn flakes-Kelloggs-USDA
Cereal-corn Flakes-lo Na
Cereal-corn Flakes-Ralston
Cereal-corn Grits-white-enr-cooked
Cereal-corn Grits-white-unenr-ckd
Cereal-corn-shredded-add sugar
Cereal-cracklin bran-USDA
Cereal- Cracklin oat bran-Kelloggs
Cereal- Cream of rice-cooked
Cereal- Cream of wheat-inst
Cereal- Cream of wheat-pac size
Cereal- Cream of wheat-reg-hot
Cereal- Crisp rice-lo Na
Cereal- Crispy rice
Cereal- Crispy wheats + raisins
Cereal-f arina-enr-cooked/salt
Cereal-f arina-enr-dry
Cereal-fortified oat flakes
Cereal-froot Loops-Gen Mills
Cereal-frosted flakes-Kellogg

Cereal-frosted flakes-Ralston
Cereal-frosted mini wheats-Kelloggs
Cereal-frosted rice krispies-Kelloggs
Cereal- Granola-homemade
Cereal- Granola-nature val
Cereal- Grape nuts flakes-Post-USDA
Cereal- Grape nuts-Post-USDA
Cereal- Heartl+ natural-plain
Cereal- Honey bran
Cereal- Honey nut cheerios-Gen Mills
Cereal- Honeycomb-Post
Cereal- King Vitaman
Cereal- Kix
Cereal- Life-plain/cinnamon
Cereal- Lucky charms-USDA
Cereal- Malt O meal-cooked
Cereal- Maypo-cooked-hot
Cereal- Nutri Grain-barley
Cereal- Nutri Grain-corn
Cereal- Nutri Grain-rye
Cereal- Nutri Grain-wheat
Cereal- Oat Bran-cooked
Cereal- Oat Bran-Kelloggs
Cereal- Oat Bran-Quaker
Cereal- Oat Bran-raisin/spice-hot
Cereal- Oatmeal-cooked
Cereal- Oatmeal-raw
Cereal- oats-apple/cinnamon-Quaker
Cereal-oats-bran/raisin-Quaker-pac
Cereal-oats-cinnamon/spice-Quaker
Cereal-oats-maple/sugar-Quaker-pac
Cereal-oats-plain-Quaker-inst-pac
Cereal-oats-puffed-add sugar
Cereal-oats-reg-dry
Cereal-Product 19-Kelloggs
Cereal-Raisin bran-Kelloggs
Cereal-Raisin Bran-Post
Cereal-Raisin Bran-Ralston
Cereal-Ralston-cooked-hot
Cereal-Rice Chex
Cereal-Rice Krispies-Kelloggs
Cereal-rice-puffed-add sugar
Cereal-rice-puffed-plain
Cereal-roman meal-cooked
Cereal-Special K-Kelloggs
Cereal-Sugar Corn Pops-Kelloggs
Cereal-Sugar Smacks-Kelloggs
Cereal-Super Sugar Crisp-Post
Cereal-Tasteeos
Cereal-Team-USDA
Cereal-Toasties-Post
Cereal-Total-Gen Mills
Cereal-Trix-Gen Mills
Cereal-Wheat Chex
Cereal-wheat flakes-add sugar
Cereal-wheat germ-sugar/honey
Cereal-wheat germ-toasted
Cereal-wheat-puffed-add sugar
Cereal-wheat-puffed-plain
Cereal-wheat-rolled-cooked-hot
Cereal-wheat-shredded-biscuit
Cereal-wheat-whole meal-cooked-hot
Cereal-Wheatena-cooked
Cereal-Wheaties
Cereal-whole wheat natural

Combination Foods

Beef potpie-home recipe-1/3 of 9" pie
Beef stew-+ vegetables
Beef-raviolios-can + meat sauce
Burrito-beans + cheese
Cheese souffle-home recipe
Chicken a la king-cooked-home
Chicken + noodles-cooked-recipe
Chicken chow mein-can
Chicken chow mein-home recipe
Chicken potpie-baked-home recipe
Chili con carne w/beans-can
Chili + beans-can
Chimichanga-beef
Chop suey-+ beef + pork-home recipe
Corn dog-plain
Corned beef hash-can
Enchilada-cheese
Enchirito-cheese/beef/bean
Frijoles-beans + cheese
Hamburger-single meat/cheese/bacon
Hot dog-plain + bun-generic
Macaroni & cheese-enrich-can
Macaroni & cheese-enrich-recipe
Meatloaf-+ celery + onions
Nachos-cheese
Omelet-two eggs-ham + cheese
Pizza-cheese-baked
Pizza-cheese/meat/vegetable
Pizza-pepperoni-baked
Pork/Beans + frankfurters-can
Pork/Beans + sweet sauce-can
Pork/Beans + tomato sauce-can
Salad-carrot raisin-home recipe
Salad-chef-+ ham + cheese
Salad-chicken
Salad-coleslaw
Salad-crab
Salad-fruit-can-juice pack
Salad-fruit-can-water pack
Salad-green salad-tossed
Salad-macaroni
Salad-m+arin orange gelatin
Salad-potato
Salad-taco
Salad-three bean-Alex
Salad-three bean-can-Del Monte
Salad-tuna/celery/mayo/pickle/egg
Salad-waldorf gelatin
S+Wich-blt-+ mayo
S+Wich-club
S+Wich-ham + cheese
S+Wich-roast beef + cheese
S+Wich-roast beef-plain
S+Wich-steak
S+Wich-submarine-roast beef
S+Wich-submarine-+ coldcuts
Spaghetti/tomato/cheese-can
Spaghetti/tomato/cheese/home recipe
Spaghetti/tomato/meat-can
Spaghetti/tomato/meat/home recipe
Spinach souffle
Taco

Dairy Products

Cheese food-american-pasteur
Cheese spread-american-processed
Cheese-american-pasteur process
Cheese-blue
Cheese-blue-crumbled-unpacked
Cheese-brick
Cheese-brie
Cheese-camembert-wedge
Cheese-caraway
Cheese-cheddar-cut pieces
Cheese-cheddar-inch cubes
Cheese-cheddar-lo fat-lo Na
Cheese-cheddar-shredded
Cheese-cheshire
Cheese-colby
Cheese-cottage-1% lofat-unpacked
Cheese-cottage-2% lofat-unpacked
Cheese-cottage-4% fat-lg curd
Cheese-cottage-4% fat-sm curd
Cheese-cottage-dry curd-uncreamed
Cheese-cottage-+ fruit-unpacked
Cheese-cream
Cheese-edam
Cheese-feta-ounce
Cheese-fontina
Cheese-garlic-lofat-lo Na-pauly
Cheese-gjetost
Cheese-gouda
Cheese-gruyere
Cheese-limburger
Cheese-monterey jack
Cheese-monterey jack-lofat-lo Na
Cheese-mozzarella-from skim milk
Cheese-mozzarella-made whole milk
Cheese-muenster
Cheese-neufchatel
Cheese-parmesan-grated
Cheese-pimento-processed
Cheese-port du salut
Cheese-provolone
Cheese-ricotta-made + part skim milk
Cheese-ricotta-made + whole milk
Cheese-romano
Cheese-roquefort
Cheese-swiss
Cheese-swiss-lofat-lo na-pauly
Cheese-swiss-pasteur process
Cheese-tilsit
Cream-coffee-table-light-fluid

Cream-half & half-milk + cream-fluid
Cream-imitation-liq-non dairy-froz
Cream-imitation-non dairy-powd
Cream-sour-cultured
Cream-sour-half & half
Cream-sour-imitation
Cream-sour-imitation-nf dry milk add
Cream-whipped-imit-non dairy-froz
Cream-whipped-imit-non dairy-powd
Cream-whipped-imitation-pressurized
Cream-whipped-imitation-pressurized
Cream-whipping-heavy-unwhipped-fluid
Cream-whipping-light-unwhipped-fluid
Milk-1% fat-lofat-fluid
Milk-1% fat-nonfat milk solids add
Milk-1% fat-protein fortified
Milk-2% fat-fluid-protein fortified
Milk-2% fat-lofat-fluid
Milk-2% fat-nonfat milk solids add
Milk-buttermilk-cultured-fluid
Milk-buttermilk-dried-sweet cream
Milk-choc-1% fat-fluid
Milk-choc-2% fat-fluid
Milk-choc-whole-vit d
Milk-condensed-sweetened-can
Milk-eggnog-commercial
Milk-evaporated-skim-can
Milk-evaporated-whole-can
Milk-goat-whole-fluid
Milk-human-whole-mature
Milk-imitation
Milk-indian buffalo-whole
Milk-malted-choc flavor-made
Milk-malted-natural flavor-made
Milk-nonfat/skim-fluid
Milk-nonfat/skim-instized-dried
Milk-nonfat/skim-instized-envelope
Milk-nonfat/skim-milk solids add
Milk-nonfat/skim-protein fortified
Milk-soy-fluid
Milk-whole-dry
Milk-whole-lo Na
Milk-whole-reg-3.3% fat-fluid
Milkshake-choc-thick
Milkshake-vanilla-thick
Mocha mix-non dairy
Whey-acid-dry
Whey-acid-fluid
Whey-sweet-dry
Whey-sweet-fluid
Yogurt-fruit flav-lofat-add solids
Yogurt-coffee-lofat-Dannon
Yogurt-plain-lofat-milk solids add
Yogurt-plain-nonfat-milk solids add
Yogurt-plain-whole milk-no solids

Desserts

Brownie-choc icing-from froz
Brownie-comm prep-1-3/4x3/4"
Brownie-+ nuts-home recipe
Cake-angel food-dry mix-prep
Cake-coffee-made from mix
Cake-devils food + icing-from mix
Cake-gingerbread-made from mix
Cake-pineapple upside down-recipe
Cake-pound-butter-home recipe
Cake-sheet-no icing-home recipe
Cake-sheet-plain-uncook white icing
Cake-sponge-home recipe
Cake-strawberry shortcake
Cake-streusel type-+ icing-from mix
Cake-white/choc icing-home recipe
Cake-yello/choc icing-home recipe
Cheesecake-comm prep
Cheesecake-Wt Watchers-froz
Cobbler-Wt Watchers-froz
Cookie-choc chip-from home recipe
Cookie-choc chip-from mix
Cookie-choc chip-lo Na
Cookie-fig bar
Cookie-fudge-lo Na
Cookie-gingersnap/ home recipe
Cookie-macaroon
Cookie-oatmeal/raisin-from mix
Cookie-peanut butter-from mix
Cookie-plain-made from mix
Cookie-s+wich-choc/vanilla
Cookie-sugar-from mix
Cookie-vanilla wafer
Cupcake + choc icing
Cupcake-devils food + choc icing
Cupcake-no icing
Custard-baked
Danish pastry-apple
Danish pastry-cheese
Danish pastry-cinnamon-enr
Danish pastry-plain
Doughnut-cake
Doughnuts-yeast-glazed
Eclair-custard + choc icing
Froz yogurt-fruit varieties
Fruit bar-oat bran-nuts-Health Val
Fruit pie-fried
Fruitcake-dark-home recipe
Gelatin dessert-made
Gelatin-d zerta-lo calorie-made
Gelatin-Jello-sugar free-made
Granola bar
Granola clusters-Nature Val
Ice cream sundae-caramel
Ice cream sundae-hot fudge
Ice cream sundae-strawberry
Ice cream-french vanilla-soft serve
Ice cream-vanilla-hardened-10% fat
Ice cream-vanilla-rich-hard-16% fat
Ice milk-vanilla-hardened
Ice milk-vanilla-soft serve
Pie crust-baked-from home recipe
Pie crust-baked-made from mix
Pie-apple-home recipe
Pie-banana cream-home recipe
Pie-blueberry-home recipe
Pie-boston cream-home recipe
Pie-cherry-home recipe
Pie-choc cream-home recipe
Pie-custard-home recipe
Pie-lemon meringue-home recipe
Pie-mince-home recipe
Pie-peach-from home recipe
Pie-pecan-home recipe
Pie-pumpkin-home recipe
Popsicle
Pudding-banana cream-inst mix-Jello
Pudding-butterscotch-inst mix-Jello
Pudding-choc-cooked/mix & milk
Pudding-choc-home recipe-starch
Pudding-choc-inst-from mix
Pudding-choc-sugar free-2% milk
Pudding-coconut cream-inst mix-Jello
Pudding-corn
Pudding-lemon-inst mix-Jello
Pudding-rice + raisins
Pudding-tapioca cream-home recipe
Pudding-vanilla (blancmange)
Pudding-vanilla-sugar free-+ 2% milk
Sherbet-orange
Toaster pastries/pop tarts
Turnover-apple
Turnover-cherry
Turnover-lemon
Twinkie-Hostess

Eggs

Egg substitute-froz
Egg substitute-liq
Egg substitute-powd
Egg-duck-whole-fresh-raw
Egg-fried in butter-whole-lg-chicken
Egg-hard cooked-no shell-lg-chicken
Egg-poached-whole-lg-chicken
Egg-raw-white-lg-chicken
Egg-raw-whole-lg-chicken
Egg-raw-yolk-lg-chicken
Egg-scrambled/milk/butter

Fast Foods

Arby's-beef + cheese s+wch
Arby's-chicken breast s+wich
Arby's-club s+wich
Arby's-ham + cheese s+wich
Arby's-roast beef s+wich
Arby's-super roast beef s+wich
Arby's-turkey deluxe
Arthur Treacher-chicken s+wich
Burger King-croissant-egg + cheese
Burger King-croissant-egg/chese/ham
Burger King-Whopper hamburger
Chicken-breast + wing-breaded-fried
Chicken-drumstick+thigh-bread-fried
Churchs chicken-white meat