ผลของการเสริมสารครีเอทีนและให้ฮอร์โมนเอสโตรเจนทดแทน ร่วมกับการออกกำลังกายต่อการทำงานของกล้ามเนื้อหัวใจ ในแฮมสเตอร์ที่ถูกตัดรังไข่

นางเกษรา รักษ์พงษ์สิริ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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EFFECTS OF CREATINE SUPPLEMENTATION AND ESTROGEN REPLACEMENT IN COMBINATION WITH EXERCISE TRAINING ON CARDIAC FUNCTION IN OVARIECTOMIZED HAMSTERS

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เกษรา รักษ์พงษ์สิริ: ผลของการเสริมสารครีเอทีนและให้ฮอร์โมนเอสโตรเจนร่วมกับการออกกำลังกายต่อการทำงานของกล้ามเนื้อหัวใจในแฮมเตอร์ที่ถูกตัดรังไข่ (EFFECTS OF CREATINE SUPPLEMENTATION AND ESTROGEN REPLACEMENT IN COMBINATION WTH EXERCISE TRAINING ON CARDIAC FUNCTION IN OVARIECTOMIZED HAMSTERS) อ. ที่ปรึกษา : ผศ. น.ส.พ. ดร. สุวรรณเกียรติ สว่างคุณ, 170 หน้า.

เป็นที่ทราบกันดีว่า อุบัติการ และความรุนแรงของโรคหัวใจ มีความเกี่ยวข้องกับสตรีที่อยู่ในวัยหมดประจำเดือน ครีเอทีน (Cr) และฟอสโฟครีเอทีน (PCr) มีบทบาทสำคัญต่อพลังงานของกล้ามเนื้อ จากการทบทวนวรรณกรรมที่ผ่านมาแสดงให้เห็นว่า การเสริม ครีเอทีน, การให้ฮอร์โมนเอสโตรเจน (E_2) ทดแทน หรือการออกกำลังกายให้ผลดีต่อการทำงานของหัวใจ แต่ยังไม่มีการศึกษาถึงผลของการเสริม Cr และให้ E_2 ทดแทนร่วมกับการไม่ออกกำลังกายและการออกกำลังกาย ต่อการทำงานของหัวใจในภาวะหัวใจล้มเหลวที่เกิดจากการขาด E_2 การทดลองครั้งนี้ทำการตัดรังไข่ทั้งสองข้างในแฮมเตอร์เพศเมียพันธ์ Golden Syrian จำนวน 100 ตัว และแบ่งออกเป็นสองกลุ่ม ได้แก่ กลุ่มไม่ออกกำลังกาย และกลุ่มออกกำลังกาย แต่ละกลุ่มแบ่งย่อยออกเป็นกลุ่มควบคุม (C) และกลุ่มที่ได้รับสารต่างๆ วันละหนึ่งครั้ง ได้แก่ กลุ่มขาด Cr (Cr-, β-GPA 200 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม), กลุ่มเสริม Cr (Cr+, Cr monohydrate 200 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม), .กลุ่มให้ E_2 (E_2, 17 β-estradiol 30 ไมโครกรัมต่อน้ำหนักตัว 1 กิโลกรัม) และกลุ่มเสริม Cr ร่วมกับให้ E_2 (Cr+E_2, Cr monohydrate 200 มิลลิกรัมและ 17 β-estradiol 30 ไมโครกรัมต่อน้ำหนักตัว 1 กิโลกรัม) สำหรับสัตว์ทดลองที่อยู่ในกลุ่มออกกำลังกาย จะถูกฝึกให้วิ่งในวงล้อ (running wheel) เป็นเวลา 9 สัปดาห์ และวัดค่าเมดาโบลิซึมในขณะออกกำลังกาย (exercise metabolic rate, EMR) ทุกๆสัปดาห์โดยใช้ closed- circuit calorimeter เมื่อครบกำหนดการทดลอง ทำการเก็บตัวอย่างเนื่อเยื่อหัวใจ เพื่อวิเคราะห์หา 1) พลังงานของหัวใจจากปริมาณ Cr, PCr, TCr (Cr+PCr), โปรตีนที่นำ Cr เข้าสู่เซลล์ (CrT) และเอนไซม์ creatine kinase (CK) 2) การทำงานของหัวใจ (cardiac function) จาก QT-c interval, left ventricular developed pressure (LVDP) และ maximum rate of rise (dP/dt_{max}) และ 3) ภาวะ oxidative stress จากปริมาณ reduced glutathione (GSH), oxidized glutathione (GSSG), และระดับเอนไซม์ glutathione peroxidase (GPx) และทำการเก็บเลือดจากหัวใจเพื่อตรวจระดับ IGF-I ในซีรัม (serum IGF-I) ผลการทดลองพบว่า การออกกำลังกาย (10 นาทีต่อวัน เป็นเวลา 5 วันต่อสัปดาห์) สามารถเพิ่มพลังงานสำรองของหัวใจจากภาวะ oxidative stress ในสัตว์ทดลองที่ขาด E_2 และพบว่าการเสริม Cr หรือให้ E_2 ทดแทนร่วมกับการออกกำลังกายในสัตว์ทดลองที่ขาด E_2 มีผลเพิ่มการทำงานของหัวใจ, เพิ่มการสะสมปริมาณ metabolic phosphate ผ่าน Cr เมดาโบลิซึม, และเพิ่มระดับ serum IGF-I มากกว่าการเสริม Cr หรือให้ E_2 ทดแทนเพียงอย่างเดียว อีกทั้งพบว่า การให้ E_2 ร่วมกับการออกกำลังกายมีผลเพิ่มระดับ antioxidant reservation มากกว่าการให้ E_2 ทดแทนเพียงอย่างเดียว และนอกจากนั้นยังพบว่า การเสริม Cr และให้ E_2 ทดแทนร่วมกับการออกกำลังกาย ในสัตว์ทดลองที่ขาด E_2 ให้ผลดีที่สุด โดยมีผลเพิ่มโปรตีน CrT และตัวแปรทุกตัวที่ควบคุมการทำงานของหัวใจ

การทดลองครั้งนี้แสดงให้เห็นว่า การเสริม Cr และให้ E_2 ทดแทนร่วมกับการออกกำลังกาย ให้ผลป้องกันภาวะขาด E_2 ต่อการทำงานของหัวใจในแฮมเตอร์ ซึ่งแสดงให้เห็นประโยชน์ต่อการนำไปใช้ในการรักษาภาวะขาด E_2 ในสตรีที่อยู่ในวัยหมดประจำเดือน

สาขาวิชา สรีรวิทยา ลายมือชื่อนิสิต.....

ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา.....

4689656120: MAJOR PHYSIOLOGY
KEY WORD: CREATINE/ ESTROGEN/ CARDIAC FUNCTION/ GROWTH HORMONE/ INSULIN-LIKE GROWTH FACTOR-I/ OXIDATIVE STRESS/ LANGENDORFF/ METABOLIC RATE

KEDSARA RAKPONGSIRI: EFFECTS OF CREATINE SUPPLEMENTATION AND ESTROGEN REPLACEMENT IN COMBINATION WITH EXERCISE TRAINING ON CARDIAC FUNCTION IN OVARIECTOMIZED HAMSTERS. THESIS ADVISOR: ASST. PROF. SUWANAKIET SAWANGKOON, D.V.M. Ph.D., 170 pp.

Menopause in elder women is known to be related with the incidence and severity of many cardiovascular diseases. Creatine (Cr) and its phosphorylated form (PCr) also play an important role in muscle energetic. Reviews of previous data showed that Cr supplementation, estrogen (E_2) replacement, or exercise training alone demonstrated positive effects on cardiac function, but there is no systematic studies on the influence of combining for Cr supplementation and E_2 replacement (Cr+E_2) in non-exercise and exercise trainings on cardiac functions against failing hearts due to E_2 deficiency. In the present study, a hundred female Golden Syrian Hamsters were ovariectomized and divided into 2 groups of non exercise and exercise-trained animals. Each group was further separated into the control and 4 treatments of Cr depletion (Cr-, β-GPA 200 mg /kg BW.), Cr supplementation (Cr+, Cr monohydrate 200 mg/kg BW.), E_2 replacement (E_2, 17β-estradiol 30 μg/kg BW.), and Cr supplementation combined with E_2 replacement (Cr+E_2, Cr monohydrate 200 mg/kg BW. plus β-estradiol 30 μg/kg BW.) once daily. The 9-week wheel-running exercise was induced to the exercise-trained group after ovariectomy, and exercise metabolic rate (EMR) was measured weekly by using closed circuit calorimeter. After 9 weeks, all animals were sacrificed to determine 1) myocardial energy from the contents of Cr, PCr, total Cr (TCr), Cr transporter (CrT) protein, and CK activities, 2) cardiac function from QT-c interval, left ventricular developed pressure (LVDP), the maximum rate of pressure rise (dp/dt_{max}) and 3) markers of oxidative stress from reduce glutathione (GSH), oxidized glutathione (GSSG), and an antioxidant enzyme, glutathione peroxidase (GPx). Blood samples were also drawn to measure serum IGF-1. The data showed that exercise training (10 min a day/ 5 day a week for 9 weeks) in estrogen-deficient animals could restore myocardial reserve against oxidative damage. In addition, Cr supplementation or E_2 replacement combined with exercise training yielded more valuable results for estrogen-deficient animals demonstrated by greater cardiac reserve function, greater accumulation of myocardial energy metabolic phosphate reservation via Cr metabolism, and higher level of serum IGF-I than Cr supplementation or E_2 replacement alone. Moreover, E_2 replacement combined with exercise training has been shown a greater improvement in antioxidant reservation than E_2 treatment alone. Furthermore, Cr supplementation plus E_2 replacement together with exercise training yielded the most valuable results for estrogen-deficient animals demonstrated by a greater improvement in all parameters regulating cardiac functions.

The present study demonstrates tnat creatine supplementation and estrogen replacement combined with exercise training provide protective effects on cardiac functions in estrogen-deficient hamsters, which provide valuable data for therapeutic uses against estrogen deficiency in menopausal women.

Field of study Physiology Student's signature .....

Academic year 2007 Advisor's signature .....

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AGAT	L-arginine: glycine amidinotransferase
ALS	Amyotrophic Lateral Sclerosis
Arg	Arginine
APS	Ammonium persulphate
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
ADP	Adenosine diphosphate
BMR	Basal metabolic rate
BP	Blood pressure
BPs	binding proteins
BSA	Bovine serum albumin
BW	Body weight
CK	Creatine kinase
Gly	Glycine
Ca^{2+}	Ionized calcium
$[Ca^{2+}]i$	Intracellular ionized calcium
CAL	Catalase
CM	Cardiomyopathy
Cr	Creatine
Cr-	Creatine depletion
Cr+	Creatine supplementation
cCr	Cyclocreatine
Crn	Creatinine
CrT	Creatine transport

CHF	Congestive heart failure
CVD	Cardiovascular disease
°C	Degree Celsius
CO_2	Carbon dioxide
$COOH^-$	Carboxyl group
Cm	Centimeter
cDNA	Complementary Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
DTNB	5, 5-dithiobis-2-nitrobenzoate
dH_2O	Deionized water
dP/dt max	Maximum rate of rise
E_2	Estrogen
ECG	Electrocardiogram
EDTA	Ethylene-diamine-tetraacetic acid
EE_2	Ethinyl E_2
EMR	Exercise metabolic rate
ER	Estrogen receptor
EPR	Electroparamagnetic resonance
ERα	Estrogen receptor-α
ERβ	Estrogen receptor-β
ERαKO	Estrogen receptor-α knockout
ETOH	Ethanol
FSH	Follicle-stimulating hormone
GAA	Guanidinoacetic acid
GAMT	S-adenosyl-L-methionine: N-guanidinoacetate
β-GPA	β-guanidinopropionic acid
g	Gram

GH	Growth hormone
GHRH	Growth hormone releasing hormone
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GMP	Guanosine monophosphate
hcCr	Homocyclocreatine
H^+	Hydrogen ion
^1H NMR image	Hydrogen-1 nuclear magnetic resonance image
$HClO_4$	Perchloric acid
HCl	Hydrochloric acid
HDL	Heavy density lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid
HR	Heart rate
HRP	Horseradish peroxidase
H_2O_2	Hydrogen peroxide
IGF-I	Insulin like growth factor-I
IgG	Immunoglobulin G
IL-1β	Interleukin-1 β
IL-6	Interleukin-6
IP.	Intraperitoneal
IU	International unit
kDa	Kilo dalton
$KHCO_3$	Potassium hydrogencarbonate
kg	Kilogram
KH_2PO_4	Potassium dihydrogenphosphate
Km	Michaelis constant

L	Liter
LDL	Low density lipoprotein
LH	Luteinizing hormone
LVDP	Left ventricular developed pressure
M	Molar
Met	Methionine
Mg^{2+}	Ionized magnesium
MI	Myocardial infarction
MES	2-(N-morpholino)-ethane sulfonic acid
MOPS	3-(N-morphalino)-propane sulfonic. acid
MRFs	Myogenic regulatory growth factor
mRNA	Messenger ribonucleic acid
mmHg	Millimeter mercury
ml	Milliliter
min	Minutes
mg	Milligram
mM	Millimolar
N	Normality
N_2	Nitrogen
NH_2	Amino group
β-NAD	β-Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NaOH	Sodium hydroxide
Na_2CO_3	Sodium dihydrogen carbonate
Na_2EDTA	Diethylene-diamine-tetraacetic acid (sodium salt)

NAD^+	Nicotinamide adenine dinucleotide
NaN_3	Sodium azide
NO	Nitric oxide
NOS	Nitric oxide synthase
ng	Nanogram
nm	Nanometer
nmol	Nanomolar
OD	Optical density
$ONOO^\bullet$	Peroxynitrite
O_2	Oxygen
O_2^-	Superoxide
PCr	Phosphocreatine
^{31}P NMR	Phosphorus-31 nuclear magnetic resonance
RMR	Resting metabolic rate
ROS	Reactive oxygen species
RPP	Rate pressure product
R.Q.	Respiratory quotient
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SOD	Superoxide dismutase
SSA	Sulfosalicylic acid
TCr	Total creatine
TNF-α	Tumor necrosis factor-α
TMB	Tetramethylbenzidine
UW	Uterine weight
V_{max}	Maximum rate of reaction

VSMCs	Vascular smooth muscle cells
VO_{2max}	Maximum oxygen uptake
μl	Microliter
μmol	Micromolar
μg	Microgram

CHAPTER I

INTRODUCTION

BACKGROUND AND RATIONALE

It is known that the incidence of cardiovascular disease (CVD) differs significantly between genders, in part, because of differences in risk factors and hormones (Epstein, 1999). The gender difference of morbidity and mortality from heart diseases has been previously documented. Premenopausal women have a lower risk of CVD than age-matched men, whereas after menopause the ratio of mortality from heart disease in female was higher than that in male at the same age (Kannel et al., 1976). Epidemiological studies have demonstrated that the incidence of CVD is low in premenopausal women, but rises in postmenopausal women. However, the incidence in postmenopausal women is reduced to the same level in premenopausal women after received estrogen (E_2) therapy (Barrett-Connor, 1997). Menopause, the most common metabolic syndrome, may in part be attributable to E_2 deficiency inducing to CVD. Moreover, it has been shown that more than 80 of menopausal women suffered with metabolic syndrome die from cardiovascular impairment (Lombard et al., 2002). In according to other studies, they have been previously emphasizing that the incidence and severity of many cardiovascular diseases in women were associated with a reduction in E_2 (Nathan and Chaudhuri, 1997; Snow and Seddon, 2000). Cardiomyopathy (CM), a heart-muscle degenerative disease resulting in impaired cardiac functions, may partially be related to E_2 deficiency (Shlipak et al., 2001; Wenger, 2002), leading to an alteration in a variety of physiological parameters regulating cardiac function. Liu et al. (2004) studied in rats with cardiac ischemia and found that cardiac functions, especially left ventricular pressure (LVP) and maximal rate of pressure rise and fall ($\pm$dP/dt), were reduced after ovariectomy and E_2 could significantly restore those cardiac functions. Similarly, Zhai and coworkers (2000) suggested that ovariectomized rat hearts were associated with more severe myocardial damage and cardiac dysfunction following ischemia-reperfusion injury than hearts of ovariectomized rats with exogenous E_2 administration. Moreover, in a later study, a reduce myocardial necrosis was demonstrated in ovariectomized rats with E_2 replacement. In contrast to deficiency, E_2 replacement therapy has long been reported to have beneficial cardiovascular

protective effects, including inhibition of response to vessel injury, limiting myocardial injury, and promoting cardiac remodeling after myocardial infarction. Besides improving cardiovascular risk factor, such as the lipid profile, E_2 also has direct effects on the myocardium, the endothelium, and the vascular smooth muscle.

Recent clinical trials have reported conflicting results concerning the cardioprotective effects of E_2 replacement. Beer and coworkers (2002) proposed that the contents of adenosine triphosphate (ATP), phosphocreatine (PCr), and inorganic phosphate during ischemia were not affected after chronic E_2 therapy. Similarly, cardiac mechanical functions after 15-min global ischemia and 30-min reperfusion were not affected in cases of ovariectomy or E_2 replacement (Peng et al., 2004).

However, many results have confirmed that E_2 deficiency may increased the risk of cardiac dysfunction and cardiovascular diseases, but this risk may be reversible with E_2 replacement therapy (Martin et al., 1993; Kolodgie et al., 1997). In the same manner as impairment of cardiac function from E_2 deficiency, depletion of creatine (Cr) in cardiac muscle leading to a reduction in cardiac performance has been reported by many researchers. Previous experiments indicated that the failing myocardium is characterized by changing in cardiac high-energy phosphate metabolism, in which contents of phosphocreatine (PCr) and total creatine (TCr) are depleted by up to 50% (Neubauer et al., 1992). The higher intracellular Cr levels may increase the myocardial energy reserve with increased PCr and phosphoryl transfer via creatine kinase (CK) system (Neubauer et al., 1995). Chronic feeding with a Cr transporter inhibitor (β-GPA) for 8 weeks in intact rats led to more than 70 % reduction of PCr and Cr stores, and 28 % reduction of mechanical function in left ventricular developed pressure (LVDP) (Neubauer et al., 1999). Similarly, studies in failing hearts of humans and animals demonstrated a reduction in the CK system and its products, namely Cr and PCr contents (Conway et al., 1991). In contrast to Cr depletion, an increase in intracellular Cr may protect the heart in a variety of ways, including a reduction in the occurrence of arrhythmia, a protection of cardiac tissue metabolic stress, and reduction in plasma cholesterol and triglycerides (Ruda et al., 1988; Earnest et al., 1996). Although the majority of studies on Cr have been focused on skeletal muscle of healthy subjects, little is known about the effect of Cr on cardiac muscle in pathologic conditions. It is known that cardiac muscles are dependent on adequate levels of ATP to function properly, and depleted cardiac Cr levels are found in chronic heart failure. The researchers have investigated a supplementation with Cr in order to improve mechanical cardiac function, and some studies have demonstrated positive therapeutic results in various clinical applications. They suggested that supplementation with high energy precursors such as Cr

appeared to be highly effective, low cost approach to improve myocardial energy and performance in patients with heart diseases (Dangott et al., 2000; Parise et al., 2001). A previous study has been reported that creatine transporter (CrT) content and/or its activity might be a major determinant of the myocardial Cr pools, and this suggested that a decrease in the energy reserve in the failing heart might be a direct consequence of the loss of CrT protein (Neubauer et al., 1999). Furthermore, an increase in the intracellular Cr content in the failing myocardium and other tissues in response to the elevated plasma Cr levels after chronic Cr feeding has been proposed to be due to either an inactivation of the CrT or a decrease in the CrT content (Horn et al., 1998). In their conclusion, Cr supplementation may play a cardioprotective effect under condition of Cr depletion via the activity of CrT proteins (Boehm et al., 2003). However, transport kinetics of Cr and its analog in failing hearts will need to be studied further.

Therefore, it can be concluded that both Cr and E_2 may play an important role in improving the cardiac mechanical performance against failing hearts.

Besides the mechanical cardiac function, other studies concerning the beneficial effects of Cr or E_2 on cardiac growth via growth hormone/insulin like growth factor-I (GH/IGF-I) level have been previously reported. Reviews of many data have shown that most of the indirect effects of the growth-promoting and metabolic effects of GH are mediated by the generation of IGF-1 (Froesch et al., 1985). In contrast to GH, IGF-I levels are more reliable and stable, presumably because IGF-I is almost totally bound by plasma binding protein which prolongs its half-life and therefore IGF-I level may predict the response of GH level (Lee et al., 1990). Previously, experimental studies suggest that GH and IGF-I have stimulatory effects on myocardial contractility by inhibiting the injury of myocardial cells, by which is possibly mediated by changes in the intracellular calcium handling (Bueke et al., 1995; Caron et al., 1997; Davani et al., 2003). The regulation of plasma levels of GH and IGF-I by the ovarian steroid is well known (Suliman et al., 2001). Previous data indicated that E_2 promoted GH secretion resulting in an increase in GH level and thus stimulating the production of IGF-I (Zofkova, 2003). Friend and coworkers (1996) reported that serum GH concentration is declined significantly in post-menopausal women but may be provoked by E_2 replacement. However, there are controversial data about the level of GH and IGF-I in pre and post E_2 deficiency. For example, Suliman et al. (2001) studied in ovariectomized rats and found a decrease in GH but no effect on IGF-I level, while E_2 replacement increased both GH and IGF-I levels. On the other hand, Ammann et al (2000) investigated the model of E_2 deficiency induced protein undernutrition, and found that decreases in bone mineral mass and

strength might be related to the decreased plasma IGF-I. In addition, a previous study reported that transdermal E_2 therapy can increase IGF-I level, without a change in GH secretion. The possible explanation is that the difference in the response of GH/IGF-I levels may depend upon the dosage and the route of E_2 replacement (Lieman et al., 2001). Similarly, Cr supplementation improved muscle performance may be explained by a previous study that Cr stimulates the growth and protein accumulation in cultured myogenic cells (Ingwall, 1972). Experiments in animals have shown that Cr supplementation may result in enhanced satellite cell activity (Dangott et al., 2000). A previous study reported that the locally produced IGF-1 isoforms (IGF-1Ea and IGF-1Ec) may play an important role in the hypertrophic process both in young and aging human by stimulating the proliferation and differentiation of myogenic satellite cells into myoblasts, which are subsequently fused with myofibers and provided new nuclei to the muscle cell (Harridge, 2003)**.** Thus, it is possible that Cr can elevate satellite cell activation, which amplifies the hypertrophic response. Similarly, another study has indicated that Cr induced hypertrophy of cultured cell is partially mediated by overexpression of IGF-I and myogenic regulatory growth factor (MRFs), a marker of hypertrophic response occurred in the skeletal muscle tissue (Louis et al., 2004). Recently, Omerovic et al. (2003) further investigated the effect of GH on myocardial CrT protein and found that GH increased the expression of CrT and decreased levels of plasma IL-1β after myocardial infarction (MI) in rats. They explained that the increased CrT expression by GH action may have the protective effect on myocardial energy metabolism during myocardial ischemia and remodeling by preventing Cr depletion and stimulating mitochondrial oxidative phosphorylation. Therefore, it can be concluded that Cr or E_2 may play protective role in failing hearts by stimulating the growth factor, GH/IGF-I.

Another evidence from experimental and clinical studies has been reported about the role of oxidative stress in the pathogenesis induced cardiac dysfunction (Singal et al., 1998). The result suggested that reactive oxygen species (ROS) could exert a direct inhibitory effect on the myocardial function *in vivo* and *in vitro*. The degree of oxidative stress and the severity of myocardial damage may be dependent on the imbalance between the excess production of ROS and the antioxidant defense of the heart (Shiomi et al., 2004). For E_2 , its antioxidant characteristics have been demonstrated *in vitro* as well as *in vivo* studies in both rat and human. Although the mechanism by which it acts as an antioxidant has not been fully determined, E_2 is believed to have a high antioxidant capacity and membrane stabilizing properties (Subbiah et al., 1993). A previous study reported that E_2 can induce antioxidant defenses by stimulating the antioxidant enzyme expression, e.g.

glutathione peroxidase (GPx) (Massafra et al., 1998) and reducing superoxide (O_2^-) and/or peroxynitrite ($ONOO^-$) (Hernandez et al., 2000). The antioxidant effect of E_2 is also suggested by Arnal and coworkers (1996) that it may be due to estrogen receptor (ER) mediated changes in the expression of genes regulating the local production and degradation of O_2^-. Another study by Yuan et al. (1992) examined the effect of ROS on mitochondrial CK activity in rat hearts, and showed that ROS might inhibit mitochondrial CK activity by modifying sulfhydryl group of the enzyme protein. In agreement with the antioxidant effect of E_2, Cr has been found in several studies to possess antioxidant properties in protecting a variety of very toxic free radicals. For instance, PCr administered intravenously either before/during cardiac artery ligation or 30 min post-ligation (Sharov et al, 1987) could reduce the necrotic zone compared to control. Other studies in patients with congestive heart failure showed that Cr supplementation improved skeletal muscle metabolism with reductions in ammonia and lactate accumulation and lower total plasma cholesterol and triglycerides. These results were similar in human and rodents, and may suggest a therapeutic benefit of Cr supplement against oxidative stress (Persky and Brazeau, 2001). Earlier studies demonstrated a protective role of the Cr precursor, arginine (Arg), against oxidative stress imposed by oxidized LDL in cells, scavenged O_2^- generated via xanthine oxidase, impeded copper-induced lipoprotein oxidation, and slowed O_2^-, release by endothelial cells and aortic rings (Matthews et al., 1998; Vergnani et al., 2000). Previous findings are consistent with the potential function of Cr as an O_2^- anion quencher and suggest that Cr may act indirectly in promoting antioxidative function since Arg is the substrate of Cr formation (Wu and Meininger, 2000). The antioxidant effect of Cr is recently proposed to act as an oxidant scavenger primarily against radical ions of O_2^- and $OONO^-$ but not of H_2O_2 suggesting that the direct antioxidant effect of Cr was lower than physiological levels of reduced glutathione (GSH), but was additive to GSH properties (Lawler et al., 2002). Thus, it can be concluded that Cr and E_2 may potentially exert an antioxidant effect against oxidative stress in failing hearts.

The reviews from previous findings can be concluded that Cr depletion and E_2 deficiency are possibly related to CM and failing hearts. These were consistent with a number of studies which have been earlier reported. For example, Stassijns and coworkers (1999) studied the mechanism of CM hamsters on the diaphragm and reported that force-generating capacity was depressed, and serum IGF-I was markedly decreased. The possible involvement of ROS in inducing myocardial oxidative stress by decreasing enzymatic antioxidant defense during the early stage of CM has also been reported by many studies (Cheung et al., 2000**;**

Ichihara et al., 2006). On the contrary, Cr supplementation or E_2 replacement could restore the alteration seen in failing hearts by improving cardiac performance, preserving myocardial energy via GH/IGF-I level, and increasing antioxidant level. Up to this point, it can be concluded that Cr supplementation or E_2 replacement may play a protective role in cardiac function against failing hearts although the mechanism by which Cr or E_2 acting as cardioprotective factors in the same manner or differ from each other have not been fully determined. Interestingly, Cr or E_2 itself demonstrated beneficial effects on cardiac functions, it is then questionable whether the combination treatment of Cr+ E_2 would indicate the combined physiological effects or not. At present, there is no study available on the beneficial effect of the combined treatment of Cr and E_2 on cardiac functions against failing hearts. Therefore, the combined effect of Cr supplementation and E_2 replacement on cardiac function should be study to investigate the benefit and the mechanism against failing hearts.

As described above, Cr supplementation or E_2 replacement may play a role in the capacity of cardiac function. Similarly, a regular physical exercise has long been shown to elicit positive adaptations which results in an improvement of myocardial function under physiological stress. The beneficial effect of exercise training includes an improvement of cardiac performance by changing in the intrinsic myocardial contractile function (Mole, 1978), increasing myocardial enzyme activity (Chesky et al., 1983), as well as modifying the cardiac isomyosin distribution (Morris et al., 1990). Improvement of cardiac performance from exercise training could be varied by many factors, including setting, type of activity, duration, and intensity of the program. Additionally, endurance exercise protects rats from oxidative stress by raising levels of antioxidant enzymes and reducing oxidant production in both skeletal and cardiac muscles (Powers et al., 1994; Leeuwenburgh and Heinecke., 2001). Moreover, a significant impact of physical exercise on the GH/IGF-I axis has been reported (Merchav et al., 1988). Relative by few studies have examined the influence of E_2 replacement in combination with exercise training. Earlier studies reported that estrogen replacement in postmenopausal women increased higher grip strength than those who did not receiving E_2. Similarly, recent studies also support the preventive effects of exercise training on the molecular alterations in the heart of ovariectomized rats (Cauley et al., 1987; Bupha-Intr and Wattanapermpool, 2003). Therefore, the beneficial effect of E_2 with exercise training should be further study to clarify the combined physiological effects on cardiac functions and to investigate its mechanism against failing hearts.

Although much is known about the influence of Cr in muscle performance, especially in skeletal muscle, less is known about the beneficial effect of Cr on

cardiac performance. Stroud and coworkers (1994) concluded that Cr supplementation may influence the pattern of substrate utilization and improve performance during a more prolonged, sub-maximal exercise. A potential explanation for these findings may be that Cr supplementation alters the energy cost of muscle force production (ATP cost) at the onset of exercise by increasing PCr content in skeletal muscle. Additionally, exercise enhanced blood flow which leads to an increase in the translocation of CrT to the skeletal muscle membrane resulting in stimulating uptake of Cr (Perky and Brazeau, 2001). Similarly, Cr supplementation was reported to induce an increase in myogenic regulatory factors (MRFs), and possibly to induce leg-muscle hypertrophy which may be GH-mediated improvement in muscle performance (Hespel et al., 2001). As Cr is formed by the substrate, Arg, an O_2^- anion quencher, Cr may be readily accessible to radicals and reactive oxygen and nitrogen species generated during exercise, and could play an important supportive role with antioxidant production of GSH (Lawler et al., 2002). A recent research has shown a combined physiological effect of Cr supplementation in animals with swimming exercise over 21–day training induces an increase in the cardiac RNA content, suggesting an increase in the metabolic efficiency of the heart as a result of an increase in the muscle PCr or the cardiac muscle protein synthesis (McClung et al., 2003). However, the beneficial effect of Cr has been observed mainly in short-term supplementation since there are limited data with long-term study and its exact mechanism is currently unclear. Therefore, the combined effect of Cr with exercise training in a long term study on cardiac performance is of interesting to investigate its beneficial effect and its mechanism against failing hearts.

Taken together, all reviews from previous data showed that Cr supplementation, E_2 replacement and exercise training individually demonstrated important roles in cardiac function. However, it is unclear whether combining effect of Cr or E_2 with exercise training will provide more benefit on cardiac functions than that of Cr, E_2, or exercise alone. At present, there is no systematic studies on the influence of combining for Cr and E_2 (Cr+E_2) or combining for Cr+E_2 with exercise training on cardiac functions against failing hearts induced by E_2 deficiency. The present study aims to determine the beneficial effects of these regards and their mechanisms on physiological parameters regulating cardiac functions against failing hearts induced by E_2 deficiency in ovariectomized hamsters.

RESEARCH QUESTIONS.

1. What is the effect of Cr supplementation and E_2 replacement on physiological parameters regulating cardiac functions in non exercise and exercise-trained ovariectomized hamsters ?
2. Do changes in the physiological parameters regulating cardiac functions in non exercise and exercise-trained ovariectomized hamsters involve an alteration of serum IGF-I levels ?
3. Do the Cr supplementation and E_2 replacement in non exercise and exercise-trained ovariectomized hamsters have protective effects against oxidative stress induced myocardial damage?

RESEARCH OBJECTIVES

1. To determine the effects of Cr supplementation and E_2 replacement on physiological parameters regulating cardiac functions in non exercise and exercise-trained ovariectomized hamsters. These parameters were determined as follows:
   -Oxygen consumption : metabolic rate
   -Myocardial energy : Content of Cr, PCr, TCr, CrT protein and CK activity.
   -Cardiac functions : QT-c interval, left ventricular developed pressure (LVDP) and the maximum rate of pressure rise (dP/dt_{max}).
2. To examine the effects of Cr supplementation and E_2 replacement on serum IGF-I levels in non exercise and exercise-trained ovariectomized hamsters. The parameter was measured as follows:
   -Level of serum IGF-I.
3. To examine the protective effects of Cr supplementation and E_2 replacement in non exercise and exercise-trained ovariectomized hamsters against oxidative stress induced myocardial damage. These parameters were determined as follows:
   -Concentration of reduced glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG ratio.
   -Activities of glutathione peroxidase (GPx).
   -EC_{50} of cardiac functions

HYPOTHESIS

1. The Cr supplementation and E_2 replacement cause changes in physiological parameters regulating cardiac functions in non exercise and exercise-trained ovariectomized hamsters.
2. The changes in the physiological parameters regulating cardiac functions in non exercise and exercise-trained ovariectomized hamsters involve an alteration of serum IGF-I levels.
3. The Cr supplementation and E_2 replacement in non exercise and exercise-trained ovariectomized hamsters have protective effects against oxidative stress induced myocardial damage.

KEY WORDS

estrogen
creatine
cardiac function
insulin like growth factor-I
oxidative stress
exercise
cardiomyopathy
langendorff apparatus
metabolic rate

EXPECTED BENEFIT AND APPLICATION

The results provide further supporting data for the clinical uses of Cr supplementation and E_2 replacement in combination with exercise training as a cardioprotection and treatment against E_2 deficiency in menopausal women.

CHAPTER II

THEORY AND LITERATURE REVIEW

GENDER AND CARDIOVASCULAR DISEASE

While it is recognized that gender differences exist in cardiovascular disease, it has only recently been appreciated that significant gender differences also exit in cardiovascular function. Previous workers have noted estrogen and androgen receptors in the myocardium of rats and baboons and suggest that sex hormones may influence the heart differently in males, and females (Krieg et al., 1978; McGill et al., 1980). These include differential response to vasoactive agents and differences in the activity of enzyme associated with cardiac lysosomes and the inner mitochondrial membrane (Altura, 1975; Koenig et al., 1982). Sex differences in cardiac responses to physical training programs have been reported that gonadectomy in prepubertal male and female animals was associated with depressed left ventricular function and contractile performance, decreases in ventricular myosin ATPase activities, and shift in the myosin heavy chain isoenzymes from a predominant V1 toward a V3 pattern (Schaible et al., 1981). These alterations were prevented in males by replacement with testosterone and estrogen (E_2) in female. (Scheuer et al., 1987). With this background, it is logical to speculate that the sex hormone play a physiological role in cardiac function. Previous review has been reported that the most obvious gender-related difference in physiological ageing is the menopause in women (Hayward et al., 2000). The rate of coronary heart disease in women between puberty and menstrual has been reported much lower than in age-match men. In addition, gender gap in cardiovascular disease has long been recognized and reported the fact that women have a lower incidence of cardiovascular disease before menopause but lose this gender advantage with the onset of menopause indicates that ovarian hormones, primarily E_2 plays a pivotal role in reducing risk for cardiovascular disease (Farhat et al., 1996) and suggests the possible cardioprotective effects of E_2 (Cavasin et al., 2003)

ESTROGEN

Steroidal E_2 are the cholesterol derivatives comprising a group of structurally related, hormonally active molecules that control sex and growth characteristic (Hall

et al., 2001; Babiker et al., 2002). Steroidal hormones are fat-soluble (lipophilic) molecules that are essential for the growth, differentiation, and function of many tissues in humans and other vertebrate animals. The three major naturally occurring E_2 in women are estradiol, estriol, and estrone. In the body these are all produced from androgens through actions of enzymes. From menarche to menopause the primary E_2 is 17β-estradiol. In postmenopausal women more estrone is present than estradiol. Estradiol is produced from testosterone and estrone from androstenedione. Estrone is weaker than estradiol. A range of synthetic and natural substances have been identified that also possess estrogenic activity. E_2 is produced primarily by developing follicles in the ovaries, the corpus luteum, and the placenta. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) stimulate the production of E_2 in the ovaries. Some E_2 are also produced in smaller amounts by other tissues such as the liver, adrenal glands, and the breasts. These secondary sources of estrogen are especially important in postmenopausal women. Synthesis of E_2 starts in theca interna cells in the ovary, by the synthesis of androstenedione from cholesterol. Androstenedione is a substance of moderate androgenic activity. This compound crosses the basal membrane into the surrounding granulosa cells, where it is converted to estrone or estradiol, either immediately or through testosterone. The conversion of testosterone to estradiol, and of androstenedione to estrone, is catalyzed by the enzyme aromatase. While E_2 is present in both men and women, they are usually present at significantly higher levels in women of reproductive age. They promote the development of female secondary sex characteristics, such as breasts, and are also involved in the thickening of the endometrium and other aspects of regulating the menstrual cycle. In males, E_2 regulates certain functions of the reproductive system important to the maturation of sperm and may be necessary for a healthy libido. Estradiol levels vary through the menstrual cycle, with levels highest just before ovulation. By the age of 50-55, women normally pass through menopause resulting in ovarian exhaustion of follicles and a precipitous decline in ovarian steroids. Menopause is physiologic cessation of menses due to decreasing ovarian function. In general, menopause is established when menses have not occurs for a year. As ovaries age, response to pituitary gonadotropins (follicle-stimulating hormone, FSH and luteinizing hormone, LH) decreases accompany with an initially resulting in shorter follicular phases (thus shorter menstrual cycles), fewer ovulations, decreases progesterone production, and more irregularity in cycles. Eventually, the follicle fails to respond and does not produce E_2. Without E_2 feedback, circulating levels of LH and FSH rise substantially. Circulating level of E_2 and progesterone are markedly reduced. The androgen androstenedione is reduced by

half, but testosterone decreases only slightly because the stroma of the postmenopausal ovary continues to secrete substantial amounts. Androgen are convert to E_2 in the periphery, especially in fat cells and skin, accounting for most of the circulating E_2 in postmenopausal women (Hall et al., 2001; Babiker et al., 2002). This transitional phase, during which a woman passes out of the reproductive stage, begins before menopause. It is termed of perimenopause, although many persons refer to it as menopause (Hayward et al., 2000). Additionally, E_2 belongs to a class of steroid hormones that regulate target cells upon binding to intracellular and membrane receptors. Estrogen receptor (ER) is classically a ligand-dependent transcription factor, it is also modulates the activity of signaling cascades at the membrane and in the cytoplasm via various second messengers, such as receptor-mediated protein kinases (Ho and Liao, 2002). Both subtype of ER, ERα and ERβ, are members of the nuclear receptor superfamily. They are synthesized from separate genes and are structurally and functionally distinct. ERβ is 96 % identical to classic ERα in its DNA-binding domain but lacks homology (18%) in the N-terminal transactivation domain lead to speculation that the two ERs may differentially regulate various genes (Beato et al., 1995). In the classical model of E_2 action, binding of the steroid agonist to the receptor initiates receptor dimerization, nuclear translocation, and gene transcription. The alternative pathway involves the activation of receptors at either membrane or cytosolic locations as well as interaction with ligand or voltage-gated ion channels (Nadal et al., 2000). Activation of both membrane and cytosolic targets can lead to the activation of kinase cascades thought to be responsible for the rapid time course (from seconds to minutes) of the primary effect that is too fast to involve either RNA synthesis or protein translocation.

ESTROGEN AND CARDIAC FUNCTION

A large number of studies of disparate design are reasonably consistent in demonstrating that women with early bilateral oophorectomy are at increased risk of coronary heart disease (Samaan and Crawford, 1995). The evidence for an increase in risk among women with a surgical menopause but without bilateral oophorectomy is inconclusive, but such an effect, if any, could plausibly be explained by decreased ovarian function in many of those women as a consequence of the surgery. These findings, taken together with the lack of excess risk in women with bilateral oophorectomy who take replacement E_2, support the view that the increased risk is due to E_2 deficiency (Stampfer et al., 1991). Previous study concluded that, women have less heart disease than men until the age of the menopause; it is assumed that

menopause constitutes a significant cardiovascular milestone in term of physiology, as well as pathology (Hayward et al., 2000). There have been reported that the incidence and severity of many diseases are associated with the reduction of E_2 in women. Menopause metabolic syndrome may in part be attributable to E_2 deficiency which have been encompassed a range of condition known to be cardiovascular disease (Liu et al., 2004). Women can live more than a third of their lives in an E_2 deficient state; however, overwhelming evidence suggests that postmenopausal E_2 loss can have negative effects on the brain, bone and cardiovascular system (Samaan and Crawford, 1995; Kannel et al 1976). Menopause is frequently studied in light of the effect it apparently has known cardiovascular risk factors, and many studies have found an increase in total or low-density lipoprotein cholesterol, as well as a decrease in high density lipoprotein. Long-term epidemiological studies has shown that the incidence of coronary heart disease to be relatively low among premenopausal women, with a sharp rise with the occurrence of menopause (Wenger et al., 1993). Although earlier studied showed binding of radiolabeled 17β-estradiol to the heart, the presence of ERs, required for the genomic effects on DNA and protein synthesis, was demonstrated only in cardiac myocytes and fibroblast (Grohe et al., 1997). Because of this ERs localization, E_2 has been proposed to play an important role in cardiac hypertrophy and remodeling after myocardial infarction (Pelzer et al., 1996). Recent researches suggest that E_2 effects in vascular cells, and possibly in the myocardium, depend on the relative expression of ERα and ERβ (Ho and Liao, 2002). However, the roles of ERα and / or ERβ in cardiac functions are not known. The cardiovascular importance of estrogen has been probed with receptor gene deletion or mutation studies. A young man with a homozygous disruption in the ERα gene resulting in the expression of a truncated receptor lacking DNA and hormone-binding domains developed premature coronary artery disease and impaired brachial endothelium-dependent vasodilation. However, this is only a single case study and should be viewed with caution because other genes may also be affected. Early studies in ovariectomized mice demonstrated that E_2 inhibits intimal and medial vascular smooth muscle proliferation, suggesting a direct protective effect of estrogen on endothelial and vascular smooth muscle cells (VSMCs). In subsequent carotid injury studies, E_2 inhibited medial thickening and VSMC proliferation in wild-type and ERα knockout (ERαKO) mice, implying that the protective effect of E_2 could be mediated in an ERα-independent manner. Furthermore, in ERα and ERβ double-knockout mice, E_2 inhibited only VSMC proliferation, suggesting instead that a retained splice variant of ERα that lacked only

the amino-terminal activation function domain could mediate partial protection. This quandary was resolved with the production of complete ERα null mice, which exhibit increased medial area, VSMC proliferation and deposition of proteoglycans in response to vascular injury. Similarly, hearts from ERαKO mice subjected to global ischemia and reperfusion exhibit greater global ischemia and a higher incidence of arrhythmias. Hearts from ERαKO mice also have higher calcium accumulation, implying that E_2 inhibits calcium influx and attenuates the harmful effects of calcium overload during myocardial ischemia/reperfusion. The mechanism of these effects may involve NO, which ameliorates coronary dysfunction and reduces tissue edema by decreasing microvascular permeability, insomuch as hearts from ERαKO mice demonstrate decreased nitric oxide (NO) release. ERα also mediates the neuroprotective effects of E_2 after cerebral ischemia, as demonstrated by greater stroke sizes in ovariectomized ERαKO mice subjected to permanent cerebral ischemia. In addition, there is growing evidence that ERβ may also have an important function in the vasculature. ERβ expression is induced in VSMCs after vascular injury, and ERβ knockout mice exhibit hypertension and ion channel dysfunction in VSMCs (Ho and Liao, 2002). A recent study by Jankowski and coworkers (2001) demonstrated that ERα expression rises from low level in newborns in a specific manner, whereas ERβ mRNA decreases in the adult rat hearts. In contrast, ERβ expression is low but ERα mRNA levels are relatively high. Age-dependent increases in ERα suggest that this receptor plays a role in heart maturation and the high ERα expression suggest its functions as a predominant estrogenic mediator in the rat heart.

ESTROGEN DEFICIENCY AND REPLACEMENT THERPY

Loss of ovarian hormones has been reported to associate with a dramatic increase in the incidence of cardiovascular disease, which is a major source of cardiac death in post-menopausal women (Bush et al., 1988). Experimental evidence has shown that E_2 deficiency increases the risk for developing cardiovascular diseases (Xu et al., 2004). In addition to their role in female reproductive function, E_2 have beneficial actions on unrelated tissues. ERs are present in cardiac myocytes, arterial smooth muscle cells, and endothelial cells. Oral E_2 therapy alters systemic factors, such as lipoproteins, that are associated with a decreased risk for cardiovascular diseases (Furman et al., 1958). Reviews of the data suggest that E_2 increases vasodilation by rapid nongenomic effects and inhibits the response of blood vessel to injury and the development of atherosclerosis by long-term actions

genomic effects (Epstein, 1999). Several cardiovascular mechanisms of estrogen have been proposed. One explanation for the observed vasodilatory effects of E_2 is that E_2 may exert a positive inotropic effect, possibly by improving cardiovascular biomechanics (Samaan and Crawford, 1995). The effect of E_2 replacement therapy on vasodilation and contractility has been examined in a series of studies (Pines et al., 1992). E_2 has long been reported a beneficial cardiovascular protective effects, including, vasodilation, inhibition of response to vessel injury, limiting myocardial injury and promoting cardiac remodeling after myocardial infarction. In addition to improving cardiovascular risk factor, such as the lipid profile, E_2 also has direct effects on the myocardium, endothelium and vascular smooth muscle. In previous study on short-term E_2 treatment, cardiac out put is increased predominantly through increased in heart rate, probably through a reflex-mediated response to systemic vasodilation. However, during long-term treatment, cardiac output appears to be augmented through elevation of stroke volume (Samaan and Crawford, 1995). Previous reviews data conclude that the rapid effect of E_2 influences endothelial-derived NO and through NO-mediated increase in cyclic guanosine monophosphate (GMP), promotes the vasodilation of vascular smooth muscle (Wellman et al., 1996). Additionally, the longer-term effects of E_2 are mediated by ER α and ER β, or both, cause the changes in vascular-cell gene and protein expression (Binko and Majewski, 1998; Epstein, 1999). In agree with cardioprotective effect of E_2, many studies have been reported cardiac dysfunction after E_2 deficiency. Liu et al., (2004) studied in post-ischemic cardiac function, found that cardiac functions (LVP and $\pm$ dP/dt_{max}) was reduced after ovariectomy where as E_2 significantly improved those cardiac functions. Improvement of mechanical function in ischemic/reperfusion injury after E_2 replacement therapy, however, did not include preservation of high energy phosphate metabolism (Beer et al., 2002). In contrast, changes in the concentrations of high-energy phosphate metabolites were measured by ^{31}P nuclear magnetic resonance (NMR) spectroscopy of surviving rat uteri from 0-48 h following E_2 administration. They found a parallel increase in the ratio of ATP to creatine phosphate (CP) concentrations and suggested that estrogen can also affect the apparent CK equilibrium by modulating [free Mg^{2+}] (Liao et al., 1996). Additionally, Zhai and coworkers (2000) suggest that hearts of ovariectomized rats are associated with more severe myocardial damage and cardiac dysfunction following ischemia-reperfusion injury than hearts of those in ovariectomized rats administered exogenous E_2. In a later study, ovariectomy in female rats with E_2 replacement was shown to reduce myocardial necrosis. Collins et al. (1996) has been previously reported an evidence that E_2 has calcium antagonistic properties which may be

involved in the long-term protective effect of E_2 on the cardiovascular system. Similarly, study of Curl and coworkers (2003), investigated the effects of ovariectomy (OVX) and 17β-estradiol replacement on $[Ca^{2+}]i$ in rat freshly isolated cardiac myocytes. These data demonstrate clear differences in peak $[Ca^{2+}]i$ and the amplitude of the Ca^{2+} transient between OVX female rat cardiac myocytes compared with intact and 17β-estradiol-replaced OVX female rat cardiac myocytes. Thus, they suggested that E_2 may play a long-term role in limiting Ca^{2+} entry into the cardiac myocyte. Recent study by Cavasin et al. (2003) reported the opposing effects between E_2 and testosterone on cardiac function in myocardial infarction (MI). Their data suggest that E_2 prevents deterioration of cardiac function and remodeling after MI, but testosterone worsens cardiac dysfunction and remodeling and has a pronounced effect, when estrogen levels are reduced. Moreover, a number of studies have been reported that E_2 replacement in ovariectomized rats improved contractile functions following the ischemic condition (Martin et al., 1993; Kolodgie et al., 1997). Furthermore, previous review further concluded a cardiovascular protection role for E_2 is that E_2 may exert some of its beneficial effect on plasma lipoproteins by decreasing LDL cholesterol and increasing HDL cholesterol. Moreover, another data has been supported that E_2 may also exert its beneficial effects through a direct action on blood vessels and could be explained by many possible reasons. Firstly, the vascular wall contains specific, high-affinity receptors for E_2. Secondly, E_2 affects vascular tone both in vivo and in vitro. Thirdly, E_2 inhibits the remodeling associated with vascular injury. Finally, cell culture studies indicate that E_2 directly affects proliferation of both endothelial and vascular smooth muscle cells (Farhat et al., 1996). Then, reviews from those data can be concluded that E_2 deficiency associated an increased risk for cardiac dysfunction developing cardiovascular diseases, and may be reversible by E_2 replacement therapy.

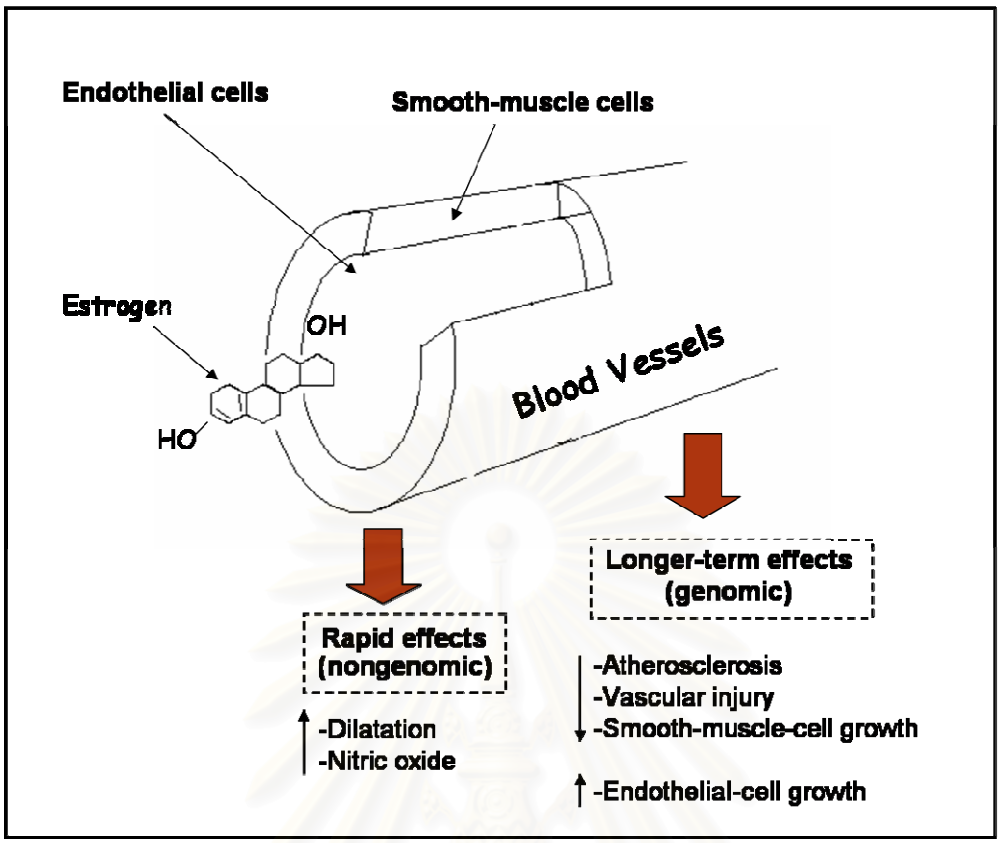


Figure 2.1 Short-term and long-term actions of estrogen. Estrogen has both short-term vasodilatory effects and long-term actions that provide protection against vascular injury and prevent atherosclerosis. Both effects are mediated by direct actions on vascular endothelial cells and smooth-muscle cells. The short-term effects of estrogen on the blood vessel wall influences the bioavailability of endothelial-derived nitric oxide and through nitric oxide-mediated increases in cyclic guanosins monophosphate, causes the relaxation of vascular smooth-muscle cell without any changes in gene expression (nongenomic effects). Conversely, the long-term effects involved changes in vascular-cell gene and protein expression (genomic effects) that are mediated by estrogen receptor, which are ligand-activated transcription factors (Modified from Epstein, 1999).

CREATINE

Creatine (Cr) is a nutrient that is made in our bodies from a combination of 3 different amino acids: arginine (Arg), glycine (Gly) and methionine (Met). Cr is an essential component in providing our muscle cells with the energy that is required for movement, especially those movements that need a quick release of energy or exertion. The average person's body contains approximately 120 grams of Cr stored as PCr. Certain foods such as beef, herring and salmon, are fairly high in Cr. Cr

exists in 2 differences in the body, as free chemically unbound Cr and as phosphocreatine (PCr). The PCr form is the active form in muscles. When muscle contract, the muscular fuel that is used for the contraction is a compound called ATP which provides its energy by releasing one of its phosphate molecules. Once the phosphate molecule is released, adenosine triphosphate (ATP) now becomes adenosine diphosphate (ADP). Unfortunately our bodies do not have a large reserve of ATP, and in order to keep muscle cell energized, more ATP must be produced. PCr carries out this role by donating its phosphate molecule to ADP enabling it to become ATP again, where it can be reused for fueling the muscle. This regeneration of ADP to ATP enables muscles to work at peak levels for a longer of time. In contrast to the constancy of the ATP concentration, the total Cr pool and hence the PCr and free Cr concentration are not constant but change with age, substrate, and work load. In hearts of adult small animals (rat, rabbit, guinea pig), total Cr content is about 90 nmol/mg cardiac protein, where as in large animal hearts, such as ferret and dog, the total Cr content is about140 nmol/mg cardiac protein. In blood-perfused isolated animal hearts and in hearts in situ, the PCr content is close to two times greater than ATP content (Wyss and Kaddurah-Daouk, 2000). Previously, Weiss et al. (1990) using ^{1}H image-aided ^{31}P (NMR) spectroscopy, showed that the PCr-to-ATP ratio for normal human myocardium is also close to 2. Usage of PCr to rephosphorylate ADP (and the reverse) is catalyzed by creatine kinase (CK) according to the following formula

$$PCr^{2-} + ADP^{-} + H^{+} \longrightarrow ATP^{2-} + Cr$$

CK is the quite essential example of an isozyme system. The CK group of isoenzymes consists of cytosolic dimers, BB-CK (brain-specific), MM-CK (muscle-specific) and a heterodimer of both of these subunits, MB-CK, which is only present in cardiac muscle cytosol. In addition, there are two mitochondrial-specific CKs (Mi-CK) positioned in the intermembrane space, termed Mib-CK (specific for sarcomeric muscle) and Mia-CK (the ubiquitous isoform). Mitochondrial CK isoenzymes form cube-like octamers with a four-fold symmetric structure, two identical top and bottom faces, and a channel through the center of the molecule. All CK isoenzymes catalyze the reversible transfer of the phosphate group of ATP to the guanidino group to Cr to yield ADP and PCr. The pathways of Cr metabolism in mammals involve many reactions and enzymes. The transfer of the amino group of Arg to Gly to yield L-ornithine and guanidinoacetic acid (GAA) represents the first of two steps in the biosynthesis of Cr and is catalyzed by L-arginine: glycine amidinotransferase (AGAT) (Van et al., 1972). GAA, by the action of S-adenosyl-L-methionine: N-guanidinoacetate (GAMT) is then methylated at the amidino group to give Cr. In the

course of evolution, both AGAT and GAMT seem to have evolved with the appearance of the lampreys. These enzyme activities were not detected in invertebrates, whereas they are found in most but not all vertebrates examined. Some invertebrate species (some annelids, echinoderms, hemichordates, and urochordates) nevertheless contain significant amounts of Cr, PCr, and CK, particularly in spermatozoa. This implies that these species either accumulate Cr from the environment or from the feed, or that the enzymes for Cr biosynthesis in these animals escaped detection so far. Many of the lower vertebrates (fish, frogs, and birds) have AGAT and GAMT in their livers and often also in the kidneys. In mammals, pancreas contains high levels of both enzymes, whereas kidneys express fairly high amounts of AGAT but relatively lower levels of GAMT. On the contrary, livers of all mammalian species tested so far contain high amounts of GAMT but display only low levels of Cr and almost completely lack CK activity. Although livers of cow, pig, monkey, and human also have high amounts of AGAT, livers of common laboratory animals such as the rat, mouse, dog, cat and rabbit were reported to lack AGAT activity. Because muscle has virtually no Cr-synthesizing capacity, Cr has to be taken up from the blood against a large concentration gradient by a saturable, Na^+ and Cl^- dependent creatine transporter (CrT) that spans the plasma membrane (Walker, 1979). Investigation of CrT across the plasma membrane has been discovered in the DNA and gene sequencing of the CrT from rabbit, rat, mouse, human and electric ray (Torpedo). These DNA sequencing approaches have shown that CrT are composed of 611-636 amino acid residues and have a calculated Mr. of 70 kDa. Additionally, CrT expression has been studied further and found that the presence of two different gene products expressed in various tissues corresponding to two major polypeptides of ~55 and ~70 kDa has been described. The two polypeptides are most likely generated by alternative splicing. This assumption is supported by the fact that antibodies generated against the N-and C-terminal region of the cDNA-derived CrT polypeptide sequence, all recognize the same two proteins with molecular masses of ~55 and ~70 kDa on Western blotting technique (Guimbal and Kilimann, 1994). An immunofluorescence studies have indicated that a high degree of intracellular CrT localization is found mainly in the mitochondria. The CrT is members of the Na^+ dependent "neurotransmitter" transporter family. They are most closely related to the GABA/taurine/betain transporter subfamily (46-53 % amino acid sequence identity), while the homology to Glycine, catecholamine, and serotonin transporters is somewhat less pronounced (38-44 %) All CrT lack a hydrophobic NH_2-terminal signal sequence, and display 12 putative transmembrane domains like other members of the Na^+ dependent neurotransmitter transporter family (Nash et al.,

1994). Consequently, the NH_2 and COOH termini of the polypeptide chain are probably both directed toward the cytosol. By classical biochemical means, a saturable uptake mechanism for Cr was identified in rat and mouse skeletal muscles, human fibroblast, human uterine, and calf aortic smooth muscle cells. In addition to the saturable component of C uptake, kinetic analysis often revealed a second component, displaying a Km for Cr of 1.3 mM or being saturable (Loike et al., 1986). In the light of a Cr concentration in the serum of 25-50 μM, it seems to be irrelevant for Cr uptake in vivo and may represent passive diffusion of Cr across the plasma membrane. Expression of CrT mRNA seems to be highest in kidneys, hearts and skeletal muscles and somewhat lower in brains, small and large intestines, testes, seminal vesicles and adrenals. Only very low amounts or no CrT mRNA at all are found in ovaries, uteri, livers, lungs, and spleens (Nash et al., 1994). A fairly good correlation seems to exist between the CrT mRNA level and total activity which correlates with the tissue concentration of total Cr which were accepted by 2 possible explanations. Firstly, the kidney displays a much higher CrT content than expected from its CK activity, which might be due to an involvement of the CrT in the reabsortion of Cr from the primary urine. Secondly, the liver has a considerably lower CK activity than expected from its Cr content, which may be an expression of strict separation between Cr-synthesizing and CK-expressing tissues in the body (Berlet et al., 1976)

The most past (up to 94 %) of Cr is found in muscular tissues. The daily demand for Cr is met either by intestinal absorption of dietary Cr or by de novo Cr biosynthesis. The first step of Cr biosynthesis probably occurs mainly in the kidney, whereas the liver is likely to be the principal organ accomplishing the subsequent methylation of GAA to Cr. It must be stressed that the detailed contribution of different bodily tissues (pancreas, kidney, liver, testes) to total Cr synthesis is still rather unclear and may vary between species (Walker, 1997). Cr exported from the liver and transported through the blood may then be taken up by the Cr-requiring tissues. Finally, inside the cell, ATP, ADP, Cr and PCr have to diffuse or to be transported through intracellular membranes to be able to contribute to high-energy phosphate transport between mitochondria and sites of ATP utilization. The highest levels of Cr and PCr are found in skeletal muscles, hearts, spermatozoa, and photoreceptor cells of the retina. Intermediate levels are found in brains, brown adipose tissues, intestines, seminal vesicles, endothelial cells, and macrophages, and only low levels are found in lungs, spleens, kidneys, livers, blood cells, and serum (Boroujerdi and Mattocks, 1983). The degradation of Cr and PCr in mammalian is, for the most part, a spontaneous, nonenzymatic process. In vitro, the

equilibrium of the reversible and nonenzymatic cyclization of Cr to creatinine (Cm) is both pH dependent and temperature dependent. Cr is favored at high pH and low temperature, whereas Crn is favored at elevated temperatures and in acidic solutions. In both directions, the reaction is monomolecular. Starting with pure Cr solutions, 1.0-1.3% of the Cr per day is converted into Crn at pH 7.0-7.2 and 38 ^{o}C. In vitro studies on the stability of PCr revealed that this high energy phosphate compound is acid labile, yielding Pi and either Cr or Crn upon hydrolysis. Both the rate of PCr hydrolysis and the ratio of Cr to Crn formed depend on temperature and pH. In contrast to these in vitro studies, experiments with ^{15}N-labeled compound clearly showed that the conversion of Cr into Crn in vivo is an irreversible process (Bloch and Schoenheimer, 1939). In accordance with in vitro studies, an almost constant fraction of the body Cr (1.1% / day) and PCr (2.6% / day) is converted nonenzymatically into Crn in vivo, giving an overall conversion rate for the total Cr pool (Cr+PCr) of 1.7% /day. Consequently, in a 70 kg man containing 120g of total Cr, roughly 2g /day are converted into Crn and have to be replaced by Cr from the diet or de novo biosynthesis. With the assumption of an average content in muscle of 30 Mm of total Cr and a quantitative uptake of the compound by the digestive tract, this loss could be compensated by ingestion of 500g raw meat per day. Because Crn is a very poor substrate of the CrT, because no other specific saturable uptake mechanism exists for Crn, and because Crn, most likely due to its nonionic nature is membrane permeable, Crn constantly diffuses out of the tissues into the blood and is excreted by the kidneys into the urine. Because the rate of nonenzymatic formation of Crn from Cr is nearly constant, and because>90 % of the total bodily Cr is to be found in muscle tissue, 24-h urinary Crn excretion is frequently used as a rough measure of total muscle mass (Guimbal and Kilimann, 1994). Using radiolabeled Crn, Boroujerdi and Mattocks (1983) showed that in rabbits, some Crn is converted into Cr, Arg, and GAA. Additional routes of Crn degradation become favored in states of renal insufficiently and seem to be relevant for human pathology.

CREATINE AND CARDIAC FUNCTION

Although much is known about the influence of Cr in skeletal muscle, less in known about the effect of Cr on cardiac muscle. Cardiac muscle has been reported to contain large amounts of PCr and CK. According to the classical view of muscle energetic, the CK reaction is near equilibrium in muscle and functions to provide an energy store in the form of PCr. In addition, the intracellular distribution of specific CK isoenzyme on mitochondria and myofibrils suggest that PCr and Cr may be important in the transport of high-energy phosphate from site to site within muscle

cells (Bessman and Carpenter, 1985). The immediate source of chemical energy for myocardial contraction is ATP, which is synthesized predominantly by three reactions: oxidative phosphorylation, glycolysis and the CK reaction. In the beating heart, the unidirectional flux through the CK reaction (Cr/PCr) in an order of magnitude greater than the rate of ATP utilization (Meyer et al., 1984). Many studies have been reported that Cr/PCr is necessary as an energy buffering and transferring system to avoid large fluctuations of cellular ATP/ADP levels. The ability of a cell to do work is directly related to its ATP status and the health of the mitochondria. Even small changes in ATP can have profound effects on the tissues' ability to function properly. For Cr metabolism, it is converted to PCr, which is necessary for ATP production to generate energy. When ATP loses a phosphate molecule and becomes ADP, it must be converted back to ATP to produce energy. When ATP depleted, it can be recharged by PCr. An increased pool of PCr means faster and greater recharging of ATP, which means more work can be performed. Cr appears to be effective for maintaining or raising ATP levels (Wyss and Kaddurah-Daouk, 2000). In patients with heart failure, extensive dilation and decreased systolic function were shown. An early clinical sign of heart failure is a diminished tolerance for exercise. The cause and pathogenesis of heart failure have not been yet clearly defined, although many hypotheses have been proposed. Among them, the hypothesis that failing myocardium is energy depleted is gaining renewed interest (Katz, 1991). In several animal models of heart failure, it has been shown that the products of the CK reaction namely ATP and PCr are decreased (Ingwall et al., 1985; Neubauer et al., 1995). Similarly, another previous experimental indicated that the failing myocardium is characterized by changes in cardiac high-energy phosphate metabolism; contents of PCr and total Cr are depleted by up to 50% and higher intracellular Cr levels might increase the myocardial energy reserve with increased PCr and phosphoryl transfer via CK system (Neubauer et al., 1999).

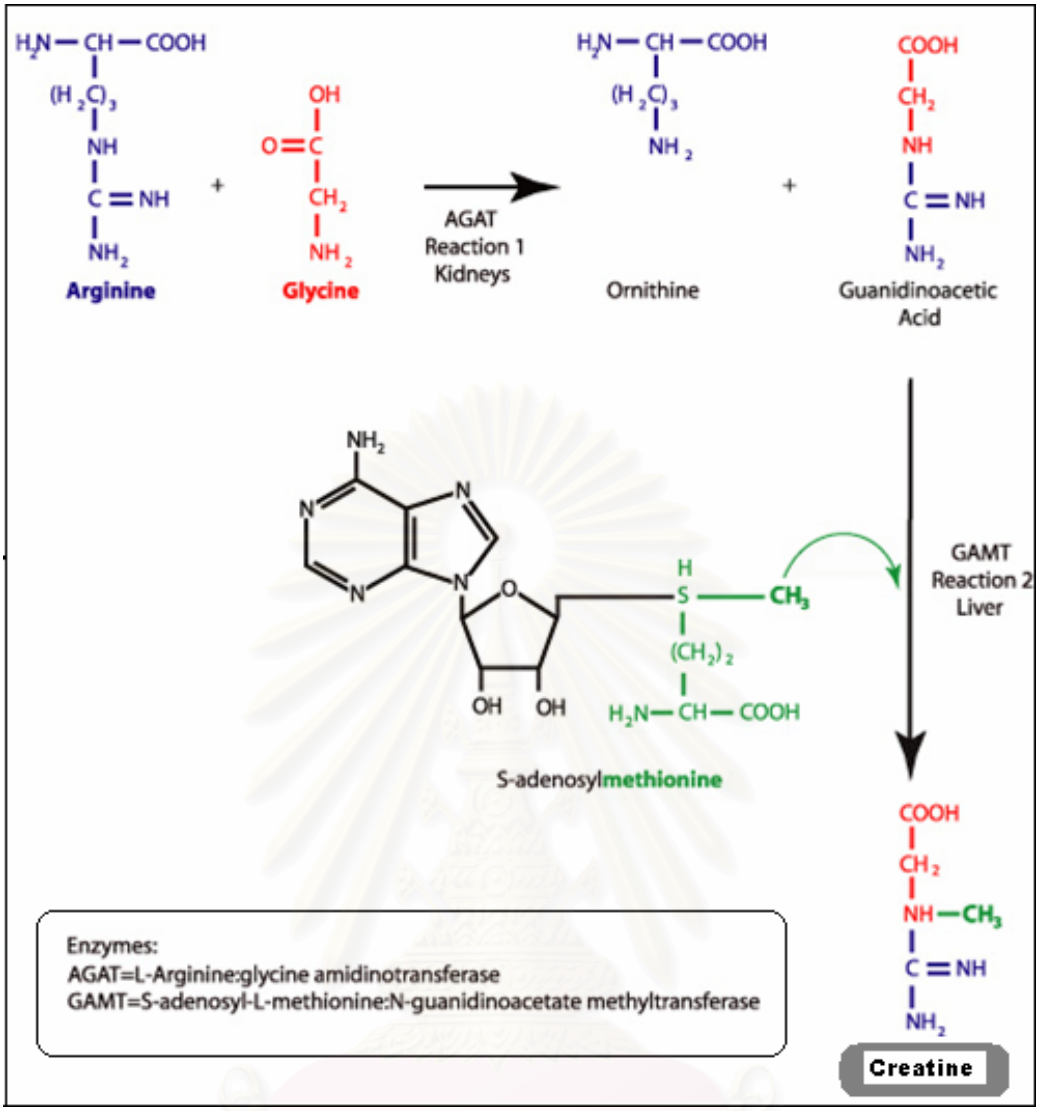


Figure 2.2 Pathway of creatine biosynthesis. Creatine is biosynthesized from the amino acids, arginine, glycine and methionine. The transfer of the amidino group of arginine to glycine to yield L-ornithine and guanidinoacetic acid (GAA) represents the first of two steps in the biosynthesis of Cr and is catalyzed by L-arginine:glycine amidinotransferase (AGAT). GAA, by the action of S-adenosyl-L-methionine:N-guanidinoacetate methytransfererase (GAMT), is then methylated at the amidino group to give Cr (Modified from Wyss and Kaddurah-Daouk, 2000).

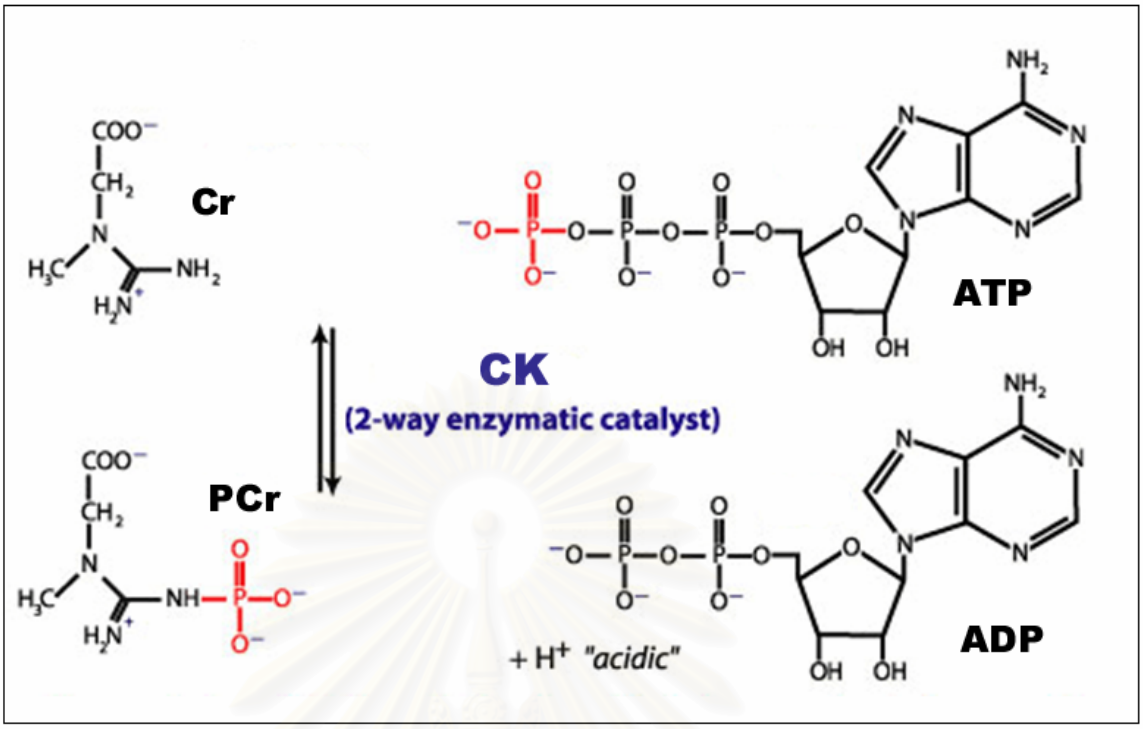


Figure 2.3 Enzymatic interconversion of PCr and ATP. ATP is the energy storage molecule of the cell. After donating its energy to the contractile apparatus of muscle, ATP becomes ADP. During intense physical exertion ATP is rapidly recreated from ADP by the donation of a phosphate group from PCr. CK is the enzyme responsible for transferring the phosphate groups (red) between PCr and ATP. The upward reaction indicates during strenuous exercise when energy (ATP) is needed to use for movement. The downward reaction occurs during moment at rest and recreates of PCr reservation (Modified from Wyss and Kaddurah-Daouk, 2000).

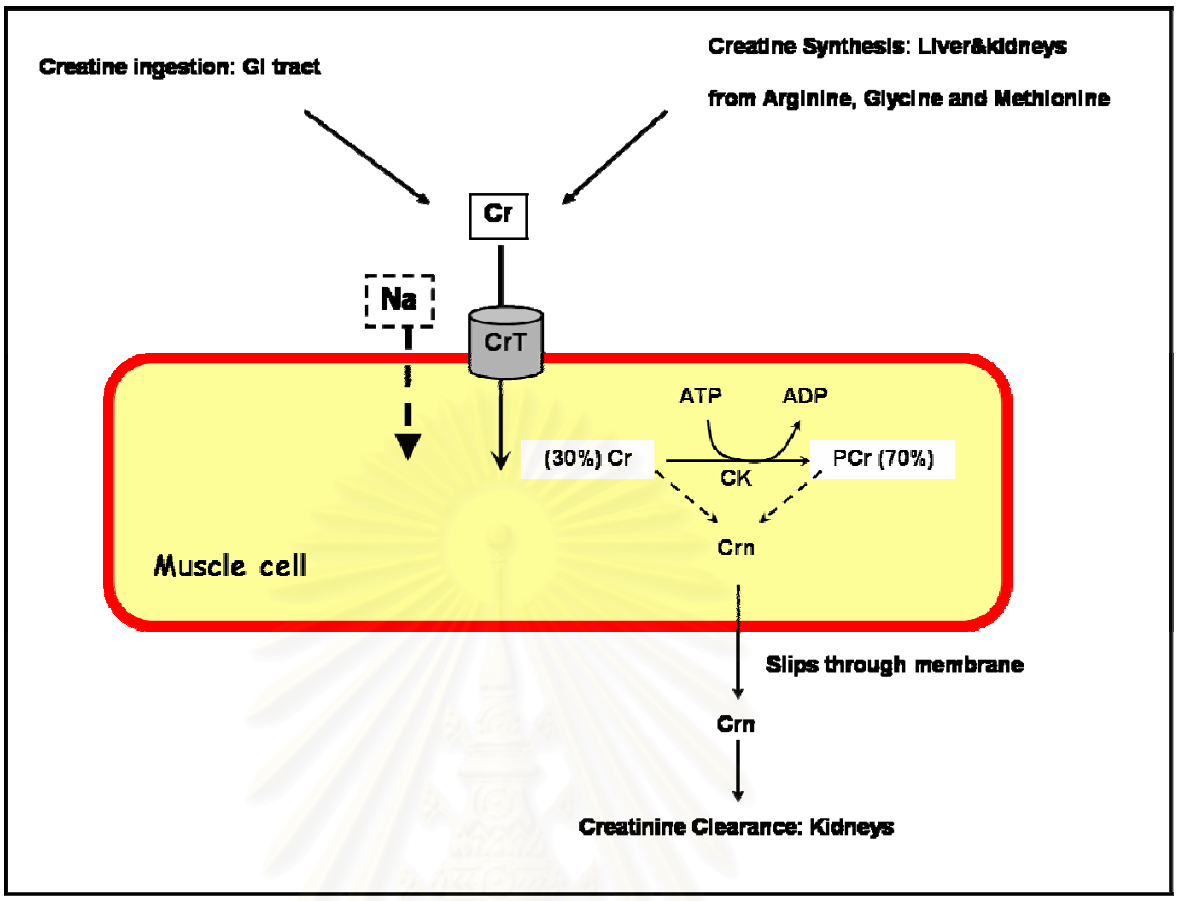


Figure 2.4 Creatine transport. Cr uptake into muscle cell is affected by a specific, saturable, sodium (Na+) dependent creatine transporter (CrT), with a Km (Na+) of 55 mM and a suggested transport stoichiometry of 2 Na+ for 1 Cr. Due to its electrical charge, Cr becomes trapped within the muscle cell when transported within. PCr is also more charged and is trapped when produced. In contrast, creatinine (Crn) lacks electrical charges at physiological pH and hence is able to diffuse out of the muscle cell in to the blood and is excreted by the kidneys into the urine (Modified from Wyss and Kaddurah-Daouk, 2000).

CREATINE DEPLETION

Administration of Cr analogs has been widely used as an experimental means of depleting tissue Cr and PCr in vivo, with the final gold to impair the physiological functions of the CK system. In animals fed with β–guanidinopropionic acid (β-GPA), cyclocreatine (cCr), or homocyclocreatine (hcCr), the accumulation of these Cr analogs within the tissues is paralleled by a decline in intracellular [Cr] and [PCr]. In rat skeletal muscle, β-GPA is accumulated by a saturable process displaying kinetic properties almost indistinguishable from Cr transport. Therefore,

the possible explanations for the effect of Cr analogs in vivo investigations have been concluded to be related to completely inhibit Cr biosynthesis, completely prevent Cr uptake by muscle and nerve, completely and specifically inhibit CK activity in vivo, or completely replace Cr and PCr with the synthetic phosphagen possessing markedly different thermodynamic and kinetic properties relative to PCr (Walker, 1979). The extent to which a synthetic phosphagen is accumulated within a given tissue and the degree to which PCr and Cr are replaced depend on a variety of factors (Roberts and Walker, 1983): the maximal concentration of the Cr analog that can be tolerated in the diet, the average concentration of this analog in the serum maintained by the rates of uptake (intestine) and excretion (kidney), the Vmax/Km values for uptake and release of the Cr analog into and from a given tissue, the fraction of the Cr analog that is phosphorylated, by CK (to yield the synthetic phosphagen), the Vmax/Km values for the synthetic phosphagen and for the Cr analog. Among the Cr analogs, β-GPA has been used frequently for studying the relevance of CK system for cellular energetics in various tissues. Most of previous studies have been performed on skeletal muscle where Cr analog (β-GPA) administration caused a series of functional changes. For example, β-GPA administration decreases developed muscle tension as well as the rates of tension development and relaxation. Cr analog affected cardiac function has been investigated by a number of researches. Studied in failing cardiomyopathic (CM) hearts of humans and animals could be observed large changes in the CK system. Total CK activity (Vmax) and total Cr and PCr contents are lower in failing than in nonfailing hearts (Conway et al., 1991; Neubauer et al., 1992). Since the velocity of the CK reaction is directly proportional to the product of CK activity (V_{max}) and [PCr], the decrease in total enzyme activity coupled with a decreased guanidino substrate content combine to decrease CK reaction velocity and hence the energy reserve of the heart. The previous studied was undertaken to test the hypothesis that in heart failure the decrease in the ability to resynthesize ATP through the CK reaction contributes to the inability of the heart to maintain its normal function and contractile reserve and suggested that impaired CK reaction velocity may contribute to the decreased cardiac reserve observed in the failing heart then impaired ability to increase rate pressure product (RPP) in response to increased stress. They concluded that the observation provide further support for the hypothesis that a decrease in energy reserve via the CK system contributes to reduced cardiac function in the failing heart. In chronic treatment, β-GPA feeding over a period of 8 weeks in intact rats led to 70 % to 80 % reduction of PCr and Cr stores, and 28 % reduction of mechanical function in left ventricular developed

pressure (LVDP) (Neubauer et al., 1999). Recently study examined how β-GPA treatment affects mechanical function, high energy phosphate metabolism, mortality in the rat chronic myocardial infarction (MI) model together with their findings of 100 % mortality in the acute MI study, may suggest that chronically failing heart can, over a period of weeks, adapt to a further depletion of PCr content, thereby maintaining LV contractile function at a minimal level necessary for survival, whereas the acutely infarcted heart cannot adapt to 65% PCr depletion with in a 24-hour period. However, it is unclear why the failing myocardium accumulates higher levels of β-GPA than the normal heart, suggesting Cr and Cr analog transport kinetics in heart failure will need to be studied further. For their conclusion, animals with predepleted myocardial PCr content cannot survive an acute MI, whereas PCr depletion induced later during heart failure development causes loss of ATP homeostasis (Horn et al., 2001).

CREATINE SUPPLEMENTATION

In contrast to Cr depletion, an increases in intracellular Cr protects the heart in a variety of ways including, reduces the occurrence of arrhythmia, protects cardiac tissue metabolic stress, and reduces plasma cholesterol and triglycerides have been reported by a number of studies (Ruda et al., 1988; Earnest et al., 1996). Although the majority of studies on Cr have on skeletal muscle in healthy subjects, recent evidence indicates Cr may be useful in the treatment of certain such as Huntington's, Parkinson's, Duchene muscular dystrophy, neuromuscular disorders and congestive heart failure. Patients with diseases that resulted in atrophy or muscle fatigue secondary to impaired energy production may benefit from Cr supplementation. The true mechanism by which Cr can be effective in these diseases are unclear but the theorized mechanisms of increased energy in the form of PCr, increased muscle accretion, and stabilization of membranes may be influential (Matthews et al., 1999). The possible mechanisms after being ingested with Cr have been reported. Cr is absorbed into the bloodstream, most likely by the amino acid transporter and usually reaches a maximum plasma concentration in less than two hours (Persky and Brazeau, 2001). While blood levels are elevated, the CrT actively transports Cr into the skeletal muscles, brains and cardiac muscles. At this point, there are a variety of mechanisms by which Cr may exert its ergogenic effects and beneficial effects have been proposed. Cr modulated energy metabolism by operatating as an energy and pH buffer during working. CK catalyzes a reaction between free Cr and phosphor ions (from the breakdown of ATP to ADP), resulting in PCr, which is locked into the muscle cell due to its strong negative charge. The PCr

can then react with ADP to form ATP during working, and during rest periods more PCr is generated. All of this equates to more energy during sets and faster recovery between sets (Persky and Brazeau, 2001). Supplementing with Cr may have an anabolic effect which has been shown to increases intracellular water retention making the muscle appear larger. Hyperhydration stimulates protein synthesis and inhibits protein breakdown, and cell volume has a correlation with catabolism in a variety of diseases (Waldegger et al., 1997). Many studies have confirmed that Cr supplementation prevents protein catabolism (Parise et al., 2001). There is also evidence that Cr increases satellite cell mitotic activity (Dangott et al., 2000). Because it is known that heart cells are dependent on adequate levels of ATP to function properly, and that cardiac Cr levels are depressed in chronic heart failure. The researchers have looked at supplemental Cr to improve heart function and overall symptomology in certain of heart disease and have demonstrated positive therapeutic results in various clinical applications. They suggested that supplementing with high energy precursors such as Cr appears to be a highly effective, low cost approach to helping patients with heart diseases live more quality of lives, and perhaps extend their life spans (Dangott et al., 2000; Parise et al., 2001). However, research has recently focused on the clinical application of Cr in rodents and humans, and therefore there is a limited amount of information available on the relationship between, the rodent studies and human studies. Although studies involving rodent offer credence in the therapeutic use of Cr, the results may not fully explain the usefulness in humans. Rodents typically have a higher blood Cr level than humans and do not response to supplementation in the same manner as humans. For examples, rat fed a 3% Cr diet for 40 days showed little increase in skeletal muscle total Cr levels with large increases in total Cr in liver and kidney (Marescau et al., 1986). Therefore, the distribution processes in the rodent may differ from humans and may cause same differences. Previous study has been suggested that CrT content and/or activity may be a major determinant of the myocardial Cr and, hence, PCr content correlates well with decreased myocardial Cr (and Pr) content in human, rat and dog failing hearts, suggesting that a decreased energy reserve in the failing heart might be a direct consequence of loss of CrT protein (Neubauer et al., 1999). Furthermore, the failing of the myocardium and other tissues to substantially increase intracellular Cr content in response to elevated plasma Cr levels caused by chronic Cr feeding has been proposed to be due to either an inactivation of the CrT or a decrease in CrT content (Horn et al., 1998). It has get to be showed how CrT activity and Cr content are related, and the precise mechanism by which the subcellular distribution and activity of the CrT are regulated remains unclear.

Previous studies showed that two distinct pools of the CrT protein exist in the cardiomyocyte, one associated with the major mitochondrial compartment and a second with the minor plasma membrane. The intracellular Cr concentration is an important bioenergetics parameter in cardiac muscle. Although Cr update is known to be via a NaCl dependent CrT, its localization regulation is poorly understood (Snow and Murphy, 2001). Recent experiment was to study the relationship between intracellular Cr content, subcellular CrT pools, and CrT uptake kinetic under condition of Cr supplementation or depletion. They investigated CrT kinetics in isolated perfused hearts of rats which were fed Cr or β-GPA, by using cardiomyocytes, measured CrT content in plasma membrane or in total lysates. They have been suggested that the regulation of CrT during Cr supplementation is due to a decrease in the number of active CrT protein than any kinetic regulation, such as phophorylation, in addition, activity under conditions of Cr supplementation and depletion are regulated by the plasma membrane CrT content while a predominantly mitochondrial pool of CrT remains constant. In their conclusion, Cr supplementation may play a cardioprotective effect under condition of Cr depletion which is regulated by the CrT proteins (Boehm et al., 2003).

GROWTH HORMONE AND CARDIAC FUNCTION

Growth hormone (GH), a poly peptide protein hormone of 190 amino acid, release via effects on the hypothalamic secretion of somatostatin (SRIF) and growth hormone releasing hormone (GHRH), stimulating production of insulin like growth factor I (IGF-I). GH-releasing hormone (GHRH), a peptide of 44 amino acids, which stimulates GH release; somatostatin, which can exist both as 14 and 28 amino acid peptides, and inhibits GH secretion (Duello and Halmi, 1979). GH secretion is regulated by negative feedback and neural control mechanisms. GH also has anti-insulin effects (Wehrenberg and Giustina, 1992). IGF-I is a single chain protein with 70 amino acid, promotes glucose transfer through cell membrane as a source of fuel for cells, which is an insulin-like growth effect. In addition, IGF-I may act directly on the pituitary to inhibit GHRH-stimulated secretion of GH. Both GH and IGF-I inhibit GH secretion after intraventricular injection by promoting hypothalamic somatostain release. Presumably, physiological concentrations of GH and IGF-I reaching the hypothalamus in the bloodstream act in the same way (increase in somatostatin tone). In addition, IGF-I may act directly on the pituitary to inhibit GHRH-stimulated secretion of GH. GH secretion can be augmented or inhibited by a number of neurogenic, metabolic, and hormonal influences (Volterrani et al., 1997). During most of the day, plasma GH levels of normal adults are < 5 ng/ml, with one or two sharp

spikes 3 to 4 hours after meals. The most consistent period of GH secretion for both children and young adults occurs about 1 hour after the onset of deep sleep (Veldhuis, 1995). Reviews of many data have shown that GH exerts its effects on myocardial tissue via direct and indirect mechanisms. Most of the indirect effects of the growth-promoting and metabolic effects of GH are mediated by the generation of insulin-like growth factor (IGF-1) (Froesch et al., 1985). The concept has emerged that GH acts by stimulating the local production of IGF-I, which in turn promotes tissue growth by paracrine or autocrine mechanisms. Most of the IGF-I is synthesized in the liver and kidney and circulates in plasma hound to protein carriers (IGFBPs), although locally synthesized IGF-I in other tissues also appears to be important in mediating GH effects (Grinspoon et al., 1995). IGF-I is structurally homologous to proinsulin, which may explain many of its insulin-like effects. In contrast to GH, IGF-I levels are more reliable and stable, presumably because IGF-I is almost totally bound by plasma binding protein which prolong its half-life. Moreover, IGF-I level may predict the response for GH level and also reported to be an ideal marker for GH activity (Lee et al., 1990). IGF-I have been reported to be the key player in growth promoting activity including stimulation of both differentiation and proliferation of myoblast. It also stimulates amino acid uptake and protein synthesis in muscle and other tissues (Buerke et al, 1995). Evidence is also accumulating that IGF-I is specifically involved in the control of cardiac tissue growth. The GH receptor gene is expressed in the myocardium to a greater extent than many other tissues (Mathews et al., 1989). Previous study reported that GH and IGF-I are involved in several physiological processes such as the control of muscle mass and function, body composition and regulation of nutrient metabolism. Moreover, experimental studies suggest that GH and, IGF-I have stimulatory effects on myocardial contractility, possibly mediated by changes in intracellular calcium handling (Caron et al., 1997). Patients with acromegaly have an increased propensity to develop ventricular hypertrophy and cardiovascular diseases; impaired cardiac efficiency can also he observed in patients with GH deficiency (Carroll et al., 1998). Furthermore, cardiac myocytes of rats express the IGF-I receptor and more importantly, IGF-I increases the size of cultured cardiomyocytes and simultaneously induces muscle specific gene expression. Previous data strongly suggest that IGF-I promotes cardiac hypertrophy. IGF-I mRNA expression is increased in the rat myocardium after pressure overload, secondary to either banding of the ascending aorta or to experimental renal hypertension. Interestingly, IGF-I expression is more pronounced in those segments of the myocardium that are particularly subjected to mechanical stress (Wahlander et al., 1992). It is noteworthy that IGF-I is the principal but not the only mediator of GH

action on cardiac tissue (Volterrani et al., 1997). Moreover, IGF-I may exert cardioprotective effects via inhibition of injury in myocardial cells have been also reported (Buerke et al., 1995; Davani et al., 2003)

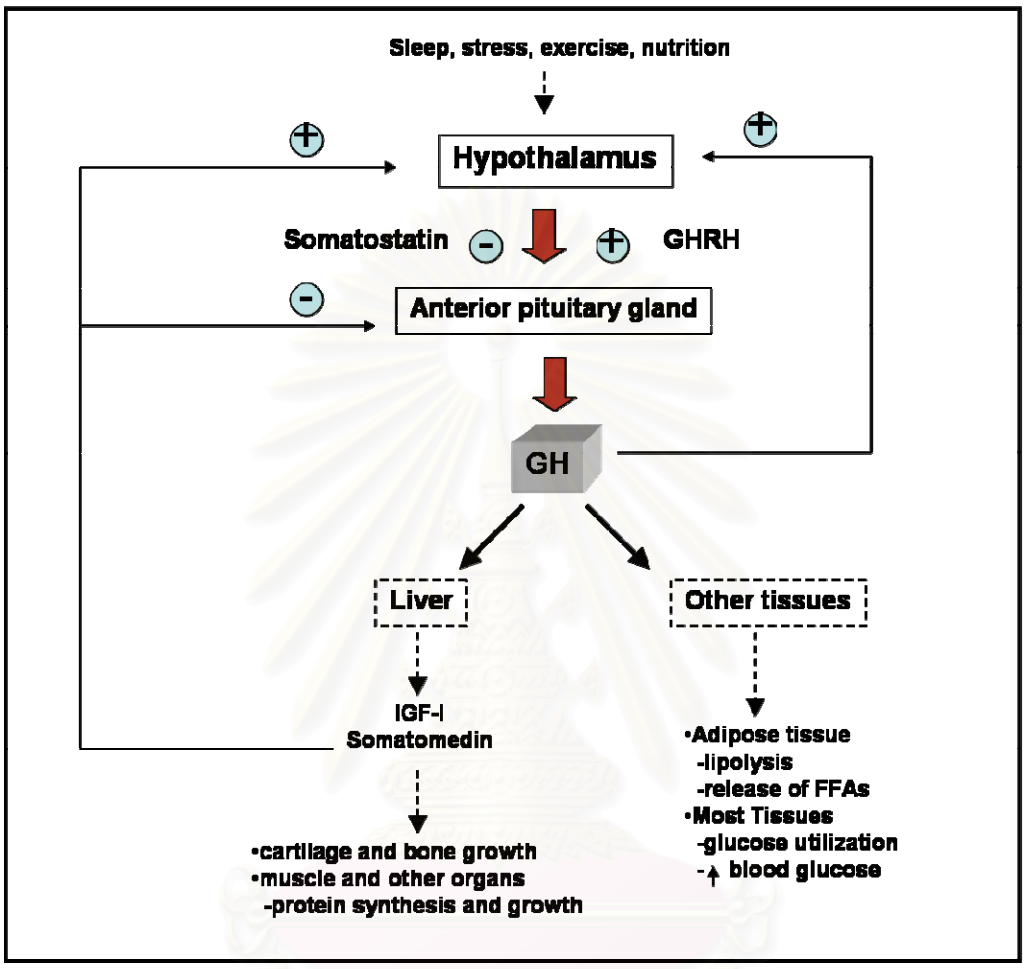


Figure 2.5 Control of Growth hormone secretion. Growth hormone (GH) known as somatotropin, is a protein hormone of about 190 amino acids that is synthesized and secreted by cells called somatotrophs in the anterior pituitary. Production of GH is modulated by many factors, including stress, exercise, nutrition, sleep and growth hormone itself. The primary controllers are two hypothalamic hormones, growth hormone releasing hormone (GHRH) and Somatostatin (SS). GH is primarily released in pulsed that take place during the beginning phases of sleep and converted in the liver to its powerful growth promoting metabolite, somatomedin or insulin-like growth factor I (IGF-I) (Modified from Bern and Levy, 1993).

ESTROGEN AND GROWTH HORMONE

GH levels, similar as IGF-I levels, are higher in childhood and they rise during puberty. IGF-I production is significantly stimulated by sex hormones. In addition, circulating IGF-I fluctuates in women during the menstrual cycle (Zofkova, 2003). Endogenous E_2 has been shown to influence GH secretion in a variety of models. E_2 administration to ovariectomized animals is associated with an increase in GH pulse amplitude (Dutour et al, 1997). Recent study by Berry et al. (2003) reports that development of prepubertal mammary is controlled through the combined actions of GH, E_2 and locally derived growth factor of IGF-I. In female of preovulatory phase, level of plasma IGF-I and GH increase simultaneously that results in an increase of GH axis drive (Ovesen et al., 1998). In contrast, Friend and coworkers (1996) reported that serum GH concentration decline significantly in post-menopausal women, but can be stimulated by E_2 replacement. However, there are controversy data about the level of GH and IGF-I in pre and post E_2 deficiency. Suliman et al. (2001) studied in femur bone, found that ovariectomized rats decreased GH and had no effect on IGF-I levels but supplementation with E_2 increased GH and IGF-I levels. Conversely, Ammann et al. (2000) investigated the model of protein undernutrition in estrogen deficiency, found that the decrease of bone mineral mass and strength might be related to decreased plasma IGF-I. A high circulating IGF-I level have been observed in estrogen-treated postmenopausal women, especially in those with very low initial IGF-I values (Zofkova, 2003). In addition, previous study reported that transdermal E_2 can increase IGF-I levels, without a change in GH secretion. The possible explanation is that serum exogenous E_2 exerts disparate effects on the somatotropic axis that appeared to be dependent upon dosage and router (Lieman et al., 2001). Recent result demonstrated that E_2 administration at low and high dose significantly increased GH serum level in young and middle age rats. In contrast, IGF-I levels were decreased with the high dose of E_2. Their findings are in agreement with several studies. For example, Duursma et al. (1984) reported an increase in GH concentration and a decrease in circulating IGF-I in postmenopausal women, which received 20 μg ethinyl E_2 (EE_2) for 3 weeks. Similar results were obtained in another study after administration of 20 μg ethinyl E_2 (EE_2) for 15 days (Dawson-Hughes et al., 1986). Additionally, Yonezawa and coworkers (2005) investigated a modulation of GH pulsatility by sex steroids in female goats. They found that subcutaneous injection of E_2 to ovariectomized goats increased the GH pulse amplitude and suggested the pulsatile pattern of GH

secretion in goats varies with levels and thereby affects IGF-I secretion during estrous cycle.

CREATINE AND GROWTH HORMONE

Cr supplementation amplifies the adaptive response to resistance training both in female and male subjects, resulting in greater increases in maximal muscle strength and fat free mass (Brose et al., 2003) in parallel with greater increases in muscle cross-sectional area (Becque et al., 2000) compared to placebo intake. Notably, not all subjects respond with elevated muscle cell Cr content following Cr loading (~10%), particularly not individuals with initially high muscle Cr concentration (Greenhaff et al., 1994). Cr loading initially gives rise to an increased retention of water in the body, along with fluid shifts into the muscle fibers due to elevated osmotic gradients caused by the increase in intracellular Cr concentration (Guimbal and Kilimann, 1993). Data exist to suggest that this initial osmotic-induced increase in muscle fiber volume provide a stimulus for increased cellular protein synthesis (Haussinger et al., 1992). Previous results confirm that Cr stimulates growth and protein accumulation in myogenic cells in culture (Ingwall et al., 1972). Since an increased production of myogenic growth factors (MRFs) seems to explain the elevated hypertrophic response occurred in the muscle tissue. The MRFs are a family of skeletal muscle-specific transcription factors that regulate the expression of several skeletal muscle genes (Lowe et al., 1998). The family is com-posed of four members: myogenin, MRF4, MyoD and Myf5. Previous studies have been reported exercise induced changes in MRFs and IGF-I expression, at both the mRNA and protein level suggesting MRFs and IGF-I might be such regulatory proteins (Aagaard et al., 2000). Another study in animal experiments has indicated an increased autocrine production of IGF-1 by the muscle itself in response to chronic muscle loading (Yang et al., 1996). While circulating IGF-1 and GH do not seem important for muscle hypertrophy or to maintain muscle mass during adulthood (Hameed et al., 2003), locally produced IGF-1 may play an important role in the hypertrophic process both in young and aging individuals (Harridge, 2003). Locally produced IGF-1 isoforms (IGF-1Ea and IGF-1Ec) stimulate the proliferation and differentiation of myogenic satellite cells into myoblasts, which subsequently fuse with myofibers and provide new nuclei to the muscle cell. An increased mRNA and protein MRFs have been observed following combined training and Cr intake (Aagaard et al., 2000). Although resistance training results in increased mRNA and protein content of MRFs (MyoD, myogenin and MRF4), this increase is substantially accelerated when training is combined with Cr intake (Willoughby and Rosene, 2003). They suggest that a rise

in MRF with Cr supplementation may not elicit an enhanced hypertrophic response, rather it increases the sensitivity of the muscle cell to the resistance training stimulus, which in turn contributes to the accelerated hypertrophy. Likewise, Hespel et al. (2001) examined the effect of oral Cr supplementation on MRF expression during human leg immobilization and rehabilitation. Their results showed that MRFs was increased after rehabilitation in placebo but not under Cr supplementation, while MRF-4 protein expression was increased with Cr but not with placebo. Animal experiments have shown that Cr supplementation may result in enhanced satellite cell activity (Dangott et al., 2000). It is possible that combined Cr intake and resistance training leads to elevated satellite cell activation compared to resistance training alone, which amplifies the hypertrophic response. Similarly, recent study proposed that Cr induced hypertrophy of cultured cell is at least partially mediated by over expression of IGF-I and MRFs (Louis et al., 2004). As it known that Cr is not synthesized in cardiomyocytes but is rather taken up from circulating blood through an active process mediated by specific Cl- and Na+-dependent CrT in the membrane. Abnormalities in Cr/PCr metabolism that ultimately results in myocardial Cr depletion together with numerous negative biological effects of proinflammatory cytokines such as interleukin-1 β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are contributing factors to deteriorate in cardiac function and structure through the continuous process of pathologic cardiac remodeling. Recently, Omerovic et al. (2003) further investigated the effect of GH on myocardial CrT protein, and found that GH increased the expression of CrT and decreased levels of plasma IL-1β during post-infarct remodeling in rats. These mechanisms may be responsible for the previously reported beneficial effects of GH on myocardial energy metabolism and preservation of cardiac function in the settings of post-infarct remodeling and congestive heart failure (CHF). They also explain that increased CrT expression by GH action may have protective effect on myocardial energy metabolism during myocardial ischemia and cardiac remodeling by preventing Cr depletion and stimulating mitochondrial oxidative phosphorylation.

OXIDATIVE STRESS AND CARDIAC FUNCTION

Reactive oxygen species (ROS) are molecules with unpaired electron in their orbit (Urso and Clarkson, 2003). As a consequence, these molecules are very unstable and highly reactive, and they tend to initiate chain reactions that results in irreversible changes in lipids or proteins. Generation of ROS species (superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-)) and nitrogen species (nitric

oxide (NO) and peroxynitrite ($ONOO^-$) are formed under both physiological and pathological condition in mammalian tissues. When O_2^- is produced in concert with NO, they rapidly react to form the highly reactive molecule $ONOO^-$. $ONOO^-$ is an important mediator of lipid peroxidation and protein nitration, including oxidation of LDL, which has dramatic proatherogenic effects. In the absence of immediately accessible NO, is rapidly dismutated to the more stable ROS, H_2O_2 by Superoxide dismutase (SOD), which is then converted to H_2O_2 by either catalase (CAL) or glutathione peroxidase (GPx). The effects of O_2^- and H_2O_2 on vascular function depend critically on the amounts produced. When formed in low amounts intracellular, they can act as intracellular second messengers, modulating in the function of biochemical pathways mediating such response as growth of vascular smooth muscle cells (VSMC) and fibroblasts. Higher amounts of ROS can cause DNA damage, significant toxicity, or even apoptosis, as demonstrated in endothelial cells and smooth muscle cells (Baim et al., 1982). Two of most important sources in the normal vessel are though to be cytochrome P450 and the membrane-associated NAD(P)H oxidases. NAD(P)H oxidase that are similar in structure to the neutrophill respiratory burst NAD(P)H oxidase, but produce less O_2^- for a longer time, has been identified in vascular cells. One important aspect of ROS production by at least the VSMC NAD(P)H oxidase in that it occurs largely intracellularly, making it ideally suited to modify signaling pathways and gene expression. ROS generation can result in modification of lipid; protein and DNA (Niwa et al., 2002). There are three major lines of evidence that implicate ROS in the pathogenesis of myocardial reperfusion injury : 1) ROS can be detected in post-ischemic myocardium, 2) exposure of myocardium to exogenous ROS results in myocardial tissue dysfunction that is comparable to that elicited by ischemic reperfusion, and 3) pretreatment of animals with anti-oxidant enzymes or genetic overexpression of these enzymes in experimental animals affords protection against reperfusion injury. A number of different experimental approaches have been use to detect ROS production in the post-ischemic myocardium. Electroparamagnetic resonance (EPR) spectroscopy is one of the most widely use methods for monitoring ROS generation in the heart. Studies based on the technique have clearly demonstrated a rapid and profound increase in ROS production after reperfusion of ischemic myocardium (Lawson et al., 1999). Zweier and colleagues (1988) were among the first to measure oxygen free-radical formation directly in isolated, Krebs buffer-perfusion rabbit heart under baseline conditions, during ischemic, and after reperfusion. They noted a make and distinct EPR spectra in the ischemic reperfused heart that was consistent with the maximum oxidant production at 10 to 30 second after reperfusion (Garlick et al.,

1987) Their study suggested that O_2^- is the predominant ROS produced after reperfusion, and that endothelial cells represent an important source of the ROS. In addition, ROS have been shown to exert a direct inhibitory effect on myocardial function in vivo and in vitro. Indeed, exposure of the normal myocardium to ROS-generating systems or H_2O_2 alters myocardium function in a fashion that mimics reperfusion injury, including persistent cellular loss of K^+, depletion of high energy phosphate, elevated [Ca^+]; loss of systolic force development, a progressive increase in diastolic tension, depressed metabolic function, and arrhythmias (Zweier et al., 1988). Evidence from experimental and clinical studies suggests the role of oxidative stress in the pathogenesis of heart dysfunction (Singal et al., 1998). These ROS are efficiently detoxified by endogenous enzymatic free radical scavengers, such as SOD, GPx, and CAT (Fowkes, 1996). The degree of oxidative stress and the severity of myocardial damage might depend on the imbalance between excess production of ROS and the antioxidant defense in the heart (Shiomi et al., 2004).

ESTROGEN AND MYOCARDIAL OXIDATIVE STRESS

The antioxidant characteristics of E_2 have been demonstrated in *vitro* as well as in *vivo* in both rat and human investigations, although the mechanism by which it acts as an antioxidant have not been fully determined. E_2 are believed to have a high antioxidant capacity and membrane stabilizing properties (Subbiah et al., 1993). All estrogen has a phenolic hydroxyl group at position 3 and a methyl group at position 13. The presence of the phenol group give E_2 showed an antioxidant property by scavenging oxygen free radicals. As lipophilic hormones, E_2 can interact directly with membrane phospholipids to decrease membrane fluidity accompanied with increase membrane stabilization against peroxidation, and finally diminish membrane disruption (Wiseman and Quinn, 1994). Previous study reported that E_2 can induce antioxidant enzyme expression by stimulating the antioxidant defense of GPx (Massafra et al., 1998). Some observations suggest that estrogen effects on NO production could play an important role in modulation of cardiac response to ischemic reperfusion condition (Node et al., 1997). A recent report indicates that estrogen is known to have antioxidant properties, which could play a role in reducing O_2^- and or $ONOO^-$ (Hernandez et al., 2000). In postmenopausal women, both long-term and short-term administration of E_2 can decrease the oxidation of low density lipoprotein (LDL) cholesterol (Farhat et al., 1996). This antioxidant effect of E_2 is suggested by Arnal and coworkers (1996) that may be due to ER-mediated changes in the expression of genes for enzyme regulating the local production and degradation of superoxide. In agree with these finding, the results of Zhai and coworkers (2000)

indicated that the hearts of ovariectomy following ischemia-reperfusion showed more myocardial contraction bands, more severe myofibrilar destruction and more prominent mitochondria damage than ovariectomized hearts replacement with E_2. Additionally, the current study proposed to examine myocardial antioxidant and oxidative stress change in mal and female rats after castration and suggested that E_2 may have an antioxidant role in cardiac muscle, while testosterone did not (Barp et al., 2002).

CREATINE AND MYOCARDIAL OXIDATIVE STRESS

Because Cr involve in energy production and acts as a shuttle of ATP from the inner mitochondria to the cytosol (Persky and Brazeau, 2001). Many researches have previously focused on the useful of Cr in diseases of mitochondria where energy production is altered (Haman et al., 1995). Oxidative stress from excession of free radical is fundamental mechanism of mitochondrial injury in myocardium (Banerjee et al., 1991). Additionally, mitochondrial CK activity has been demonstrated to be related to cardiac function. Therefore, the depression in mitochondrial CK activity may lead to disruption of energy utilization and dysfunction of the heart (Mekfhi et al., 1996). Several studies have provided direct evidences that oxygen free radicals are produced in myocardial ischemia follow by reperfusion resulted in mechanical dysfunction (Haman et al., 1995). In agree with this information, Yuan et al. (1992) examine the effect of oxygen free radical on mitochondrial CK activity in rat heart and found that oxygen free radicals may inhibit mitochondrial CK activity by modifying sulhydrl group in the enzyme protein. On the contrary, Cr has been found in several studies to possess antioxidant properties, protecting against a variety of very toxic free radicals. In animal studies, the effect of Cr on cardiac tissue has been previously investigated. A study by Sharov et al. (1987) showed a protective effect on PCr on cardiac tissue following ischemia. Using rabbit heart, PCr was administered intravenously either before or during cardiac artery ligation or 30 min post-ligation and found a reduction in necrotic zone under both PCr treatment compared to the control. Ruda et al. (1988) found that PCr administration reduced ventricular arrhythmia after acute myocardial infarctions, but the effect of Cr on cardiac tissue are still unclear. Other studies have also shown PCr to posse's anti-arrhythmic activities. Feeding Cr to healthy rats or rats after myocardial infarction failed to increase intramuscular Cr. In human studies, Gordon et al. (1995) investigated the effect on ingestion of Cr in patients with congestive heart failure in a double-blind, placebo-controlled study (10 days). They found an ejection fraction at rest and at work did not change but increase exercise performance in regard to both

strength and endurance (Becque et al., 2000). Another study in patients with congestive heart failure showed that Cr supplementation improve skeletal muscle metabolism with reductions in ammonia and lactate accumulation. In 1999, Neubauer et al., showed that hearts with dilated CM had 50 % less total Cr compare with healthy hearts as well as 30 % less CrT (Gordon et al., 1995). Cr supplementation also has been shown to lower total plasma cholesterol and triglycerides. These results were similar in human and rodents and may suggest a therapeutic benefit of Cr supplement against oxidative stress (Persky and Brazeau, 2001). Another study by Rando et al. (1998) proposed that Cr is a downstream product of the amino acids Gly and Arg producing GAA and also ornithine, which feeds back into the urea cycle. Arg is also a substrate for the nitric oxide synthase (NOS) family and can increase production of nitric oxide, a free radical that modulates metabolism, contractility and glucose uptake in skeletal muscle. Likely, Vergnani et al. (2000) demonstrated a protective role for the Cr precursor, Arg, against oxidative stress imposed by oxidized LDL in cells. Additional data indicate that Arg may be able to quench free radicals such as O_2^-. Matthews et al. (1998) proposed that Cr supplemental provides direct or indirect antioxidant protection against metabolic damage with Huntington's disease. For example, Arg, a substrate for Cr formation can scavenge O_2^- generated via xanthine oxidase, impede copper-induced lipoprotein oxidation, and slow O_2^-, release by endothelial cells and aortic rings. Wu and Meininger (2000) proposed that the antioxidant effect of Arg might protect against heart disease. The preceding findings are consistent with the potential function of Cr as an O_2^- anion quencher and antioxidant since Cr is produced from Arg and Gly. Health status may be a critical factor in the efficacy of Cr. For example, it has been postulated that patients with neuromuscular disease, often characterized by increased oxidative stress might benefit a greater extent from Cr supplementation than healthy subjects (Reid et al., 1994). Cr supplementation has been shown potential to be efficacious in treatment of oxidative stress and damage with pathologies that result in muscle wasting including amyotrophic lateral sclerosis (ALS) (Strong and Pattee, 2000) It is also possible that Cr may act indirectly promote antioxidant function. For example, If Cr levels in muscle cells were increased, then, less Arg would be required for energy metabolism and more available for nitric oxide production by nitric oxide synthase. The antioxidant effects of Cr and Arg following Cr supplementation might be beneficial in ameliorating cardiovascular disease (Wu and Meininger, 2000). To confirm the antioxidant effect of Cr, recent study by Lawler et al. (2002) found that Cr displayed a significant ability to act as an antioxidant scavenger primarily against radical ions of O_2^- and $OONO^-$ but not of H_2O_2. Their data are claimed to be the first demonstration

of a direct antioxidant property for Cr. They explained a direct antioxidant effect for Cr was lower than physiological levels of reduced glutathione (GSH), but was additive with reduced glutathione. Thus, they suggest a potential for Cr may exert a protective effect against neuromuscular diseases that cause muscle wasting and cardiovascular disease.

EXERCISE TRAINING AND CARDIAC FUNCTION

The capacity for performing endurance exercise depends on the ability of the heart to augment its output to the exercising muscles and the ability of these muscles to utilize oxygen from the delivered blood. The increase in cardiac output during maximal upright exercise is typically 4 to 6 fold in healthy subjects. This is accomplished by a 2 to 4 fold increase in heart rate and a 20% to 50% augmentation of stroke volume. The stroke volume increase is accomplished both by use of the Frank-Starling mechanism to maintain left ventricular (LV) end diastolic volume and by more complete LV emptying to reduce end-systolic volume. Both enhanced LV contractility and peripheral vasodilation contribute to the more complete LV emptying observed during exercise (Pina et al., 2003). The beneficial effects of exercise training include an improvement in cardiac performance by improving in the intrinsic myocardial contractile function (Mole, 1978) and myocardial enzyme activity (Chesky et al., 1983) as well as modifying the cardiac isomyosin distribution (Morris et al., 1990). The exercise training programs has varied by such factors as setting (supervised training), type of activity (running, walking, cycling), duration (from 8 weeks to 3 months), and intensity (from low to moderate). Most of improvement occurs by week 3 but can continue up to 6 months. The cardiovascular system adapts to exercise training by minimizing the energy cost of the work. To provide the necessary work, increases in pressure work and heart rate are minimized in favor of augmented stroke volume, then inotropic function and blood flow occur. Moreover, cardiac myocyte metabolism adapts to the demands of training to provide a more efficient and better sustained energy supply. The increase in peak of oxygen uptake (VO_2) correlated with other improvements, such as an increase in muscle mitochondria and decrease ventilation (Pina et al., 2003). Although, exercise can create an imbalance between oxidant and antioxidant levels, a situation known as oxidative stress (Leeuwenburgh and Heinecke, 2001), on the other hand, endurance exercise training protects rats from exercise induce oxidative stress, raising levels of antioxidant enzyme and reducing oxidant production in both skeletal and cardiac muscle (Powers et al., 1994; Leeuwenburgh and Heinecke, 2001). Physical exercise has a significant impact on the GH/IGF-I axis. However, data from studies evaluating

IGF-I response to exercise are controversial. While some studies have demonstrated no change in circulating IGF-I levels, in many others, exercise induced a transient increase in IGF-I levels resulting from acute release of IGF-I from its binding proteins (BPs) (Rosenfeld et al., 1990). Furthermore, IGF-I response depends on exercise type, intensity, and duration as well as training status (Schwarz et al., 2007). Growing evidence suggest that like IGF-I, FGF-2 plays an important role in exercise-induced muscle hypertrophy and angiogenesis. Another previous reported that the stimulatory effect of GH/IGF-I axis increased exercise capacity (Merchav et al., 1988). Likely, recent research also suggests exercise raining induced secretion of GH plays a significant role in the regulation of fatty acid metabolism (Kanaley et al., 2001). Examples of human physical activities are jogging, swimming, cycling, resistance exercise, or weight lifting. The animal models that mimic human physical activity are few. Some are running of rats on motor-driven treadmills. In contrast, the cardiovascular response of rats to swimming is opposite to the response seen in human swimming. During, swimming, the heart rate and mean arterial pressure in rats decreases where as heart rate can increase to near-maximal values during swimming in human. It can be suggested the swimming rat model dose not mimic human swimming (Baldwin, 1985). Another animal model of increased contractile activity that mimic human physical activity is running that included treadmill and voluntary wheel running (Booth and Thomason, 1991). For force treadmill running, Moraska et al. (2000) concluded the benefit of this running type. Firstly, it can control duration, intensity and frequency of running. Secondly, treadmill running animals can increase in oxidative capacity of skeletal and cardiac muscle. Thirdly, however, treadmill running animals may elicit adaptations indicative of chronic stress such as adrenal gland hypertrophy, suppression in lymphocyte proliferation and antigen-specific IgM production. Voluntary wheel running has also reported about its benefit by a number of studies. Voluntary wheel running improves survival (long living), increases mean life-span, and prevents stress-induced behavioral depression and immunosuppression as well as decreases in body weight (Holloszy et al., 1985; Sherwimen, 1998; Engesser-Cesar et al., 2005). Additionally, long-term wheel running has also showed an antioxidant effect by decreasing lipid peroxidation accompany with an increase in antioxidant enzymes of CAT, GPx activity

ESTROGEN AND EXERCISE

Regular physical exercise has long been shown to elicit positive adaptations that results in improve myocardial function under physiological stress and in certain pathogenesis (Booth and Thomason, 1991). A beneficial outcome of

exercise training has been indicated in patients with heart failure (Belardinelli et al., 1999). Moreover, many investigators examining the effect of exercise training on the contractile performance of cardiac muscle cells demonstrated the increased tension generating capacity of the myocardium (Wisloff et al., 2001). Exercise training have been reported to be related to a variety of cellular adaptation against ischemic-reperfusion injury, including a more developed myocardial vasculature, a greater high energy reservation, a reduced coronary resistances, an enhanced antioxidant defense capacity and an improve Ca^{2+} sensitivity (Moore et. al, 1993). However, a small number of studies have investigated the effects of estrogen on exercise performance. Previous study have demonstrated that rodents treated with high E_2 can sustain less muscle damage and disruption during and following exercise training (Persky et al., 2000). Sotiriadou and coworkers (2003) concluded that E_2 may have a protective effect on muscle tissue possibly due to their antioxidant and membrane stabilizing properties. In 1987, Cauley and coworkers reported that estrogen replacement in postmenopausal women increased higher grip strength than those who did not receiving estrogen. Additionally, in ovariectomized rats, exercise training has been reported to prevent the changes in cardiac myofilament Ca^{2+} activation (Malhotra et al., 1990). Recent research also supports the preventive effects of exercise training on the molecular alterations of heart in ovariectomized rats (Bupha-Intr and Wattanapermpool, 2003). However, there is continuing some controversy as to unclear whether estrogen has any effect on cardiac performance. In training groups of postmenopausal women receiving estrogen, Barrett-Connor et al. (1995) found no significant changes in electrocardiography (ECG) signal responses to exercise. Similarly, in an exercise group, there is not significantly different of heart rate and blood pressure in postmenopausal women treated with E_2 (Rosano et al., 1993).

CREATINE AND EXERCISE

Cr has a long history as a safe and effective sports supplement. Previous reviews have focused primarily on the improvements in exercise performance seen in human subject's ingestion of Cr (Earnest et al., 1995; Casey et al., 1996; Volek et al., 1999). Cr is a dietary supplement purposed to improve exercise performance and increase fat-free mass. One beneficial effect of Cr supplementation in young and healthy man is reported to enhance muscle fiber size (Volek et al., 1999) as well as increase lean body mass (Green et al., 1996). Previous study suggested that Cr loading of 20 g daily for 5 days is used to rapidly increase muscle Cr levels, and muscle Cr levels can also be increased more

gradually with a daily maintenance dose of 2-5 grams (Harris et al., 1992; Hultman et al., 1996). Stroud and coworkers (1994) concluded that Cr supplementation may influence the pattern of substrate utilization and improve performance during more prolonged, sub-maximal exercise. However, the beneficial effect of Cr has been observed mainly in short-term supplementation, although there are limited research data with long-term supplementation. Recently, the effect of oral Cr supplementation on contractile performance and metabolism of cardiac muscle have become on area of major interest in exercise physiology. Dietary Cr supplementation has been shown to increase muscle PCr concentration and improve muscle performance during intermittent high intensity exercise (Jobobs, 1991; Balsom et al., 1994; Greenhalff et al., 1994). A potential explanation for these findings may be that Cr supplementation alters the energy cost of muscle force production (ATP cost) at the onset of exercise by increasing muscle PCr concentration. Additionally, exercise has also shown stimulatory effects on Cr-uptake, suggesting that the exercise may enhance blood flow which leads to increase the translocation of CrT to the muscle membrane (Perky and Brazeau, 2001). Hespel et al. (2001) investigated the effect of oral Cr supplementation during leg immobilization on muscle volume and function. They concluded that oral Cr supplementation stimulated muscle hypertrophy during rehabilitative strength training. Similarly, in human studies, effects of Cr supplementation have been reported to induce muscle hypertrophy which might be GH-mediated improvement in muscle performance (Perky and Brazeau, 2001). The antioxidant of Cr in exercise condition has been previously proposed by other studies. Cr supplementation may blunt increased hypoxanthine efflux from muscle observed with repeated bouts of high intensity exercise (Balsom et al., 1993) As Cr in solution is widely dispersed throughout the sarcoplasm, Cr may be readily accessible to radicals and reactive oxygen and nitrogen species generated during exercise and could play an important supportive role (Lawler et al., 2002). Previously, swim training rats combined with Cr supplementation can increase the combined expression of two myocardial CK isoforms (BB and MB). Recent research has shown that Cr supplementation in animals with swimming exercise over 21–day training induces an increase in cardiac RNA content, suggesting an increase in the metabolic efficiency of cardiac as a result of increase in muscle PCr or cardiac muscle protein synthesis (McClung et al., 2003). In agree with information as mentioned above, our pilot study in hamster heart can be concluded that combined Cr supplementation with exercise training for 14-day has beneficial effect on high energy phosphate contents (Cr, PCr and TCr), however, mechanism of these combining effects have to be investigated further.

CARDIOMYOPATHY

Cardiomyopathies (CM) are diseases of the myocardium associated with cardiac dysfunction. The CM is now classified by the dominant pathophysiology or pathogenetic factors that can be classified as follow.

Dilated cardiomyopathy

This condition is characterized by dilatation and impaired contraction of the left or both ventricles. It may be idiopathic, familial/genetic, viral (Why et al., 1994) and/or immune (Carforio et al., 1994), alcohol/toxic or associated with recognized cardiovascular disease where the degree of myocardial dysfunction is not explained by the abnormal loading conditions or the extent of ischemic damage. Histology is nonspecific. Presentation is usually with heart failure which is often progressive. Arrhythmias, thromboembolism and sudden death are common and may occur at any stage.

Hypertrophic cardiomyopathy

This condition is characterized by left and/or right ventricular hypertrophy, which is usually asymmetric and involves the interventricular septum (Wigle et al., 1985). Typically, the left ventricular volume is normal or reduced. Systolic gradients are common. Familial disease with autosomal dominant inheritance predominates. Mutations in sarcomeric contractile protein genes cause disease . Typical morphological changes include: myocyte hypertrophy and disarray surrounding areas of increased loose connective tissue.

Restrictive cardiomyopathy.

This entity is characterized by restrictive filling and reduced diastolic volume of either or both ventricles with normal or near normal systolic function and wall thickness. Increased interstitial fibrosis may be present. It may be idiopathic or associated with other disease (e.g. amyloidosis, endomyocardial disease with or without hypereosinophilia

Arrhythmogenic right ventricular cardiomyopathy

The disorder is characterized by progressive fibro fatty replacement of right ventricular myocytes, initially with typical regional and later global right and some left ventricular involvement with relative sparing of the septum (McKenna et al., 1994). Familial disease is common with autosomal dominant inheritance and incomplete penetrance, a recessive form is described. Presentation with arrhythmias and sudden death is common, particularly in the young (Thiene et al., 1988).

Unclassified cardiomyopathy

Unclassified DM includes a few cases which do not fit readily with any group (e.g. fibroelastosis, no compacted myocardium, systolic dysfunction with minimal

dilatation, mitochondrial involvement). Some diseases may present with features of more than one type of CM (i.e. amyloidosis, systemic hypertension). It is recognized that arrhythmias and conduction disease may be primary myocardial disorders. At this time, however, it was elected not to include them as CM.

Syrian hamster provides a good model for experimental study of CM (Kobayashi et al., 1987) and suggests to be more closely related to human CM (Stassijns et al., 1999). Free radicals are known to be increased at the onset (40-90 days) of CM (Mailer et al., 1991). Na+/K+ ATPase (Proschek and Jasmine, 1982) and calcium loading of mitochondria are decreased while calcium deposition in myocytes (Farber, 1982) is noted. Diseased hearts at late stages of CM showed fewer mitochondria and defective oxidative phosphorylation (Hoppel et al., 1982). The response of sarcolemmal adenylate cyclase to hormonal control is decreased as is adenosine-mediated feedback control (Bohm et al., 1986). In agree with previous study (Li et al., 1998) which was found that myocardial protein oxidation, a marker of oxidative stress, was markedly increased during the late stages of hamster CM where as myocardial antioxidant defense, GPx activity was significantly decreased. Previously, the skeletal muscle changes seen in CM could be associated with the reduction in IGF-I serum levels (Burbach et al., 1987). Moreover, another study aimed to elucidate changes in diaphragm and their mechanism in CM and found that heart failure in hamsters with dilated CM depressed the force-generation capacity and impaired histological properties of muscle (Stassijns et al., 1999).In several animal models of heart failure, it has been shown a reduction of ATP, Cr substrate pools and CK activity (Ingwall et al., 1985; Neubauer et al., 1995; Nascimben et al., 1996). As it known that the velocity of the CK reaction changes in parallel with cardiac performance, increases with elevated work and decreases with ischemia or hypoxia in rat hearts (Ingwall, 1993). Langendorff-perfused failing hearts of CM hamsters and turkeys have been demonstrated to have lower total Cr content and CK activity to perform less cardiac work (Khuchua et al., 1989; Liao et al., 1996). Likely, E_2 deficiency in ovariectomized animals impaired cardiac performance has been previously reported by many studies (Kolodgie et al., 1997; Liu et al., 2004). Similar to cardiac dysfunction after estrogen deficiency and Cr depletion, contractile function of CM hamsters has been studied at a cellular level, and it has been shown that the peak force under the isometric condition and external work were decreased (Nishimura et al., 2005). In addition, Stupka and Tiidus (2001) investigated the effects of ovariectomy and E_2 on ischemia-reperfusion injury in hindlimbs of female rats and conclude that ovariectomy possibly related to changed circulating IGF-I levels can influence indexes of muscle damage. Additionally, Horn et al. (2001)

proposed that chronic Cr substrate pools depletion is associated with increased mortality and loss of ATP in heart failure rats. In agree with this finding, many studies have been also reported a decrease in serum IGF-I level, a growth factor could be seen in heart failure animals (Volterrani et al., 2000). Similarly, Stassijns and coworkers (1999) studied the mechanism of CM hamsters on the diaphragm and reported that force-generating capacity was depressed, and serum IGF-I was markedly decreased. The possible involvement of free radicals and antioxidant enzymes in the early stage of CM has been reported in many studies (Cheung et al., 2000; Ichihara et al., 2006.). Similar to an induce in myocardial oxidative stress by decreasing enzymatic antioxidant defense, SOD in E_2 deficiency female rats (Barp et al., 2002), a report studied by many researchers (Mailer et al., 1991; Li et al, 1998; Ichihara et al., 2006) showed an increase in concentration of mitochondria free radicals in CM hamster than those in the control. Likewise, a decrease in Cr level may induce an increased in antioxidant effect against myocardial oxidative stress (Lawler et al., 2002).

LANGENDROFF APPRATUS

The visible Heart is a live beating heart functioning outside of the body under simulated physiologic conditions. The isolated heart apparatus is an experimental simulation of the donor recipient, providing oxygen and metabolic substrates for the heart to survive. Since the coronary system of the heart relies on the pressure, which enables the transport of oxygen and metabolic substrates to the heart, initially the heart must externally assisted after transplantation. This is accomplished by pumping fluid directly into the coronary system and removing fluid from the heart's chambers, a technique called the Langendorff mode of perfusion. The isolated perfused heart system, as originated by Oscar Langendorff (1895), has become a predominant technique in pharmacological and physiological research to produce an isolated mammalian heart full contractile activity. The technique allows the examination of cardiac contractile strength (inotropic effects), heart rate (chronotropic effects) and vascular effects without the complications of an intact animal model. From its simple beginning, the technique and equipment has evolved to include both constant pressure and constant flow models in both recirculating and non-recirculating modes (Langendorff, 1895).

Basic Principles

. The basic goal of the Langendorff method is to provide an isolated heart with oxygen and metabolites via a single cannula inserted into the ascending aorta Oxygenated blood or a perfusate is pumped down the aorta towards the heart

by means of an external pump. At the base of the aorta is an ostium (hole) which feeds blood under pressure into the coronary arteries. Langendorff maintained the isolated heart through the use of a reservoir containing a physiological solution that was elevated above the heart to create a pressure head. This reservoir was connected to the heart via a tube to the aortic cannula. When the reservoir was opened, the perfusate was forced through the base of aorta into the coronary bed. This is often termed as a "retrograde" perfusion, as the perfusate flows directly into the aorta rather than in the normal situation where blood enters the aorta from the left ventricle. In the Langendorff technique, perfusate solution does not flow via the normal ventricular circulatory pathway. The perfusate in langendorff mode flows in a retrograde fashion in to aorta and exits via the coronary arteries, where as the perfusate in a working heart enters the left atrium and is ejected from the left ventricle into the aorta. Therefore, this system does not permit the left ventricle to generate pressure-volume work that is representative of typical cardiac function.

Donor Heart Preparation

. To maximize the capabilities of the perfused heart system, it is important to decide on the design of the experiment, the donor heart, its maintenance and instrumentation. The selection of the heart is often based on the typical or unique response of the donor organ to pharmacological or physiological stimuli, or on selected metabolic or biochemical events. The most common donors are mice, rats, rabbits, guinea pigs, ferrets and hamsters.

Perfusate Preparation

. Once the donor heart is selected, an appropriate perfusate solution is required to provide the isolated heart with nutrients and oxygen. The heart is a metabolically demanding organ and, therefore, the choice of solution is important for the survival of the tissue and successful investigation. All solutions will have to be aerated, as the oxygen consumption of most mammalian hearts is considerable. As the solubility of oxygen in saline solutions is much lower than that of blood, the oxygen tension is normally about 550-600 mmHg at 37 ^{o}C to ensure adequate delivery to the cardiac cells. Preparation of perfusate is designed primarily for use with saline solutions that do not contain protein components. In this system, the solution must be buffered, either with the traditional carbonate buffers such as Krebs-Henseleit, or Tyrodes or with variations of these formulas using HEPES or MES buffers. Due to the lower viscosity of blood-free media, the flow rates used in a Langendorff system are nearly twice as large as with whole blood. Glucose (normally 5-10 mM) is necessary and, depending on the experimental design, other substrates can be used such as pyruvate, lactate, fatty acids, amino acids, etc. The ionic

components of the solutions are also important and vary with the species; potassium and calcium are the most variable and critical of the ions. Calcium carbonate or calcium carbonate crystals may form in the solution if it is not maintained at the correct pH. The bubbling of the solution with carbogen is not only important in oxygenating the solution but is also important in maintaining the pH.

Experimental Preparation

. As the heart is a highly metabolically active organ, it requires a constant supply of oxygen and nutrients. Therefore, the time in which it takes to remove and mount the heart is very important. An extended period (> 30 seconds) of reduced oxygen and/or nutrients will significantly affect cardiac tissue (at body temperature), in particular its survival and experimental responses. After inducing anesthesia, the donor should be placed in a dissecting tray near the isolated heart apparatus. To facilitate last removal and mounting of the organ, extra sets of sutures and instruments should be positioned close to hands. Cardiac removal may be performed as a surgical procedure by intubating the donor and artificially ventilating the animal. Ventilating the animal provides more time to prepare and remove the heart as the animal and heart will continue to be oxygenated once the thorax has been opened.

The constant flow system requires the use of a pump to perfuse the heart at a set flow rate. Once the flow is constant, syringe pumps can be used to conveniently tritrate the heart with drugs or other agents. The experimental effects on coronary vessel diameter are measured as a change in perfusion pressure. This type of system setup is often used when conducting experiments in which a limitation of substrate or oxygen availability to the cardiac tissue is provided. The coronary vessels can dilate or constrict, i.e. auto-regulate, but the total supply of oxygen and substrate is controlled by the flow rate. In the constant pressure system, the pressure head is kept constant by adjusting the reservoir level or through the use of a pump and overflow system. These types of experiments will result in changes in flow that can be measured volumetrically, or with fraction collectors, electromagnetic flow probes, drop counters, ultrasonic flowmeters etc. In the constant pressure system, changes in vascular resistance can increase or decrease the supply of oxygen and substrate supplied to the heart. In the working heart system, both the aorta and the left atrium are cannulated and the atrial pressure (preload) and aortic resistance (afterload) are regulated experimentally as the heart circulates the perfusate solution. In using any of these systems, the experimenter may choose to have a non-recirculating (single pass) system or a recirculating system. A single pass system is useful when applying several agents in sequence and then allowing their effects to

dissipate as the agent is washed out of the heart. This approach is also useful when measuring the uptake or release of various drugs, neurotransmitters or metabolites. A recirculating system is useful when it is necessary to reduce the total volume of perfusate when using expensive drugs or substrates. If recirculation continues for 15-30 minutes or more, denatured proteins released from the heart will accumulate in the perfusate.

Experimental Measurement

. There are a number of physiological parameters that can be measured in the perfused heart preparation. The simplest measurement of contractile force is made using a force transducer attached to the apex of the heart with a pulley in between the heart and the transducer. In this system, a measurable amount of force is lost in a rotational motion as the heart contraction. Cardiac electrical activity can he measured using surface monopolar or bipolar electrodes. Microelectrodes implanted in the surface myocytes can also be used for electrical measurements. Oxygen consumption can be measured with dual oxygen electrodes, one placed in the perfusate stream entering the heart, the other monitoring the effluent leaving coronary sinus. This effluent can be bypassed through the use of a peristaltic pump and then transferred to the second oxygen electrode. Radiolabelled compounds can be used for metabolic studies, the release or uptake of various ions or substrates. Optical studies have been performed on the fluorescence of endogenous or exogenous fluorescent compounds (Dehnert, 1988).

ECG, LVDP and $\pm$ dP/dt were used as indices of cardiac mechanical function. Some abnormalities have the ability to delay cardiac repolarization, an effect that is manifested on the surface electrocardiogram (ECG) as prolongation of the QT interval. The QT interval represents the duration of ventricular depolarization and subsequent repolarization, beginning at the initiation of the Q wave of the QRS complex and ending where the T wave returns to isoelectric baseline. QT interval prolongation creates an electrophysiological environment that favours the development of cardiac arrhythmias. Because of its inverse relationship to heart rate, the QT interval is routinely transformed (normalized) by means of various formulae into a heart rate independent “corrected” value known as the QTc interval. The QTc interval is thus intended to represent the QT interval at a standardized heart rate. QTc values higher than normal are associated with increased risk of serious heart rhythm abnormalities (Torsades de Pointes), such as ventricular fibrillation, which can result in sudden death. Another index for cardiac performance, it is easier to consider LVDP during the early period of isovolumic contraction of cardiac contraction-relaxation cycle. During the period of isovolumic contration, the preload

and afterload are constant, and the maximal rate of pressure generation should be an index of the inotropic index which is calculated by max dP/dt, where P is LVDP, t is time, and d indicates rate of change. This index is not fully independent of the preload, which when increased will enhance the contractile state by length-activation (Opie, 1997; Davey et al., 2000; Hamlin et al., 2003)

In the present work, we tested the mechanical damage induced by exogenously administered H_2O_2 in the isolated hamster hearts. We designed the langendorff apparatus in constant coronary pressure at 80 mmHg with re-circulating system to measure the parameters for cardiac functions including HR, LVDP and $dP/dt_{max.}$ Similar to experimental design in our study, other studies measured cardiac performance in the perfused heart preparation in a langendorff technique. Previous reports studied the effect of Cr on cardiac functions and measured parameter of cardiac performance in LVDP and heart rate by retrograde perfusion in the langendorff mode at a constant coronary perfusion pressure (Horn et al., 1998, 1999, 2001; Vona-Davis et al., 2002). Likewise, other studies were design to assess the effects of E_2 replacement therapy on mechanical function after cardiac ischemic reperfusion injury by isolated and perfused hearts in working mode with langendorff apparatus (Fraser et al., 1999; Beer et al., 2002; Liu et al., 2004). Moreover, to determine whether the drug administration or supplementation is effective against the mechanical and metabolic damage induced by exogenous administrated by H_2O_2 in the isolated work heart was also started in the langendorff perfusion technique (Naohiro and Akiyoshi, 1996; Hill and Singal, 1997; Merrill, 2002).

METABOLIC RATE

Metabolic rates (MR) are important components of the energetic budget of the animals. To estimate different types of MR diverse specific apparatuses have been developed. Measurements of metabolic rates (MR) based on oxygen consumption are a useful method to estimate the energy requirements of free-living animals (Peters, 1983). These measurements are fundamental for quantitative analyses and are a part of our understanding of the ecosystem energetics. Energy is expended in basal metabolism, physical activity, and thermogenesis. Basal energy expenditure arises from metabolic processes necessary for sustenance of life processes, and is measured as basal metabolic rate (BMR). The release of energy in this state is sufficient for the functioning of the vital organs, such as the heart, lungs, brain and the rest of the nervous system, liver, kidneys, sex organs, muscles and skin. BMR is minimum amount of energy needed to sustain life, often termed the “metabolic cost

of living" (Vander et al., 1994). An accurate BMR measurement requires that the person's sympathetic nervous system is not stimulated and measured after waking up in the morning, 10-12 hours after a meal. For convenience, resting metabolic rate (RMR), accounts for 65-75% of total energy expenditure, can be measured anytime of the day, 3-4 hours after a meal, and at rest for at least 30 minutes. During the measurement of either BMR or RMR, the individual has to be at as complete a mental and physical rest as possible, awake but relaxed, and in a comfortable environment. Metabolic rate can be measured by gas analysis using the methods of direct or indirect calorimetry, and predictive equations. Indirect calorimetry calculates heat that living organisms produce from their production of carbon dioxide and nitrogen waste (frequently ammonia in aquatic organisms, or urea in terrestrial ones), or from their consumption of oxygen but heat generated by living organisms may also be measured by direct calorimetry, in which the entire organism is placed inside the calorimeter for the measurement . BMR decreases with age and with the loss of lean body mass. In contrast, Increase cardiovascular exercise and muscle mass can increase BMR. Illness, previously consumed food and beverages, environmental temperature, and stress levels can affect one's overall energy expenditure, and can affect one's BMR as revealed by gas analysis. Studies of energy metabolism using both methods provide convincing evidence for the validity of the respiratory quotient (R.Q.) ratio, which measures the inherent composition and utilization of carbohydrates (1), fats (0.696) and proteins (0.818) as they are converted to energy substrate units that can be used by the body as energy. Additionally, the early work of Harris and Benedict (1918) showed that approximate values could be derived using body surface area (computed from height and weight). Therefore, BMR is calculated from $VO_{2\ (STP)}$x$RQ_{(average)}$/surface area and expressed as cal/m^2/hr (McCarter and Palmer,1992). The metabolism of an animal is estimated by determining rates of carbon dioxide production (VCO_2) and oxygen consumption (VO_2) of individual animals, either in a closed or an open-circuit respirometry system. Two measures are typically obtained: standard (SMR) or basal metabolic rate (BMR) and maximal rate (VO_{2max}). SMR is measured while the animal is at rest (but not asleep) under specific laboratory (temperature, hydration) and subject-specific conditions (e.g., size or allometry), age, reproduction status, post-absorptive to avoid thermic effect of food (White and Seymour, 2005). VO_2max is typically determined during aerobic exercise at or near physiological limits (Weibel and Hoppeler, 2005). In contrast, field metabolic rate (FMR) refers to the metabolic rate of an unrestrained, active animal in nature (Nagy, 2005). Whole animal metabolic rates refer to these measures without correction for body mass. If SMR or BMR values are divided by the

body mass value for the animal, then the rate is termed mass-specific. It is this mass-specific value that one typically hears in comparisons among species.

Closed-circuit system

. Respirometry depends on a "what goes in must come out" principle (Frappell et al., 1989). Consider a closed system, an animal was place into an air-tight container. The air sealed in the container initially contains the same composition and proportions of gases that were present in the room: 20.95% O_2, 0.03% CO_2, water vapor (the exact amount depends on air temperature), 71% (approximately) N_2, and a variety of trace gases making up the rest (see Earth's atmosphere). As time passes, the animal in the chamber produces CO_2 and water vapor, but extracts O_2 from the air in proportion to its metabolic demands. Therefore, as long as we know the volume of the system, the difference between the concentrations of O_2 and CO_2 at the start when we sealed the animal into the chamber (the baseline or reference conditions) compared to the amounts present after the animal has breathed the air at a later time must be the amounts of CO_2/O_2 produced/consumed by the animal. The operating principal for measuring the volume of the consumed O_2 in closed respirometry systems is based on a constant air pressure and chemical content inside a metabolic chamber. This can be achieved by exchanging the exhaled CO_2 by O_2, which is a result of lowering the air pressure by withdrawing the CO_2 and restoring the pressure inside the metabolic chamber by injection of O_2 from an external source. One of the very first closed type respirometer was designed by Morrison (1951). It permits measurements of resting and basal metabolism rates (RMR and BMR). Recently, the aim of the work is to develop an advanced type of a closed respirometer, which permits accurate measurement of a variety of metabolic rates in small mammals (Dimitrov et al., 2005). Moreover, MR in small animals was measured using indirect calorimetry with closed-circuit metabolic chamber has been reported by a other studies (Tomasi and Horwitz, 1987; Dinulescu et al., 1998; Nespolo et al., 2002; Liu et al., 2003)

Open-circuit system

For an open-system, direct measurement of VO_2, design constraints include washout characteristics of the animal chamber and sensitivity of the gas analyzers (Withers, 2001). However, the basic principle remains the same. The primary distinction between an open and closed system is that the open system flows air through the chamber (i.e., air is pushed or pulled by pump) at a rate that

constantly replenishes the O_2 depleted by the animal while removing the CO_2 and water vapor produced by the animal. The flow rate must be high enough to ensure that the animal never consumes all of the oxygen present in the chamber while at the same time, the rate must be low enough so that the animal consumes enough O_2 for detection. For examaple, a 20 g animal, flow rates of about 200 ml/min through 500 ml containers would provide a good balance. At this flow rate, about 40 ml of O_2 is brought to the chamber and the entire volume of air in the chamber is exchanged within 5 min. For other smaller animals, chamber volumes can be much smaller and flow rates would be adjusted down as well. Note that for warm-blooded or endothermic animals (birds and mammals), chamber sizes and or flow rates would be selected to accommodate their higher metabolic rates.

CHAPTER III

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The study was performed in female Golden Syrian Hamsters weighing 100-120 grams obtained from National Laboratory Animal Centre, (Mahidol University, Salaya, Nakhonpathom). The animals were housed in a room in which the temperature was maintained at 24±2°C, which provided a 12:12 dark/light cycle. The animals were given standard chow and water *ad libitum*.

CHEMICALS

Chemical agents

Tiletamine chlorhydrate and Zolazepam chlorhydrate (Zoletil®) was purchased from Virbac, France. Heparin (500 i.u./ml) was obtained from Leo pharmaceutical product, Denmark. Creatine monohydrate powder, β-guanidinopropionic acid powder (β-GPA), 17 β-estradiol, β-Nicotinamide adenine dinucleotide (β-NAD), reduced Glutathione (GSH), Glutathione reductase from bake's yeast and 2-vinylpyridine were purchased from Sigma, USA. Soda lime pellets with indicator grade was obtained from Merck, USA. 2,3,Butanedione (diacetyl) and α-napthol were obtained from Fluka, USA. CK-NAC liquid UV was purchased from Human Diagnostics GmbH, Germany. The OCTEIA Rat/mouse IGF-I immunoassay kit was obtained from Immunodiagnostic system Ltd, UK. Protease inhibitor cocktails and 10 % Tween 20 were purchased from Sigma, USA. Modified Lowey protein assay kit and SuperSignal® West Pico kit were purchased from Pierce, USA. Prestained protein molecular markers, Laemmli sample buffer, ammonium persulfate, 40% acrylamide/Bis, glycine, TEMED, methanol, sucrose, filter paper, nitrocellulose membrane, and CL-XPosure™ Film 5 x 7 inches were purchased from Pierce, USA.

Antibodies

Rabbit anti-Human Creatine Transporter (CT1, CRT, Catalog No. C7911-05) was purchased from US Biological, USA. Goat anti-rabbit IgG horseradish peroxidase-conjugate (Catalog No.31460, Pierce, USA), α-Actin mouse monoclonal antibody (Catalog No. 03-3100, Zymed, USA), and goat anti-mouse IgG horseradish peroxidase-conjugate (Catalog No. 31430, Pierce, USA).

EXPERIMENTAL DESIGN

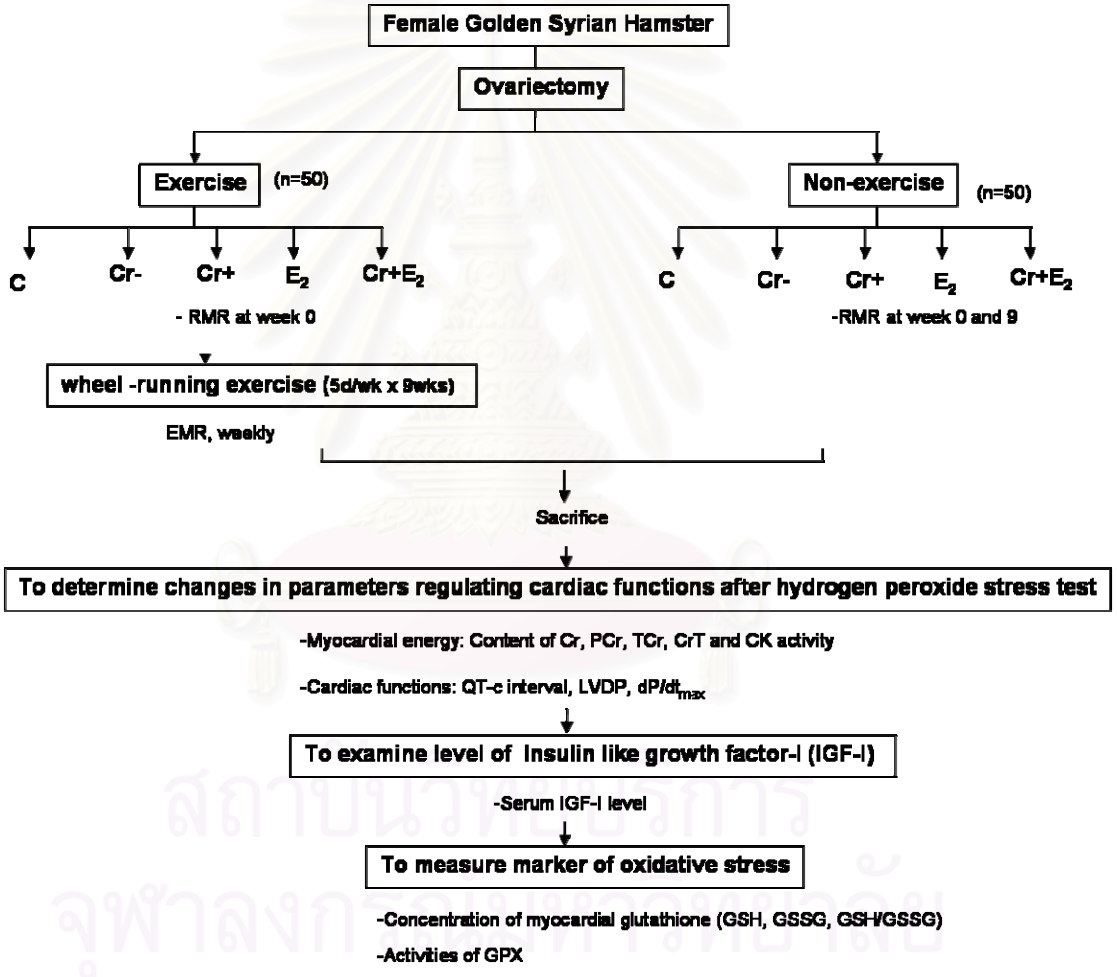


Figure 3.1 Experimental design

This study was approved by the Institutional Laboratory Animal Care and Use Committee of the faculty of Veterinary Science, Chulalongkorn University. All female Golden Syrian Hamsters weighing between 100-120 grams were ovariectomized 7 days before they were divided into two groups of non exercise (n = 50) and exercise (n = 50). Each group was further divided into the control and 4 treatments as follows:

1. Control group (control).

(Animals were gavaged with sucrose solution and injected subcutaneously with propylene glycol once daily).

2. Cr depletion (Cr).

(Animals were gavaged with 200 mg /kg BW of β-GPA in sucrose solution once daily)

3. Cr supplementation (Cr+)

(Animals were gavaged with 200 mg/kg BW of Cr monohydrate in sucrose solution once daily)

4. Estrogen replacement (E_2)

(Animals were injected subcutaneously with 30 μg/kg BW of 17 β-estradiol in propylene glycol once daily)

5. Cr supplementation combined with estrogen replacement ($Cr+E_2$)

(Animals were gavaged with 200 mg/kg BW of Cr monohydrate in sucrose solution and injected subcutaneously with 30 μg/kg BW of 17 β-estradiol in propylene glycol once daily)

Surgical Operation

After 7-day adaptation period, the animals were anesthetized with Tiletamine chlorhydrate and Zolazepam chlorhydrate (Zoletil®), at a dose of 60 mg/kg intraperitoneally (IP), and surgical procedures of ovariectomy were done as follows.

1) When animal is properly anesthetized, and reflexes were checked, skins were shaved on both sides of the flank, and cleaned with 70% alcohol and Providone iodine.

2) Surgical procedures were performed under aseptic technique. A 5 mm. A dorsal incision was made through the skin. The incision was centered between the

dorsal hump and the base of the tail. The skin was separated from the underlying muscle.

3). Rather than cutting the muscle, inserted the tip of a double blunt/blunt iridectomy or tissue scissors just through the muscle layer, and separated the muscle fibers by opening the scissors in a dorsal/ventral direction.

4) Located the ovarian fat pad which was visible in the abdominal cavity.

5) Hold the edge of the incision rim with a small rat toothed forceps and pulled the fat pad through the incision with a blunt forceps. The ovary would be attached to the under side of the fat pad.

6) A mosquito forceps was placed at the boundary between the oviduct and uterus and a ligature placed just below the hemostat. After removing the ovary and oviduct with a scissors, released the hemostat and maked sure no bleeding occurs. Return the uterine horn to the abdominal cavity. Sutured the abdominal wall with absorbable sutures, 4-0 vicryl (Ethicon, Piscataway, NJ). The skin incision was closed with silk.

7) Returned the animal to its cage and monitor recovery from anesthesia as described below.

Anesthesia Recovery Monitoring

During recovery from anesthesia, the following clinical parameters must be monitored at a minimum of 15 minute intervals until the animal is recovered. To protect the animal from hypothermia, animals were placed on a heating blanket wrapped with a towel (while still allowing visible monitoring) to conserve body temperature. Respiratory rate, movement and ability to maintain sternal recumbancy were observed. After fully recovered from anesthesia, the animals were returned to the vivarium, and the post-operative care was done daily.

Experimental protocol

To determine the effect of Cr supplementation and estrogen replacement on physiological parameters regulating cardiac functions in non exercise and exercise-trained ovariectomized hamsters.

After recovery, all animals were measured resting metabolic rate (RMR) at day 6 after ovariectomy by using closed circuit calorimeter (a metabolic chamber) at ambient temperature of 23 $\pm$ 2^{o}C.

For exercise-trained animals, the 9-week running program was introduced to animals at day 7 after ovariectomy. The exercise protocol is set to run for 10 min/day

for 5 days/week in a running wheel which had a 17-cm in diameter. The running wheel was connected to an electronic box that recorded the number of revolutions made by the wheels. The intensity of the session was adjusted by gradually increasing the number of rounds per minute (rpm) of the running wheel from 0 and maintained at 40 rpm for 5 minute. After that, the intensity was gradually decreased by adjusting numbers of rpm running wheel until stop. After resting for 1 minute, the second session was started and trained as similar to the first session. Adequacy of the exercise-running program was determined by heart rate measurement before and after exercise sessions with ECG transmitter that placed at the back of the animal and connected to an ECG receiver. These equipments were attached to computer software to record the heart rate and ECG signals.

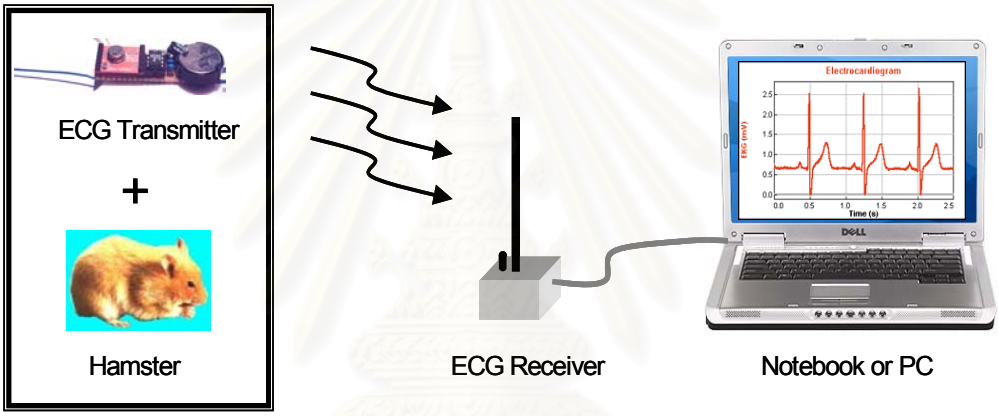
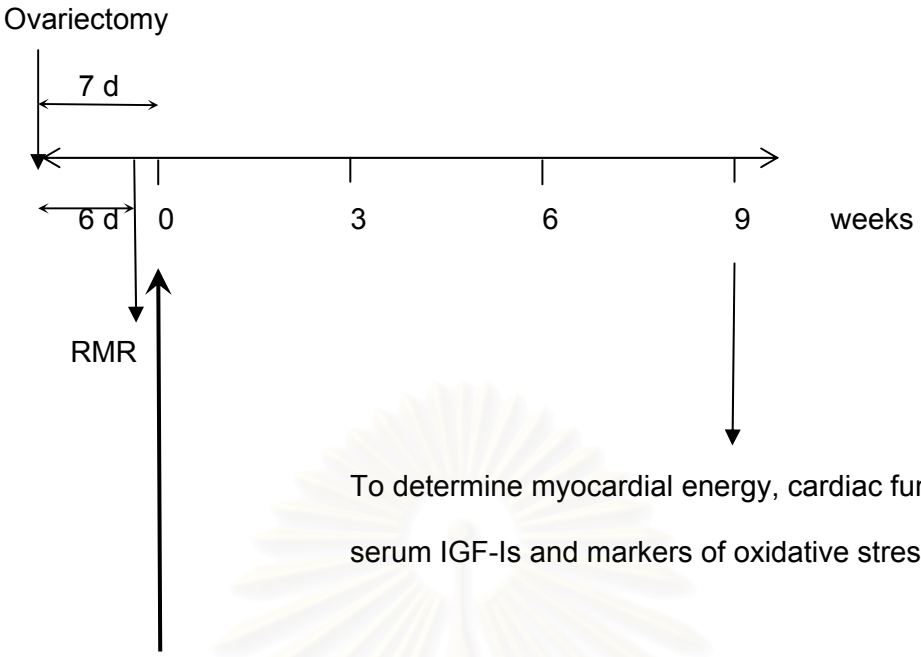


Figure 3.2. Equipments for heart rate measurement

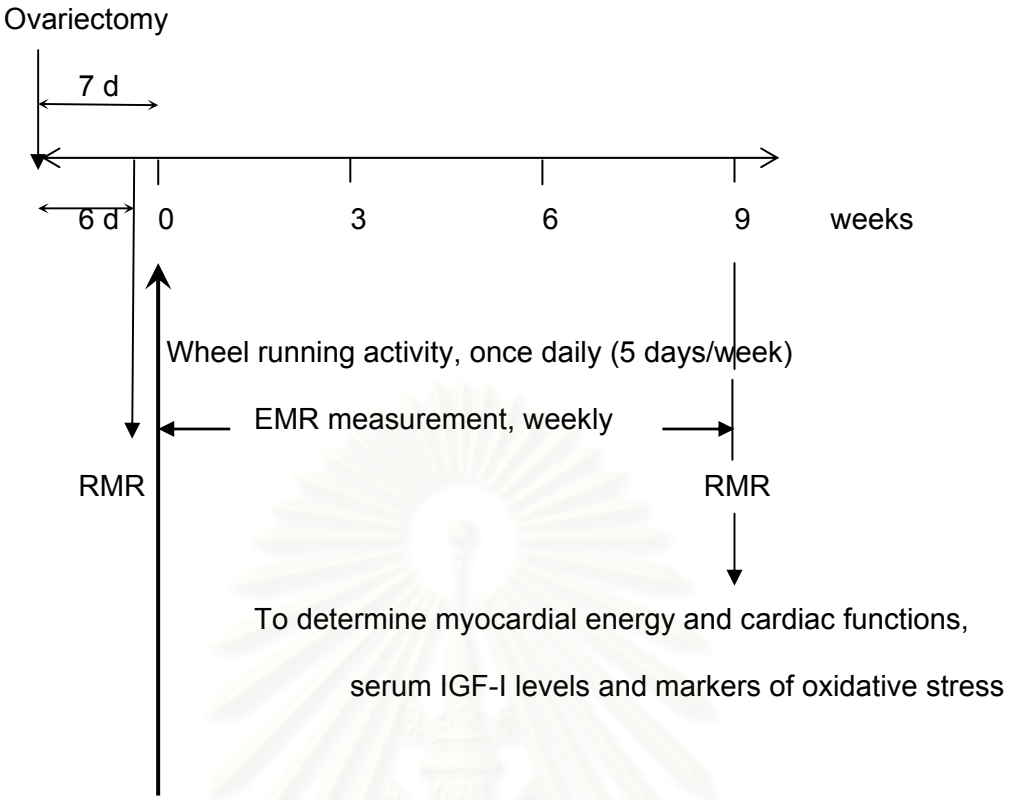
All exercise-trained animals received treatments similar to the non-exercise animals, and gavaged 30 min before the wheel-running exercise. In exercise animals, we measured exercise metabolic rate (EMR) weekly by using closed circuit calorimeter containing running wheels. The animals ran in the running wheels at 40 rpm for 5 minutes after that oxygen consumption and EMR were calculated. RMR was also measured before EMR at week 9 of all exercise-trained animals.

After 9 weeks, all animals were determined myocardial energy from the contents of Cr, PCr, TCr, CrT protein and CK activities. Cardiac functions were determined from QT-c interval, Left ventricular developed pressure (LVDP), and the maximum rate of pressure rise (dP/dt_{max})



- Sucrose solution and propylene glycol were gavaged and injected subcutaneously once daily in the control group.
- Cr monohydrate (200 mg/kg BW) in sucrose solution was gavaged once daily for Cr supplementation (Cr+)
- β-GPA (200 mg /kg BW) in sucrose solution was gavaged once daily for Cr depletion (Cr-).
- 17β-estradiol (30 μg/kg BW) in propylene glycol was injected subcutaneously once daily for E_2 replacement (E_2).
- Cr monohydrate (200 mg/kg BW) in sucrose solution was gavaged and 17β-estradiol (30 μg/kg BW) in propylene glycol was injected subcutaneously once daily for Cr supplementation combined with E_2 replacement group (Cr+E_2)

Figure. 3.3 Diagram of the experimental protocol in non exercise ovariectomized hamsters, RMR = resting metabolic rate, BW=body weight, d=day



- Sucrose solution and propylene glycol were gavaged and injected subcutaneously once daily for control group.
- Cr monohydrate (200 mg/kg BW) in sucrose solution was gavaged once daily for Cr supplementation group.
- β-GPA (200 mg /kg BW) in sucrose solution was gavaged once daily for Cr depletion group.
- 17β-estradiol (30 μg/kg BW) in propylene glycol was injected subcutaneously once daily for E_2 replacement group.
- Cr monohydrate (200 mg/kg BW) in sucrose solution was gavaged and 17β-estradiol (30 μg/kg BW) in propylene glycol was injected subcutaneously once daily for Cr supplementation combined with E_2 replacement group.

Figure. 3.4 Diagram of the experimental protocol in exercise-trained ovariectomized hamsters, RMR, EMR = resting metabolic rate and exercise metabolic rate, BW=body weight, d=day.

To examine the effects of Cr supplementation and estrogen replacement on serum IGF-I level in non exercise and exercise-trained ovariectomized hamsters.

After 9 weeks of experimental period, blood samples were drawn after dissection of the heart from the thorax for measurement of serum IGF-I level by ELISA technique

To study the effects of Cr supplementation and estrogen replacement on markers of oxidative stress in non exercise and exercise-trained ovariectomized hamsters.

After 9 wk of experimental period, right ventricles of all animals were excised for measurement of the concentration of myocardial glutathione (GSH, GSSG and GSH/GSSG) and antioxidative enzyme, glutathione peroxidase (GPx), by spectrophotometric technique.

METABOLIC RATE MEASUREMENT

RMR was measured 3-4 hours after a meal, and at rest for at least 30 minutes. The RMR in non exercise and exercise-trained animals were followed these procedures.

1) A metabolic chamber of 1000 ml containing soda lime (CO_2 absorbent) was connected to injected syringe, O_2 balloon, and water manometer. Instruments in this system were tested before measurement by injection air from external source to the system. If water level in water manometer could be maintained for at least 2-3 min, the measurement was ready to start.
2) The animal was weighed and placed into the sealed metabolic chamber.
3) Amount of known O_2 was injected into the system
4) While the animal in the chamber consumed O_2, water level in water manometer was returned to the normal level.
5) As time passed, the animal in the chamber produced CO_2 and water vapor, but extracted O_2 from the air in proportion to its metabolic demand resulting an equal of water level in a manometer
6) Since production of CO_2 was absorbed by CO_2 absorbent, soda lime. Therefore, animal inspired O_2 from the sealed chamber determined O_2 volume of the system (O_2 consumed by the animal).

7) Chamber temperature and air pressure were also recorded. For volume compensation, each 1 ^{O}C of increased temperature resulted in an increase in air volume for 15 ml. Total volume of O_2 consumed by the animal was calculated using the following equation.

Total volume of O_2 consumed (ml) = total O_2 volume+ compensated volume

O_2 consumption at STP $P_1V_1/T1 = P_2V_2/T_2$

Where P_1= air pressure

V_1 = total volume of O_2 consumed

T_1 = temperature at STP.

Then, RMR were calculated using the following equation.

$$\textbf{RMR (cal/m}^2\textbf{/h)} = \frac{\text{R.Q. x Oxygen at STP (liter/h)}}{\text{surface area [hamster weight (g)}^{2/3} \times 10] \text{ (m}^2\text{)}}$$

For EMR, it was measured only in the exercise-trained animals. The procedures were similar to the RMR measurement except that the O_2 of the system was measured during animal was running at 40 rpm for 5 minutes in the running wheels, which was contained in sealed metabolic chamber. Oxygen consumption and EMR were calculated by the same equation as RMR.

DETERMINATION OF BODY WEIGHT AND UTERINE WEIGHT

Body weights (BW) of the animals were determined daily. Uterine weight (UW), indicating deficiency of sex hormones after removal of ovaries, were determined immediately after sacrifice.

DETERMINATION OF CHANGES IN CARDIAC FUNCTION AFTER HYDROGEN PEROXIDE STRESS TEST

After 9-week, all animals in exercise and non-exercise groups were measured the QT-c interval, LVDP and dP/dt_{max} before and after hydrogen peroxide stress test in isolated working heart model by using the langendorff apparatus (Langendorff, 1895; Dehnert, 1988).

Isolated Heart Preparations by Langendorff Apparatus

Principle: The isolated perfused heart system, as originated by Oscar Langendorff in 1895, has been a well-known technique in pharmacology and physiology research. The technique allows the examination of cardiac contractile strength, heart rate, and vascular effects without complications like in an intact animal model. The equipment and technique include both constant pressure and constant flow models in both recirculating and non-recirculating modes. Landendorff method provides an isolated heart with oxygen and metabolites through the use of a reservoir containing a physiological solution via a single cannula inserted into the ascending aorta. When the reservoir was opened, the constant 'retrograde' perfusion of the aorta keeps the aortic valve closed and allows for fluid flow directly into the aorta and exits via the coronary arteries during the diastolic period. Throughout this procedure, the left chamber was filled with fluid to created ventricular pressure and force of contraction.

Preparation of the heart

After anesthesia with Tiletamine chlorhydrate and Zolazepam chlorhydrate, animals were intra-peritoneally injected with heparin at a dose of 5000 IU/kg prior to surgery in order to reduce the formation of emboli/clots in the cardiac vasculature.

After exposing the heart by a sternotomy and cutting and retracting the rib cage, two loose ties were placed around the aorta. One tie was used to manipulate the aorta and the other was to secure the aorta to the cannula. An extension catheter with perfusate solution (Krebs-Henseleit (KH) buffer (mM): 118 NaCl, 4.7 KCl, 1.75 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 11.2 Glucose and equilibrated with 95% O_2 5% CO_2 to a pH of 7.3 to 7.4, maintained at 37^{o}C) was placed on the cannula for ease of preparation. This cannula was connected to the perfusion apparatus, and a slow stream of perfusate was permitted to flow through the aortic cannula, removing any air bubbles. The posterior vena cava was then clamped above the diaphragm and the heart flooded with ice cold perfusate to reduce its activity. The pulmonary artery was then cut, followed by a cut across the aorta. The cannula was then inserted and secured with the ties. The tip of the aortic cannula should not be inserted below the base of the aorta, as the ostium might be occluded (coronary perfusion restricted) or the aortic valve might be damaged. Once perfusion had commenced, the heart was removed from the animal and the cannula was disconnected from the extension tube and placed in the apparatus. To measure ventricular contractile force, a saline-filled balloon catheter was inserted into the left ventricle to measure left ventricular

developed pressure. A balloon, made of latex, should be slightly larger than the maximum expanded volume of the ventricle to avoid effects of measuring the resistance of the balloon to stretch. The balloon was secured to a plastic or stainless steel tube that was connected to a pressure transducer and connected to a physiograph (Grass Model 79, Grass instruments Co., Mass., USA). The balloon-tip was inserted through the left atrium, and bipolar electrodes were attached to the right atrium and apex for ECG monitoring. In any of these cases, the appropriate amount of resting force or pressure required to maintain the heart on the ascending limb of the Starling curve should be ensured to avoid overstretching the heart muscle.

The right ventricle was collected, weighed and rapidly frozen in liquid nitrogen before storage at –70 ^{o}C until further analysis for myocardial energy and markers for oxidative stress.

Preparation of the perfusion

. Once mounted on the apparatus, the heart was checked to have strong contraction within seconds after perfusion. The pressure of the perfusate in a constant pressure system was used at a constant pressure of 80 mmHg. As previously shown, the perfusion system was allowed maintenance of hearts in a steady state for at least 90 min with changes of < 5% for all mechanical and metabolic parameters (Neubauer et al., 1995). The heart was stabilized for 10 min. by perfusing oxygenated buffer through the canulated aorta.

After the baseline, data was collected for 5 minutes before calculated volumes of a stock solution of hydrogen peroxide in K-H buffer were added incrementally to the perfusate to final concentration of 0.01 0.05, 0.10 and 0.2, mmol/l hydrogen peroxide with recirculating perfusion mode (Merrill, 2002). The heart was perfused 10 min for each concentration before the next concentration was added. Parameters of cardiac function (HR, LVDP, dP/dt_{max}) were recorded at the end of each of the 15 second periods for subsequent comparison. After finished the experimental period, heart weights (HW) were determined.

All data were recorded and transfer to a computer installed with Biopac system software (Biopac system, Inc, California). HR, RR and QT intervals were determined by the apparent QRS complex in a minute. The QT-c values were calculated by the Bazett's method (QT-c = QT interval / (RR interval) $^{1/2}$) (Bazett , 1920). LVDP was defined as the pressure difference between systolic pressure and diastolic pressure. Dp/dt_{max} was measured at each wave of LVDP at the period of isovolumic contraction (Tosaki et al., 1998).

DETERMINATION OF MYOCARDIAL ENERGY

METABOLIC PHOSPHATE CONTENTS

Principle: The colour developed of absorption spectra occurred when guanidine compound of Cr react with a mixture of diacetyl and α-napthol high energy phosphate contents of Cr, PCr and TCr. These contents were determined as indicators of myocardial energy reservation following a protocol described previously (De Saedeleer and Marechal, 1984; McClung et al., 2003) by adapting the nonenzymatic technique.

***Assay procedure*:** Cardiac muscle was homogenized using a Polytron (Glas-COL, Indiana 47802) at 60% of maximum setting (4x10 sec each) in homogenization buffer (0.5 M $HClO_4$ in 1 mM EDTA) and centrifuged for 2 minutes at 10,000 rpm (4^{o}C). The sample for Cr concentration was neutralized in an alkaline solution (2.1M $KHCO_3$ in 0.3M MOPS). The sample for TCr concentration was heated 10 min at 65 ^{o}C in 0.4 N HCl and neutralized with 2N NaOH. The supernatant was re-centrifuged (3 minutes at 10,000 rpm, 4^{o}C. The reaction tube contained: 0.25 ml of sample; 0.5 ml of diluted diacetyl (1:2,500); 1 ml of 1% α-napthol (10 g of α-napthol dissolved in 1000 ml of an alkaline solution containing 60 g of NaOH and 160 g of Na_2CO_3). The reaction was allowed to proceed in the dark for 40 min. The color developed of supernatant was determined by the spectrophotometric measurement at 520 nm (Shimudzu UV1201, Japan). The concentration of the stand Cr (μmol/tube) is plotted versus absorbance and the concentration of the unknown are read from the standard curve. The PCr concentration was calculated by the difference between TCr and Cr for each sample. All assays were expressed as μmole/mg protein.

PROTEIN CONCENTRATION

Principle: Protein concentration was assessed by Lowry's method (Lowry et al., 1951). The first step is the reaction of protein with cupric ions (Ca^{2+}) in alkaline medium, leading to reduction of cupric ions to cuprous ions (Ca^{+}). The second step is reduction of Folin-Ciocalteu reagent with the alkaline copper-protein complex, giving a characteristic blue reaction colour. Absorbance was measured with a spectrophotometer (Shimudzu UV1201, Japan) at 750 nm and expressed as mg protein/gram tissue.

Assay procedure: Each frozen heart tissue (10 mg) was homogenized on ice with a homogenizer (Polytron, Glas-COL, Indiana 47802)) in homogenization buffer (1M Tris pH 7.4, 0.5 M EDTA, 3M NaCl, 10% Tritonx-100, 20% SDS, and 5% v/v protease inhibitor cocktail, Sigma, USA). The homogenates were centrifuged at

12,000 g for 20 min at 4˚C. The pellet containing cell nuclei and tissue debris was discarded. The protein concentration of the supernatant was collected and aliquot into a sterile microcentrifuge tube, kept at -70˚C until use. Five standard dilutions in duplicate (0, 50, 100, 200, and 500 µg/ml) were prepared by dissolving the stock standard BSA solution (2 mg/ml). Prepare 1X (1 N) Folin-Ciocalteu reagent by diluting the supplied 2X (2 N) reagent 1:1 with ultrapure water. Because the diluted reagent is unstable, prepare only as much 1X Folin-Ciocalteu reagent as will be used in a day. Pipette 0.1 ml of each standard and unknown sample replicate into an appropriately labeled test tube. At 15-sec intervals, add 0.5 ml of working reagent of Modified Lowry Reagent (Bio-rad, USA) to each test tube. Mixed well and incubated each tube at room temperature for exactly 10 min. Exactly at the end of each tube's 10-min incubation period, add 50 µl of prepared 1X Folin-Ciocalteu Reagent, immediately vortex to mix the contents and allow to stand at room temperature for 30 min. Read absorbance with the spectrophotometer (Shimudzu UV1201, Japan) at 750 nm and expressed as mg protein/gram. The concentration of the stand BSA (µg/tube) is plotted versus absorbance and the concentration of the unknown are read from the standard curve.

CREATINE KINASE ACTIVITIES

Principle: CK isoenzymes catalyze the reversible transfer of the phosphate group of ATP to the guanidine group of Cr to yield ADP and PCr (Liao et al., 1996). CK was assayed spectrophotometrically by coupling the CK reaction to the hexokinase and glucose-6-P dehydrogenase reactions (Headrick, 1998). Total CK activity was determined as the indicator of high-energy phosphate turn-over rate and performed following a modified protocol described previously (Banerjee et al., 1991).

***Assay procedure*:** Cardiac tissue was weighed, and 10 volumes of cold isotonic extraction buffer (250 mM sucrose, 50 mM imidzole acetate, 10 mM Mg acetate, 4 mM KH_2PO_4, 2mM EDTA, 50µM N-acetylcysteine as antioxidant, and 12.5 µM sulfur in 0.8% ETOH to inhibit adenylate kinase, final buffer (pH 7.6) was added to the frozen tissue. Tissue was initiated in a homogenizer (Polytron Glas-COL, Indiana 47802) at 60% of maximum setting (4x10 sec each) followed by centrifugation at 2,000xg for 5 min. The supernatant was again centrifuged for 10 min at 20,000xg, and the final supernatant was used for assaying CK activity. The assay was performed with CK-NAC UV liquid (Biochemica and Diagnotica mbH Germany) on spectrophotometer at 340 nm (Shimudzu UV1201, Japan) in cuvettes maintaining at 25^{o}C and expressed as µmol/min/mg tissue protein following this equation.

CK activity= delta OD/min x (Vi/Vs)

3333 x mg protein of reaction solution

where; 3333=factor to get the activity in the sample at 340 nm, 27^{o}C

Vi=volume of incubation mixture

Vs=volume of enzyme sample

DETERMINATION OF CREATINE TRANSPORTER PROTEIN BY WESTERN BLOT ANALYSIS

Principal: Immuno-blotting is a widely used and powerful technique for the detection and identification of protein using antibodies. The process involves the separation of sample proteins by polyacrylamide gel electrophoresis (PAGE). The most widely used method for qualitative analysis of a protein mixture is called **"**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) by using the buffer system of Laemmli (1970). With this method, it is possible to determine both the purity and the relative molecular weight of an unknown isolated protein or proteins. In the process, proteins migrate in response to an electrical field through pores in the gel matrix and separate based on molecular size. The separated proteins were transferred from the gel onto a thin support membrane. The membrane binds and immobilizes the proteins in the same pattern as in the original gel. The membrane (or "blot") is then exposed to a solution containing antibodies that recognize and bind to the specific protein of interest. The antibodies bound to the membrane are detected by any of a variety of techniques, usually involving treatment with a secondary antibody.

Assay procedure**:** CrT was determined as the myocardial Cr pool reservation following a modified protocol described previously (Walzel et al., 2002; Boehm et al., 2003) as followed.

Preparation of apparatus

The reagents used in preparing the gel should be fresh or removed from the refrigerator and allowed to warm at lease one hour prior to prepare for the gel. First of all, the sandwich plates were set up for casting the gel. A sandwich consists of two rectangular glass plates: the outer is 10.1 cm (width) x 8.3 cm (height) and the inner is 10.1 cm (width) x 7.3 cm (height) separated by spacers of 0.75 mm thickness (Mini-Protein® 3 cell, Bio Rad, Canada). In order to prepare a flawless gel, one containing has no air bubble or debris, the glass plates must be

perfectly cleaned and dry with absolute ethanol. The casting clamps were used to mount the outer and inner glass plates facing together.

Preparation of SDS gel

For separating gel, 10% acrylamide (2.5 ml of 1.5 M Tris pH 8.8, 0.1 ml of 10% SDS, 3.3 ml of 40 % acrylamide mixed, 4.07 ml of dH_2O, 0.1 ml of 10 % APS, 0.01 ml of TEMED) was carefully filled into the space of sandwich plates from bottom to top with no air bubbles. The height of the gel was adjusted by the comb, approximately 1 cm below the bottom edge. The top layer was filled with 1 ml of distilled water. The gel was allowed to polymerize at room temperature for 40 min. After polymerization, the water was drained off and excess liquid was removed with a piece of filtered paper. The selected comb (number of wells, thickness same as spacers) was gently inserted, then 4% acrylamide solution (1.25 ml of 0.5 M Tris pH 6.8, 0.05 ml of 10% SDS, 0.85 ml of 40 % acrylamide mixed, 2.85 ml of dH_2O, 0.025 ml of 10 % APS, 0.005 ml of TEMED) was filled into the space for making the stacking gel. It should be made sure that no air bubbles formed around the teeth the comb, as they will impede the migration and separation of the proteins. The gel was allowed to polymerize for 1-1.5 hours at room temperature.

Preparation of tissue samples

During polymerizing the stacking gel, equal amounts of total protein from 1 part of each sample were mixed with 4 parts of sample buffer (1.042 ml of 1.5 M of Tris pH 6.8, 2.5 ml of glycerol, 0.5 g of 10% SDS, 0.3856 DTT, 0.0025 g of bromophenol blue, adjust volume to 5 ml by dH_2O). The mixers were heat for 5 min at 100 ºC in a boiling water bath (Thermostatic bath/circulator; Grant W6, UK). The amount of total protein used for total CrT was 50 μg.

Electrophoresis

After polymerization is complete, the comb was gently removed. The wells were filled with running buffer (0.05 M Tris, 0.384 M glycine, 0.1 % SDS). The gel was mounted in the electrophoresis apparatus and the upper buffer chamber was filled with running buffer. Removed any air bubbles trapped at the bottom of the wells. This will disrupt the electrical circuit and an uneven electrophoresis. Each protein sample (50 μg/lane) was loaded into the bottom of each well. The molecular weight markers (Pierce, USA) were also loaded. Then, the running buffer was poured into the lower chamber. The electrophoresis apparatus was attached to an electric power supply (PowerPac™ HC, Bio-Rad, USA) and turned on at 125 volt. Small bubbles should start to be produced and rise off the electrode wire at the bottom of the lower chamber. If no bubbles appear then there is

an electrical circuit problem. The gel was run until the dye front reaches the bottom of gel. The power supply was turned off. Then, the gel from the glass plate was removed and placed into the transfer buffer (5.82 g of Tris pH 8.3, 4.348 g of glycine, 0.370 g of SDS, 800 ml of dH_2O, 200 ml of 100 % methanol)

Protein transfer

A nitrocellulose membrane (Trans-Blot®, Bio-Rad, USA) and two sheets of absorbent filter paper were cut into the same size of the gel. The membrane, filter papers, and support pads were soaked in the transfer buffer for 10 min. The transfer cassette was assembled by lying the black side down, then plate a support pad, filter paper, gel, membrane, filter paper, and support pad. Lock and put the complete transfer cassette into the transfer tank containing transfer buffer by facing the membrane side to the positive electrode (anode, red electrode) and transferred for 120 min at 100 volt. After transfer, the membrane was removed from the cassette and immersed into the blocking solution.

Membrane Blocking

The membrane was blocked with 5% fat-free milk powder in TTBS buffer (150 mM NaCl, 25 mM Tris, 0.05% Tween, 3% BSA, pH 7.5) for 1 hour at room temperature with gentle rotation on a platform rocker (Orbital Shaker, UK). This process will reduce the background of non-specific binding site with irrelevant protein. After blocking, the membrane was washed for 30 min by TTBS buffer.

Detection of bound antibody

The dilution of primary antibody, rabbit anti human CRT peptide antibodies was prepared in TTBS buffer (1:3,000) for 2 hours at room temperature. The membrane, in a clean plastic box, was incubated with the respective anti-CRT peptide antibodies overnight at 4 ^{o}C on a platform rocker. After incubation, the primary antibody solution was discarded. The membrane was washed with 20 ml of TTBS buffer for 20 min 2 times. The secondary antibody was prepared in TTBS buffer. The goat anti rabbit IgG conjugated HRP antibody at 1:20,000 dilution was used for CrT. The goat anti mouse IgG conjugated HRP antibody at 1:20,000 dilution was used for α-actin. The membrane was incubated in the secondary antibody for 1 hour at room temperature on a platform rocker. After that, the blotting membrane was washed again with TTBS buffer for 20 min 2 times on a platform rocker.

Protein detection and analysis

The blotting membrane was placed on a cleaned glass plate. The detection reagent (SuperSignal® West Pico kit, Pierce, USA) was prepared by

mixing equal parts of the stable peroxide solution and the luminal/enhancer solution, and then overlay the reagent directly on the membrane surface carrying the protein. After incubation for 1 min. at room temperature, the excess reagent was drained off, and wrapped by a piece of saran wrap. It is necessary to work quickly once the membrane has been exposed to the detection solution. In a dark room, the membrane was placed, protein side up, in a X-ray film cassette. The light was turned off and a sheet autoradiography film (CL-XPosure^{TM} Film 5 x 7 inches, Pierce, USA) was carefully placed on the top of the membrane, then the cassette was closed and exposed for a certain period for 5 min for total CrT and 3 min for α-actin. Each intensity band was quantified by Scion image software and scanned the intensity by using high resolution scanner (Genius, Color page–HR 7x Slim, USA) linked to a computer analysis system (Belta, mollis-mini-151, Thailand). The intensity values for both CrT bands (55-kDa and 70-k-Da polypeptide bands) were added together for calculation of absolute CrT protein content. The intensity ratio of each studied protein to α-actin was calculated and expressed as % of total CrT protein.

DETERMINATION OF INSULIN LIKE GROWTH FACTOR-I

Principle: In the IGF-I immunoassay kit, a purified monoclonal anti-Rat IGF-I is coated onto the inner surface of polystyrene microtitre wells. The pre-treated, diluted sample is then incubated, together with biotinylated polyclonal rabbit anti–rat IGF-I, in antibody coated wells and shaken for 2 hours at room temperature. The wells are washed and enzyme (horseradish peroxidase) labeled avidin is added which binds to the biotin complex. After a further wash, a single component chromogenic substrate (a formulation of tetramethyl-benzidine) is added to develop colour. The absorbance of the stopped reaction is read in a microtitre plate reader, colour intensity developed being directly proportional to the level of IGF-I present in the sample.

***Assay procedure*:** Blood was drawn immediately before excision of the heart from the thorax. Serum was obtained by centrifugation at 2,000xg for 10 min at 4^{o}C and stored at -70^{o}C (Stassijns et al., 1999). IGF-I was determined as a growth promoting metabolite of growth hormone. Serum IGF-I was measured by OCTEIA Rat/mouse IGF-I immunoassay kit (Immunodiagnostic system Ltd, UK). Firstly, 25 μl of each control (lyophilized rat serum) or sample (hamster serum) was added to appropriately labelled tubes. Releasing reagent (proprietary reagent for dissociating IGF-I from binding proteins) for 100 μl was added to each tube, vortexed all tubes, and incubated at 18-28 ^{o}C for 10 minutes. Sample diluent (phosphate-buffered saline

containing protein and 0.05% sodium azide) for 1 ml was added to each tube and mixed all tubes. Control and sample tubes were then ready to assay. Each of calibrator (lyophilized phosphate buffer containing rat IGF-I and 0.05% sodium azide) or diluted control or diluted sample for 25 μl were added to the appropriate wells of the antibody coated plate (microplate with anti-rat IGF-I monoclonal antibody linked to the inner surface of the polystyrene wells, 12 x 8 well strips in a foil pouch with desiccant in duplicate) within a period of 30 minutes to minimize assay drift. After that, anti-rat IGF-I biotin (phosphate buffered saline containing rabbit anti-rat IGF-I polyclonal antibody labelled with biotin, protein, proprietary stabilizers and preservative) for 100 μl was added to all wells. Then, covered the plate with an adhesive plate sealer and incubate the plate on an orbital microplate shaker (500-700 rpm) at 18-28 ^{o}C for 2 hours. Washed all wells for three times by adding 250 μl of wash solution (phosphate buffered saline containing Tween) to all wells and decanted the contents of the wells by inverting sharply. The inverted plate was tapped firmly on absorbent tissue to remove excess wash solution before proceeding to the next step. Enzyme conjugate (phosphate buffered saline containing avidin linked to horseradish peroxidase, protein, enzyme stabilizers and preservative) for 200 μl was added to all wells and incubated at 18-28 ^{o}C for 30 minutes. Repeated washing all wells again by wash solution as described above. Next, TMB substrate (a proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide) was added for 200 μl to all wells and incubated at 18-28 ^{o}C. After 30 minutes, Stop solution (5M hydrochloric acid) for 100 μl was added to all wells. The absorbance of each well was measured at 450 nm (reference 650 nm) using the microplate reader within 30 min after adding the stop solution. The mean absorbance for each calibrator (0, 271, 492, 1031, 2086, 3821 ng/ml), control and unknown samples were calculated from the calibration curve and expressed the values as ng/ml (Stassijns et al., 1999).

DETERMINATION OF MARKERS FOR OXIDATIVE STRESS

CONCENTRATION OF TOTAL GLUTATHIONE

Principle: Glutathione is assayed by an enzymatic recycling procedure in which it is sequentially oxidized by 5,5'-dithiobis (2-nitrobensoic acid) (DTNB) and reduced by NADPH in the presence of glutathione reductase. Myocardial oxidized (GSSG) and reduced (GSH) glutathione levels were measured by the modified method of glutathione reductase/DTNB recycling assay (Griffith, 1980; Kannan et al., 2004). The extent or rate of DTNB formation is monitor at 412 nm. The rate of TNB

formation is followed at 412 nm and is proportional to the sum of GSSG+GSH present. The selective determination of GSSG requires 2-vinypyridineis, a reagent for derivitization of the GSH (Griffith, 1980).

***Assay procedure*:** Myocardial tissue was homogenized at 60% of maximum setting (4x10 sec each) in nine volumes of 5% sulfosalicylic acid (SSA). The tissue homogenate was mixed with 20% SSA (3:1) and centrifuged for 10 min at 10000xg. The supernatant of total glutathione was assayed in a 1-ml cuvette containing 50 μl of supernatant. The following solutions was then be added with assay mixture: 150 μl of stock buffer (143 mM sodium phosphate in 6.3 mM Na_2EDTA, pH 7.5), 650 μl of 0.248 mg NADPH in stock buffer and 100 μl of 6 mM DTNB. The reaction was initiated by addition of 10 μl glutathione reductase (266 U/ml) and performed on a spectrophotometer at 412 nm (Shimudzu UV1201, Japan) for 4 min every 20 min at 25^{o}C. GSH was used as a standard and was assay in parallel under the same condition as the tissue sample.

For the GSSG assay, it was measured by treatment of the sulfosalicylic acid supernatant with 10 μl of 2-vinylpyridine and triethanolamine. The solution was vigorously mixed, and the final pH of the solution was adjusted to between 6 and 7. After 60 min, the derivatized sample was assayed as described above in the DTNB-GSSG reductase recycling assay. GSSG was used as a standard and sample blanks containing only 2-vinylpyridine was also run. GSH value was calculated as the difference between total (GSSG+GSH) and GSSG concentrations. Values were expressed as nmol per mg protein.

ACTIVITY OF GLUTATHIONE PEROXIDASE

Principle: GPx catalyzes the reduction of hydroperoxides, including hydrogen peroxides, by reduced glutathione and functions to protect the cell from oxidative damage. In the presence of NADPH and glutathione reductase, the oxidized glutathione is immediately convert to the reduced from with a concomitant oxidation of NADPH to NAD^{+}. Then the decrease in absorbance at 340 nm was determined following a modified protocol described previously (Kankofer, 2002).

***Assay procedure*:** Tissue was homogenized at 60% of maximum setting (4x10 sec each) in nine volume of 75 mM phosphate buffer, pH 7 and centrifuged for 25 min at 20,000xg. The supernatant of GPx activity was assayed in a 3-ml cuvette containing 2.0 ml of 75 mM phosphate buffer, pH 7. The following solutions was then be added: 50 μl. of 60 mM glutathione, 100 μl glutathione reductase solution (30 U/ml), 50 μl of 120 mM NaN_3, 100 μl of 15 mM Na_2EDTA, 100 μl of 3 mM NADPH ,

and 100 μl of cytosolic fraction. The reaction was started by the addition of 100 μl of 7.5 mM hydrogen peroxide, and the conversion of NADPH to NADP was monitored by a continuous recording of decreases in absorbance at 340 nm at 1-min intervals for 5 min. GPx activity was expressed as nmol of reduced NADPH oxidized to $NADP^+$ per min /mg protein following this equation (Paglia and Valentine, 1967; Hill and Singal, 1997).

$$\textbf{GPx activity} = \frac{\text{delta OD/min x (Vi/Vs)}}{6.22\times10^6 \text{ x mg protein of reaction solution}}$$

where; 6.22×10^6=molar extinction coefficient for NADPH at 340nm

Vi=volume of incubation mixture, Vs=volume of enzyme sample

DATA ANALYSIS

All data are presented as mean and standard deviation (mean ± SD). The comparison data between exercise and non-exercise groups were analyzed using two way analysis of variance (Two-way-Anova) in combination with the post hoc (Student–Newman-Keuls method) The non-parametric method was used if data had failed normality, and two way analysis of variance on rank was used instead. The level of significance for all analysis was set at $p<0.05$. Statistical analysis was performed with Sigma Stat software, version 2.0 (Jandel Scientific, San Rafael, CA, USA.).

CHAPTER IV

RESULTS

Effects of Cr supplementation and E_2 replacement on body weight (BW), % heart weight/body weight (HW/BW) ratio and % uterine weight/body weight (UW/BW) ratio in non exercise and exercise-trained ovariectomized hamsters

As shown in Figure 4.1, BW, % of HW/BW ratio and % of UW/BW ratio in non exercise and exercise-trained ovariectomized hamsters were determined and compared. In non exercise ovariectomized hamsters, BW in control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2) and Cr supplementation combined with E_2 replacement groups (Cr+E_2) were 153.5$\pm$9.44g, 151$\pm$9.07g, 168$\pm$16.02g, 157.5$\pm$12.3g and 153$\pm$11.35g respectively. The slightly increases of BW were observed in exercise-trained ovariectomized animals in control and all treatments in Cr-, Cr+, E_2 and Cr+E_2 which were 163$\pm$6.75g, 164$\pm$9.66g, 175$\pm$15.09g, 175$\pm$14.34g and 161.5$\pm$8.18g respectively. However, there was no significant difference in BW among C, Cr-, Cr+, E_2 and Cr+E_2 between non exercise and exercise-trained ovariectomized hamsters.

In non exercise ovariectomized hamsters, % HW/BW ratio in control, Cr-, Cr+, E_2 and Cr+E_2 were 0.25$\pm$0.05, 0.27$\pm$0.03, 0.25$\pm$0.04, 0.26$\pm$0.04, and 0.28$\pm$0.04, respectively. The slightly increases in % HW/BW ratio were observed in exercise-trained ovariectomized animals in control and all treatments in Cr-, Cr+, E_2 and Cr+E_2 which were 0.29$\pm$0.02, 0.28$\pm$0.02, 0.3$\pm$0.03, 0.29$\pm$0.03, and 0.31$\pm$0.03, respectively. However, there was no significant difference in % HW/BW ratio among C, Cr-, Cr+, E_2 and Cr+E_2 between non exercise and exercise-trained ovariectomized hamsters.

In contrast to HW and % HW/BW ratio, % UW/BW ratio in non exercise and exercise-trained ovariectomized hamsters were significantly increased from 0.062$\pm$0.004 and 0.068$\pm$0.008 in control to 0.1$\pm$0.03 and 0.1$\pm$0.02 in Cr+ treatment ($P<0.05$). In addition, % UW/BW ratio in non exercise hamsters was significantly increased to 0.23$\pm$0.01 and 0.24$\pm$0.01 in E_2 treatment. Similarly, % UW/BW ratio in exercise-trained ovariectomized hamsters was also significantly increased to 0.26$\pm$0.01, 0.25$\pm$0.01 in Cr+E_2 treatments ($P<0.05$). However, there was no significant difference in % UW/BW ratio between non exercise and exercise-trained ovariectomized hamsters.

Effects of Cr supplementation and E_2 replacement on metabolic rate in non exercise and exercise-trained ovariectomized hamsters

Resting metabolic rate (RMR) of animals was measured in non exercise and exercise-trained ovariectomized hamsters before starting the experiment (week 0). RMR at week 0 in non exercise and exercise-trained ovariectomized hamsters of control, Cr-, Cr+, E_2, and Cr+E_2 were 15.78±0.55 and 16.79±0.7 cal/m^2/h, 15.71±0.69 and 16.8±0.55 cal/m^2/h, 15.57±0.68 and 16.38±0.88 cal/m^2/h, 15.7±0.77 and 16.86±0.81 cal/m^2/h, 15.45±0.76 and 17.00±1.14 cal/m^2/h, respectively.

As shown in Figure 4.2, RMR at week 0 and week 9 in exercise-trained ovariectomized hamsters were determined and compared. RMR at week 0 and week 9 of control, Cr-, Cr+, E_2, and Cr+E_2 were 16.79±0.7 and 17.91±2.81 cal/m^2/h, 16.80±0.55 and 18.08±3.26 cal/m^2/h, 16.38±0.88 and 18.50±3.77 cal/m^2/h, 16.86±0.81 and 17.44±3.11 cal/m^2/h, 17.00±1.14 and 18.66±2.4 cal/m^2/h, respectively. However, there was no significant difference of RMR at week 0 and week 9 among C, Cr-, Cr+, E_2 and Cr+E_2 treatments in exercise-trained ovarietomized hamsters.

Concerning the exercise metabolic rate (EMR), we determined EMR in the difference week of measurement only in the exercise-trained animals of the control and all treatments. As shown in Figure 4.3, RMR and EMR at the 9^{th} week of exercise-trained ovariectomized hamsters were determined and compared. RMR in control, Cr-, Cr+, E_2 and Cr+E_2 were 17.91±2.81, 18.08±3.26, 18.5±3.77, 17.44±3.11 and 18.66±2.4 cal/m^2/h, respectively. On the other hand, a marked increase in EMR at week 9 of exercise-trained ovariectomized hamsters compare to RMR in all treatments of control, Cr-, Cr+, E_2 and Cr+E_2 which were 27.29±2.57, 24.85±3.18, 27.89±2.62, 28.35±3.75 and 30.58±3.5 cal/m^2/h, respectively ($P<0.05$). However, there was no significant difference of EMR among Cr-, Cr+, E_2 and Cr+E_2 treatments in exercise-trained ovarietomized hamsters.

Oxygen economy of interest in the current study was the oxygen uptake of submaximal running exercise (10 min.) normalized for distance traveled which was expressed in milliliters of oxygen per kilogram body mass per meter of distance traveled (mlO_2/kg/m) (Harris et al., 2003). In our study, oxygen economy of exercise-trained ovariectomized hamsters at the 9^{th} week in C, Cr-, Cr+, E_2 and Cr+E_2 treatment were 1.08±0.18, 1.09±0.24, 1.07±0.16, 1.07±0.15 and 1.10±0.16 mlO_2/kg/m, respectively (Table 4.1). However, there was no significant difference of oxygen economy among Cr-, Cr+, E_2 and Cr+E_2 treatments in exercise-trained ovarietomized hamsters.

Effects of Cr supplementation and E_2 replacement on cardiac functions of QT-c interval, LVDP and dP/dt_{max} in non exercise and exercise-trained ovariectomized hamsters

As shown in Table 4.2, cardiac functions of Q-Tc interval, LVDP and dP/dt_{max} in control and all treatments in non exercise and exercise-trained ovariectomized hamsters for 9 weeks were determined and compared. QT-c interval of non exercise ovariectomized hamsters in C, Cr-, Cr+, E_2 and Cr+E_2 were 0.214±0.009, 0.208±0.008, 0.215±0.007, 0.211±.01 and 0.21+.007 msec., respectively. The slightly decrease in QT-c interval was observed in exercise-trained ovariectomized animals in control and all treatments of Cr-, Cr+, E_2 and Cr+E_2 which were 0.197±0.007, 0.196±0.008, 0.196±0.009, 0.199±0.01, and 0.198±0.006 msec., respectively. However, there was no significant difference in QT-c interval in among C, Cr-, Cr+, E_2 and Cr+E_2 treatments between non exercise and exercise-trained ovariectomized hamsters.

LVDP of non exercise ovariectomized hamsters in C, Cr-, Cr+, E_2 and Cr+E_2 were 58.23±5.52, 57.19±7.09, 57.75±.7.21, 57.92±.7.82 and 59.23±7.45 mmHg., respectively. The slightly increase in LVDP was observed in exercise-trained ovariectomized animals in control and all treatments in Cr-, Cr+, E_2 and Cr+E_2 which were 69±10.83, 69.55±10.26, 68.28±13.11, 70.95±12.74, and 71.11±12.41 mmHg, respectively. However, there was no significant difference in LVDP among C, Cr-, Cr+, E_2 and Cr+E_2 treatments between non exercise and exercise-trained ovariectomized hamsters.

DP/dt_{max} of non exercise ovariectomized hamsters in C, Cr-, Cr+, E_2 and Cr+E_2 were 1834.08±189.15, 1831.82±168.59, 1879.73±.173.30, 1860.53±.209.7 and 1851.40±174.27 mmHg/min., respectively. The slightly increase in dP/dt_{max} was observed in exercise-trained ovariectomized animals in control and all treatments in Cr-, Cr+, E_2 and Cr+E_2 which were 2230.29±204.64, 2174.91±187.83, 2289.02±263.44, 2258.09±218.64, and 2274.1±239.03 mmHg/min, respectively. However, there was no significant difference in dP/dt_{max} among C, Cr-, Cr+, E_2 and Cr+E_2 treatments between non exercise and exercise-trained ovariectomized hamsters.

Effects of Cr supplementation and E_2 replacement on EC_{50} derived from the dose response curve of cardiac functions of Q-Tc interval, LVDP and dP/dt_{max} against H_2O_2 stress test in non exercise and exercise-trained ovariectomized hamsters

When myocardial electrical properties and contractile properties of QT-c interval, LVDP and dP/dt_{max} in control and all treated groups in non exercise and exercise-trained ovariectomized hamsters for 9 weeks were plotted against various concentration of H_2O_2, it was showed as EC_{50} derived from the dose response curves of QT-c interval, LVDP and dP/dt_{max}, respectively in Figure 4.4-4.6. Although the QT-c interval of both non exercise and exercise-trained ovariectomized hamsters were progressively prolonged when increasing the concentration of hydrogen peroxide in the control, Cr+, E_2 and Cr+E_2 replacement, but the prolongation QT-c interval could be seen at quite low dose of H_2O_2 in Cr- treatment at the 9^{th} week (Figure 4.4). Additionally, EC_{50} derived from the dose response curves of QT-c intervals in non exercise and exercise-trained ovariectomized hamsters was significantly decreased from 0.06$\pm$0.024 and 0.067$\pm$0.015 mM. in the control to 0.017$\pm$0.003 and 0.02$\pm$0.003 mM. in the Cr- treatment ($P<0.05$, Table 4.3). However, it did not showed a significant difference in EC_{50} derived from the dose response curves of QT-c interval between non exercise and exercise-trained ovariectomized hamsters in Cr+, E_2, Cr+E_2 treatments.

LVDP was progressively decreased with increasing the concentration of H_2O_2 in the control and all treatments of non exercise and exercise-trained ovariectomized hamsters (Figure 4.5). In addition, EC_{50} derived from the dose response curves of LVDP in non exercise and exercise-trained ovariectomized hamsters were reduced from 0.057$\pm$0.008 and 0.104$\pm$0.01 mM. in the control to 0.016$\pm$0.008 and 0.038$\pm$0.006 mM. in the Cr- treatment at the 9^{th} week ($P<0.05$, Table 4.3). Moreover, EC_{50} of LVDP in the control and other treatments of ovariectomized hamsters in non exercise group was remarkably lower than the exercise-trained group ($P<0.05$). Interestingly, EC_{50} of LVDP in Cr- treatment of non exercise ovariectomized hamsters was the highest compared to other treatments ($P<0.05$).

A similar pattern was also observed in plotting of dP/dt_{max} against various concentrations H_2O_2. As shown in Figure 4.6, dP/dt_{max} was progressively decreased with increasing the concentration of H_2O_2 in all groups of non exercise and exercise-trained ovariectomized hamsters. Additionally, EC_{50} derived from the dose response curves of dP/dt_{max} in non exercise and exercise-trained ovariectomized hamsters was decreased from 0.053$\pm$0.008 and 0.103$\pm$0.021 mM. in the control to 0.023$\pm$0.005 and 0.038$\pm$0.003 mM. in Cr- treatment ($P<0.05$, Table 4.3). Moreover, EC_{50} of

dP/dt_{max} in the control and other treatments of ovariectomized hamsters in non exercise group was remarkably lower than the exercise-trained group ($P<0.05$). Interestingly, EC_{50} of dP/dt_{max} of Cr- treatment in non exercise ovariectomized hamsters was the highest as compared to other treatments ($P<0.05$).

Effects of Cr supplementation and E_2 replacement on metabolic phosphate contents (Cr, PCr, TCr), CK enzyme activity and CrT protein in non exercise and exercise-trained ovariectomized hamsters

Metabolic phosphate contents and activities of CK of the control, Cr-, Cr+, E_2 and Cr+E_2 in non exercise and exercise-trained ovariectomized hamsters were determined and compared (Figure 4.7a-d). Metabolic phosphate contents (Cr, PCr, and TCr) were measured as markers for myocardial energy reserve for cardiac function. In control of non exercise and exercise-trained ovariectomized hamsters, the Cr content in cardiac muscle was 0.043$\pm$0.005 and 0.048$\pm$0.004 μmol/mg protein. Cr- treatment caused a decrease in Cr content at the 9th week (0.026$\pm$0.003 μmol/mg protein) in non exercise ovariectomized animals as compared to the control and other treatment ($P<0.05$) as shown in Figure 4.7a. On the contrary, it did not showed a significant difference of Cr content in Cr- treatment at the 9th week in exercise-trained ovariectomized hamsters. In addition, marked increases in Cr contents, approximately 2 times of that in the control could be observed only in non exercise ovariectomized hamsters treated with Cr+ and Cr+E_2 ($P<0.05$). Moreover, Cr contents in exercise-trained ovariectomized hamsters were increased and the statistical significances were detected in animals treated with Cr+, E_2 and Cr+E_2 compared to those in the non exercise animals ($P<0.05$). PCr contents of the ovariectomized control in non exercise and exercise-trained animals were 0.017$\pm$0.005 and 0.016$\pm$0.002 μmol/mg protein as shown in Figure 4.7b. Similar to the changes of the Cr contents, the PCr content of Cr- at the 9th week was decreased only in non exercise ovariectomized hamsters compared to the control ($P<0.05$). In contrast, it did not showed a significant difference of PCr content of Cr- at 9 weeks in exercise-trained ovariectomized hamsters. Additionally, a marked increase in the PCr content, approximately 2 times of that in the control could be observed only in treatments with Cr+ and Cr+E_2 in non exercise ovariectomized hamsters ($P<0.05$). Moreover, PCr content in exercise-trained ovariectomized hamsters were higher than the non exercise-trained animals, and statistical significances were detected in treatments with Cr+, E_2 and Cr+E_2 ($P<0.05$). TCr contents, calculated from the combination of Cr and PCr, of the control ovariectomized non exercise and exercise

trained groups were 0.060±0.009 and 0.065±0.006 μmol/mg protein as shown in Figure 4.7c. In agree with the changes of Cr and PCr contents, the TCr content in non exercise-trained ovariectomized hamsters was decreased in Cr- treatment at the 9th week as compared to the control ($P<0.05$). In contrast, there was no significant difference of TCr content of Cr- treatment at the 9th week in exercise-trained ovariectomized hamsters ($P>0.05$). Likewise, other treatments with Cr+ and Cr+E_2 in non exercise-trained ovariectomized hamsters caused increases in TCr contents, approximately 2 times of that in the control ($P<0.05$). In addition, TCr contents in exercise-trained ovariectomized hamsters treated with Cr+, E_2 and Cr+E_2 were higher than those in the non exercise-trained animals ($P<0.05$). Interestingly, metabolic phosphate contents (Cr, PCr and TCr) in both non exercise and exercise-trained ovariectomized hamsters were demonstrated increases in accumulation of the contents in treatment with Cr+ compared to others.

For the CK activity, an important enzyme in Cr reaction involving in high-energy phosphate metabolism of cells was shown in Figure 4.7d. CK activities of the control ovariectomized hamsters in non exercise and exercise trained groups were 5.07±0.33 and 5.41±0.49 μmol/min/mg protein. Cr- treatment at the 9th week did not showed a significant difference in the CK activity in both non exercise and exercise-trained ovariectomized hamsters. In agree with an increase in myocardial energy reservation via Cr metabolism which is observed in the non exercise-trained ovariectomized hamsters treated with Cr+ and Cr+E_2, their energy metabolism also showed the higher CK activities in these groups as compared to the control, which were 10.95±2.51, and 13.22±1.78 μmol/min/mg protein ($P<0.05$). Additionally, the CK activities were further increased in exercise-trained animals treated with Cr+, E_2 and Cr+E_2 at 9 weeks as compared to those in the non exercise-trained animals, which were 16.07±1.70, 11.2±0.94 and 17.7±1.61 μmol/min/mg protein, respectively. Interestingly, it seem likely that the ovariectomized hamsters treated with Cr+ in both non exercise and exercise-trained groups could increased the CK activities compared to other treatments.

As shown in Figure 4.8, myocardial CrT protein in control and all treatments in non exercise and exercise-trained ovariectomized hamsters were determined and compared. Two major protein bands with an apparent mobility of 70 and 55 kDa were added together for absolute myocardial CrT protein. In non exercise ovariectomized hamsters, myocardial CrT proteins which were expressed as ratio of myocardial CrT protein per α-actin in the control, Cr-, Cr+, E_2 and Cr+E_2 were 0.28±0.02, 0.17±0.01, 0.33±0.05, 0.34±0.03 and 0.34±0.03, respectively. A marked decrease in myocardial

CrT protein was observed in Cr- treatment of non exercise ovariectomized hamsters as compared to the control and other treatments but not in exercise-trained ovariectomized hamsters. In addition, animals treated with Cr+, E_2 and Cr+E_2 also did not show any significant differences compared to the control in non exercise-trained ovariectomized hamsters. On the other hand, exercise-trained ovariectomized hamsters treated with Cr+, E_2 and Cr+E_2 increased myocardial CrT protein as compared to those in non exercise-trained animals, approximately 2 times of that in the control ($P<0.05$). Moreover, myocardial CrT protein was further increased in the exercise-trained ovariectomized hamsters treated with Cr+E_2 ($P<0.05$). Interestingly, it seems that the highest level of myocardial CrT protein was found in the combination treatment of Cr+ and E_2 in exercise-trained animals when compared to those in Cr+ or E_2 treatments alone.

Effects of Cr supplementation and E_2 replacement on markers for oxidative stress of myocardial glutathione (GSH, GSSG and GSH/GSSG) and GPx enzyme activity in non exercise and exercise-trained ovariectomized hamsters

As shown in Figure 4.9 (a-d), markers for oxidative stress changes, concentrations of myocardial total glutathione (GSH and GSSG and GSH/GSSG) and myocardial GPx enzyme activity, were determined and compared. Myocardial GSH concentration of ovariectomized hamster in non exercise and exercise-trained groups were reduced from 6.19$\pm$0.89 and 6.76$\pm$1.09 nmol/mg protein in the control to 1.97$\pm$0.6 and 5.54$\pm$0.62 nmol/mg protein in Cr- animals ($P<0.05$) (Figure 4.9a). In addition, the GSH concentration in exercise-trained ovariectomized hamsters treated with E_2 and Cr+E_2 were increased approximately more than 3 times of that in the control and showed significant differences when compared to those in non exercise-trained animals ($P<0.05$). The concentrations of myocardial GSSG in non exercise and exercise-trained ovariectomized hamsters at the 9th week were 1.67$\pm$0.46 and 1.83$\pm$0.34 nmol/mg protein in the control as shown in Figure 4.9b. Moreover, there were no significant differences of myocardial GSSG concentrations between control and all treatments as well as between non exercise and exercise-trained animals ($P>0.05$).

When the concentration of total glutathione was demonstrated as the ratio of GSH/GSSG, there was an evidence that non exercise ovariectomized hamsters had a depletion of GSH/GSSG in Cr- treatment at the 9th week ($P<0.05$) (Figure 4.9c) but not in the other treatments. In addition, GSH/GSSG ratio in exercise-trained ovariectomized hamsters treated with E_2 and Cr+E_2 were increased, approximately

more than 3 times of that in the control and showed a significant difference when compared to those in non exercise-trained animals ($P<0.05$).

For the activity of antioxidant enzyme, GPx activity in the control and all treatments in non exercise and exercise-trained ovariectomized hamsters were determined and compared (Figure 4.9d). In the control ovariectomized hamsters of non exercise and exercise-trained groups, GPx activity were 15.21$\pm$2.41 and 17.7$\pm$2.74 nmol/min/mg protein. In agree with the depletion of the myocardial GSH concentration and GSH/GSSG ratio, GPx activity was also declined in Cr- treatment at the 9th week in non exercise-trained ovariectomized hamsters as compared to the control ($P<0.05$).). In contrast, the non exercise-trained ovariectomized hamsters treated with Cr+, E_2 and Cr+E_2 did not showed a significant difference in GPx activities compared to the control. In addition, GPx activities in exercise-trained ovariectomized hamsters treated with E_2 and Cr+E_2 were increased approximately more than 3 times of that in the control and showed a significant difference when compared to those in non exercise-trained animals ($P<0.05$). Interestingly, it seems that the concentration of myocardial GSH, GSH/GSSG ratio, and GPx activity were higher in exercise-trained ovariectomized animals treated with E_2 and Cr+E_2 compared to other treatments.

Effects of Cr supplementation and E_2 replacement on serum IGF-I levels in non exercise and exercise-trained ovariectomized hamsters

As shown in Figure 4.10, serum IGF-I levels in the control and all treatments in non exercise and exercise-trained ovariectomized hamsters were determined and compared. Serum IGF-I levels in the control of non exercise and exercise-trained ovariectomized hamsters were 333.78$\pm$101.39 and 378.67$\pm$110.19 ng/ml. A marked decrease in the serum IGF-I level was observed only in Cr- treatment of non exercise ovariectomized hamsters ($P<0.05$), but not in exercise-trained animals. Although, slightly increases in IGF-I were observed in non exercise-trained animals treated with Cr+, E_2, and Cr+E_2, but the statistical significances were not detected. In contrast, marked increases in serum IGF-I level were observed in exercise-trained ovariectomized hamsters treated with Cr+, E_2 and Cr+E_2 which were 744.89$\pm$69.67, 1255.44$\pm$50.58 and 1239.06$\pm$140.6 ng/ml, respectively ($P<0.05$). Interestingly, it seems that the higher level of IGF-I could be seen in exercise-trained animals treated with E_2 and Cr+E_2 when compared to other treatments. Therefore, it suggested that a beneficial combination effect of E_2 and Cr+E_2 with exercise training is more potent on the GH-induced IGF-I level than others.

Table 4.1 Effects of Cr supplementation and E_2 replacement on the O_2 consumption, distance traveled in running wheel and the O_2 economy at the 9th week in exercise-trained ovariectomized hamsters.

Group	O_2 consumption (mlO_2/kg)	Distance travel in running wheel (m)	O_2 economy ($mlO_2/kg/m$)
C (n=10)	17.37±1.79	16.28±2.08	1.08±0.18
Cr- (n=10)	15.79±2.19	14.89±2.62	1.09±0.24
Cr+ (n=10)	17.36+1.95	16.39+1.67	1.07+0.16
E_2 (n=10)	17.65±2.58	16.44±0.34	1.07±0.15
Cr+E_2 (n=10)	19.54±2.51	17.83±1.10	1.10±0.16

The data are presented as mean ± SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2).

Table 4.2 Effects of Cr supplementation and E_2 replacement on cardiac functions of QT-c interval, LVDP and dP/dt_{max} at the 9^{th} week in non exercise and exercise-trained ovariectomized hamsters.

Group	QT-c interval (msec)		LVDP (mmHg)		dP/dt_{max} (mmHg/min)	
	Non exercise	Exercise	Non exercise	Exercise	Non exercise	Exercise
C (n=10)	0.214±0.009	0.197±0.007	58.23±5.52	69.00±10.83	1834.08±189.15	2230.29±204.64
Cr- (n=10)	0.218±0.008	0.196±0.008	57.19±7.09	69.55±10.26	1831.82±168.59	2174.91±187.83
Cr+ (n=10)	0.215±0.007	0.196±0.009	57.75±7.21	68.28±13.11	1879.73±173.30	2289.02±263.44
E_2 (n=10)	0.211±0.010	0.199±0.010	57.92±7.82	70.95±12.74	1860.53±209.70	2258.09±218.64
$Cr+E_2$ (n=10)	0.210±0.007	0.198±0.006	59.23±7.45	71.11±12.41	1851.40±174.27	2274.10±239.03

The data are presented as mean ± SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement ($Cr+E_2$).

Table 4.3 Effects of Cr supplementation and E_2 replacement on EC50 values derived from the dose-response curves of QT-c interval, LVDP and dP/dt_{max} after hydrogen peroxide stress test at the 9th week in non exercise and exercise-trained ovariectomized hamsters.

Group	EC50 of QT-c interval (mM)		EC50 of LVDP (mM)		EC50 of dP/dt max (mM)	
	Non exercise	Exercise	Non exercise	Exercise	Non exercise	Exercise
C (n=10)	0.060±0.024[a]	0.067±0.015[a]	0.057±0.008[a]	0.104±0.010[c]	0.053±0.008[a]	0.103±0.021[c]
Cr- (n=10)	0.017±0.003[b]	0.020±0.003[b]	0.016±0.008[b]	0.038±0.006[d]	0.023±0.005[b]	0.038±0.003[d]
Cr+ (n=10)	0.067±0.020[a]	0.067±0.011[a]	0.059±0.011[a]	0.109±0.008[c]	0.064±0.014[a]	0.108±0.008[c]
E_2 (n=10)	0.064±0.011[a]	0.069±0.014[a]	0.064±0.013[a]	0.124±0.013[c]	0.070±0.014[a]	0.110±0.007[c]
Cr+E_2 (n=10)	0.065±0.017[a]	0.075±0.011[a]	0.065±0.008[a]	0.126±0.016[c]	0.072±0.012[a]	0.110±0.006[c]

The data are presented as mean ± SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2). Values with different superscripts are significantly different among treatments in non exercise and exercise-trained ovariectomized hamsters ($P<0.05$).

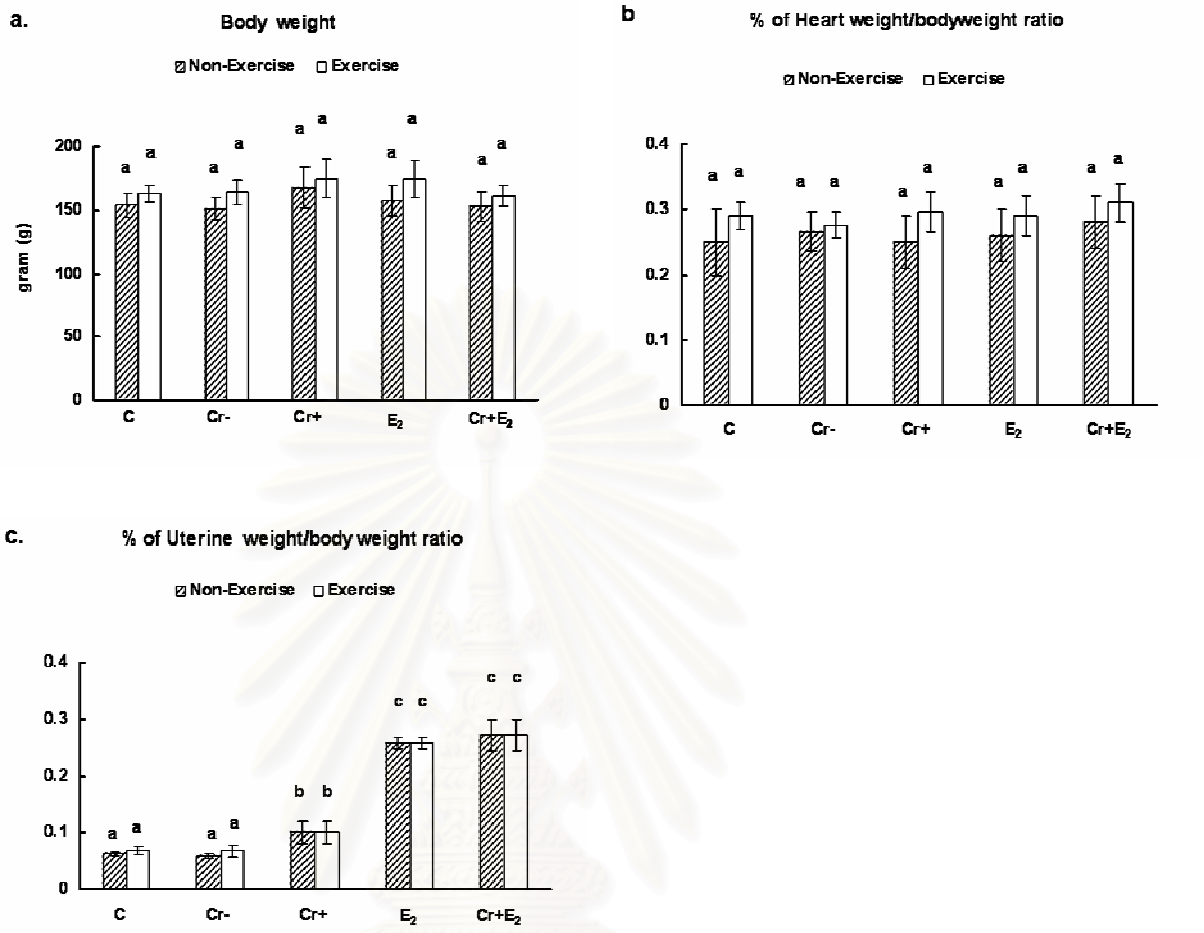


Figure 4.1 Effects of Cr supplementation and E_2 replacement on (a) BW, (b) % HW/BW ratio and (c) % UW/BW ratio at the 9th week in non exercise and exercise-trained ovariectomized hamsters. The data are presented as mean $\pm$ SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2). Values with different superscripts are significantly different among treatments in non exercise and exercise trained-ovariectomized hamsters ($P<0.05$).

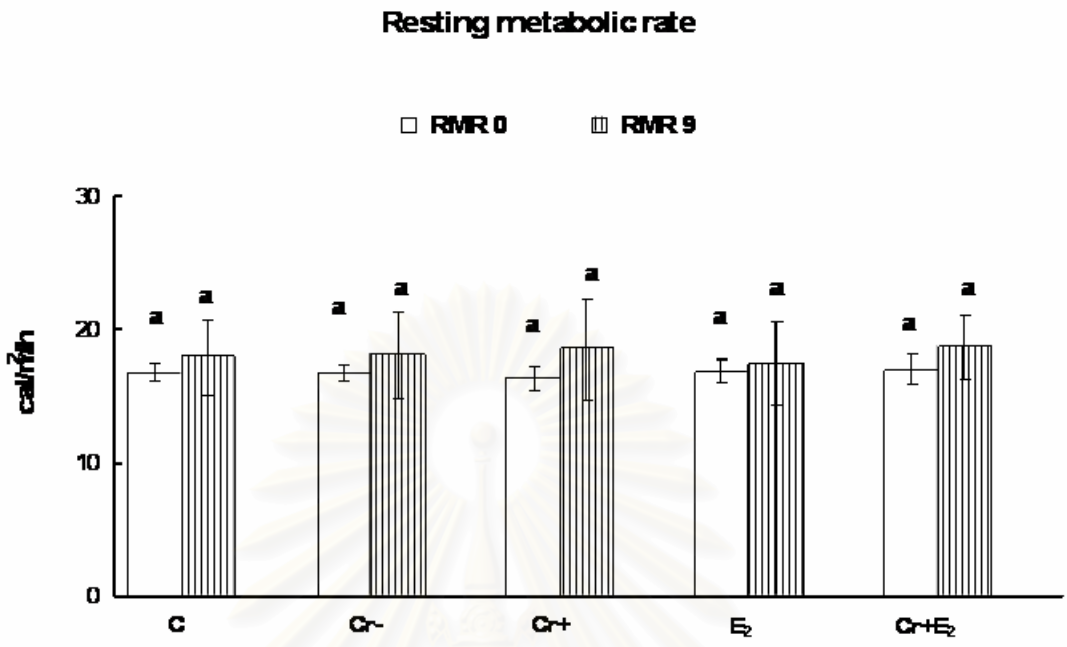


Figure 4.2 Effects of Cr supplementation and E_2 replacement on RMR (at week 0 and week 9) in exercise-trained ovariectomized hamsters. The data are presented as mean $\pm$ SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2).

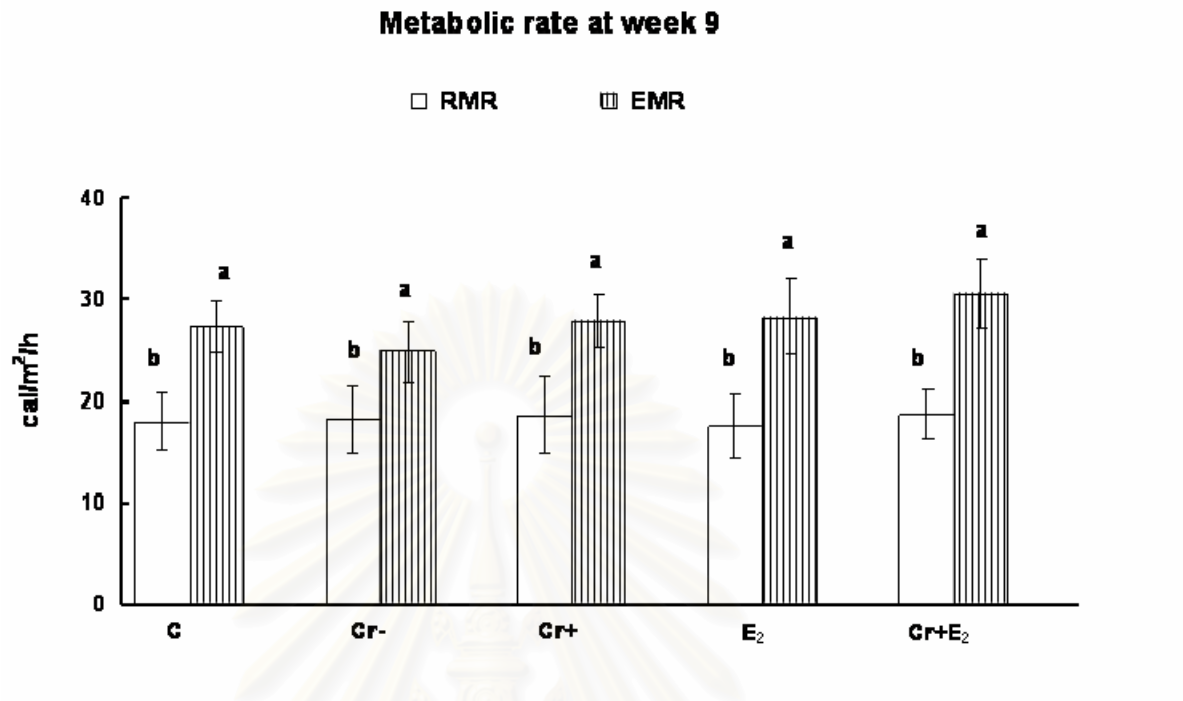


Figure 4.3 Effects of Cr supplementation and E_2 replacement on RMR and EMR at the 9th week in exercise-trained ovariectomized hamsters. The data are presented as mean $\pm$ SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2). Values with different superscripts are significantly different between RMR and EMR of exercise- trained ovariectomized hamsters. ($P<0.05$).

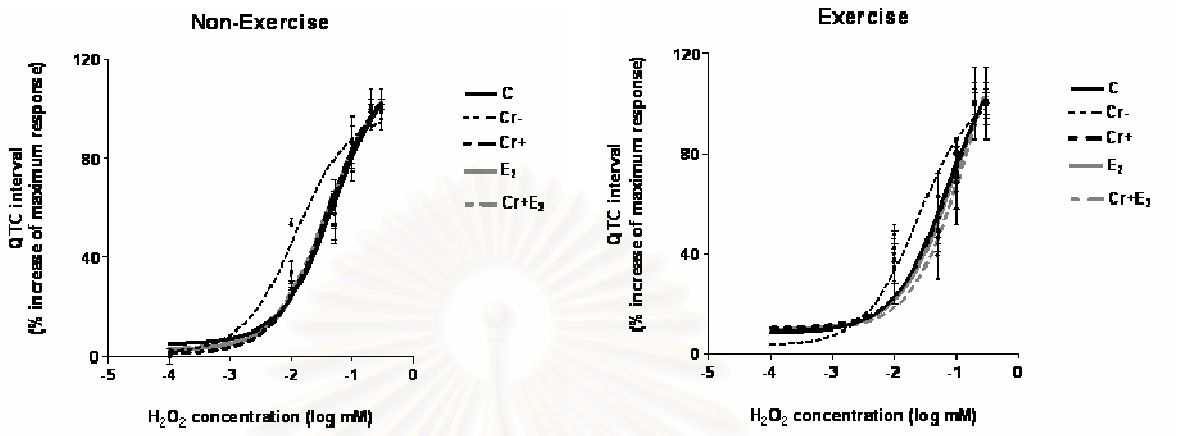


Figure 4.4 Effects of Cr supplementation and E_2 replacement on EC50 values derived from the dose-response curves of QT-c interval after hydrogen peroxide stress test at the 9th week in non exercise and exercise-trained ovariectomized hamsters. The data are presented as mean $\pm$ SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2).

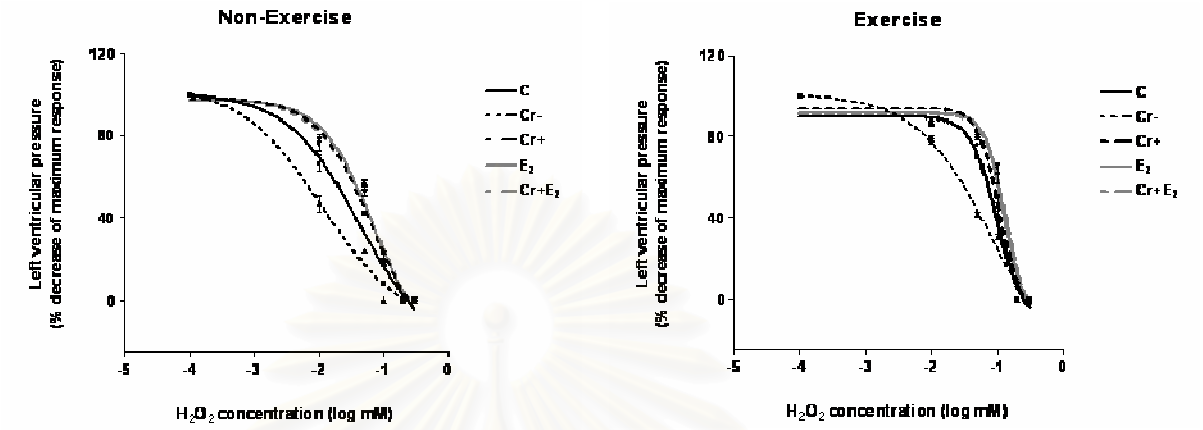


Figure 4.5 Effects of Cr supplementation and E_2 replacement on EC50 values derived from the dose-response curves of LVDP after hydrogen peroxide stress test the 9th week in non exercise and exercise-trained ovariectomized hamsters. The data are presented as mean $\pm$ SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2).

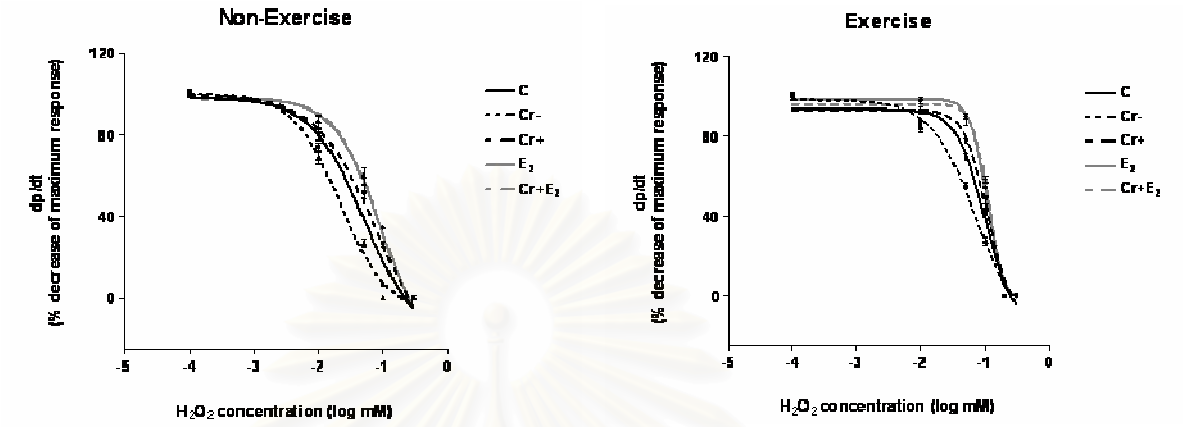


Figure 4.6 Effects of Cr supplementation and E_2 replacement on EC50 values derived from the dose-response curves of dP/dt_{max} after hydrogen peroxide stress test at the 9^{th} week in non exercise and exercise-trained ovariectomized hamsters. The data are presented as mean $\pm$ SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement ($Cr+E_2$).

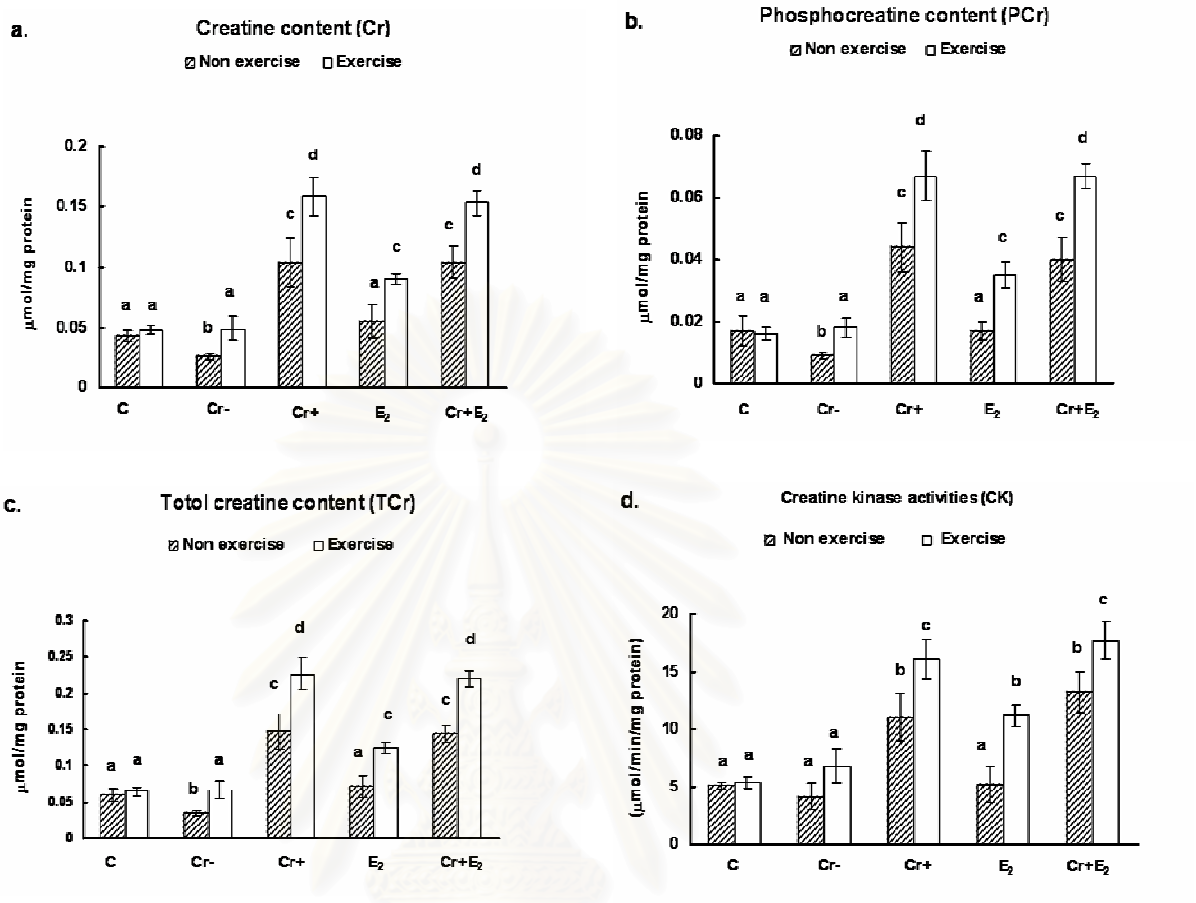


Figure 4.7 Effects of Cr supplementation and E_2 replacement on contents of (a) Cr, (b) PCr, (c) TCr and (d) enzyme activity of CK at the 9th week in non exercise and exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2). Values with different superscripts are significantly different among treatments in non exercise and exercise-trained ovariectomized hamsters ($P<0.05$).

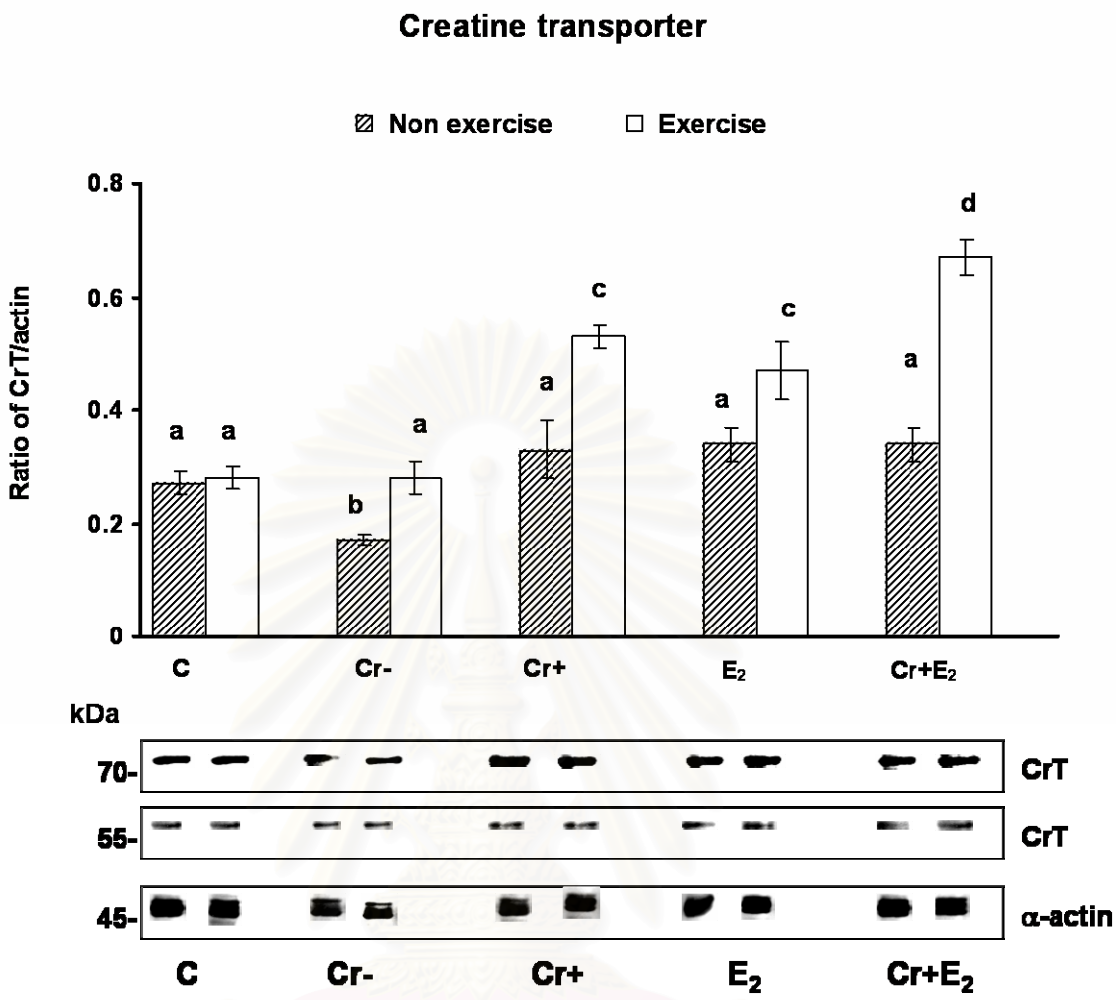


Figure 4.8 Effects of Cr supplementation and E_2 replacement on myocardial CrT protein at the 9th week in non exercise and exercise-trained ovariectomized hamsters. The upper panel is histogram bars showing the results of densitometric analyses from pooled data (n = 10/group). Values were normalized by the corresponding optical density for α-actin, used as internal control. The lower panel shows the results of immunoblots analyzed, and probed with antibodies against total CrT (55-kDa and 70-kDa) or α-actin (45 kDa). The data are presented as mean $\pm$ SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2). Values with different superscripts are significantly different among treatments in non exercise and exercise-trained ovariectomized hamsters ($P<0.05$).

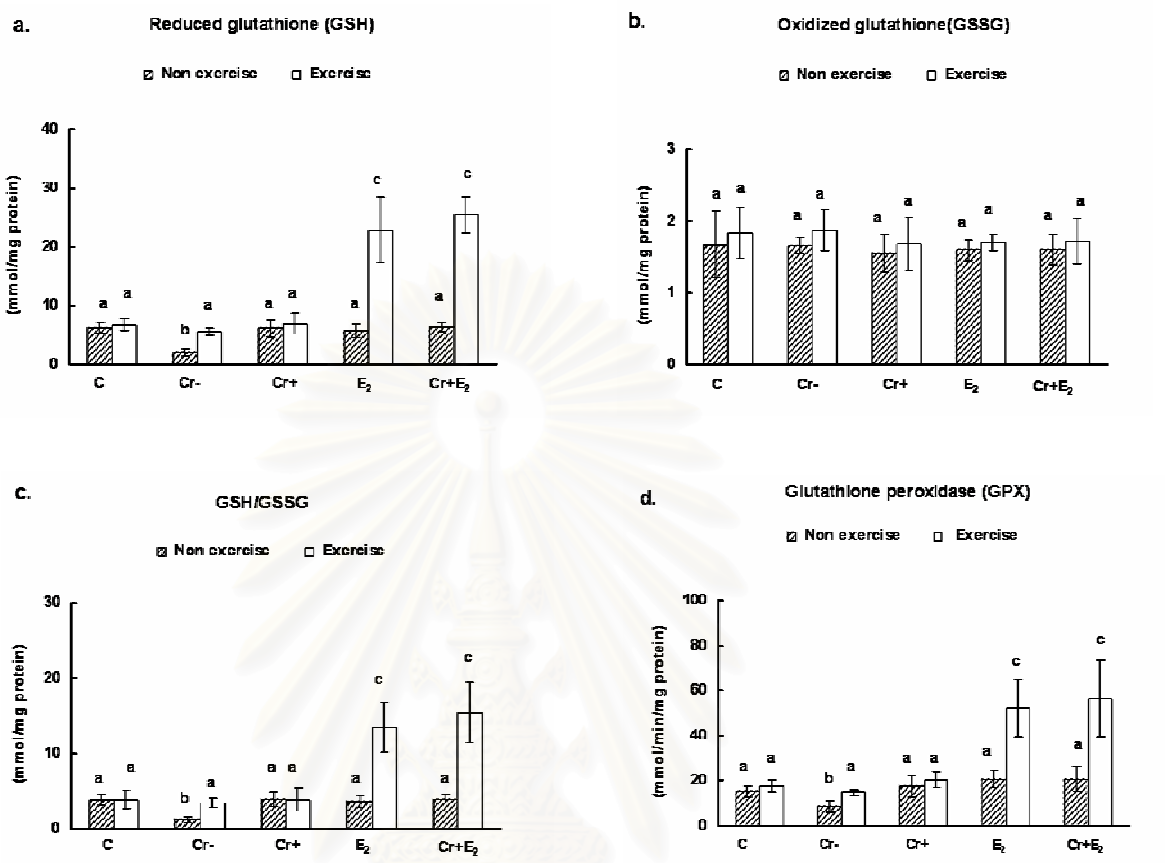


Figure 4.9 Effects of Cr supplementation and E_2 replacement on contents of (a) GSH, (b) GSSG, (c) GSH/GSSG ratio and (d) enzyme activity of GPX at the 9th week in non exercise and exercise-trained ovariectomized hamsters. The data are presented as mean $\pm$ SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2). Values with different superscripts are significantly different among treatments in non exercise and exercise-trained ovariectomized hamsters ($P<0.05$).

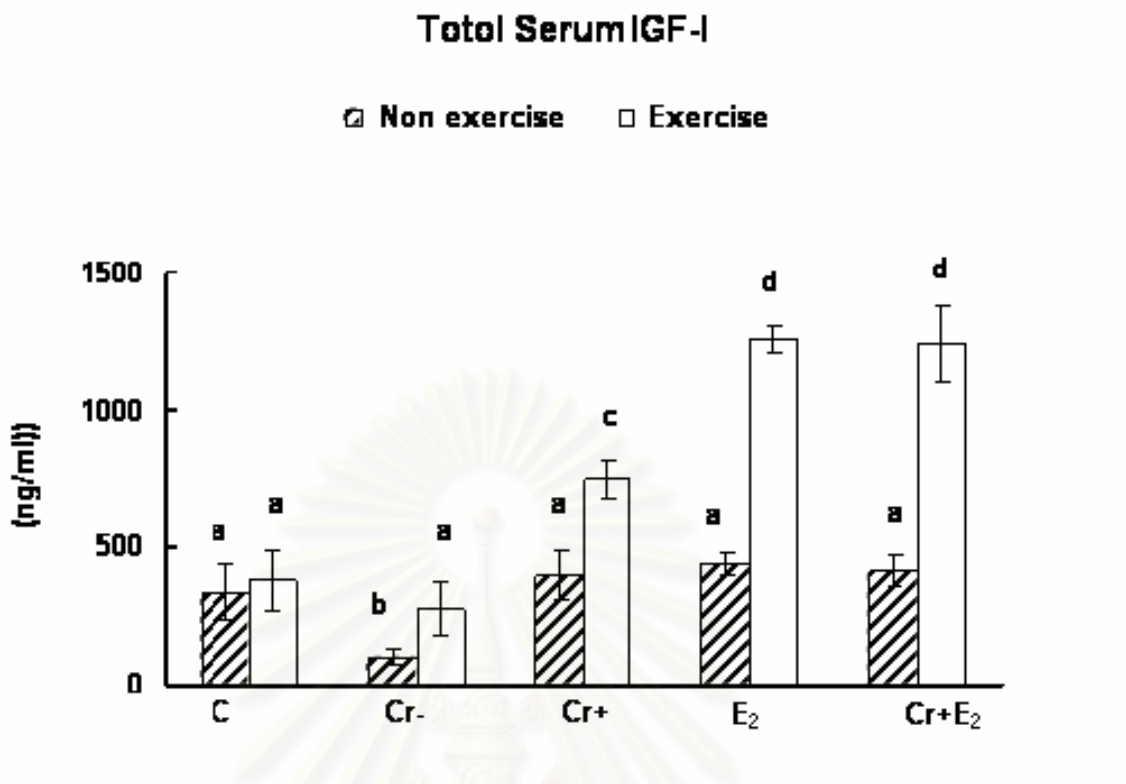


Figure 4.10 Effects of Cr supplementation and E_2 replacement on serum IGF-I levels at the 9th week in non exercise and exercise-trained ovariectomized hamsters. The data are presented as mean $\pm$ SD. Control (C), Cr depletion (Cr-), Cr supplementation (Cr+), E_2 replacement (E_2), Cr supplementation combined with E_2 replacement (Cr+E_2). Values with different superscripts are significantly different among treatments of non exercise and exercise-trained ovariectomized hamsters ($P<0.05$).

CHAPTER V

DISCUSSION

Effects of Cr supplementation and E_2 replacement on body weight (BW), % heart weight/body weight (HW/BW) ratio and % uterine weight/body weight (UW/BW) ratio in non exercise and exercise-trained ovariectomized hamsters

The results in the present study demonstrated that Cr- treatment as well as other animals treated with Cr+, E_2 and Cr+E_2 at the 9^{th} week has no influence on BW and % of HW/BW ratio compared to the control. It is possible that animals appeared to tolerate our treatment regimens, with no sign of distress even. All groups in non exercise ovariectomized hamsters were not different from those in the exercise-trained animals. Chronic exercise training (more than 6-8 weeks) have been reported a significant reduction in BW (Baldwin et al., 1980). In addition, it seems to be that chronic exercise training (more than 90 minutes daily and up to 6 weeks) may induce a significant degree of cardiac hypertrophy by increasing HW/BW ratio, reflecting a decrease in BW and an increase in HW (Kaplan et al., 1994). In our study, it was apparent that wheel running exercise at the sub-maximal intensity (10 minutes/day, 5 day/week for 9 weeks) did not observed body weight loss or cardiac hypertrophy.

In contrast, non exercise and exercise-trained ovariectomized hamsters treated with Cr+ significantly increased % UW/BW ratio. Our results indicate that Cr+ treatment for 9 weeks was more affected the weight of uterus than those in the heart. Relatively few studies have examined the influence of Cr directly on the uterus weight. However, Cr+ treatment has been shown to increase intracellular water retention from the anabolic effect and induce the muscular hypertrophy (Haussinger et al., 1994). One possible explanation is that Cr+ treatment may act as an osmotic agent to increase cellular over-hydration resulting in an increase in UW. However, when E_2 and Cr+E_2 were treated in exercise-trained ovariectomized hamsters, there was a significant increase in % UW/BW ratio and therefore hypertrophy of the uterus. From previous study, uterine muscle which has often been used for evaluating the status of E_2 in female has been reported to be atrophy after sex hormones deficiency (Chu et al., 1999). Our study is in agreement with the earlier reports that uterus in ovariectomized animals becomes hypertrophies by E_2 replacement (Ren et al., 2003). However, all treatments in non exercise ovariectomized hamsters were not significantly different from those in exercise-trained animals. Similar to BW and %

HW/BW ratio, our study indicated that sub-maximal exercise training has also no influence on % UW/BW ratio.

Effects of Cr supplementation and E_2 replacement on metabolic rate in non exercise and exercise-trained ovariectomized hamsters

RMR was measured in non exercise and exercise-trained ovariectomized hamsters before starting the experiment (week 0). RMR and EMR at the 9th week were measured only in exercise-trained animals. All metabolic rates were measured from O_2-consumption determination by indirect calorimetry following the procedure similar to those small mammal studies (Nespolo et al, 2002). The factors that regulate MR, the major component of daily energy expenditure for up to 70 % of the caloric utilization, are age, size, digestive condition, thermal condition, activity level and experimental condition (Dinulescu et al., 1998). In the present study, there was no significant difference of RMR at the beginning and the 9th week among control and all treatments in exercise-trained ovarietomized hamsters.

In addition, the results demonstrated that EMR at the 9th week of exercise-trained ovariectomized hamsters in the control and all treatments were significantly increased compared to RMR ($P<0.05$). It is well recognized that aerobic exercise training induced structural adaptation serve to increase in the myocardium's maximum power and energy-consuming capacity (Stuewe et al., 2000). Thus, it was apparent that wheel-running exercise in our study (10 minutes/day, 5 day/weeks for 8 weeks) enough to elicit an alteration of oxygen consumption. A previous report studied the metabolic rate in resting condition and during running exercise and found that oxygen supply and consumption were significantly increased during exercise with increasing exercise intensity and duration (Holloszy et al., 1985). Our results of exercise metabolic rate calculated from O_2-consumption were consistent with the previous reports that exercise training induced an increase in O_2-uptake in accompany with an increase metabolic rate during exercise (Dohm et al., 1994). A possible explanation is that the long-term exercise training for 9 weeks in our study could increase the high energy phosphate capacity system which is reinforced by an increase in O_2-uptake providing for cardiac energy-consuming capacity. In addition, EMR calculated from O_2-consumption in exercise-trained ovariectomized hamsters of all treatments were not significantly differences from the control. Concerning the oxygen economy, we also determined in exercise-trained ovariectomized hamsters at week 9 of experiment. In our study, the result demonstrated that oxygen economy in all treatments was not significantly altered compared to the control. Thus, it is suggested that treatments in our study has no effect on EMR and oxygen economy among Cr-, Cr+, E_2, Cr+E_2 in exercise-trained hamsters.

Effects of Cr supplementation and E_2 replacement on cardiac functions of QT-c interval, LVDP and dP/dt_{max} in non exercise and exercise-trained ovariectomized hamsters

In the present work, QT-c interval, LVDP and dP/dt_{max} were recorded as indices of electrical and mechanical functions similar to other previous studies reported (Naohiro and Akiyoshi, 1996; Merrill, 2002; Hamlin et al., 2003). Our results demonstrated that QT-c interval, LVDP and dp/dt_{max} of non exercise and exercise-trained ovariectomized hamsters in all treatments of Cr-, Cr+, E_2 and Cr+E_2 were not significantly different from the control. Although a slightly shorter in the QT-c interval in accompany with higher LVDP and dP/dt_{max} could be observed in exercise–trained ovariectomized hamsters compared to non exercise animals but the results did not show any statistical significances. As a result, a possible explanation for our result is that all treatments with or without exercise training in our study has no direct influence on the cardiac contractility. This suggestion might, at least, provide an explanation for the results found in our study.

Effects of Cr supplementation and E_2 replacement on EC_{50} derived from the dose response curve of cardiac functions of QT-c interval, LVDP and dP/dt_{max} against H_2O_2 stress test in non exercise and exercise-trained ovariectomized hamsters

In the present work, we tested the electrical and mechanical damages induced by exogenously administered H_2O_2 in the isolated hamster hearts. The results were expressed as EC_{50} derived from the dose response curves of QT-c interval, LVDP and dP/dt_{max} of non exercise and exercise-trained ovariectomized hamsters treated with Cr-, Cr, E_2 and Cr+E_2. In our study, Cr- treatment at the 9^{th} week in both of non exercise and exercise-trained ovariectomized hamsters were more affected to EC_{50} of electrical and mechanical parameters than those in other treatments. Along with a reduction in EC_{50} value of QT-c interval, EC_{50} of both LVDP and dP/dt_{max} were also reduced after Cr- treatment for 9 weeks. Our results showed that the prolongation of QT-c interval and a reduction in LVDP and dP/dt_{max} of Cr-treatment at the 9^{th} weeks responded to quite low dose of H_2O_2. Recently, it has been reported that prolongation of QT-c interval found in pathologic hearts of ventricular hypertrophy and heart failure (Davey et al., 2000). In addition, previous study have reported a decrease in the total enzyme activity coupled with a decrease in Cr substrate content, reflecting a decrease in energy of the heart found in both the Cr depletion and the dilated cardiomyopathy animals (Liao et al.,1996). The mechanical function of LVDP was depressed by 33 % and 32 % in the Cr depletion

and myocardial infarction rats. These may suggest that the mechanical damage was induced by energy depletion and oxidative stress (Horn et al., 2001). Therefore, it implies that depletion of Cr induced electrical and mechanical damage in response to an increase in oxidative stress. In contrast to Cr- treatment, EC_{50} values of QT-c interval, LVDP and dP/dt_{max} in other animals treated with Cr+, E_2 and Cr+E_2 of both in non exercise and exercise-trained animals were not significantly altered compared to the control. Our results were in agreement with the other studies found that the ejection fraction at rest and at work was not changed in Cr ingestion in human with congestive heart failure (Gordon et al., 1995). In addition, the antioxidant properties of E_2 have been reported in *vivo* and in *vitro* studies elsewhere as well as demonstrated in our study (Subbiah et al., 1993). The present study indicated that Cr+ or E_2 protect myocardial electrical and mechanical damages against oxidative stress. Interestingly, EC_{50} values in all treatments of exercise-trained ovariectomized hamster were significantly increased compared to the non exercise animals. The results showed that the prolongation of QT-c interval and reductions in LVDP and dP/dt_{max} were observed at high doses of H_2O_2. This indicates that exercise training can protect electrical and mechanical damage in response to the increased oxidative stress. Chronic exercise training also stimulated antioxidant response in the heart (Urso and Clarkson, 2003). Other investigators have shown that long-term running exercise and also swimming exercise can protect hearts from ischemic-reperfusion injury (McDonough, 1999; Leeuwenburgh and Heinecke, 2001). Therefore, our results suggested that Cr+ or E_2 combined with exercise training demonstrated a greater improvement on the myocardial reserve against oxidative damage than that of Cr+ or E_2 treatment alone.

Effects of Cr supplementation and E_2 replacement on metabolic phosphate contents (Cr, PCr, TCr), CK enzyme activity and CrT protein in non exercise and exercise-trained ovariectomized hamsters

For metabolic phosphate contents of Cr, PCr, and TCr, indicators of myocardial energy reservation via Cr metabolism, the results demonstrated that contents of myocardial Cr, PCr and TCr were decreased in β-GPA treatment (Cr-) in the non exercise ovariectomized hamster but not in the exercise animals. The present work indicated that the contents of myocardial Cr, PCr and TCr were decreased by feeding with β-GPA, but these could be restored by exercise training. Previous study has been reported that β-GPA is Cr-analogue which has been used widely as an experimental means of depleting tissue Cr and PCr (Zweier and

Jacobus, 1991). Our study was in agreement with the other studies that reported decreases in Cr and PCr contents after β-GPA feeding (Meyer et al., 1986). An improvement in myocardial Cr, PCr and TCr contents in Cr- treatment can be seen in ovariectomized exercise-trained hamster which may be due to exercise training can preserve of metabolic phosphate metabolism. In addition, non exercise ovariectomized hamsters treated with Cr+ and Cr+E_2 increased the accumulation of these contents whereas E_2-treatment alone did not show the statistical significant different. The results indicate that Cr+ treatment plays an important role on the accumulation of myocardial Cr reservation. As it known that, total Cr content of cardiomyocytes is dependent on the rate of Cr uptake, Cr retention, and Cr loss via creatinine (Wyss and Kaddurah-Daouk, 2000). Thus, our information was consistent with the earlier reports in cardiac muscle showing an increase in intracellular Cr and TCr storage in Cr+ treatment (Robinson et al., 1999), but not in E_2 treatment (Beer et al., 2002). Interestingly, the contents of Cr, PCr and TCr were highest when combined the treatment of Cr+ and Cr+E_2 with exercise training. Previous study has been reported that exercise training can influence the net uptake of Cr in to the skeletal muscle cell (Harris et al., 1992). In addition, it is well conceivable that changes in Cr metabolism contribute to cardiac contractile dysfunction (Ingwall, 1993; Liao et al., 1996). These results in our study could be shown that the combined effect of Cr supplementation and exercise training in improving myocardial energy reservation via Cr metabolism.

As mentioned earlier, CK is an important enzyme induced in energy maintenance and energy transfer in muscle and brain cells. It catalyzes the reversible transfer of a phosphate moiety between ATP and Cr (Sousa et al., 1999). Additionally, CK mechanism has been proposed to communicate changes in ATP hydrolysis to the mitochondria to adjust ATP synthesis by the oxidative phosphorylation (Saks et al., 1994). Surprisingly, the effect of 9-week Cr depletion in non exercise ovariectomized hamsters, CK activities did not change. This was different from those (Shoubridge et al., 1985) who reported that CK reaction velocity was depressed in Cr-depleted rat hearts. The discrepancy might be due to the difference in duration of depletion which can be observed in chronic depletion for up to 3 months. Similarly, Cr- treatment at 9 weeks has also no influence on CK activities in exercise-trained ovariectomized animals. These results can be suggested that the depletion of Cr pools by β-GPA feeding in our study did not relate to CK activity. Meanwhile, similar to the changes of metabolic phosphate contents, the results demonstrated that other groups of non exercise ovariectomized hamsters

treated with Cr+ and Cr+E_2 increase the activity of CK as compared to the control. This indicates that Cr+ treatment plays an important role on the activation of myocardial CK activities. In 1986, Loike and coworkers have been reported that CK kinetics seems to be a major determinant of myocardial Cr and PCr contents. Our result was similar to those evidences showing an increase in CK activity and accompanied with increase in Cr substrate pools in Cr+ treatment (Liao et al., 1996). They implied the contents of Cr and PCr suggest the capacity of the CK energy shuttle (Neubauer et al., 1995). Moreover, ovariectomized hamsters treated with Cr+ alone, E_2 alone and Cr+E_2 cause increases of CK activities in exercise animals compared to those in the non exercise animals and showed the high CK activities in animals treated with Cr+ and Cr+E_2. Exercise training also has been reported to increase in CK activities in canine myocardium (Stuewe et al., 2001). Thus, our results suggested that exercise training demonstrated a greater improvement of the CK energy shuttle.

Furthermore, previous studies have been reported that CrT functions seem to be a major determinant of myocardial Cr pools (Loike et al., 1986). Therefore, in this study, CrT protein was measured in myocardium tissue. The present data illustrate that Cr depletion for 9 weeks caused a decrease in CrT protein in non exercise ovariectomized hamsters but not in exercise-trained animals. Our results indicated that feeding with β-GPA caused a decrease in CrT protein content whereas it could be restored by exercise training. This result was consistent with the earlier study has been reported that animals treated with β-GPA were unable to maintain myocardial ATP concentration, and expression of CrT was downregulated by 25 % in β-GPA induced heart failure (Horn et al., 2001). The possible mechanism for the reduction in CrT after Cr depletion in our study may results from decreases in metabolic phosphate contents reflecting to a decreased in protein synthesis or an increased in protein catabolism. On the contrary, a restoration in CrT protein content was also suggested by exercise training, and may increase the translocation of the CrT protein to the sarcolemma similar to the effects seen between exercise and GLUT-4 translocation (Thorell et al., 1999) resulting in maintaining the content of CrT protein in the sarcolemma. Along with an increase in myocardial energy phosphate contents and CK activities, exercise-trained ovariectomized hamsters treated with Cr+, E_2 and Cr+E_2 caused an increases in CrT proteins compared to those in the non exercise animals. Our result showed that Cr+ or E_2 alone has no effect on CrT proteins. These results of Cr supplementation in our study are in the same manner as those performed by Fry and Morales (1980). Indeed, the effect of Cr on protein synthesis is

controversy in which it was enhanced in cell culture (Ingwall et al., 1972; Ingwall and Wildenthal, 1976) or unaffected in human studies (Tarnopolsky et al., 1997; Parise et al., 2000). This discrepancy seems to be associated with the difference of experimental models. One limiting of the existing information about CrT protein is that it is restricted to only two experimental models, supplementation and depletion of Cr. At present, no information is available on the effects of E_2 directly on the CrT protein content. One study reported the contents of myocardial Cr and PCr were unaffected by chronic E_2 replacement (Beer et al., 2002). These results imply that E_2 has no effect on myocardial energy reservation via Cr metabolism; then, it could not alter CrT protein. Of interest, the CrT protein was increased in the exercise animals treated with Cr+, and E_2. Our study was also in accord with other studied reported that Cr supplementation combined with exercise training increases in CrT protein (Harris et al., 1992; Robinson et al., 1999). A possible explanation for those changes is that Cr combined with exercise training was more effect on Cr-uptake resulting from enhanced blood flow (Thorell et al., 1999), increased much more energy demand for myocardium tissue leading to activation of the CrT protein synthesis, and caused an increase in the CrT protein content. Similar to the combination of Cr with exercise training, E_2 combined with exercise training also cause an increase in CrT protein. This again, may explain by enhancing of blood flow from exercise training to promote E_2 induce-protein synthesis (Reiss and Kaye, 1981), and selective on activating the CrT protein synthesis. Moreover, a greatest improvement in CrT protein was observed in exercise animals treated with Cr+E_2. Our results suggested that exercise training can stimulate the CrT protein synthesis which is much more sensitive to the combination treatment than that of the Cr+ or E_2 treatment alone.

Effects of Cr supplementation and E_2 replacement on myocardial glutathione (GSH, GSSG and GSH/GSSG ratio) and GPx enzyme activity in non exercise and exercise-trained ovariectomized hamsters

Level of reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG) is known to measure as an index of oxidative stress in ischemic-reperfusion hearts. Glutathione peroxidase, an important antioxidant enzyme that is present in relatively high amounts within the heart and demonstrates to confer greater protection against oxidative damage than either superoxide dismutase (SOD) or catalase(CAT) (Shiomi et al., 2004). In another study of the heart after 10-week constriction of aorta, glutathione peroxidase activity (GPx) was increased as well as the levels of GSH and the GSH/GSSG ratio (McDonough, 1999). In the present study, the contents of GSH, GSSG, GSH/GSSG ratio were measured, and set as the

markers of oxidative stress together with GPx activity. The present data illustrated that all treatments in the non exercise ovariectomized hamsters were not alter myocardial GSH, GSSG GSH/GSSG ratio and GPx enzyme compared to the control excepted in Cr depletion. Little is known about the effect of Cr directly on markers of oxidative stress. Depletion of Cr showed a functional impairment response to oxidative stress in heart failure induced by β-GPA administration (Kapelko et al., 1988). Our result of Cr depletion was in accord to the others, previously reported a direct cause of impaired functional performance in pathologic hearts due to oxidative damage (Park et al., 1991). Thus, this can be implied that 9-week Cr depletion in our study caused a loss of antioxidant reserve. This may cause an induction of oxidative damage to cardiac tissues. In contrast, exercise training could restore all of antioxidant levels which were decreased in Cr depletion. Our results are similar to those of Powers and coworkers (1994) who have been reported that endurance exercise training protect rats from exercise-induced oxidative stress, raising level of antioxidants and antioxidant enzymes in both skeletal and cardiac muscles. In contrast to Cr depletion, supplementation with Cr as well as replacement with E_2 in our study, were unaffected to markers of the oxidative stress and an antioxidant enzyme. It was found that a reduction in the necrotic zone under PCr administration either before or post 30-min artery ligation in rabbit hearts (Sharov et al, 1987). However, feeding Cr in another study was failed to protect the loss of energy reservation after myocardial infarction and no improvement on mechanical functions in pathologic heart failure (Persky and Brazeau, 2001). Thus, this might be the case in our study that Cr supplementation has no plus effect to the maker of oxidative stress and an antioxidant enzyme because supplementation with Cr has no significant effect on antioxidants. Likely, E_2 treatment in our experiment also showed the difference results from previous study reported that E_2 can induce antioxidant enzyme expression by stimulating the antioxidant defense of glutathione peroxidase (GPx) (Massafra et al., 1998), and may inhibit the oxidation of low-density lipoprotein by affecting the SOD enzyme (Booth et al., 2003). This discrepancy seems to be associated with the difference in route of administration in which the earlier observations can be detected in implanted application of E_2. In contrast, our study was conducted E_2 replacement by subcutaneous injection. However, another study reported the GPx enhancement in the heart, but did not change after E_2 deficiency in female rats and explained to be an example of pro-oxidant/antioxidant balance (Barp et al., 2002). The antioxidant effects of E_2 have been previously reported by Tiidus (1995) that E_2 may donate hydrogen atoms from the phenolic hydroxyl group, thus terminating the peroxidation chain reaction. Therefore, it is the possible explanation

of our study that E_2 treatment may yield a better balance between oxidants and antioxidants levels due to its antioxidant properties. Although the GSSG level was not changed in all treatment of exercise-trained ovariectomized hamsters, the myocardial GSH of animals treated with E_2 and Cr+E_2 were increased. Ji et al. (1993) examined the mechanism of glutathione change in response to exercise, and found that the total gluthathione (GSH+GSSG) and the GSH increase throughout a cycling exercise at 70% VO_{2max} to exhaustion. An increase in protection against oxidative stress in the myocardial ischemic reperfusion injury was evidenced by a significant increase in the GSH level accompany with an increase in the GSH/GSSG ratio, and prevention in the accumulation of the GSSG level (Hill and Singal, 1997; Leichtweis et al., 2001). All data as mentioned above may be the explanation in exercise-trained animals treated with E_2 and Cr+E_2 of our study. The possible mechanism has been previously reported that exercise increased blood flow may promote the antioxidant activity of E_2 resulting to counteract on the myocardial oxidant level by reducing oxidant production and increasing the activity of the antioxidant enzyme level (Persky et al., 2000; Sotiriadou et al., 2003; Urso and Clarkson, 2003). Therefore, it suggested that a combined physiological effect of E_2 and Cr with exercise training in our study showed a cardioprotective effect in improving the antioxidant reservation against oxidative damage.

Effects of Cr supplementation and E_2 replacement on serum IGF-I levels in non exercise and exercise-trained ovariectomized hamsters

As it known that GH is synthesized in the pituitary somatotrophs to regulate growth and body composition, and exerts its metabolic actions through a complicated process that is partly dependence on the IGF-I level (Leung et al., 2004). Insulin-like growth factor (IGF)-I is a small peptide growth factor similar in structure to pro-insulin which plays a major role in the control of muscle growth. IGF-I is produced by liver, under the control of GH, and is released in blood circulation as a peptide hormone. Skeletal muscle also produces IGF-I that acts as paracrine and autocrine growth factors. Overexpression of IGF-I by injection of a plasmid or a viral construct containing IGF-I cDNA into the mouse muscle has been shown to increase muscle mass (15%) and to prevent sarcopenia in old mice (Hameed et al., 2003). Additionally, the IGF-I level in a previous study has been reported to be an ideal marker for GH activity (Kao et al., 1986). In the present work, although the non exercise ovariectomized hamsters treated with Cr+, E_2 and Cr+E_2 for 9 weeks have no effect on level of serum IGF-I, a decrease in serum IGF-I level could be observed only in Cr- treatment. Our result may be explained by the previous study which has

been reported plasma IGF-I levels are generally reduce by energy restriction including nutritional deficiencies (Sticker et al., 1995). This may imply a reduction in myocardial energy phosphate in our study might be related to a decrease in serum IGF-I level. On the other hand, exercise training for 9 weeks can reverse the reduction of serum IGF-I level in Cr depletion to the control level. This indicated that exercise training in our study has a significant impact on the GH/IGF-I axis. However, data from studies evaluating IGF-I response to exercise are controversial. While some studies have demonstrated no change in circulating IGF-I levels, in many others, exercise induced a transient increase in IGF-I levels (Schwarz et al., 1996). Furthermore, IGF-I response depends on status include exercise type, intensity, and duration (Rosendal et al., 2002). Similarly, our study of exercise training induced an increase in serum IGF-I level, particularly in Cr depletion, was in accord to the previous work reported that may be resulted from acute release of IGF-I from its binding proteins (Kraemer and Ratamess, 2005). Likewise, a greater improvement on serum IGF-I levels in our study can be detected in Cr+ or E_2 treatment combined with exercise training but not in Cr or E_2 without exercise. However, Cr+ or E_2 treatment has been reported to involve in stimulating GH/IGF-I axis by other previous studies. For example, cells were incubated with 5 mM Cr, the level of IGF-I mRNA increased progressively to be multiplied by 3.7 after 72 hours suggested that Cr-induced hypertrophy of C_2C_{12} cells is at least partially mediated by overexpression of IGF-I (Louis et al., 2004). Additionally, long-term E_2 treatment for 3 months increased the level of serum IGF-I in ovariectomized rats (Bottner and Wuttke, 2006). These previously results did not similar to our results seem to be the fact that the earlier observation was conducted Cr in cell culture and replacement of E_2 was extended to 3 months of duration. The possible explanation in our study is that 9-week of Cr+ or E_2 treatment did not enough to affect the GH-stimulated serum IGF-I level. Consequently, exercise-trained ovariectomized hamsters treated with Cr+ at 9 weeks improved the level of serum IGF-I, and the highest level could be observed in the exercise training groups treated with E_2 and Cr+E_2 for 9 weeks. The possible mechanism explained by exercise training exerts powerful anabolic effects of Cr+ and also E_2 treatments on GH-activity to stimulate the level of serum IGF-I. Therefore, it is suggested that E_2 and Cr+E_2 treatments with exercise training are more potent on the GH-induced IGF-I level than the others.

It is clear from our study that exercise training (10 min a day/ 5 day a week) in estrogen-deficient animals could restore myocardial reserve against oxidative damage. In addition, Cr supplementation or E_2 replacement combined with exercise training yielded more valuable results for estrogen-deficient animals demonstrated by

greater cardiac reserve function, greater accumulation of myocardial energy metabolic phosphate reservation via Cr metabolism, and higher level of serum IGF-I than Cr supplementation or E_2 replacement alone. Moreover, E_2 replacement combined with exercise training has been shown a greater improvement in antioxidant reservation than E_2 treatment alone. Furthermore, Cr supplementation plus E_2 replacement together with exercise training yielded the most valuable results for estrogen-deficient animals demonstrated by a greater improvement in all parameters regulating cardiac function.

Currently, the mechanism of Cr supplementation with exercise training in improvement of muscle performance has become an interesting subject particularly in skeletal muscle. The mechanism thereby Cr with exercise training act on cardiac muscle may partly be explained by the following study. Wyss and Kaddurah-daouk (2000) found that Cr with exercise training may improve muscle performance in three different ways: by increasing the muscle stores of PCr which is the most important energy source for immediate regeneration of ATP in the first few seconds of intense exercise; by accelerating PCr synthesis during recovery periods, and by depressing the degradation of adenine nucleotides and possibly also the accumulation of lactate during exercise. Another possibility mechanism can be explained by exercise resulting from enhanced blood flow, and may increase the uptake of Cr into the muscle cell (Harris et al., 1992; Robinson et al., 1999). Additionally, the exercise increases the translocation of CrT protein to the sarcolemma similar to the effects seen between exercise and GLUT-4 translocation has also been reported (Thorell et al., 1999). Exercise has also shown stimulatory effects on IGF-I levels concomitant with influence on Cr uptake (Odoom et al., 1996).

Another mechanism for the effect of E_2 replacement with exercise training act on cardiac muscle may partly be explained by the following study. As it known that exercise training has been shown to elicit positive adaptations results in an increase index of cardiac function (Jin et al., 2000), and causes an improvement in cardiac performance (Belardinelli et al., 1999). Similarly, there are animal data supporting the hypothesis that the cardiovascular system is an E_2 target tissue and that E_2 may play a significant role in regulating cardiovascular cell function (Lin and Shain, 1985). Several other cardiovascular mechanisms of exercise training affected E_2 replacement have been proposed to involve multiple mechanisms such as potentiation of endothelium-derived relaxing factor, a calcium channel blocker effect, inhibition of the alpha-adrenergic response of vascular smooth muscle, and an increase in prostacyclin biosynthesis (Samaan and Crawford, 1995). E_2 may also exert a positive inotropic effect with exercise training, possibly by improving

cardiovascular biomechanics (Pines et al., 1992). Enhance expression of endothelial nitric oxide synthesis and its inducible form in the myocardium has also been observed with the administration of E_2 (Farhat et al., 1996). Previous finding suggested that E_2 may have a protective effect on muscle damage after exercise and the mechanism of this effect may be related to the antioxidant characteristic and membrane properties associated with E_2 (Sotiriadou et al., 2003). Recent finding reported another possibility that E_2 replacement and exercise shared a common pathway, which led to the improvement of β-cell function and mass, via cAMP response element binding protein activation associated with enhanced insulin/IGF-I signaling cascade (Choi et al., 2005).

Therefore, up to this point, it is worth to suggest that creatine supplementation and estrogen replacement combined with exercise training provide protective effects on cardiac functions in estrogen-deficient hamsters, which provide valuable data for therapeutic uses against estrogen deficiency in menopausal women.

CHAPTER VI

CONCLUSION

The result of the present study can be summarized as follows:

1. Exercise-trained ovariectomized hamsters in the control and all treatments increased the EMR compared to RMR, however oxygen economy was not changed among treatments. It is indicated that 9-week wheel running exercise (10 min a day/ 5 day a week) in our study increases oxygen consumption, but shows no advantage on oxygen economy among treatments
2. Cr depletion, induced by feeding β-GPA for 9 weeks, causes a decrease in metabolic phosphate contents of Cr, PCr and TCr in accompany with a decrease in the CrT protein content in cardiac muscle but it does not affect CK activities. Exercise training can restore all metabolic phosphate contents demonstrated in 9-week Cr depletion group. An accumulation of myocardial Cr substrate pools, CK activities and CrT proteins were observed in the animals treated with Cr+ and Cr+E_2 and a greater accumulation could be seen when combined the treatments were combined with exercise training. This may suggest a combined effect of Cr supplementation and exercise training in improving myocardial energy metabolic phosphate reservation via Cr metabolism.
3. Cr-, Cr+, E_2 and Cr+E_2 treatments in exercise-trained ovariectomized hamsters have no direct influence on the cardiac contractility compared to those in non exercise animals.
4. Cr depletion for 9 weeks in both non exercise and exercise-trained ovariectomized hamsters induces electrical and mechanical damages in response to increased oxidative stress by decreasing EC_{50} of QT-c interval, LVDP and dP/dt_{max} compared to other treatments. On the other hand, Cr+, E_2 and Cr+E_2 treatments combined with exercise training showed a protective effect against oxidative stress supported by EC_{50} of QT-c interval, LVDP and dP/dt_{max} which were higher than observed in those of non exercise animals. This may indicate that Cr+ or E_2 combined with exercise

training demonstrates a greater improvement in the myocardial reserve against oxidative damage than that of Cr+ or E_2 treatment alone.

5. Depletion of Cr for 9 weeks suppresses markers of oxidative stress by decreasing GSH content, GSH/GSSG ratio and GPx activity whereas these alterations can be restored by exercise training. Although the GSSG level was not changed in all treatments of non exercise and exercise-trained ovariectomized hamsters, the animals treated with E_2 and Cr+E_2 combined with exercise training caused an increase in the myocardial GSH, the GSH/GSSG ratio and the antioxidant enzyme. Therefore, it is suggested that a combined physiological effect of E_2 and Cr+E_2 with exercise training in our study shows a cardioprotective effect by improving the antioxidant reservation.

6. A decrease in serum IGF-I level could be observed only in the Cr- treatment group and it could be restored by exercise training. On the contrary , an improvement on serum IGF-I levels could be detected in Cr+ combined with exercise training group and showed the high level in the animals treated with E_2 and Cr+E_2. Therefore, it is suggested that a beneficial combination effect of E_2 and Cr+E_2 with exercise training is more potent on the GH-induced IGF-I level than others.

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APPENDIX

Appendix. I Effects of Cr supplementation and E_2 replacement on BW, % of HW/BW ratio and % of UW/BW ratio in non exercise ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	BW1	BW2	BW3	BW4	BW5	BW6	BW7	BW8	BW9	UW	HW	%HW/BW	%UW/BW
	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
C1	130	140	135	135	140	140	150	155	170	0.098	0.542	0.3188	0.0576
C2	120	120	120	120	120	130	135	140	140	0.092	0.362	0.2586	0.0657
C3	125	140	145	140	135	145	145	150	160	0.098	0.357	0.2231	0.0613
C4	120	110	110	110	115	120	120	135	150	0.091	0.356	0.2373	0.0607
C5	130	140	135	150	145	145	140	150	155	0.090	0.561	0.3619	0.0581
C6	150	150	155	160	155	155	155	155	160	0.098	0.425	0.2656	0.0613
C7	130	140	135	135	140	140	140	140	140	0.098	0.300	0.2143	0.0700
C8	120	120	120	120	120	130	135	140	150	0.092	0.312	0.2080	0.0613
C9	125	140	145	140	135	145	140	140	150	0.098	0.357	0.2380	0.0653
C10	120	110	110	110	115	120	120	135	160	0.098	0.356	0.2225	0.0613
MEAN	127.00	131.00	131.00	132.00	132.00	137.00	138.00	144.00	153.50	0.095	0.393	0.2548	0.06225
SD	9.19	14.49	15.42	16.70	13.78	11.60	11.35	7.75	9.44	0.00	0.09	0.0496	0.00375
Cr-1	140	140	140	145	140	150	150	155	140	0.098	0.400	0.2857	0.0700
Cr-2	125	135	135	135	130	140	135	130	150	0.089	0.331	0.2207	0.0593
Cr-3	120	120	120	120	120	130	130	130	170	0.087	0.423	0.2488	0.0512
Cr-4	130	135	130	140	145	150	140	135	145	0.086	0.421	0.2903	0.0593
Cr-5	115	110	110	110	110	115	120	120	150	0.075	0.420	0.2800	0.0500
Cr-6	130	120	140	130	150	145	150	150	160	0.092	0.420	0.2626	0.0575
Cr-7	120	120	120	120	120	130	130	130	155	0.087	0.323	0.2084	0.0561
Cr-8	130	135	130	140	145	150	140	135	150	0.086	0.421	0.2807	0.0573
Cr-9	115	110	110	110	110	115	120	120	140	0.075	0.420	0.3000	0.0536
Cr-10	130	120	140	130	150	145	150	150	150	0.092	0.420	0.2801	0.0613
MEAN	125.50	124.50	127.50	128.00	132.00	137.00	136.50	135.50	151.00	0.087	0.400	0.26572	0.05757
SD	7.98	10.92	11.84	12.52	16.02	13.78	11.56	12.35	9.07	0.01	0.04	0.0306	0.00569
Cr+1	135	140	140	145	140	145	140	135	140	0.095	0.351	0.2507	0.0679
Cr+2	130	155	160	160	170	170	170	170	170	0.211	0.426	0.2506	0.1241
Cr+3	120	145	145	145	155	160	165	165	170	0.213	0.359	0.2112	0.1253
Cr+4	130	145	150	150	150	150	150	150	150	0.089	0.439	0.2927	0.0593
Cr+5	150	170	170	175	180	180	180	180	180	0.200	0.421	0.2339	0.1111
Cr+6	150	170	170	175	180	185	185	185	185	0.215	0.432	0.2335	0.1162
Cr+7	120	145	145	145	155	160	165	165	170	0.213	0.459	0.2700	0.1253
Cr+8	130	145	150	150	150	150	150	150	150	0.089	0.439	0.2927	0.0593
Cr+9	150	170	170	175	180	180	180	180	180	0.200	0.321	0.1783	0.1111
Cr+10	150	170	170	175	180	185	185	185	185	0.215	0.532	0.2876	0.1162
MEAN	136.50	155.50	157.00	159.50	164.00	166.50	167.00	166.50	168.00	0.174	0.418	0.25011	0.1016
SD	12.48	13.01	12.29	14.03	15.60	15.47	16.02	17.00	16.02	0.06	0.06	0.0375	0.0278

Appendix.I (continued) Effects of Cr supplementation and E_2 replacement on BW, % of HW/BW ratio and % of UW/BW ratio in non exercise ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	BW1 (g)	BW2 (g)	BW3 (g)	BW4 (g)	BW5 (g)	BW6 (g)	BW7 (g)	BW8 (g)	BW9 (g)	UW (g)	HW (g)	%HW/BW (g)	%UW/BW (g)
E1	135	140	140	140	135	140	140	145	140	0.312	0.350	0.2500	0.2229
E2	120	125	135	140	145	140	145	145	150	0.325	0.456	0.3040	0.2167
E3	140	145	140	150	180	160	165	170	170	0.389	0.426	0.2506	0.2288
E4	130	140	140	145	140	155	160	165	170	0.400	0.420	0.2471	0.2353
E5	140	150	150	150	160	160	160	160	160	0.398	0.440	0.2750	0.2488
E6	145	150	150	150	155	155	155	155	155	0.352	0.326	0.2103	0.2271
E7	135	140	140	140	135	140	140	145	140	0.312	0.450	0.3214	0.2229
E8	120	125	135	140	145	140	145	145	150	0.325	0.456	0.3040	0.2167
E9	140	145	140	150	180	160	165	170	170	0.389	0.326	0.1918	0.2288
E10	130	140	140	145	140	155	160	165	170	0.400	0.420	0.2471	0.2353
MEAN	133.50	140.00	141.00	145.00	151.50	150.50	153.50	156.50	157.50	0.360	0.407	0.2601	0.22831
SD	8.51	8.82	5.16	4.71	17.00	9.26	10.01	10.81	12.30	0.04	0.05	0.0415	0.0097
Cr+E1	120	130	130	135	135	140	140	140	140	0.330	0.384	0.2743	0.2357
Cr+E2	120	130	140	140	135	145	150	150	145	0.321	0.361	0.2490	0.2214
Cr+E3	125	140	140	145	140	150	150	155	150	0.345	0.465	0.3100	0.2300
Cr+E4	150	160	160	170	170	170	170	170	170	0.426	0.459	0.2700	0.2506
Cr+E5	150	160	165	150	165	155	155	155	160	0.389	0.468	0.2925	0.2431
Cr+E6	150	160	165	150	165	155	155	155	160	0.395	0.459	0.2869	0.2469
Cr+E7	120	130	130	135	135	140	140	140	140	0.330	0.484	0.3457	0.2357
Cr+E8	120	130	140	140	135	145	150	150	145	0.321	0.461	0.3179	0.2214
Cr+E9	125	140	140	145	140	150	150	155	150	0.345	0.365	0.2433	0.2300
Cr+E10	150	160	160	170	170	170	170	170	170	0.426	0.359	0.2112	0.2506
MEAN	133.00	144.00	147.00	148.00	149.00	152.00	153.00	154.00	153.00	0.363	0.427	0.2801	0.23654
SD	14.76	14.30	13.98	12.74	16.12	10.85	10.33	10.22	11.35	0.04	0.05	0.0395	0.0110

Appendix.II Effects of Cr supplementation and E_2 replacement on BW, % of HW/BW ratio and % of UW/BW ratio in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	BW1	BW2	BW3	BW4	BW5	BW6	BW7	BW8	BW9	UW	HW	%HW/BW	%UW/BW
	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
C1	130	135	140	145	150	160	165	165	170	0.098	0.423	0.2488	0.05765
C2	130	140	150	155	160	170	170	170	150	0.097	0.421	0.2807	0.06467
C3	130	140	150	160	160	160	160	160	160	0.087	0.501	0.3131	0.05438
C4	135	150	160	160	170	175	175	175	160	0.109	0.420	0.2625	0.06813
C5	130	140	150	160	160	160	160	160	160	0.112	0.501	0.3131	0.07000
C6	135	150	160	160	170	175	175	175	170	0.119	0.520	0.3059	0.07000
C7	130	135	140	145	150	160	170	170	160	0.130	0.423	0.2644	0.08125
C8	130	140	150	155	160	170	170	170	170	0.125	0.521	0.3065	0.07353
C9	130	140	150	160	160	160	160	160	160	0.115	0.501	0.3131	0.07188
C10	135	150	160	160	170	175	175	175	170	0.119	0.520	0.3059	0.07000
MEAN	131.50	142.00	151.00	156.00	161.00	166.50	168.00	168.00	163.00	0.111	0.475	0.2914	0.06815
SD	2.42	5.87	7.38	6.15	7.38	7.09	6.32	6.32	6.75	0.01	0.05	0.0248	0.00772
Cr-1	120	130	140	145	150	160	160	165	170	0.098	0.491	0.2888	0.05765
Cr-2	140	155	165	155	170	180	180	185	190	0.089	0.395	0.2079	0.04684
Cr-3	140	145	150	150	160	160	160	160	160	0.081	0.452	0.2825	0.05063
Cr-4	140	145	150	150	160	160	160	160	160	0.123	0.461	0.2881	0.07688
Cr-5	140	145	150	150	160	160	160	160	160	0.120	0.455	0.2844	0.07500
Cr-6	140	145	150	150	160	160	160	160	160	0.110	0.440	0.2750	0.06875
Cr-7	140	145	150	150	160	160	160	160	160	0.115	0.452	0.2825	0.07188
Cr-8	140	145	150	150	160	160	160	160	160	0.123	0.461	0.2881	0.07688
Cr-9	140	145	150	150	160	160	160	160	160	0.120	0.455	0.2844	0.07500
Cr-10	140	145	150	150	160	160	160	160	160	0.110	0.440	0.2750	0.06875
MEAN	138.00	144.50	150.50	150.00	160.00	162.00	162.00	163.00	164.00	0.109	0.450	0.27567	0.06682
SD	6.32	5.99	5.99	2.36	4.71	6.32	6.32	7.89	9.66	0.01	0.02	0.0243	0.01113
Cr+1	140	160	150	160	160	170	170	180	180	0.189	0.415	0.2306	0.10500
Cr+2	145	150	170	170	180	190	190	200	200	0.147	0.610	0.3050	0.07350
Cr+3	140	150	145	145	150	150	150	160	170	0.156	0.513	0.3018	0.09176
Cr+4	130	140	140	145	150	150	150	170	160	0.185	0.513	0.3206	0.11563
Cr+5	140	150	145	145	150	150	150	160	170	0.176	0.521	0.3065	0.10353
Cr+6	130	140	140	145	150	150	150	170	160	0.146	0.516	0.3225	0.09125
Cr+7	140	160	150	160	160	170	170	180	180	0.189	0.519	0.2883	0.10500
Cr+8	145	150	170	170	180	190	190	200	200	0.147	0.516	0.2580	0.07350
Cr+9	140	150	145	145	150	150	150	160	170	0.156	0.523	0.3076	0.09176
Cr+10	130	140	140	145	150	150	150	170	160	0.185	0.513	0.3206	0.11563
MEAN	138.00	149.00	149.50	153.00	158.00	162.00	162.00	175.00	175.00	0.168	0.516	0.29615	0.0967
SD	5.87	7.38	11.41	10.85	12.29	16.87	16.87	15.09	15.09	0.02	0.05	0.0299	0.0151

Appendix.II (continued) Effects of Cr supplementation and E_2 replacement on BW, % of HW/BW ratio and % of UW/BW ratio in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	BW1	BW2	BW3	BW4	BW5	BW6	BW7	BW8	BW9	UW	HW	%HW/BW	%UW/BW
	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
E1	140	150	155	160	160	170	170	170	180	0.500	0.511	0.2839	0.27778
E2	130	140	145	150	150	160	160	160	170	0.452	0.502	0.2953	0.26588
E3	135	150	155	160	170	180	180	180	190	0.475	0.516	0.2716	0.25000
E4	130	135	145	150	150	150	150	150	160	0.412	0.508	0.3175	0.25750
E5	135	140	155	160	170	180	180	180	190	0.475	0.521	0.2743	0.25000
E6	130	135	145	150	150	150	150	150	160	0.412	0.518	0.3238	0.25750
E7	135	150	155	160	170	180	180	180	190	0.475	0.516	0.2716	0.25000
E8	130	135	145	150	150	150	150	150	160	0.412	0.528	0.3300	0.25750
E9	135	140	155	160	170	180	180	180	190	0.475	0.502	0.2642	0.25000
E10	130	135	145	150	150	150	150	150	160	0.412	0.508	0.3175	0.25750
MEAN	133.00	141.00	150.00	155.00	159.00	165.00	165.00	165.00	175.00	0.450	0.513	0.2950	0.25737
SD	3.50	6.58	5.27	5.27	9.94	14.34	14.34	14.34	14.34	0.03	0.01	0.0251	0.0089
Cr+E_2-1	145	150	160	155	160	160	160	170	180	0.400	0.601	0.3339	0.22222
Cr+E_2-2	140	155	160	165	170	160	160	170	170	0.408	0.501	0.2947	0.24000
Cr+E_2-3	130	135	140	140	140	150	150	150	160	0.419	0.400	0.2500	0.26188
Cr+E_2-4	130	130	130	130	140	140	145	150	160	0.406	0.513	0.3206	0.25375
Cr+E_2-5	130	135	140	140	140	150	150	150	160	0.407	0.503	0.3144	0.25438
Cr+E_2-6	130	130	130	130	140	140	145	145	150	0.408	0.492	0.3280	0.27200
Cr+E_2-7	130	135	140	140	140	150	150	150	155	0.403	0.500	0.3226	0.26000
Cr+E_2-8	130	130	130	130	140	140	145	150	160	0.402	0.503	0.3144	0.25125
Cr+E_2-9	130	135	140	140	140	150	150	150	160	0.409	0.453	0.2831	0.25563
Cr+E_2-10	130	130	130	130	140	140	145	150	160	0.409	0.494	0.3088	0.25563
MEAN	132.50	136.50	140.00	140.00	145.00	148.00	150.00	153.50	161.50	0.407	0.496	0.3070	0.25267
SD	5.40	8.83	11.55	11.79	10.80	7.89	5.77	8.83	8.18	0.01	0.05	0.0251	0.01343

Appendix. III **Effects of Cr supplementation and E_2 replacement on oxygen consumption and RMR at week 0 in non exercise ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.**

No.	BW	Oxygen	Pr.atm	Temp	Temp2	Temp2	time	Oxygen (used)	Oxygen(STP) (ml/min)	Oxygen(STP) (l/h)	RQ (cal/h)	skin surface (m2)	RMR (cal/m2/h)
	(g)	(ml)	(mmHg)	°C	°C	°K	(min)	(ml/min)					
C1	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
C2	115	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0236	16.3013
C3	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
C4	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
C5	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
C6	140	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0270	14.2978
C7	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
C8	115	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0236	16.3013
C9	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
C10	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
MEAN	121.00	20.00	764.00	1.00	26.00	299.00	24.00	1.4583	1.3385	0.0803	0.3855	0.0245	15.7817
SD	6.99	-	-	-	-	-	-	-	-	-	0.00	0.00	0.55
Cr-1	130	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0257	15.0219
Cr-2	130	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0257	15.0219
Cr-3	110	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0230	16.7916
Cr-4	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
Cr-5	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
Cr-6	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
Cr-7	130	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0257	15.0219
Cr-8	130	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0257	15.0219
Cr-9	110	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0230	16.7916
Cr-10	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
MEAN	122.00	20.00	764.00	1.00	26.00	299.00	24.00	1.4583	1.3385	0.0803	0.3855	0.0246	15.7052
SD	7.89	-	-	-	-	-	-	-	-	-	0.00	0.00	0.69
Cr+1	125	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0250	16.0903
Cr+2	120	20	764	1	26	299	25	1.4000	1.2850	0.0771	0.3701	0.0243	15.2115
Cr+3	120	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0243	16.5342
Cr+4	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
Cr+5	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
Cr+6	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
Cr+7	120	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0243	16.5342
Cr+8	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
Cr+9	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
Cr+10	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
MEAN	128.50	20.00	764.00	1.00	26.00	299.00	23.40	1.4969	1.3739	0.0824	0.3957	0.0254	15.5738
SD	10.01	-	-	-	-	-	0.70	0.04	0.04	0.00	0.01	0.00	0.68

Appendix. III (continued) Effects of Cr supplementation and E_2 replacement on oxygen consumption and RMR at week 0 in non exercise ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	BW	Oxygen	Pr.atm	Temp	Temp2	Temp2	time	Oxygen (used)	Oxygen(STP) (ml/min)	Oxygen(STP) (l/h)	RQ (cal/h)	skin surface (m2)	RMR (cal/m2/h)
	(g)	(ml)	(mmHg)	°C	°C	°K	(min)	(ml/min)					
E1	130	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0257	15.0219
E2	115	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0236	16.3013
E3	130	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0257	15.0219
E4	125	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0250	15.4199
E5	130	20	764	1	26	299	21	1.6667	1.5297	0.0918	0.4406	0.0257	17.1679
E6	140	20	764	1	26	299	21	1.6667	1.5297	0.0918	0.4406	0.0270	16.3403
E7	130	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0257	15.0219
E8	115	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0236	16.3013
E9	130	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0257	15.0219
E10	125	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0250	15.4199
MEAN	127.00	20.00	764.00	1.00	26.00	299.00	23.40	1.5000	1.3768	0.0826	0.3965	0.0253	15.7038
SD	7.53	-	-	-	-	-	1.26	0.09	0.08	0.00	0.02	0.00	0.77
Cr+E_2-1	115	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0236	17.0100
Cr+E_2-2	115	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0236	16.3013
Cr+E_2-3	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
Cr+E_2-4	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
Cr+E_2-5	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
Cr+E_2-6	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
Cr+E_2-7	120	20	764	1	26	299	24	1.4583	1.3385	0.0803	0.3855	0.0243	15.8453
Cr+E_2-8	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
Cr+E_2-9	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
Cr+E_2-10	140	20	764	1	26	299	23	1.5217	1.3967	0.0838	0.4023	0.0270	14.9194
MEAN	131.00	20.00	764.00	1.00	26.00	299.00	23.30	1.5027	1.3793	0.0828	0.3972	0.0258	15.4518
SD	11.74	-	-	-	-	-	0.48	0.03	0.03	0.00	0.01	0.00	0.76

Appendix. IV **Effects of Cr supplementation and E_2 replacement on oxygen consumption and RMR at week 0 in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.**

No.	BW	Oxygen	Pr.atm	Temp	Temp2	Temp2	time	Oxygen (used)	Oxygen(STP) (ml/min)	Oxygen(STP) (l/h)	RQ (cal/h)	skin surface (m^2)	RMR (cal/m^2/h)
	(g)	(ml)	(mmHg)	C	°C	°K	(min)	(ml/min)					
C1	125	20	764	1	26	299	23	1.522	1.397	0.084	0.402	0.025	16.090
C2	135	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.026	15.935
C3	125	20	764	1.2	26.2	299.2	23	1.652	1.515	0.091	0.436	0.025	17.458
C4	130	20	764	1.2	26.2	299.2	23	1.652	1.515	0.091	0.436	0.026	17.007
C5	120	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.024	17.237
C6	130	20	764	1.3	26.3	299.3	23	1.717	1.575	0.094	0.454	0.026	17.673
C7	125	20	764	1	26	299	23	1.522	1.397	0.084	0.402	0.025	16.090
C8	135	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.026	15.935
C9	125	20	764	1.2	26.2	299.2	23	1.652	1.515	0.091	0.436	0.025	17.458
C10	130	20	764	1.2	26.2	299.2	23	1.652	1.515	0.091	0.436	0.026	17.007
MEAN	128.00	20.00	764.00	1.14	26.14	299.14	23.00	1.61	1.48	0.09	0.43	0.03	16.79
SD	4.83	-	-	0.10	0.10	0.10	-	0.06	0.06	0.00	0.02	0.00	0.70
Cr-1	115	20	764	1.1	26.3	299.3	25	1.460	1.339	0.080	0.386	0.024	16.304
Cr-2	130	20	764	1.2	26.3	299.3	23	1.652	1.515	0.091	0.436	0.026	17.002
Cr-3	130	20	764	1.1	26.2	299.2	23	1.587	1.456	0.087	0.419	0.026	16.336
Cr-4	130	20	764	1.2	26.1	299.1	23	1.652	1.516	0.091	0.437	0.026	17.013
Cr-5	130	20	764	1.3	26.3	299.3	23	1.717	1.575	0.094	0.454	0.026	17.673
Cr-6	130	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.026	16.341
Cr-7	130	20	764	1.1	26.2	299.2	23	1.587	1.456	0.087	0.419	0.026	16.336
Cr-8	130	20	764	1.2	26.1	299.1	23	1.652	1.516	0.091	0.437	0.026	17.013
Cr-9	130	20	764	1.3	26.3	299.3	23	1.717	1.575	0.094	0.454	0.026	17.673
Cr-10	130	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.026	16.341
MEAN	128.50	20.00	764.00	1.17	26.20	299.20	23.20	1.62	1.49	0.09	0.43	0.03	16.80
SD	4.74	-	-	0.08	0.09	0.09	0.63	0.08	0.07	0.00	0.02	0.00	0.55
Cr+1	135	20	764	1	26.1	299	22	1.591	1.460	0.088	0.421	0.026	15.980
Cr+2	135	20	764	1	26.1	299	24	1.458	1.339	0.080	0.385	0.026	14.649
Cr+3	120	20	764	1.1	26.2	299	24	1.521	1.396	0.084	0.402	0.024	16.524
Cr+4	120	20	764	1.2	26.1	299	24	1.583	1.453	0.087	0.419	0.024	17.203
Cr+5	130	20	764	1.1	26.2	299	24	1.521	1.396	0.084	0.402	0.026	15.666
Cr+6	120	20	764	1.2	26.1	299	24	1.583	1.453	0.087	0.419	0.024	17.203
Cr+7	120	20	764	1.1	26.2	299	24	1.521	1.396	0.084	0.402	0.024	16.524
Cr+8	120	20	764	1.2	26.1	299	24	1.583	1.453	0.087	0.419	0.024	17.203
Cr+9	130	20	764	1.1	26.2	299	24	1.521	1.396	0.084	0.402	0.026	15.666
Cr+10	120	20	764	1.2	26.1	299	24	1.583	1.453	0.087	0.419	0.024	17.203
MEAN	125.00	20.00	764.00	1.12	26.14	299.00	23.80	1.55	1.42	0.09	0.41	0.02	16.38
SD	6.67	-	-	0.08	0.05	-	0.63	0.04	0.04	0.00	0.01	0.00	0.88

Appendix.IV (continued) Effects of Cr supplementation and E_2 replacement on oxygen consumption and RMR at week 0 in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	BW	Oxygen	Pr.atm	Temp	Temp2	Temp2	time	Oxygen	Oxygen(STP)	Oxygen(STP)	RQ	skin surface	RMR
								(used)	(ml/min)	(l/h)	(cal/h)	(m^2)	(cal/m^2/h)
	(g)	(ml)	(mmHg)	°C	°C	°K	(min)	(ml/min)					
E1	135	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.026	15.935
E2	125	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.025	16.774
E3	130	20	764	1.2	26.2	299.2	24	1.583	1.452	0.087	0.418	0.026	16.299
E4	120	20	764	1.2	26.2	299.2	23	1.652	1.515	0.091	0.436	0.024	17.939
E5	125	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.025	16.774
E6	120	20	764	1.2	26.3	299.3	23	1.652	1.515	0.091	0.436	0.024	17.933
E7	135	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.026	15.935
E8	125	20	764	1.1	26.1	299.1	23	1.587	1.456	0.087	0.419	0.025	16.774
E9	130	20	764	1.2	26.2	299.2	24	1.583	1.452	0.087	0.418	0.026	16.299
E10	120	20	764	1.2	26.2	299.2	23	1.652	1.515	0.091	0.436	0.024	17.939
MEAN	126.50	20.00	764.00	1.15	26.16	299.16	23.20	1.61	1.47	0.09	0.42	0.03	16.86
SD	5.80	-	-	0.05	0.07	0.07	0.42	0.03	0.03	0.00	0.01	0.00	0.81
Cr+E_2-1	140	20	764	1.1	26.2	299.2	24	1.521	1.395	0.084	0.402	0.027	14.901
Cr+E_2-2	135	20	764	1.2	26.1	299.1	24	1.583	1.453	0.087	0.418	0.026	15.899
Cr+E_2-3	120	20	764	1.1	26.2	299.2	24	1.521	1.395	0.084	0.402	0.024	16.513
Cr+E_2-4	120	20	764	1.2	26.1	299.1	24	1.583	1.453	0.087	0.418	0.024	17.198
Cr+E_2-5	120	20	764	1.3	26.2	299.2	23	1.717	1.575	0.095	0.454	0.024	18.648
Cr+E_2-6	120	20	764	1.1	26.3	299.3	23	1.587	1.455	0.087	0.419	0.024	17.226
Cr+E_2-7	120	20	764	1.1	26.2	299.2	24	1.521	1.395	0.084	0.402	0.024	16.513
Cr+E_2-8	120	20	764	1.2	26.1	299.1	24	1.583	1.453	0.087	0.418	0.024	17.198
Cr+E_2-9	120	20	764	1.3	26.2	299.2	23	1.717	1.575	0.095	0.454	0.024	18.648
Cr+E_2-10	120	20	764	1.1	26.3	299.3	23	1.587	1.455	0.087	0.419	0.024	17.226
MEAN	123.50	20.00	764.00	1.17	26.19	299.19	23.60	1.59	1.46	0.09	0.42	0.02	17.00
SD	7.47	-	-	0.08	0.07	0.07	0.52	0.07	0.07	0.00	0.02	0.00	1.14

Appendix. V Effects of Cr supplementation and E_2 replacement on oxygen consumption and RMR at week 9 in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	BW9	Oxygen	Pr.atm	Temp ▲	Temp2	Temp2	time	Oxygen (used)	Oxygen(STP) (ml/min)	Oxygen(STP) (l/h)	RQ (cal/h)	skin surface (m^2)	RMR ($cal/m^2/h$)
	(g)	(ml)	(mmHg)	°C	°C	°K	(min)	(ml/min)					
C1	170	20	764	1.3	26.3	299.3	20	1.975	1.811	0.109	0.522	0.031	16.995
C2	140	20	764	1.3	26.3	299.3	20	1.975	1.811	0.109	0.522	0.027	19.344
C3	160	20	764	1.3	26.3	299.3	20	1.975	1.811	0.109	0.522	0.029	17.696
C4	150	20	764	1.3	26.3	299.3	15	2.633	2.415	0.145	0.695	0.028	24.632
C5	155	20	764	1.3	26.3	299.3	24	1.646	1.509	0.091	0.435	0.029	15.062
C6	160	20	764	1.3	26.3	299.3	23	1.717	1.575	0.094	0.454	0.029	15.388
C7	140	20	764	1.3	26.3	299.3	20	1.975	1.811	0.109	0.522	0.027	19.344
C8	150	20	764	1.3	26.3	299.3	24	1.646	1.509	0.091	0.435	0.028	15.395
C9	150	20	764	1.3	26.3	299.3	21	1.881	1.725	0.103	0.497	0.028	17.595
C10	160	20	764	1.3	26.3	299.3	20	1.975	1.811	0.109	0.522	0.029	17.696
MEAN	153.50	20.00	764.00	1.30	26.30	299.30	20.70	1.94	1.78	0.11	0.51	0.03	17.91
SD	9.44	-	-	-	-	-	2.63	0.28	0.26	0.02	0.07	0.00	2.81
Cr-1	140	20	764	1	26	299	18	1.944	1.785	0.107	0.514	0.027	19.064
Cr-2	150	20	764	1	26	299	15	2.333	2.142	0.128	0.617	0.028	21.848
Cr-3	170	20	764	1	26	299	16	2.188	2.008	0.120	0.578	0.031	18.843
Cr-4	145	20	764	1	26	299	14	2.500	2.295	0.138	0.661	0.028	23.944
Cr-5	150	20	764	1	26	299	20	1.750	1.606	0.096	0.463	0.028	16.386
Cr-6	160	20	764	1	26	299	16	2.188	2.008	0.120	0.578	0.029	19.620
Cr-7	155	20	764	1	26	299	23	1.522	1.397	0.084	0.402	0.029	13.941
Cr-8	150	20	764	1	26	299	24	1.458	1.339	0.080	0.385	0.028	13.655
Cr-9	140	20	764	1	26	299	20	1.750	1.606	0.096	0.463	0.027	17.157
Cr-10	150	20	764	1	26	299	20	1.750	1.606	0.096	0.463	0.028	16.386
MEAN	151.00	20.00	764.00	1.00	26.00	299.00	18.60	1.94	1.78	0.11	0.51	0.03	18.08
SD	9.07	-	-	-	-	-	3.37	0.35	0.32	0.02	0.09	0.00	3.26
Cr+1	140	20	764	1.2	26.2	299.2	20	1.900	1.743	0.105	0.502	0.027	18.616
Cr+2	170	20	764	1.2	26.2	299.2	25	1.520	1.394	0.084	0.402	0.031	13.084
Cr+3	170	20	764	1.2	26.2	299.2	18	2.111	1.936	0.116	0.558	0.031	18.173
Cr+4	150	20	764	1.2	26.2	299.2	15	2.533	2.324	0.139	0.669	0.028	23.705
Cr+5	180	20	764	1.2	26.2	299.2	23	1.652	1.515	0.091	0.436	0.032	13.690
Cr+6	185	20	764	1.2	26.2	299.2	18	2.111	1.936	0.116	0.558	0.032	17.177
Cr+7	170	20	764	1.2	26.2	299.2	16	2.375	2.178	0.131	0.627	0.031	20.444
Cr+8	150	20	764	1.2	26.2	299.2	15	2.533	2.324	0.139	0.669	0.028	23.705
Cr+9	180	20	764	1.2	26.2	299.2	15	2.533	2.324	0.139	0.669	0.032	20.992
Cr+10	185	20	764	1.2	26.2	299.2	20	1.900	1.743	0.105	0.502	0.032	15.459
MEAN	168.00	20.00	764.00	1.20	26.20	299.20	18.50	2.12	1.94	0.12	0.56	0.03	18.50
SD	16.02	-	-	0.00	0.00	0.00	3.50	0.37	0.34	0.02	0.10	0.00	3.77

Appendix. V (continued) Effects of Cr supplementation and E_2 replacement on oxygen consumption and RMR at week 9 in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	BW9	Oxygen	Pr.atm	Temp	Temp2	Temp2	time	Oxygen	Oxygen(STP)	Oxygen(STP)	RQ	skin surface	RMR
								(used)	(ml/min)	(l/h)	(cal/h)	(m^2)	(cal/m^2/h)
	(g)	(ml)	(mmHg)	°C	°C	°K	(min)	(ml/min)					
E1	140	20	764	1.2	26.2	299.2	18	2.111	1.936	0.116	0.558	0.027	20.684
E2	150	20	764	1.2	26.2	299.2	25	1.520	1.394	0.084	0.402	0.028	14.223
E3	170	20	764	1.2	26.2	299.2	24	1.583	1.452	0.087	0.418	0.031	13.629
E4	170	20	764	1.2	26.2	299.2	16	2.375	2.178	0.131	0.627	0.031	20.444
E5	160	20	764	1.2	26.2	299.2	18	2.111	1.936	0.116	0.558	0.029	18.922
E6	155	20	764	1.2	26.2	299.2	24	1.583	1.452	0.087	0.418	0.029	14.495
E7	140	20	764	1.2	26.2	299.2	26	1.462	1.341	0.080	0.386	0.027	14.320
E8	150	20	764	1.2	26.2	299.2	17	2.235	2.050	0.123	0.590	0.028	20.916
E9	170	20	764	1.2	26.2	299.2	16	2.375	2.178	0.131	0.627	0.031	20.444
E10	170	20	764	1.2	26.2	299.2	20	1.900	1.743	0.105	0.502	0.031	16.355
MEAN	157.50	20.00	764.00	1.20	26.20	299.20	20.40	1.93	1.77	0.11	0.51	0.03	17.44
SD	12.30	-	-	0.00	0.00	0.00	3.95	0.36	0.33	0.02	0.10	0.00	3.11
Cr+E_2-1	140	20	764	1.5	26.5	299.5	20	2.125	1.947	0.117	0.561	0.027	20.799
Cr+E_2-2	145	20	764	1.5	26.5	299.5	24	1.771	1.623	0.097	0.467	0.028	16.932
Cr+E_2-3	150	20	764	1.5	26.5	299.5	25	1.700	1.558	0.093	0.449	0.028	15.891
Cr+E_2-4	170	20	764	1.5	26.5	299.5	18	2.361	2.164	0.130	0.623	0.031	20.304
Cr+E_2-5	160	20	764	1.5	26.5	299.5	16	2.656	2.434	0.146	0.701	0.029	23.784
Cr+E_2-6	160	20	764	1.5	26.5	299.5	20	2.125	1.947	0.117	0.561	0.029	19.028
Cr+E_2-7	140	20	764	1.5	26.5	299.5	24	1.771	1.623	0.097	0.467	0.027	17.333
Cr+E_2-8	145	20	764	1.5	26.5	299.5	23	1.848	1.693	0.102	0.488	0.028	17.668
Cr+E_2-9	150	20	764	1.5	26.5	299.5	24	1.771	1.623	0.097	0.467	0.028	16.553
Cr+E_2-10	170	20	764	1.5	26.5	299.5	20	2.125	1.947	0.117	0.561	0.031	18.274
MEAN	153.00	20.00	764.00	1.50	26.50	299.50	21.40	2.03	1.86	0.11	0.53	0.03	18.66
SD	11.35	-	-	-	-	-	3.03	0.31	0.29	0.02	0.08	0.00	2.40

Appendix. VI **Effects of Cr supplementation and E_2 replacement on oxygen consumption, EMR and oxygen economy at week 9 in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.**

No.	BW9	Oxygen	Pr.atm	Temp	Temp2	Temp2	time	Oxygen	Oxygen(STP)	Oxygen(STP)	RQ	skin surface	EMR	O_2/kg	no.of	distance	Economy
								(used)	(ml/min)	(l/h)	(cal/h)	(m^2)	(cal/m^2/h)	(ml/kg)	round	(m)	(ml O_2/kg/m)
	(g)	(ml)	(mmHg)	°C	°C	°K	(min)	(ml/min)									
C1	170	20	764	1.3	26.3	299.3	13	3.038	2.786	0.167	0.802	0.031	26.147	16.389	29	15.480	1.059
C2	150	20	764	1.3	26.3	299.3	13	3.038	2.786	0.167	0.802	0.028	28.422	18.574	33	17.615	1.054
C3	160	20	764	1.3	26.3	299.3	12	3.292	3.018	0.181	0.869	0.029	29.494	18.864	25	13.345	1.414
C4	160	20	764	1.3	26.3	299.3	12	3.292	3.018	0.181	0.869	0.029	29.494	18.864	33	17.615	1.071
C5	160	20	764	1.3	26.3	299.3	13	3.038	2.786	0.167	0.802	0.029	27.225	17.413	25	13.345	1.305
C6	170	20	764	1.3	26.3	299.3	14	2.821	2.587	0.155	0.745	0.031	24.279	15.218	32	17.082	0.891
C7	160	20	764	1.3	26.3	299.3	11	3.591	3.293	0.198	0.948	0.029	32.175	20.579	35	18.683	1.101
C8	170	20	764	1.3	26.3	299.3	13	3.038	2.786	0.167	0.802	0.031	26.147	16.389	26	13.879	1.181
C9	160	20	764	1.3	26.3	299.3	14	2.821	2.587	0.155	0.745	0.029	25.280	16.169	33	17.615	0.918
C10	170	20	764	1.3	26.3	299.3	14	2.821	2.587	0.155	0.745	0.031	24.279	15.218	34	18.149	0.838
MEAN	163.00	20.00	764.00	1.30	26.30	299.30	12.90	3.08	2.82	0.17	0.81	0.03	27.29	17.37	30.50	16.28	1.08
SD	6.75	-	-	-	-	-	0.99	0.25	0.23	0.01	0.07	0.00	2.57	1.79	3.89	2.08	0.18
Cr-1	170	20	764	1	26	299	14	2.500	2.295	0.138	0.661	0.031	21.535	13.498	24	12.811	1.054
Cr-2	190	20	764	1	26	299	14	2.500	2.295	0.138	0.661	0.033	19.996	12.077	34	18.149	0.665
Cr-3	160	20	764	1	26	299	13	2.692	2.471	0.148	0.712	0.029	24.148	15.446	25	13.345	1.157
Cr-4	160	20	764	1	26	299	14	2.500	2.295	0.138	0.661	0.029	22.423	14.341	33	17.615	0.814
Cr-5	160	20	764	1	26	299	12	2.917	2.677	0.161	0.771	0.029	26.160	16.732	21	11.210	1.493
Cr-6	160	20	764	1	26	299	14	2.500	2.295	0.138	0.661	0.029	22.423	14.341	24	12.811	1.119
Cr-7	160	20	764	1	26	299	11	3.182	2.920	0.175	0.841	0.029	28.538	18.253	31	16.548	1.103
Cr-8	160	20	764	1	26	299	11	3.182	2.920	0.175	0.841	0.029	28.538	18.253	26	13.879	1.315
Cr-9	160	20	764	1	26	299	11	3.182	2.920	0.175	0.841	0.029	28.538	18.253	35	18.683	0.977
Cr-10	160	20	764	1	26	299	12	2.917	2.677	0.161	0.771	0.029	26.160	16.732	26	13.879	1.206
MEAN	164.00	20.00	764.00	1.00	26.00	299.00	12.60	2.81	2.58	0.15	0.74	0.03	24.85	15.79	27.90	14.89	1.09
SD	9.66	-	-	-	-	-	1.35	0.30	0.28	0.02	0.08	0.00	3.18	2.19	4.91	2.62	0.24
Cr+1	180	20	764	1.2	26.2	299.2	11	3.455	3.169	0.190	0.913	0.032	28.625	17.604	35	18.683	0.942
Cr+2	200	20	764	1.2	26.2	299.2	11	3.455	3.169	0.190	0.913	0.034	26.684	15.843	26	13.879	1.142
Cr+3	170	20	764	1.2	26.2	299.2	12	3.167	2.905	0.174	0.837	0.031	27.259	17.086	34	18.149	0.941
Cr+4	160	20	764	1.2	26.2	299.2	12	3.167	2.905	0.174	0.837	0.029	28.383	18.154	25	13.345	1.360
Cr+5	170	20	764	1.2	26.2	299.2	12	3.167	2.905	0.174	0.837	0.031	27.259	17.086	32	17.082	1.000
Cr+6	160	20	764	1.2	26.2	299.2	10	3.800	3.485	0.209	1.004	0.029	34.060	21.784	31	16.548	1.316
Cr+7	180	20	764	1.2	26.2	299.2	12	3.167	2.905	0.174	0.837	0.032	26.240	16.137	30	16.014	1.008
Cr+8	200	20	764	1.2	26.2	299.2	12	3.167	2.905	0.174	0.837	0.034	24.460	14.523	31	16.548	0.878
Cr+9	170	20	764	1.2	26.2	299.2	11	3.455	3.169	0.190	0.913	0.031	29.737	18.639	32	17.082	1.091
Cr+10	160	20	764	1.2	26.2	299.2	13	2.923	2.681	0.161	0.772	0.029	26.200	16.757	31	16.548	1.013
MEAN	175.00	20.00	764.00	1.20	26.20	299.20	11.60	3.29	3.02	0.18	0.87	0.03	27.89	17.36	30.70	16.39	1.07
SD	15.09	-	-	0.00	0.00	0.00	0.84	0.25	0.23	0.01	0.07	0.00	2.62	1.95	3.13	1.67	0.16

Appendix. VI (continued) Effects of Cr supplementation and E_2 replacement on oxygen consumption, EMR and oxygen economy at week 9 in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	BW9	Oxygen	Pr.atm	Temp	Temp2	Temp2	time	Oxygen	Oxygen(STP)	Oxygen(STP)	RQ	skin surface	EMR	O_2/kg	no.of	distance	Economy
								(used)	(ml/min)	(l/h)	(cal/h)	(m^2)	(cal/m^2/h)	(ml/kg)	round	(m)	(ml O_2/kg/m)
	(g)	(ml)	(mmHg)	°C	°C	°K	(min)	(ml/min)									
E1	180	20	764	1.2	26.2	299.2	12	3.167	2.905	0.174	0.837	0.032	26.240	16.137	30	16.014	1.008
E2	170	20	764	1.2	26.2	299.2	13	2.923	2.681	0.161	0.772	0.031	25.162	15.771	31	16.548	0.953
E3	190	20	764	1.2	26.2	299.2	12	3.167	2.905	0.174	0.837	0.033	25.311	15.287	30	16.014	0.955
E4	160	20	764	1.2	26.2	299.2	13	2.923	2.681	0.161	0.772	0.029	26.200	16.757	31	16.548	1.013
E5	190	20	764	1.2	26.2	299.2	13	2.923	2.681	0.161	0.772	0.033	23.364	14.111	30	16.014	0.881
E6	160	20	764	1.2	26.2	299.2	10	3.800	3.485	0.209	1.004	0.029	34.060	21.784	31	16.548	1.316
E7	190	20	764	1.2	26.2	299.2	10	3.800	3.485	0.209	1.004	0.033	30.373	18.345	32	17.082	1.074
E8	160	20	764	1.2	26.2	299.2	12	3.167	2.905	0.174	0.837	0.029	28.383	18.154	31	16.548	1.097
E9	190	20	764	1.2	26.2	299.2	10	3.800	3.485	0.209	1.004	0.033	30.373	18.345	31	16.548	1.109
E10	160	20	764	1.2	26.2	299.2	10	3.800	3.485	0.209	1.004	0.029	34.060	21.784	31	16.548	1.316
MEAN	175.00	20.00	764.00	1.20	26.20	299.20	11.50	3.35	3.07	0.18	0.88	0.03	28.35	17.65	30.80	16.44	1.07
SD	14.34	-	-	0.00	0.00	0.00	1.35	0.40	0.37	0.02	0.11	0.00	3.75	2.58	0.63	0.34	0.15
Cr+E_2-1	180	20	764	1.5	26.5	299.5	14	3.036	2.782	0.167	0.801	0.032	25.130	15.464	35	18.683	0.827
Cr+E_2-2	170	20	764	1.5	26.5	299.5	15	2.833	2.596	0.156	0.748	0.031	24.365	15.272	33	17.615	0.867
Cr+E_2-3	160	20	764	1.5	26.5	299.5	12	3.542	3.245	0.195	0.935	0.029	31.713	20.283	32	17.082	1.187
Cr+E_2-4	160	20	764	1.5	26.5	299.5	13	3.269	2.996	0.180	0.863	0.029	29.273	18.723	35	18.683	1.002
Cr+E_2-5	160	20	764	1.5	26.5	299.5	12	3.542	3.245	0.195	0.935	0.029	31.713	20.283	35	18.683	1.086
Cr+E_2-6	150	20	764	1.5	26.5	299.5	11	3.864	3.540	0.212	1.020	0.028	36.117	23.602	35	18.683	1.263
Cr+E_2-7	155	20	764	1.5	26.5	299.5	12	3.542	3.245	0.195	0.935	0.029	32.391	20.937	35	18.683	1.121
Cr+E_2-8	160	20	764	1.5	26.5	299.5	12	3.542	3.245	0.195	0.935	0.029	31.713	20.283	34	18.149	1.118
Cr+E_2-9	160	20	764	1.5	26.5	299.5	12	3.542	3.245	0.195	0.935	0.029	31.713	20.283	30	16.014	1.267
Cr+E_2-10	160	20	764	1.5	26.5	299.5	12	3.542	3.245	0.195	0.935	0.029	31.713	20.283	30	16.014	1.267
MEAN	161.50	20.00	764.00	1.50	26.50	299.50	12.50	3.43	3.14	0.19	0.90	0.03	30.58	19.54	33.40	17.83	1.10
SD	8.18	-	-	-	-	-	1.18	0.30	0.27	0.02	0.08	0.00	3.50	2.51	2.07	1.10	0.16

Appendix.VII Effects of Cr supplementation and E_2 replacement on cardiac functions and EC_{50} of QT-c interval, LVDP and ±dp/dt in non exercise ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	HR (beats/min)					QT interval (sec)					QTc (sec)						LVP (mmHg)						dp/dt_{max} and dp/dt_{min} (mmHg/min)											
	Hydrogen peroxide (mmol/l)					Hydrogen peroxide (mmol/l)											Hydrogen peroxide (mmol/l)						Hydrogen peroxide (mmol/l)											
	0	0.01	0.05	0.1	0.2	0	0.01	0.05	0.1	0.2	0	0.01	0.05	0.1	0.2	EC50	0	0.01	0.05	0.1	0.2	EC50	0		0.01		0.05		0.1		0.2		EC50
C1	292.00	240.00	234.00	240.00	108.00	0.14	0.15	0.16	0.18	0.19	0.229	0.242	0.242	0.270	0.282	0.072	58.64	49.99	31.38	6.35	0.00	0.049	2031.62	-1995.29	1687.87	-781.96	977.60	-701.97	105.02	-90.59	0.0	0.0	0.067
C2	294.00	312.00	306.00	312.00	156.00	0.14	0.14	0.16	0.17	0.18	0.215	0.227	0.242	0.254	0.266	0.062	68.10	41.91	36.32	8.45	0.00	0.058	1732.40	-1794.31	1487.37	-885.97	1028.83	-602.24	105.20	-79.68	0.0	0.0	0.047
C3	325.00	234.00	240.00	336.00	158.00	0.13	0.15	0.15	0.17	0.18	0.207	0.232	0.233	0.261	0.273	0.083	56.32	53.64	32.70	6.25	0.00	0.067	1956.59	-1720.84	1533.04	-920.63	1063.60	-685.46	150.68	-89.68	0.0	0.0	0.056
C4	274.00	204.00	210.00	210.00	152.00	0.13	0.15	0.15	0.17	0.18	0.207	0.229	0.234	0.261	0.269	0.064	56.43	45.64	32.70	4.36	0.00	0.051	1975.82	-1720.98	1483.77	-521.44	913.70	-865.27	104.65	-69.09	0.0	0.0	0.043
C5	299.00	240.00	234.00	240.00	146.00	0.14	0.14	0.16	0.17	0.18	0.216	0.227	0.242	0.254	0.266	0.066	58.56	61.91	31.32	7.16	0.00	0.067	1532.40	-1894.31	1387.37	-785.97	978.83	-602.24	147.68	-59.68	0.0	0.0	0.053
C6	395.00	313.00	307.00	311.00	156.00	0.13	0.14	0.17	0.17	0.18	0.207	0.217	0.264	0.261	0.270	0.014	51.32	58.64	35.70	5.23	0.00	0.051	1775.66	-1960.84	1443.04	-712.63	1363.60	-565.46	164.65	-79.09	0.0	0.0	0.053
MEAN	313.17	257.17	255.17	274.83	146.00	0.13	0.15	0.16	0.17	0.18	0.214	0.229	0.243	0.260	0.271	0.060	58.23	51.95	33.35	6.30	0.00	0.057	1834.08	-1847.76	1503.74	-768.10	1054.36	-670.44	129.65	-77.97	0.0	0.0	0.053
SD	43.33	44.89	41.08	51.11	19.10	0.01	0.01	0.01	0.00	0.00	0.009	0.008	0.011	0.006	0.006	0.024	5.52	7.63	2.15	1.43	0.00	0.008	189.15	119.80	102.62	142.54	159.84	109.09	27.65	11.95	0.0	0.0	0.008
Cr-1	250.00	210.00	204.00	210.00	45.00	0.13	0.16	0.17	0.18	0.19	0.200	0.252	0.260	0.273	0.284	0.018	59.38	45.80	18.87	0.00	0.00	0.021	2034.69	-1893.82	1241.48	-1210.28	605.02	-309.59	0.00	0.00	0.0	0.0	0.019
Cr-2	295.00	228.00	234.00	240.00	58.00	0.14	0.16	0.17	0.18	0.19	0.218	0.253	0.259	0.272	0.284	0.014	70.01	39.82	18.89	0.00	0.00	0.013	1867.40	-1855.75	1340.47	-1410.60	405.20	-349.68	0.00	0.00	0.0	0.0	0.017
Cr-3	247.00	228.00	234.00	240.00	55.00	0.13	0.16	0.17	0.18	0.19	0.212	0.254	0.262	0.274	0.286	0.018	50.88	39.78	18.85	0.00	0.00	0.025	1961.26	-1959.61	1542.88	-1210.54	654.68	-259.68	0.00	0.00	0.0	0.0	0.030
Cr-4	344.00	288.00	210.00	336.00	58.00	0.13	0.16	0.17	0.18	0.19	0.212	0.248	0.257	0.269	0.281	0.019	56.00	29.80	18.87	0.00	0.00	0.007	1833.16	-1725.33	1441.78	-1109.97	504.65	-359.09	0.00	0.00	0.0	0.0	0.022
Cr-5	250.00	210.00	204.00	210.00	53.00	0.12	0.16	0.17	0.18	0.19	0.196	0.254	0.262	0.274	0.286	0.020	50.88	39.78	18.85	0.00	0.00	0.021	1561.26	-1959.61	1542.88	-1210.54	587.68	-349.68	0.00	0.00	0.0	0.0	0.028
Cr-6	259.00	238.00	234.00	240.00	68.00	0.13	0.16	0.17	0.18	0.19	0.212	0.248	0.257	0.269	0.281	0.013	56.00	26.80	18.87	0.00	0.00	0.007	1733.16	-1725.33	1241.78	-1029.97	564.65	-379.09	0.00	0.00	0.0	0.0	0.020
MEAN	274.17	233.67	220.00	246.00	56.17	0.13	0.16	0.17	0.18	0.19	0.208	0.252	0.260	0.272	0.284	0.017	57.19	36.96	18.87	0.00	0.00	0.016	1831.82	-1853.24	1391.88	-1196.98	553.65	-334.47	0.00	0.00	0.0	0.0	0.023
SD	38.59	28.83	15.49	46.48	7.52	0.01	0.00	0.00	0.00	0.00	0.008	0.003	0.002	0.002	0.002	0.003	7.09	7.17	0.01	0.00	0.00	0.008	168.59	106.77	138.48	127.78	87.82	43.06	0.00	0.00	0.0	0.0	0.005
Cr+1	210.00	210.00	228.00	234.00	112.00	0.13	0.14	0.16	0.18	0.18	0.209	0.225	0.247	0.274	0.270	0.068	55.64	41.61	35.45	10.23	0.00	0.069	1596.57	-1931.86	1622.94	-1389.08	1079.75	-826.40	569.91	-384.88	0.0	0.0	0.085
Cr+2	318.00	192.00	186.00	210.00	96.00	0.13	0.15	0.16	0.17	0.18	0.209	0.228	0.245	0.258	0.275	0.059	70.42	49.64	45.47	15.53	0.00	0.054	2095.32	-1828.14	1752.16	-1387.20	1184.45	-826.83	469.91	-384.88	0.0	0.0	0.066
Cr+3	240.00	108.00	288.00	300.00	64.00	0.14	0.15	0.16	0.18	0.18	0.220	0.233	0.254	0.268	0.271	0.032	59.19	51.91	40.63	14.93	0.00	0.068	1816.55	-1712.38	1677.58	-1198.67	1065.78	-894.53	586.57	-270.52	0.0	0.0	0.042
Cr+4	211.00	218.00	228.00	234.00	92.00	0.14	0.15	0.17	0.15	0.18	0.221	0.233	0.255	0.235	0.275	0.081	59.17	41.93	40.27	14.97	0.00	0.064	1826.31	-1969.56	1774.93	-1196.20	1052.00	-749.29	586.14	-170.54	0.0	0.0	0.061
Cr+5	319.00	199.00	189.00	210.00	96.00	0.13	0.14	0.16	0.18	0.18	0.209	0.225	0.247	0.274	0.270	0.081	50.64	61.61	45.45	16.25	0.00	0.041	1946.31	-1731.86	1422.94	-1089.08	979.75	-826.40	569.91	-384.88	0.0	0.0	0.067
Cr+6	245.00	278.00	274.00	300.00	104.00	0.13	0.13	0.16	0.18	0.18	0.209	0.197	0.245	0.273	0.270	0.082	51.42	53.64	45.47	15.10	0.00	0.057	1995.32	-1928.14	1572.16	-1387.20	884.45	-826.83	469.91	-484.88	0.0	0.0	0.063
MEAN	257.17	200.83	232.17	248.00	94.00	0.13	0.14	0.16	0.17	0.18	0.213	0.223	0.249	0.264	0.272	0.067	57.75	50.06	42.12	14.50	0.00	0.059	1879.73	-1853.66	1637.12	-1274.57	1041.03	-825.05	542.06	-346.76	0.0	0.0	0.064
SD	49.64	54.83	42.18	41.68	16.35	0.00	0.01	0.00	0.01	0.00	0.006	0.014	0.005	0.015	0.002	0.020	7.21	7.58	4.09	2.15	0.00	0.011	173.30	114.52	129.74	130.23	101.07	45.99	56.37	109.82	0.0	0.0	0.014

Appendix.VII (continued) Effects of Cr supplementation and E_2 replacement on cardiac functions and EC_{50} of QT-c interval, LVDP and ±dp/dt in non exercise ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	HR (beats/min)					QT interval (sec)					QTc (sec)						LVP (mmHg)						dp/dt_{max} and dp/dt_{min} (mmHg/min)											
	Hydrogen peroxide (mmol/l)					Hydrogen peroxide (mmol/l)											Hydrogen peroxide (mmol/l)						Hydrogen peroxide (mmol/l)											
	0	0.01	0.05	0.1	0.2	0	0.01	0.05	0.1	0.2	0	0.01	0.05	0.1	0.2	EC50	0	0.01	0.05	0.1	0.2	EC50	0		0.01		0.05		0.1		0.2		EC50
E1	256.00	132.00	150.00	180.00	90.00	0.14	0.15	0.15	0.18	0.189	0.218	0.229	0.237	0.274	0.286	0.051	71.55	54.12	42.79	19.40	0.00	0.048	2020.34	-1995.25	1676.41	-1375.27	1206.70	-926.93	707.25	-474.90	0.0	0.0	0.068
E2	300.00	294.00	294.00	276.00	115.00	0.14	0.15	0.15	0.18	0.172	0.219	0.233	0.237	0.273	0.260	0.065	58.62	54.24	49.73	25.64	0.00	0.074	1884.98	-1744.32	1680.10	-1377.39	1207.92	-1024.85	703.15	-475.66	0.0	0.0	0.064
E3	338.00	234.00	228.00	234.00	146.00	0.13	0.15	0.16	0.16	0.171	0.211	0.235	0.252	0.242	0.258	0.065	49.26	55.79	41.16	11.36	0.00	0.065	1852.25	-1971.79	1899.49	-1426.64	1195.54	-1003.29	711.53	-473.81	0.0	0.0	0.081
E4	304.00	204.00	234.00	198.00	150.00	0.14	0.14	0.15	0.18	0.172	0.220	0.220	0.238	0.278	0.260	0.064	51.42	53.00	43.26	20.25	0.00	0.082	1976.67	-1700.52	1658.10	-1333.57	1210.54	-944.86	708.40	-477.55	0.0	0.0	0.045
E5	298.00	234.00	228.00	234.00	116.00	0.12	0.15	0.16	0.17	0.185	0.196	0.235	0.252	0.265	0.280	0.057	57.26	55.79	49.65	9.46	0.00	0.062	1452.25	-1871.79	1699.49	-1426.64	1195.54	-1003.29	711.53	-473.81	0.0	0.0	0.082
E6	279.00	204.00	234.00	198.00	155.00	0.13	0.14	0.16	0.15	0.182	0.204	0.220	0.254	0.232	0.275	0.084	59.42	53.00	40.17	9.46	0.00	0.052	1976.67	-1900.52	1958.10	-1333.57	1210.54	-944.86	708.40	-477.55	0.0	0.0	0.078
MEAN	295.83	217.00	228.00	220.00	128.67	0.13	0.15	0.16	0.17	0.178	0.211	0.229	0.245	0.261	0.270	0.064	57.92	54.32	44.46	15.93	0.00	0.064	1860.53	-1864.03	1761.95	-1378.85	1204.47	-974.68	708.38	-475.55	0.0	0.0	0.070
SD	27.34	53.05	45.85	34.92	25.66	0.01	0.00	0.01	0.01	0.008	0.010	0.007	0.009	0.019	0.012	0.011	7.82	1.25	4.20	6.78	0.00	0.013	209.70	119.41	131.22	41.67	7.07	40.53	3.11	1.70	0.0	0.0	0.014
Cr+E2-1	304.00	234.00	258.00	245.00	146.00	0.14	0.14	0.17	0.18	0.179	0.218	0.221	0.257	0.274	0.270	0.075	70.37	59.39	47.21	10.40	0.00	0.071	2016.45	-1915.14	1663.54	-1449.15	1158.27	-606.15	707.25	-474.90	0.0	0.0	0.072
Cr+E2-2	319.00	270.00	270.00	276.00	175.00	0.13	0.14	0.16	0.17	0.179	0.206	0.223	0.242	0.260	0.270	0.071	47.18	59.35	43.06	16.44	0.00	0.061	1817.80	-1925.49	1568.20	-1339.51	1229.94	-1039.44	709.24	-476.04	0.0	0.0	0.054
Cr+E2-3	256.00	278.00	270.00	278.00	159.00	0.14	0.14	0.15	0.18	0.176	0.218	0.224	0.227	0.273	0.266	0.068	57.29	49.34	37.39	17.53	0.00	0.061	1519.61	-1831.44	1770.53	-1234.69	1011.80	-944.00	712.31	-477.22	0.0	0.0	0.085
Cr+E2-4	298.00	213.00	270.00	256.00	158.00	0.13	0.15	0.16	0.16	0.172	0.208	0.227	0.243	0.243	0.260	0.032	61.08	41.35	51.26	19.38	0.00	0.059	1917.16	-1919.18	1968.20	-1539.51	1323.29	-939.65	706.75	-475.93	0.0	0.0	0.081
Cr+E2-5	391.00	370.00	325.00	237.00	151.00	0.13	0.15	0.18	0.16	0.179	0.206	0.239	0.273	0.246	0.270	0.076	59.18	62.35	45.00	19.44	0.00	0.057	1917.80	-1725.49	1968.20	-1139.51	1529.94	-1139.44	709.24	-476.04	0.0	0.0	0.081
Cr+E2-6	395.00	371.00	345.00	259.00	164.00	0.13	0.15	0.15	0.17	0.184	0.202	0.240	0.227	0.260	0.277	0.068	60.29	59.34	43.26	13.53	0.00	0.078	1919.61	-1900.44	1670.53	-1534.69	1111.80	-1244.00	712.31	-477.22	0.0	0.0	0.061
MEAN	327.17	289.33	289.67	258.50	158.83	0.13	0.15	0.16	0.17	0.178	0.210	0.229	0.245	0.259	0.269	0.065	59.23	55.19	44.53	16.12	0.00	0.065	1851.40	-1869.53	1768.20	-1372.84	1227.51	-968.78	709.52	-476.22	0.0	0.0	0.072
SD	55.13	67.18	35.98	16.36	10.15	0.00	0.01	0.01	0.01	0.004	0.007	0.008	0.018	0.013	0.006	0.017	7.45	8.11	4.63	3.56	0.00	0.008	174.27	78.53	167.63	163.97	181.86	255.09	2.39	0.88	0.0	0.0	0.012

Appendix.VIII Effects of Cr supplementation and E_2 replacement on cardiac functions and EC_{50} of QT-c interval, LVDP and $\pm$dp/dt in exercise-trained ovariectomized hamsters. The data are presented as mean $\pm$ SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	HR (beats/min)					QT interval (sec)					QTc (sec)						LVDP (mmHg)						dp/dt_{max} and dp/dt_{min} (mmHg/min)											
	Hydrogen peroxide (mmol/l)					Hydrogen peroxide (mmol/l)					Hydrogen peroxide (mmol/l)						Hydrogen peroxide (mmol/l)						Hydrogen peroxide (mmol/l)											
	0	0.01	0.05	0.1	0.2	0	0.01	0.05	0.1	0.2	0	0.01	0.05	0.1	0.2	EC50	0	0.01	0.05	0.1	0.2	EC50	0		0.01		0.05		0.1		0.2		EC50
C1	204.00	198.00	192.00	192.00	150.00	0.13	0.13	0.14	0.16	0.16	0.200	0.208	0.215	0.241	0.240	0.084	77.54	63.96	54.26	33.48	0.00	0.101	2538.42	-1626.02	1711.36	-1661.02	1440.09	-940.49	885.57	-356.69	0.00	0.00	0.100
C2	224.00	195.00	191.00	195.00	140.00	0.12	0.13	0.14	0.15	0.16	0.184	0.207	0.221	0.222	0.240	0.071	59.78	58.04	56.37	28.60	0.00	0.091	2277.85	-1879.43	1809.66	-1559.11	1307.41	-920.82	985.01	-429.19	0.00	0.00	0.143
C3	314.00	190.00	185.00	194.00	120.00	0.13	0.13	0.14	0.15	0.16	0.200	0.209	0.213	0.225	0.239	0.072	61.14	63.97	54.08	30.67	0.00	0.095	2025.30	-1935.67	1624.11	-1759.30	1480.55	-970.04	887.79	-504.60	0.00	0.00	0.101
C4	316.00	187.00	179.00	175.00	130.00	0.13	0.13	0.15	0.15	0.16	0.200	0.207	0.227	0.233	0.240	0.039	56.84	64.25	55.08	27.79	0.00	0.109	2036.99	-1964.21	1815.73	-1665.50	1500.08	-960.29	885.20	-325.72	0.00	0.00	0.097
C5	356.00	195.00	184.00	182.00	142.00	0.13	0.14	0.14	0.15	0.16	0.200	0.224	0.221	0.222	0.240	0.074	78.01	60.04	56.37	38.60	0.00	0.111	2377.85	-1694.33	1909.66	-1659.11	1307.41	-920.82	736.50	-309.19	0.00	0.00	0.083
C6	375.00	194.00	176.00	172.00	121.00	0.13	0.14	0.14	0.15	0.16	0.200	0.225	0.213	0.225	0.239	0.064	80.71	63.97	54.08	36.67	0.00	0.118	2125.30	-1935.67	1621.11	-1759.30	1480.55	-970.04	787.79	-404.60	0.00	0.00	0.092
MEAN	298.17	193.17	184.50	185.00	133.83	0.12	0.14	0.14	0.15	0.16	0.197	0.213	0.218	0.228	0.240	0.067	69.00	62.37	55.04	32.63	0.00	0.104	2230.29	-1839.22	1748.61	-1677.22	1419.35	-947.08	861.31	-388.33	0.00	0.00	0.103
SD	69.55	3.97	6.35	10.08	12.14	0.00	0.01	0.00	0.00	0.00	0.007	0.009	0.006	0.007	0.000	0.015	10.83	2.66	1.09	4.39	0.00	0.010	204.64	143.04	116.03	75.04	88.88	23.03	87.35	72.99	0.00	0.00	0.021
Cr-1	342.00	276.00	234.00	170.00	74.00	0.12	0.13	0.15	0.16	0.17	0.191	0.209	0.226	0.243	0.251	0.021	59.88	61.54	36.61	20.85	0.00	0.043	2233.60	-1806.72	1722.71	-1571.81	1085.20	-827.32	581.70	-240.42	0.00	0.00	0.035
Cr-2	312.00	306.00	294.00	164.00	68.00	0.13	0.14	0.16	0.15	0.17	0.210	0.226	0.241	0.237	0.255	0.015	60.87	59.33	32.07	25.85	0.00	0.034	2131.25	-1715.67	1822.71	-1767.18	1185.58	-798.33	581.10	-224.16	0.00	0.00	0.042
Cr-3	216.00	240.00	252.00	152.00	72.00	0.12	0.14	0.14	0.16	0.17	0.191	0.225	0.211	0.242	0.251	0.021	59.86	62.54	36.51	23.85	0.00	0.032	2519.45	-1926.58	1929.10	-1567.44	1208.52	-755.32	698.56	-153.55	0.00	0.00	0.036
Cr-4	342.00	276.00	234.00	170.00	74.00	0.12	0.14	0.16	0.16	0.17	0.191	0.224	0.241	0.240	0.255	0.018	78.93	65.61	30.79	26.86	0.00	0.034	2033.32	-1808.64	1595.55	-1475.95	1187.28	-816.03	568.45	-194.65	0.00	0.00	0.039
Cr-5	312.00	306.00	294.00	164.00	68.00	0.13	0.13	0.15	0.16	0.17	0.203	0.210	0.226	0.240	0.255	0.021	77.87	58.33	31.07	23.85	0.00	0.041	2131.25	-1875.67	1821.71	-1771.81	1085.58	-804.33	481.71	-264.16	0.00	0.00	0.034
Cr-6	216.00	240.00	252.00	152.00	72.00	0.12	0.14	0.16	0.16	0.16	0.191	0.225	0.241	0.243	0.240	0.025	79.88	62.54	32.61	25.78	0.00	0.046	2000.60	-1900.27	1592.71	-1671.81	1085.20	-799.32	456.38	-41.00	0.00	0.00	0.039
MEAN	290.00	274.00	260.00	162.00	71.33	0.12	0.14	0.15	0.16	0.17	0.196	0.220	0.231	0.241	0.251	0.020	69.55	61.65	33.28	24.51	0.00	0.038	2174.91	-1838.92	1747.42	-1637.67	1139.56	-800.11	561.32	-186.32	0.00	0.00	0.038
SD	58.87	29.56	27.54	8.20	2.73	0.01	0.00	0.01	0.00	0.00	0.008	0.008	0.013	0.002	0.006	0.003	10.26	2.60	2.63	2.15	0.00	0.006	187.83	77.39	135.50	119.46	59.96	24.58	86.07	80.84	0.00	0.00	0.003
Cr+1	220.00	211.00	232.00	162.00	110.00	0.13	0.13	0.13	0.14	0.16	0.208	0.204	0.208	0.210	0.235	0.068	58.69	73.26	64.46	41.23	2.23	0.108	2539.23	-1912.65	1783.54	-1690.02	1596.22	-1408.43	1001.06	-540.40	108.52	-80.97	0.112
Cr+2	216.00	270.00	216.00	162.00	112.00	0.13	0.14	0.14	0.14	0.16	0.208	0.224	0.224	0.216	0.239	0.087	51.05	72.69	64.08	36.25	6.28	0.101	2270.97	-1878.98	1820.38	-1582.19	1692.80	-1382.80	931.47	-704.13	117.27	-71.31	0.100
Cr+3	246.00	222.00	228.00	145.00	96.00	0.12	0.13	0.15	0.15	0.16	0.190	0.208	0.229	0.226	0.240	0.064	79.45	71.03	67.56	45.23	2.36	0.113	2595.98	-1792.74	1700.23	-1594.44	1523.79	-1406.67	908.18	-523.94	109.35	-86.49	0.113
Cr+4	294.00	288.00	276.00	152.00	117.00	0.12	0.14	0.14	0.16	0.15	0.191	0.215	0.214	0.241	0.230	0.057	80.26	69.89	62.35	36.56	3.24	0.102	2355.84	-1867.49	1770.23	-1694.53	1522.82	-1447.29	908.36	-624.96	104.76	-80.52	0.104
Cr+5	210.00	245.00	213.00	162.00	114.00	0.12	0.15	0.14	0.15	0.16	0.190	0.231	0.214	0.226	0.237	0.068	79.98	68.45	60.29	38.09	4.16	0.108	2016.29	-1781.27	1800.23	-1664.44	1623.79	-1416.67	1178.18	-413.94	103.49	-86.49	0.119
Cr+6	216.00	270.00	216.00	116.00	91.00	0.12	0.13	0.14	0.16	0.17	0.191	0.199	0.214	0.241	0.256	0.058	60.23	71.23	64.23	40.56	5.47	0.121	1955.84	-1867.49	1800.23	-1604.53	1602.82	-1477.29	1008.36	-592.50	120.48	-85.19	0.101
MEAN	233.67	251.00	230.17	145.83	106.67	0.12	0.14	0.14	0.15	0.16	0.196	0.213	0.217	0.227	0.239	0.067	68.28	71.09	63.83	39.65	3.96	0.109	2285.02	-1850.10	1779.14	-1638.36	1593.71	-1423.19	989.27	-566.64	110.64	-81.83	0.108
SD	32.14	30.23	23.68	17.98	10.58	0.01	0.01	0.01	0.01	0.01	0.009	0.012	0.008	0.013	0.009	0.011	13.11	1.77	2.42	3.41	1.66	0.008	263.44	51.73	42.23	50.46	64.39	33.68	102.62	98.80	6.82	5.80	0.008

Appendix.VIII (continued) Effects of Cr supplementation and E_2 replacement on cardiac functions and EC_{50} of QT-c interval, LVDP and ±dp/dt in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	HR (beats/min)					QT interval (sec)					QTc (sec)						LVDP (mmHg)						dp/dt_{max} and dp/dt_{min} (mmHg/min)										
	Hydrogen peroxide (mmol/l)					Hydrogen peroxide (mmol/l)					Hydrogen peroxide (mmol/l)						Hydrogen peroxide (mmol/l)						Hydrogen peroxide (mmol/l)										
	0	0.01	0.05	0.1	0.2	0	0.01	0.05	0.1	0.2	0	0.01	0.05	0.1	0.2	EC50	0	0.01	0.05	0.1	0.2	EC50	0		0.01		0.05		0.1		0.2		EC50
E1	252.00	238.00	206.00	126.00	102.00	0.14	0.14	0.15	0.14	0.15	0.217	0.224	0.229	0.216	0.220	0.085	50.15	71.26	63.04	58.26	5.88	0.133	2513.26	-1787.23	1913.17	-1629.47	1868.59	-1406.87	1142.46	-610.33	147.98	-80.17	0.109
E2	267.00	265.00	204.00	144.00	136.00	0.13	0.13	0.14	0.14	0.15	0.200	0.210	0.213	0.216	0.220	0.085	77.20	67.92	65.23	38.04	7.59	0.107	2211.02	-1822.10	2111.34	-1731.45	1877.98	-1403.55	1140.14	-610.42	148.56	-95.00	0.114
E3	287.00	286.00	262.00	132.00	126.00	0.12	0.13	0.15	0.14	0.16	0.186	0.209	0.229	0.208	0.236	0.065	79.65	65.39	61.59	54.25	6.28	0.132	1946.86	-1699.70	2053.85	-1728.27	1728.93	-1583.23	1155.16	-912.73	127.70	-104.68	0.113
E4	249.00	246.00	213.00	144.00	126.00	0.13	0.13	0.14	0.15	0.17	0.202	0.209	0.213	0.231	0.251	0.068	81.36	70.68	65.00	52.36	9.26	0.136	2245.36	-1796.61	2055.95	-1627.04	1782.89	-1583.23	1054.26	-912.44	128.33	-99.46	0.104
E5	245.00	239.00	211.00	132.00	124.00	0.13	0.13	0.14	0.16	0.16	0.201	0.208	0.213	0.247	0.235	0.057	60.15	69.00	60.25	52.69	5.12	0.130	2131.69	-1987.23	1913.17	-1629.47	1868.59	-1406.87	1042.46	-610.33	117.98	-91.17	0.119
E6	248.00	246.00	203.00	132.00	102.00	0.12	0.13	0.12	0.14	0.16	0.190	0.210	0.193	0.216	0.235	0.051	77.20	67.92	62.39	38.04	5.89	0.108	2500.36	-1990.10	1911.34	-1731.45	1877.98	-1403.55	1110.40	-610.42	148.56	-105.00	0.101
MEAN	258.00	253.33	216.50	135.00	119.33	0.13	0.14	0.14	0.15	0.15	0.199	0.212	0.215	0.222	0.233	0.069	70.95	68.70	62.92	48.94	6.67	0.124	2258.09	-1847.16	1993.13	-1679.53	1834.16	-1464.55	1107.48	-711.11	136.52	-95.91	0.110
SD	16.17	18.72	22.63	7.35	14.07	0.01	0.00	0.01	0.01	0.01	0.011	0.006	0.013	0.014	0.012	0.014	12.74	2.13	1.94	8.70	1.51	0.013	218.64	117.11	90.65	55.74	63.11	91.94	48.23	156.06	13.49	9.41	0.007
Cr+E_2-1	274.00	278.00	210.00	174.00	156.00	0.12	0.12	0.13	0.14	0.15	0.190	0.194	0.202	0.215	0.229	0.069	60.20	69.56	60.64	55.23	5.89	0.132	2534.40	-1908.58	1956.64	-1756.80	1850.30	-1500.47	1254.34	-833.52	127.70	-105.68	0.116
Cr+E_2-2	246.00	262.00	222.00	180.00	154.00	0.13	0.13	0.14	0.14	0.16	0.202	0.204	0.211	0.216	0.235	0.056	75.26	70.98	61.23	48.69	6.59	0.132	2155.29	-1720.76	1856.85	-1540.85	1550.33	-1400.45	1123.69	-633.68	157.70	-93.68	0.112
Cr+E_2-3	262.00	256.00	268.00	138.00	142.00	0.13	0.13	0.13	0.14	0.15	0.202	0.210	0.202	0.215	0.229	0.085	51.25	71.23	62.31	59.32	8.26	0.138	2243.02	-1964.80	1956.45	-1606.85	1950.31	-1300.48	1253.98	-682.87	130.70	-96.68	0.103
Cr+E_2-4	275.00	278.00	213.00	170.00	146.00	0.13	0.14	0.14	0.15	0.16	0.202	0.226	0.217	0.222	0.237	0.074	81.36	69.58	60.64	46.98	9.54	0.108	1913.44	-1908.58	1995.64	-1706.80	1865.30	-1400.47	1054.26	-903.52	126.39	-91.41	0.104
Cr+E_2-5	277.00	274.00	205.00	148.00	126.00	0.13	0.13	0.14	0.15	0.16	0.202	0.204	0.218	0.222	0.235	0.081	79.65	68.54	59.86	50.13	7.45	0.140	2255.29	-1960.76	1956.85	-1690.85	1750.33	-1400.45	1255.18	-913.68	137.70	-83.68	0.110
Cr+E_2-6	279.00	248.00	211.00	174.00	135.00	0.12	0.14	0.14	0.15	0.15	0.190	0.219	0.217	0.228	0.225	0.086	78.94	65.23	60.65	50.36	9.26	0.104	2543.17	-1664.80	1756.45	-1406.85	1950.31	-1500.48	1253.98	-932.87	140.70	-106.68	0.102
MEAN	268.83	266.00	221.50	164.00	143.17	0.12	0.13	0.14	0.14	0.15	0.198	0.209	0.211	0.219	0.232	0.075	71.11	69.19	60.89	51.79	7.83	0.126	2274.10	-1854.71	1913.15	-1618.17	1819.48	-1417.13	1199.24	-816.69	136.82	-96.30	0.108
SD	12.67	12.59	23.45	16.88	11.43	0.00	0.01	0.00	0.00	0.00	0.006	0.011	0.008	0.005	0.005	0.011	12.41	2.18	0.82	4.61	1.46	0.016	239.03	128.98	89.67	128.93	151.33	75.28	88.19	128.16	11.67	8.79	0.006

Appendix. IX **Effects of Cr supplementation and E_2 replacement on contents of Cr, PCr, TCr, CrT and CK activity in non exercise ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.**

No.	Cr content (umol/mg protein)	PCr content (umol/mg protein)	TCr content (umol/mg protein)	CK activity (umol/min/mg protein)	CrT protein (ratio of CrT/α-actin)
C1	0.042	0.007	0.050	5.253	0.286
C2	0.043	0.013	0.056	4.886	0.248
C3	0.035	0.009	0.045	4.349	0.286
C4	0.037	0.019	0.056	5.234	0.277
C5	0.047	0.022	0.069	5.351	0.286
C6	0.040	0.017	0.057	4.878	0.248
C7	0.046	0.019	0.065	5.521	0.286
C8	0.045	0.018	0.063	4.970	0.277
C9	0.046	0.019	0.064	5.043	0.286
C10	0.052	0.021	0.073	5.172	0.277
MEAN	0.043	0.017	0.060	5.066	0.276
SD	0.005	0.005	0.009	0.326	0.015
Cr-1	0.026	0.010	0.036	4.278	0.168
Cr-2	0.028	0.010	0.038	5.676	0.154
Cr-3	0.027	0.009	0.036	3.119	0.167
Cr-4	0.028	0.011	0.039	3.105	0.183
Cr-5	0.026	0.010	0.036	3.592	0.168
Cr-6	0.025	0.008	0.034	6.441	0.154
Cr-7	0.022	0.008	0.030	4.560	0.167
Cr-8	0.020	0.006	0.026	3.064	0.183
Cr-9	0.029	0.009	0.039	4.272	0.167
Cr-10	0.028	0.008	0.035	3.412	0.183
MEAN	0.026	0.009	0.035	4.152	0.169
SD	0.003	0.001	0.004	1.153	0.011
Cr+1	0.119	0.044	0.163	15.065	0.325
Cr+2	0.124	0.047	0.171	14.223	0.281
Cr+3	0.113	0.029	0.143	9.289	0.307
Cr+4	0.111	0.046	0.157	10.985	0.399
Cr+5	0.113	0.050	0.163	11.976	0.325
Cr+6	0.082	0.049	0.130	9.406	0.281
Cr+7	0.071	0.029	0.100	7.145	0.307
Cr+8	0.068	0.054	0.122	12.700	0.399
Cr+9	0.116	0.045	0.161	8.788	0.307
Cr+10	0.123	0.050	0.173	9.940	0.399
MEAN	0.104	0.044	0.148	10.952	0.333
SD	0.022	0.008	0.024	2.518	0.048
E1	0.049	0.018	0.067	5.216	0.327
E2	0.055	0.016	0.071	4.953	0.320
E3	0.053	0.019	0.072	4.888	0.389
E4	0.049	0.018	0.067	4.703	0.316
E5	0.047	0.012	0.059	3.707	0.327
E6	0.052	0.018	0.070	5.576	0.320
E7	0.052	0.020	0.072	5.225	0.389
E8	0.048	0.014	0.062	4.432	0.316
E9	0.054	0.014	0.068	4.360	0.389
E10	0.094	0.018	0.112	9.478	0.316
MEAN	0.055	0.017	0.072	5.254	0.341
SD	0.014	0.003	0.015	1.576	0.034
C+$E_2$1	0.098	0.047	0.144	12.609	0.362
C+$E_2$2	0.101	0.040	0.141	11.693	0.344
C+$E_2$3	0.132	0.036	0.168	10.016	0.294
C+$E_2$4	0.101	0.036	0.136	12.745	0.362
C+$E_2$5	0.090	0.046	0.136	12.187	0.362
C+$E_2$6	0.102	0.043	0.145	13.483	0.344
C+$E_2$7	0.101	0.053	0.154	14.746	0.294
C+$E_2$8	0.114	0.033	0.147	15.115	0.362
C+$E_2$9	0.086	0.035	0.121	16.094	0.294
C+$E_2$10	0.112	0.035	0.148	13.530	0.362
MEAN	0.104	0.040	0.144	13.222	0.338
SD	0.013	0.007	0.012	1.783	0.031

Appendix. X **Effects of Cr supplementation and E_2 replacement on contents of Cr, PCr, TCr, CrT and CK activity in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion, Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.**

No.	Cr content (umol/mg protein)	PCr content (umol/mg protein)	TCr content (umol/mg protein)	CK activity (umol/min/mg protein)	CrT protein (ratio of CrT/α-actin)
C1	0.050	0.015	0.065	5.919	C/a
C2	0.047	0.015	0.061	5.487	0.286
C3	0.041	0.014	0.054	4.928	0.291
C4	0.048	0.017	0.065	4.727	0.286
C5	0.050	0.017	0.067	5.803	0.248
C6	0.044	0.015	0.059	5.034	0.286
C7	0.050	0.017	0.067	5.093	0.291
C8	0.047	0.017	0.064	5.226	0.286
C9	0.050	0.017	0.067	5.673	0.248
C10	0.056	0.020	0.076	6.242	0.286
MEAN	0.048	0.016	0.065	5.413	0.248
SD	0.004	0.002	0.006	0.489	0.275
Cr-1	0.041	0.014	0.056	4.457	0.248
Cr-2	0.045	0.019	0.064	7.080	0.269
Cr-3	0.043	0.013	0.056	6.589	0.314
Cr-4	0.060	0.017	0.077	7.198	0.307
Cr-5	0.059	0.023	0.082	6.656	0.248
Cr-6	0.069	0.018	0.086	10.313	0.269
Cr-7	0.043	0.025	0.068	6.849	0.314
Cr-8	0.039	0.015	0.054	6.077	0.307
Cr-9	0.042	0.020	0.062	6.289	0.314
Cr-10	0.044	0.020	0.064	6.783	0.307
MEAN	0.049	0.018	0.067	6.829	0.290
SD	0.010	0.004	0.012	1.450	0.028
Cr+1	0.160	0.066	0.226	17.261	0.502
Cr+2	0.176	0.066	0.242	16.485	0.543
Cr+3	0.146	0.061	0.207	14.982	0.528
Cr+4	0.156	0.066	0.222	16.948	0.561
Cr+5	0.162	0.079	0.241	16.703	0.502
Cr+6	0.165	0.082	0.247	16.797	0.543
Cr+7	0.119	0.054	0.173	11.755	0.528
Cr+8	0.169	0.070	0.239	16.711	0.561
Cr+9	0.163	0.067	0.230	17.575	0.528
Cr+10	0.170	0.063	0.233	15.532	0.561
MEAN	0.159	0.067	0.226	16.075	0.536
SD	0.016	0.008	0.022	1.702	0.022
E1	0.090	0.039	0.128	11.759	0.408
E2	0.091	0.034	0.124	11.886	0.458
E3	0.093	0.036	0.130	12.518	0.514
E4	0.088	0.032	0.120	11.241	0.486
E5	0.082	0.030	0.112	9.111	0.408
E6	0.096	0.039	0.135	11.748	0.458
E7	0.088	0.032	0.120	11.095	0.514
E8	0.086	0.029	0.115	11.237	0.486
E9	0.095	0.035	0.130	11.057	0.514
E10	0.092	0.041	0.133	10.360	0.486
MEAN	0.090	0.035	0.125	11.201	0.473
SD	0.004	0.004	0.008	0.938	0.040
C+$E_2$1	0.150	0.063	0.214	16.416	0.692
C+$E_2$2	0.156	0.067	0.223	16.469	0.628
C+$E_2$3	0.144	0.062	0.207	17.530	0.687
C+$E_2$4	0.153	0.069	0.222	19.147	0.672
C+$E_2$5	0.140	0.063	0.203	15.299	0.692
C+$E_2$6	0.155	0.071	0.226	17.689	0.628
C+$E_2$7	0.150	0.075	0.226	18.577	0.687
C+$E_2$8	0.167	0.064	0.230	17.674	0.672
C+$E_2$9	0.143	0.065	0.208	17.135	0.687
C+$E_2$10	0.171	0.068	0.238	21.062	0.672
MEAN	0.153	0.067	0.220	17.700	0.672
SD	0.010	0.004	0.011	1.614	0.024

Appendix. XI **Effects of Cr supplementation and E_2 replacement on contents of GSH, GSSG, GSH and GPx enzyme in non exerciseand ovariectomized hamsters. The data are presented as mea C, control; Cr-, Cr depletion; Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementa combined with E_2 replacement.**

No	GSSG (nmol/mg protein)	GSH (nmol/mg protein)	GSH/GSSG	GPx (mmol/min/mg protein)
C1	2.320	6.279	2.707	17.914
C2	1.365	5.497	4.026	17.194
C3	1.256	5.985	4.766	12.408
C4	1.312	5.937	4.527	15.867
C5	2.269	7.699	3.393	13.935
C6	1.319	5.602	4.247	12.938
C7	2.391	7.148	2.989	17.952
C8	1.444	6.911	4.785	11.398
C9	1.435	4.577	3.189	17.188
C10	1.605	6.272	3.908	15.264
MEAN	1.672	6.191	3.854	15.206
SD	0.463	0.898	0.750	2.410
Cr-1	1.578	1.288	0.816	5.743
Cr-2	1.669	1.523	0.912	6.016
Cr-3	1.632	2.520	1.544	7.614
Cr-4	1.718	1.384	0.806	13.549
Cr-5	1.606	2.612	1.626	8.036
Cr-6	1.857	2.859	1.540	10.240
Cr-7	1.631	2.250	1.379	9.693
Cr-8	1.470	2.317	1.577	5.999
Cr-9	1.576	1.411	0.895	7.169
Cr-10	1.768	1.536	0.869	11.973
MEAN	1.651	1.970	1.196	8.603
SD	0.109	0.597	0.362	2.681
Cr+1	1.684	4.133	2.455	16.854
Cr+2	1.734	6.611	3.812	18.986
Cr+3	1.541	7.481	4.856	20.616
Cr+4	1.684	7.150	4.247	18.960
Cr+5	1.603	7.823	4.881	28.030
Cr+6	2.009	6.920	3.445	19.731
Cr+7	1.237	5.486	4.437	14.698
Cr+8	1.644	4.206	2.559	13.461
Cr+9	1.255	6.846	5.455	11.470
Cr+10	1.128	4.181	3.705	12.511
MEAN	1.552	6.084	3.985	17.532
SD	0.270	1.452	0.987	4.896
E1	1.638	3.416	2.085	27.093
E2	1.734	6.470	3.732	15.310
E3	1.776	7.176	4.041	16.406
E4	1.701	6.468	3.802	23.260
E5	1.489	6.190	4.157	17.496
E6	1.588	3.651	2.300	18.714
E7	1.627	5.705	3.507	20.460
E8	1.304	6.164	4.728	21.923
E9	1.630	6.010	3.688	23.780
E10	1.432	6.135	4.285	24.073
MEAN	1.592	5.739	3.633	20.851
SD	0.145	1.225	0.836	3.820
C+E1	1.735	6.660	3.839	18.990
C+E2	1.662	7.028	4.229	20.184
C+E3	1.462	6.447	4.441	29.414
C+E4	1.425	6.208	4.358	24.678
C+E5	1.778	6.513	3.664	20.951
C+E6	1.507	6.970	4.625	28.427
C+E7	1.944	4.924	2.533	15.863
C+E8	1.582	6.859	4.336	22.127
C+E9	1.162	4.904	4.221	13.504
C+E10	1.739	7.115	4.091	14.759
MEAN	1.598	6.363	4.034	20.890
SD	0.222	0.814	0.598	5.446

Appendix. X II **Effects of Cr supplementation and E_2 replacement on contents of GSH, GSSG, GSH/G and GPx enzyme in exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion; Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplentation combined with E_2 replacement.**

No	GSSG (nmol/mg protein)	GSH (nmol/mg protein)	GSH/GSSG	GPx (mmol/min/mg protein)
C1	1.743	5.929	3.401	16.913
C2	1.833	7.440	4.059	16.177
C3	2.280	5.931	2.601	17.000
C4	1.841	5.541	3.011	18.246
C5	1.353	9.187	6.791	18.085
C6	1.407	6.198	4.407	15.652
C7	1.583	6.525	4.121	16.561
C8	2.384	6.229	2.613	16.036
C9	2.096	7.102	3.389	17.188
C10	1.804	7.546	4.182	25.141
MEAN	1.832	6.763	3.857	17.700
SD	0.343	1.085	1.220	2.744
Cr-1	1.886	6.196	3.285	18.164
Cr-2	1.855	5.782	3.117	14.838
Cr-3	1.758	4.378	2.490	15.323
Cr-4	1.817	6.305	3.470	13.885
Cr-5	1.686	4.990	2.959	13.144
Cr-6	1.898	5.511	2.904	13.636
Cr-7	1.506	5.999	3.983	14.817
Cr-8	1.095	5.237	4.780	13.803
Cr-9	1.576	6.011	3.814	13.492
Cr-10	1.223	5.049	4.129	15.896
MEAN	1.630	5.546	3.493	14.700
SD	0.281	0.623	0.685	1.503
Cr+1	1.684	4.636	2.753	16.854
Cr+2	1.752	6.658	3.800	21.678
Cr+3	2.411	4.295	1.781	20.095
Cr+4	1.684	9.162	5.441	20.697
Cr+5	2.078	8.328	4.007	22.721
Cr+6	1.671	9.807	5.869	18.586
Cr+7	1.108	6.662	6.013	20.095
Cr+8	2.092	7.322	3.500	28.204
Cr+9	2.128	6.704	3.150	17.825
Cr+10	2.049	5.177	2.527	16.839
MEAN	1.866	6.875	3.884	20.359
SD	0.363	1.850	1.459	3.389
E1	1.784	22.876	12.825	66.446
E2	1.813	28.764	15.866	54.663
E3	1.670	15.794	9.455	68.531
E4	1.778	13.949	7.847	53.062
E5	1.591	26.557	16.690	34.624
E6	1.561	18.383	11.776	58.212
E7	1.577	28.509	18.077	35.316
E8	1.576	20.424	12.957	35.500
E9	1.839	30.198	16.419	58.411
E10	1.808	23.940	13.245	55.779
MEAN	1.700	22.939	13.516	52.054
SD	0.115	5.683	3.289	12.634
C+E1	1.573	26.109	16.601	74.283
C+E2	2.045	26.541	12.976	42.611
C+E3	1.849	25.182	13.621	73.161
C+E4	2.124	27.523	12.960	78.855
C+E5	1.802	23.655	13.130	44.581
C+E6	1.994	26.541	13.309	44.827
C+E7	1.619	27.997	17.294	43.287
C+E8	1.449	17.610	12.156	40.827
C+E9	1.060	27.474	25.914	42.749
C+E10	1.616	26.990	16.699	77.652
MEAN	1.713	25.562	15.466	56.283
SD	0.319	3.068	4.113	17.065

Appendix. XIII Effects of Cr supplementation and E_2 replacement on serum IGF-I level in non-exercise and exercise-trained ovariectomized hamsters. The data are presented as mean ± SD. C, control; Cr-, Cr depletion; Cr+, Cr supplementation; E_2, E_2 replacement; Cr+E_2, Cr supplementation combined with E_2 replacement.

No.	Non exercise animals	Exercise-trained animals
	Serum IGF-I level (ng/ml)	
C1	386.00	449.83
C2	361.00	376.00
C3	194.33	456.83
C4	272.67	300.67
C5	487.67	487.67
C6	301.00	201.00
MEAN	**333.78**	**378.67**
SD	**101.39**	**110.19**
Cr-1	119.33	231.00
Cr-2	119.33	201.00
Cr-3	119.33	264.33
Cr-4	102.67	232.67
Cr-5	79.33	462.67
Cr-6	56.00	264.33
MEAN	**99.33**	**276.00**
SD	**26.42**	**94.49**
Cr+1	411.00	797.67
Cr+2	414.33	807.67
Cr+3	272.67	731.00
Cr+4	374.33	707.67
Cr+5	364.33	796.00
Cr+6	551.00	629.33
MEAN	**397.94**	**744.89**
SD	**90.83**	**69.67**
E1	436.00	1194.33
E2	436.00	1331.00
E3	437.67	1279.33
E4	512.67	1212.67
E5	401.00	1279.33
E6	402.67	1236.00
MEAN	**437.67**	**1255.44**
SD	**40.50**	**50.58**
C+E1	377.67	1211.00
C+E2	397.67	1356.00
C+E3	434.33	1286.00
C+E4	372.67	1341.00
C+E5	516.00	972.67
C+E6	377.67	1267.67
MEAN	**412.67**	**1239.06**
SD	**55.54**	**140.60**

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