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# Effect of creatine pyruvate supplement, a novel compound, on lipid and protein metabolism in male rats

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ABSTRACT

The study was conducted to assess the effects of creatine pyruvate (Cr-Pyr) on lipid and protein metabolism in male rats. Healthy adult male Sprague-Dawley (SD) rats (40) were randomly assigned to control group (CG) and 3 experiment groups (EG) administrated with 750 ($EG_1$), 1500 ($EG_2$), or 3000 ($EG_3$) mg/kg Cr-Pyr daily for 42 d. The fat mass in male rats of EGs was lower and relative muscle weight was significantly higher in $EG_2$ and $EG_3$ than CG. The content of serum HDL-C and hepatic TC was significantly lower in $EG_2$, and male rats of $EG_3$ had significantly lower hepatic TG as compared to those in CG. Supplementation of male rats with 3000 mg/kg Cr-Pyr significantly decreased the serum leptin concentration but increased insulin. Serum IGF-1 was thought statistically not significantly difference but its level elevated in EGs compared to CG. The expressions of HSL in fat and peroxisome proliferators-activated receptor $\alpha$ mRNA in liver were enhanced in $EG_2$ and $EG_3$ than CG. Supplementation of Cr-Pyr resulted in a significant increase in muscle protein content in EGs. Hepatic GPT was increased in EGs, but no effect on hepatic GOT activity. Supplementation of Cr-Pyr resulted in significant increase in muscle myogenin mRNA expression, while muscle mRNA abundance of myostatin (MSTN) was unaffected. The results described herein demonstrated that supplementation of Cr-Pyr was accompanied by significant elevated levels of lipolysis and increased muscle protein synthesis through the regulation of various metabolic parameters in male rats.

**Key words**: Creatine pyruvate, Lipolysis, Metabolic parameter, Protein deposit

Pyruvate, a three-carbon compound, plays a central role in the inter-conversion of carbohydrates, lipids, and amino acids in both catabolic and anabolic reactions. The dietary addition of pyruvate plus dihydroxyacetone (DHA) resulted in a significant decrease in body fat and increase in lean body mass in rats (Stanko and Adibi 1986). Although data suggest that pyruvate have a positive affect on body composition, the mechanism by which pyruvate augments lean body mass and decreases fat mass is unclear. Understanding the effects of pyruvate supplementation on energy metabolism and body composition is essential before drawing any definitive conclusions. Creatine (Cr), a natural ergogenic compound, increases muscle creatine and phosphorylcreatine (Persky *et al.* 2001); and Cr-supplement improved weight gain in pigs (Young *et al.* 2007). In contrast to the large number of studies on the effects of pyruvate and creatine in rats and pigs, little is known about the effect of Cr-Pyr on the fat and protein metabolism in animals. Present study was undertaken to study the effects of Cr-Pyr on lipid and protein metabolism and relative gene expression in male rats.

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## MATERIALS AND METHODS

*Animals and housing:* Male Sprague-Dawley (SD) rats, 8-week-old, weighing 220–250 g, were procured from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences, China. All experimental protocols were approved by the regional Animal Ethics Committee, and animal care and use procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University, China. Animals were maintained on standard rodent chow with free access to drinking water. Rats were acclimatized for 1 week prior to starting of the experiment.

Male rats (40) were randomly assigned to 3 experiment groups (EG) administrated with 750 ($EG_1$), 1500 ($EG_2$), or 3000 ($EG_3$) mg/kg Cr-Pyr by gavage each morning, respectively and the control group (CG) administrated with equal volume physiological saline until sacrificed at 42 d. Daily feed consumption and weekly body weights were recorded. At the end of the trial rats were deprived of feed for 12 h, weighed and sacrificed. The epididymis adipose

tissue and left leg muscle were dissected and weighed.

*Measurement of metabolic parameters:* Blood samples were allowed to clot at 4°C, centrifuged at 3000 × g for 10 min, blood serum was separated and stored at –20°C till further analysis. Liver, fat and muscle were collected and stored at –70°C. Blood samples were analyzed for triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and non-esterified fatty acids (NEFA), using commercial kits. Liver lipid content was determined on homogenized liver samples using a mixture of chloroform and methanol (2:1 v/v), according to Folch *et al.* (1957). Approximately 100 mg of liver was homogenized on ice with 1 ml of physiological saline. An aliquot of these homogenates was used to determine glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) using commercial kits. The contents of blood glucose, urea nitrogen, creatinine and total protein in serum were determined using commercial kits.

*Measurement of metabolic hormones:* The serum concentrations of insulin, glucagons and leptin were measured with radioimmunoassay (RIA) using a commercial kit. IGF-I levels were measured by enzyme-linked immunosorbent assays. All assays were performed in a blinded manner, and control samples provided by the manufacturer were included in each run. IGF-I was separated from its binding proteins in serum prior to measurement. The antibodies used showed no cross-reactivity with other components of the IGF axis.

*RNA extraction:* Total RNAs were extracted from the tissue samples using isolation reagent according to the manufacturer's protocols. Total RNA concentration was quantified by measuring the absorbance at 260 nm using a biophotometer. Ratios of absorption (260/280 nm) of all preparations were between 1.8 and 2.0. Aliquots of RNA samples were subjected to electrophoresis through 2% agarose formaldehyde gel to verify their integrity.

*Real-time quantitative RT-PCR:* Reverse transcription was performed using the extracted RNA (2 µg) in a final volume of 25 µl containing 10 units of MMLV reverse transcriptase, 1 mmol/litre dNTP mixture, 40 units of recombinant RNase inhibitor and 21 µmol/litre random primers (6 bp) and 3 mmol/litre $MgCl_2$ in sterilized water and buffer supplied by the manufacturer. After incubation at 37° for 60 min, the mixture was heat treated at 95° for 5 min and quickly cooled on ice. An aliquot of cDNA samples was mixed with 25 µl master mix, in the presence of 5 pmol of each forward and reverse primer for fatty acid synthase (FAS), sterol regulatory element binding protein-1c (SREBP-1c), peroxisome proliferators-activated receptors α (PPARα), carnitine palmitoyl transferase-I (CPT-I), hormone-sensitive lipase (HSL), myogenin (MyoG) and myostatin (MSTN) mRNA (Table 1), and then it was subjected to PCR for 45 cycles under standard conditions. As an internal control, the same RT products were also subjected to PCR in the presence of a second pair of primers specific to rat β-actin RNA. All samples were analyzed in duplicate using the sequence detection system and programmed to conduct one cycle (95° for 1 min) and 45 cycles (95° for 20 sec, 62° for 30 sec and 72° for 30 sec) or one cycle (95° for 1 min) and 45 cycles (95° for 20 sec, 64° for 30 sec and 72° for 30 sec). Results (fold changes) were expressed as 2-ΔΔCt with ΔΔCt = (Ct ij-Ct ß-actinj)-(Ct i1-Ct ß-actin1), where Ct ij and Ct ß-actinj are the Ct for gene i and for ß-actin in a pool or a sample (named j) and where Ct i1 and Ct ß-actin1 are the Ct in pool 1 or sample 1, expressed as the standard. The primes used

Table 1. Oligonucleotide PCR primers

| Gene | Genbank accession number | Primers sequence(5'–3') | Orientation | Product size[bp] |
|---|---|---|---|---|
| ß-actin | NM031144 | CCCTGTGCTGCTCACCGA ACAGTGTGGGTGACCCCGTC | ForwardReverse | 186 |
| Liver | | | | |
| FAS | NM017332 | GAGGAAGGCCATCGAGTACAGGAAGACAAAGGCACAGAGG | ForwardReverse | 436 |
| SREBP-1c | AF286470 | CGCTACCGTTCCTCTATCATGCCTCGGCTATGTG | ForwardReverse | 434 |
| PPARα | NM013196 | CTGGAAAGTCCCTTATCTGCTTTAGCCGAATAGTT | ForwardReverse | 346 |
| CPT-I | NM031559 | ATGGAGGTTGTCTACGAGTGAAGCCTACTGTTGTT | ForwardReverse | 441 |
| Adipose tissue | | | | |
| FAS | NM017332 | GAGGAAGGCCATCGAGTACAGGAAGACAAAGGCACAGAGG | ForwardReverse | 436 |
| HSL | NM012859 | AGGGCGATGAGGGACTTGGGTCTATGGCGAAT | ForwardReverse | 230 |
| Muscle | | | | |
| MyoG | NM017115.2 | AGCATCACGGTGGAGGACGGTGGGTAGTCTGGC | ForwardReverse | 200 |
| MSTN | NM019151.1 | GCCGTCAAGACTCCTACAACATCAATACTCTGCCAAATACC | ForwardReverse | 139 |

Cr-Pyr, creatine pyruvate;TG, triglyceride;TC, total cholesterol;LDL-C, low density lipoprotein- cholesterol; HDL-C, high density lipoprotein- cholesterol; NEFA, non-esterified fatty acids; GPT, glutamic-pyruvic transaminase; GOT, glutamic-oxaloacetic transaminase; FAS, fatty acid synthase;SREBP-1c, sterol regulatory element-binding proteins-1c; CPT-I, carnitine palmitoyl transferase I; PPARα, peroxisome proliferators-activated receptors α; HSL, hormone-sensitive lipase; MyoG, myogenin; MSTN, myostatin.

were determined as published guidelines (Zhu *et al.* 2007) or designed by Primes Premier 5.

*Statistical analysis:* The experimental data were expressed as the mean±SE, and differences were considered significant when P<0.05, as tested by one-way analysis of variance (ANOVA), using the Statistical Packages for Social Science 16.0 (StatSoft, Inc., USA) and Excel 2003 in Microsoft. Replicate was considered as the experimental unit for the entire index determined.

## RESULTS

*Growth performance and carcass composition*

Although no significantly difference in initial body weight were observed among CG and EGs, final body weight decreased significantly (P < 0.01) when rats supplemented with 3000 mg/kg Cr-Pyr (Table 2). The feed intake of male rats in $EG_1$ and $EG_2$ was lower (P < 0.01) than CG. The fat mass in male rats of EGs, though statistically nonsignificantly lower than those of CG. In contrast, relative leg muscle weight was significantly higher in $EG_2$ and $EG_3$ (P<0.05) than CG (Table 2). Feed conversion ratio between CG and EGs was similar with a mean value of during the experimental period (P>0.05) (Data not shown).

*Lipid metabolites*

HDL-C was (P < 0.05) elevated in $EG_2$, but there were no significant differences in serum TC, TG, LDL-C or NEFA (P>0.05) among rats supplement different doses of Cr-Pyr during the entire experimental period (Table 3). The content of hepatic TC was lower (P<0.01) in $EG_2$, and male rats of $EG_3$ had significantly lower (P<0.05) hepatic TG as compared to those in CG (Table 3).

*Serum metabolic hormones:* Supplementation of male rats with 3000 mg/kg Cr-Pyr significantly (P < 0.05) decreased leptin (ng/mL) but increased (P < 0.05) insulin (μIU/mL).Glucagons (pg/mL) in serum between CG and EGs were similar. Serum IGF-1 (ng/mL) showed no statistically significant difference between CG and EGs, its level elevated in EGs compared to CG (Table 4).

*Lipid metabolic gene mRNA expression:* HSL mRNA expression was higher (P<0.05) in fat tissue of $EG_2$ and $EG_3$ than CG (Fig. 2). Hepatic mRNA expression levels of FAS (Fig. 3A), SREBP-1c (Fig. 3B) and CPT-I (Fig. 3C) were unaffected (P > 0.05) by Cr-Pyr supplementation. However, supplementation 1500 ($EG_2$) and 3000 ($EG_3$) mg/kg Cr-Pyr increased mRNA expression level of PPARα (P < 0.05) in rat liver (Fig. 3D).

Table 2. Effect of creatine pyruvate (Cr-Pyr) on growth performance of the male rat

| Item | Supplemental Cr-Pyr mg/kg BW | | | |
|---|---|---|---|---|
| | 0 | 750 | 1500 | 3000 |
| Initial body weight (g) | 261.5±3.5 | 262.9±3.0 | 259.5±3.2 | 257.5±3.1 |
| Body weight (g) | 424.6±12.4 | 434.2±6.7 | 411.4±8.3 | 388.0±7.4** |
| Daily feed intake (g) | 28.98±0.27 | 29.93±0.22 | 26.69±0.39** | 26.50±0.43** |
| Fat mass | 5.37±0.38 | 5.12±0.32 | 4.58±0.15 | 4.66±0.15 |
| Fat (%)† | 1.283±0.068 | 1.205±0.075 | 1.137±0.043 | 1.216±0.031 |
| Weight of muscle | 15.95±0.83 | 17.02±0.57 | 16.82±0.38 | 16.46±0.62 |
| Muscle (%)†† | 3.822±0.164 | 4.001±0.101 | 4.255±0.088* | 4.208±0.111* |

Relative percentage of either fat mass† or muscle weight†† to body weight. Level of significance: *P < 0.05; **P < 0.01.

Table 3. Effect of creatine pyruvate (Cr-Pyr) on lipid metabolites in the serum and liver of the male rat

| Item† | Supplemental Cr-Pyr mg/kg BW | | | |
|---|---|---|---|---|
| | 0 | 750 | 1500 | 3000 |
| TC(mmol/L) | 1.70±0.10 | 1.75±0.13 | 1.89±0.07 | 1.89±0.09 |
| TG(mmol/L) | 1.42±0.12 | 1.61±0.17 | 1.58±0.21 | 1.78±0.26 |
| LDL-C(mmol/L) | 0.36±0.06 | 0.56±0.15 | 0.50±0.09 | 0.31±0.09 |
| HDL-C(mmol/L) | 1.00±0.06 | 0.98±0.06 | 1.20±0.06* | 1.11±±0.08 |
| NEFA(mmol/L) | 1561.68±135.81 | 1303.21±121.81 | 1217.56±64.17 | 1317.37±124.01 |
| | | Liver | | |
| TC (μmol/g protein) | 53.83±3.29 | 52.03±2.09 | 42.25±3.05** | 50.97±2.57 |
| TG (μmol/g liver) | 8.23±0.60 | 7.61±0.34 | 8.05±0.65 | 6.64±0.45* |

†TG, triglyceride; TC, total cholesterol; NEFA, non-esterified fatty acid; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol.Level of significance: *P < 0.05; **P < 0.01.

Table 4. Effect of creatine pyruvate (Cr-Pyr) on metabolic hormones in the serum of the male rat

| Item | Supplemental Cr-Pyr mg/kg BW | | | |
|---|---|---|---|---|
| | 0 | 750 | 1500 | 3000 |
| Leptin (ng/mL) | 2.424±0.11 | 2.296±0.29 | 1.949±0.23 | 1.728±0.23* |
| Insulin (μIU/mL) | 42.97±2.83 | 39.91±0.72 | 41.38±1.19 | 55.45±8.28* |
| Glucagons (pg/mL) | 789.93±45.28 | 800.90±41.67 | 844.56±49.02 | 766.03±55.12 |
| IGF-1 (ng/mL) | 321.98±51.35 | 389.58±38.89 | 367.49±54.11 | 332.42±26.10 |

Level of significance: *P < 0.05; **P < 0.01.

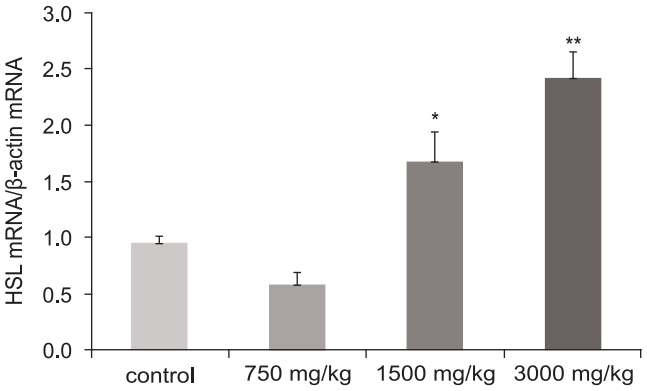


Fig. 2. Effect of Cr-Pyr on hormone-sensitive lipase and fatty acid synthase gene expression in fat tissue of the male rat (Level of significance: *P < 0.05; **P < 0.01).

*Protein metabolites and enzymes*

No significant differences were observed in urea nitrogen and creatinine between CG and EGs. In contrast, significantly (P < 0.05) higher level of serum total protein was evident in $EG_1$. Supplementation of Cr-Pyr resulted in a significant (P < 0.01) increase in the protein content of muscle (Table 5).

Hepatic glutamic-pyruvic transaminase (GPT) increased in EG1, EG2 and EG3 (Fig. 4A). However, Cr-Pyr treatment had no effect on hepatic glutamic-oxaloacetic transaminase (GOT) activity (Fig. 4B).

*Muscle protein metabolic gene mRNA expression:* Supplementation of Cr-Pyr resulted in a significant (P < 0.05) increase in muscle myogenin gene mRNA expression (Fig.5A), whereas muscle mRNA abundance of myostatin as unaffected (P > 0.05) (Fig.5B).

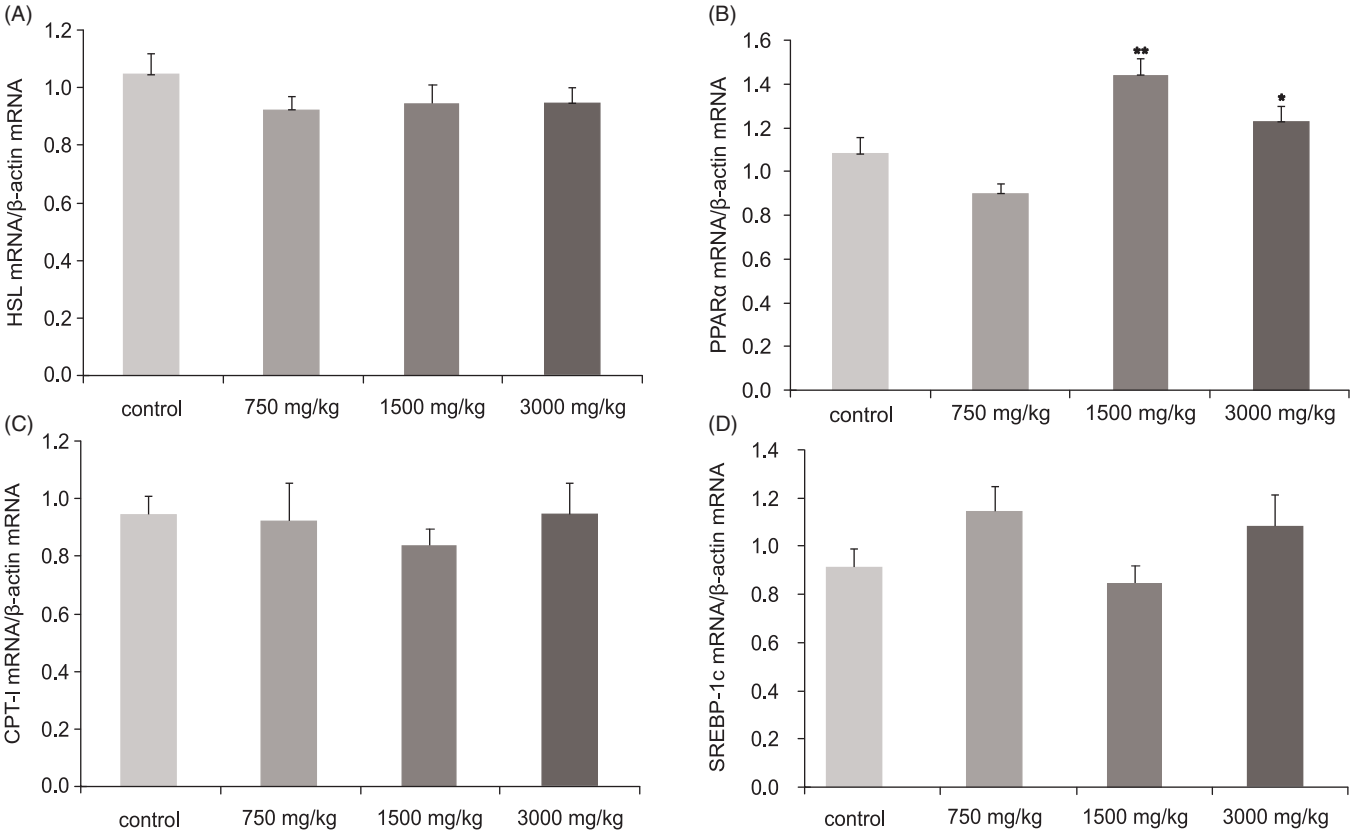


Fig. 3. Effect of Cr-Pyr on fatty acid synthase (A), sterol regulatory element-binding proteins (B), carnitine palmitoyl transferase (C) and peroxisome proliferators-activated receptors (D) gene expression in the liver of the male rat. (level of significance: *P < 0.05; **P < 0.01).

Table 5. Effect of creatine pyruvate (Cr-Pyr) on protein metabolic parameters in serum and muscle of the male rat

| Item | Supplemental Cr-Pyr mg/kg BW | | | |
|---|---|---|---|---|
| | 0 | 750 | 1500 | 3000 |
| Urea nitrogen (mmol/L) | 5.501±0.218 | 5.838±0.321 | 6.128±0.354 | 6.045±0.318 |
| Creatinine (mmol/L) | 78.125±4.706 | 84.907±6.914 | 88.344±6.319 | 77.000±3.407 |
| Total protein (mg/mL) | 75.614±3.339 | 88.006±5.236* | 78.435±2.698 | 73.486±3.179 |
| Blood glucose (mmol/L) | 4.985±0.203 | 4.950±0.212 | 5.123±0.218 | 5.680±0.560 |
| LMPC (mg/g) † | 49.279±3.630 | 57.580±2.882** | 56.746±2.327** | 57.824±1.901** |

† LMPC, leg muscle protein content. Level of significance: *P < 0.05; **P < 0.01.

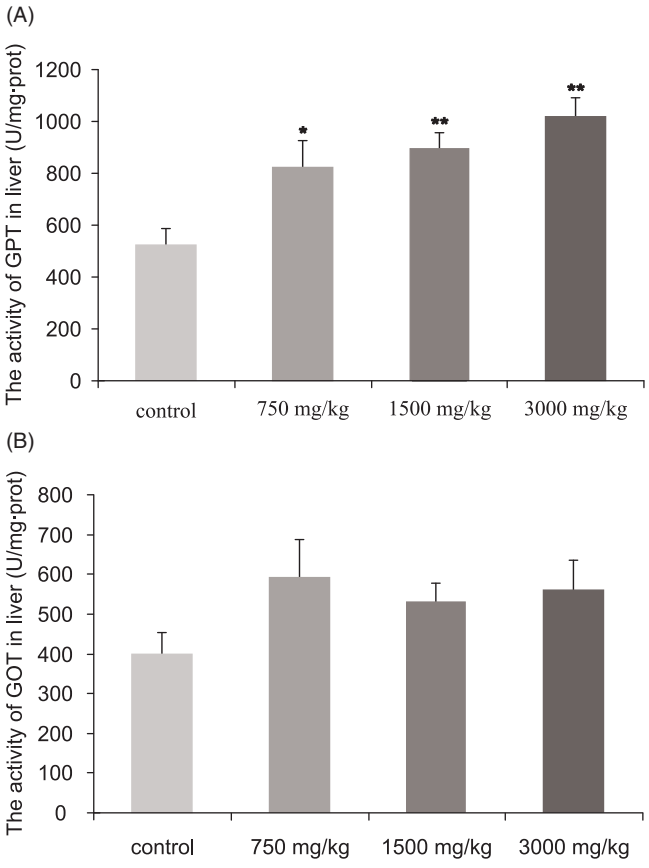


Fig. 4. Effect of Cr-Pyr on the activity of GPT (Subfig A) and GOT (Subfig B) in the liver of the male rat. (Level of significance: *P < 0.05; **P < 0.01).

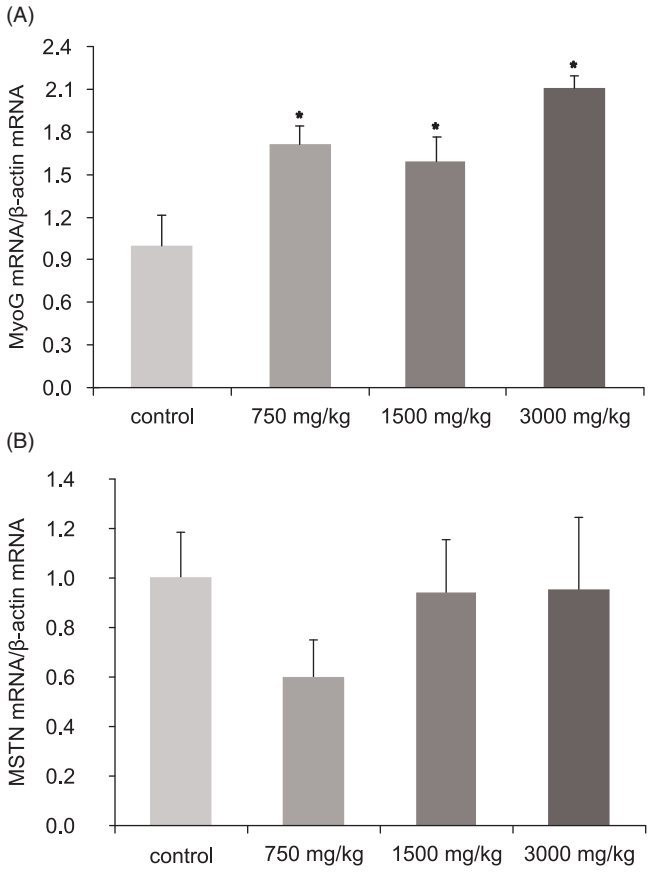


Fig. 5. Effect of Cr-Pyr on MyoG (A) and MSTN (B) gene expression in the muscle of the male rat. (Level of significance: *P < 0.05; **P < 0.01).

## DISCUSSION

The study explored the effect of creatine pyruvate (Cr-Pyr) on lipid metabolism and nitrogen metabolism in male rats. To the best of our knowledge, scarcely any reports are available to understand the effect of graded levels (750, 1500 and 3000 mg/kg) of Cr-Pyr supplementation on hormone levels, growth performance, lipid and nitrogen metabolites and enzymes, and the mRNA levels of relevant genes in male rats. The amount of supplement executed in the experiment was though on higher side, dose was increased incrementally to translate the metabolic impacts of Cr-Pyr supplementation at higher magnitude.

Significant (P<0.01) decrease in weight gain in EG3 was manifestation of significant decrease (P<0.01) in feed intake. Similarly in EG2 feed intake significantly lesser however, weight gain decline was not statistically significant. These results are consistent with the observations of Johnstone *et al.* (1989), supplemented pyruvate/dihydroxyacetone (1:1) in the diet at 10%. Cortez *et al.* (2001) also reported administration of pyruvate to rats for 3–5 weeks resulted in decreased body weight. Furthermore, rats fed Cr-Pyr showed

decreased absolute fat mass and the ratio of fat weight to body weight and higher muscle weight and the individual muscle to body weight ratio. This is more prominent in EG2 and EG3 where Cr-Pyr dose was 1500 and 3000 mg/kg. These observations are consistent with previous reports that found that pyruvate augmented lean body mass and decreased fat mass (Koh-Banerjee *et al*. 2005) and that creatine supplementation improved upper-body strength and decreased the percentage body fat in lacrosse players during preseason training (Brenner *et al.* 2000). With the reduced abdominal fat and increased leg muscle as a proportion of total carcass, we presumed that Cr-Pyr supplementation reduced the fat deposition, meanwhile, increased in muscle mass.

Significant decrease in hepatic TC in EG2 ($P< 0.01$) and TG ($P< 0.01$) in EG3 indicated change in the fat metabolism in both the groups (Zhan *et al.* 2006). However, serum TC and TG were unaffected by Cr-Pyr treatment, and the serum concentrations of free fatty acids, indicative of enhanced lipolysis, did not differ significantly among the experimental groups. Effect of Cr-Pyr on liver TG observed in this study was comparable to supplements of pyruvate, DHA and/or riboflavin (Goheen *et al*. 1981). Interestingly Cr-Pyr supplementation increased serum HDL-C content in $EG_2$ while the LDL-C content was unchanged. Earlier reports also indicated that pyruvate promotes fat loss and increases HDL-C (Olson *et al.* 1991). Based on these observations, it is clear that Cr-Pyr supplementation increased lipolysis however, mechanism behind metabolic shift in substrate utilization is not clear.

Pyruvate has been documented to have various functions in the regulation of lipid metabolism, including increased utilization of fat and an elevation in the resting metabolic rate (Cortez *et al.* 2001). Supplementation of Cr-Pyr in $EG_2$ and $EG_3$ resulted in a significant up-regulation of HSL expression in adipose tissue. This is consistent with results of Cortez *et al*. (2001), who found that supplemented pyruvate and DHA increased fat utilization in rats. HSL, an enzyme that catalyses the hydrolysis of triglycerides into NEFAs and monoglycerides, those are further metabolized to NEFA and glycerol by monoglyceride lipase (Haemmerle *et al*. 2002). The plasma NEFA concentration reflects a balance between adipose tissue lipolysis and uptake (oxidation and esterification) by the peripheral tissues. It is worth noting that supplementation with Cr-Pyr had no effect on serum NEFA, which suggested that NEFA are oxidized by peripheral tissues in male rats. Similar to the result for HSL, hepatic PPARα mRNA expression also significantly increased in $EG_2$ and $EG_3$. Rocchi and Auwerx (2000) demonstrated that PPARα can increase the rate of ß-oxidation by increasing the expression of several PPARα target genes. Based on the above results, we could speculate that Cr-Pyr may increase the expression of the lipolytic gene HSL, which subsequently promotes triacylglycerol mobilization and expresses PPARα, resulting in a higher rate of ß-oxidation and reduced fat accumulation.

In this study, supplementation of 3000 mg/kg Cr-Pyr decreased serum leptin and increased plasma insulin, but it had no effect on glucagons. Leptin is secreted by adipose tissue and play an important role in mammalian feed intake regulation, energy metabolism and reproduction (Sun *et al.* 2006). It is well established that the pancreatic hormones insulin and glucagons regulate intermediary metabolism in animals, and Taouis *et al.* (2001) have reported that glucagons may exert its effect on liver leptin by elevating intracellular cyclic AMP. Moreover, Schwartz *et al*. (2000) have demonstrated the interaction of leptin and insulin increases energy expenditure and the basal metabolic rate, resulting in a reduction in body weight. Study revealed that Cr-Pyr promoted lipolysis while elevated plasma insulin increased the metabolic rate through a feed back mechanism that is responsive to decreased serum leptin levels which lead to stabilization of serum glucose.

Cr-Pyr supplementation (Table 5) significantly increased total leg muscle protein. Cr-Pyr also increased the ratio of muscle weight to body weight in male rats, which suggested that Cr-Pyr stimulated protein synthesis. Supplementation with CMH increased weight gain, and that this was due to an increase in muscle protein synthesis. In addition, pyruvate was found involved in the inter-conversion of amino acids by influencing transamination pathways. In our study, only GPT activity increased, while the activity of GOT was unaffected by Cr-Pyr. The higher level of GPT following Cr-Pyr treatment may have been due to the availability of substrate for protein synthesis. Most amino acids may undergo transamination reactions, and transaminase enzymes, which are both cytosolic and mitochondrial, can be induced by high protein diets.

Creatine supplementation increased protein synthesis in ovine myogenic satellite cells (Vierck *et al.* 2003) and chick embryos (Young *et al.* 2007) are mainly due to increased IGF-1 levels which stimulate the proliferation and protein synthesis in muscle cells (Oksbjerg *et al.* 2006). The level of IGF-I in plasma from rats was not significantly affected by Cr-Pyr supplementation, but rats supplemented with 750, 1500 and 3000 mg/kg Cr-Pyr had 20.99%, 14.14% and 3.24% higher levels of the hormone than were evident in the CG rats, respectively. This was accompanied by an increase in relative leg muscle weights. In addition, the protein content of leg muscle was significantly increased by adding Cr-Pyr. Besides IGF-I, MyoG expression level is closely related to muscle growth and MSNT act as a negative regulator of muscle development (Louis *et al.* 2004). Although MSTN gene mRNA expression was unaffected, supplementation with Cr-Pyr significantly increased MyoG gene mRNA expression in muscle. Young *et al.* (2007) reported that supplementation with CMH decreased the expression of MNST in swine muscle. Other studies suggested that CMH

may increase differentiation and protein synthesis (Vierck *et al.* 2003).

In summary, supplementation of Cr-Pyr was accompanied by significant elevated levels of lipolysis and increased muscle protein synthesis in male rats. This study improves understanding of the mechanism of the Cr-Pyr effect in the rat and could be useful in the prevention of obesity-related problems and in exploring ways to improve muscle growth. However, elucidation of the precise mechanism involved in the regulation of Cr-Pyr will require further investigation.

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