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# Effects of the Combination of Glucose, Chromium Picolinate, and Vitamin C on Lipid Metabolism in Steers*

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**ABSTRACT :** This study was conducted to examine the effects of glucose, chromium picolinate (CrP), and vitamin C (Vit C) on lipid metabolism in Korean native steers fitted with indwelling catheters. A total of 12 Korean native steers were randomly allocated to the following treatments: 1) normal control diet, 2) same as 1) +250 g of glucose by intravenous (IV) infusion, 3) same as 2)+13.5 g CrP administered orally, and 4) same as 3)+2.52 g Vit C by IV infusion. Glucose, Vit C, and CrP treatments were administered for five days. At days 1 and 3, serum insulin was higher in treated animals than in those fed the control diet ($p<0.05$). Serum non-esterified fatty acid (NEFA) concentration in the steers on treatment 2), control+13.5 g CrP, was lower than those on other treatments at 90 min post-infusion on days 1 and 3 ($p<0.05$). The expression of peroxisome proliferator-activated receptor-γ (PPARγ)2, stearoyl-CoA desaturase-1 (SCD), fatty acid synthase (FAS), and glucose transporter type 4 (Glut 4) in the longissimus muscle of steers on treatment 2 was higher than those on other treatments. In conclusion, the results suggest that CrP is associated with the regulation of gene expression involved in adipogenesis. (**Key Words :** Chromium Picolinate, Glucose, Lipid Metabolism, Steers, Vitamin C)

## INTRODUCTION

The amount of adipose tissue in muscle depends primarily on the number and size of the constituent adipocytes. Although proliferation and differentiation of pre-adipocytes takes place and is almost completed in the perirenal and subcutaneous adipose tissues of cattle by the first year of age, intramuscular adipose tissue is actively proliferated and differentiated in beef steers until 14-months-of-age. Cianzio et al. (1985) reported that the number of adipocytes in the longissimus muscle increased during 13 to 19-months-of-age and correlated with beef marbling. Previous scientific literature indicated a substantial role for glucose, chromium picolinate (CrP), and vitamin C (Vit C) in intramuscular fat deposition (Smith et al., 1984; Toriisin et al., 1995).

Smith and Crouse (1984) reported that intramuscular fat deposition increased with a higher supply of glucose.

In addition, chromium (Cr) is known as an essential nutrient for humans and animals (Mertz et al., 1993). The main physiologic role of Cr is to increase insulin action or sensitivity in peripheral tissues (Anderson, 1998). In general, dietary Cr supplementation increases cellular uptake of glucose (Mooradian et al., 1997). Although, domestic animals are not considered to require dietary Vit C because they can synthesize a sufficient amount in the liver (McDowell, 1989), a dietary supplement of L-ascorbic acid-2-phosphate during the late fattening stage produced a higher marbling score in Japanese Black cattle (Ohashi et

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al., 2000). Vit C also enhanced bovine preadipocyte proliferation and differentiation *in vitro* and improved insulin secretion in broiler chickens (Toriisin et al., 1995; Sahin et al., 2003).

To our knowledge, the effect of the combination of CrP, Vit C, and glucose on lipid metabolism in steers is unknown. Therefore, this study was designed to evaluate the physiological responses of CrP, Vit C, and glucose on lipid metabolism in Korean native steers.

## MATERIALS AND METHOD

### Animals, diets, experimental design, and procedures

A total of 12 Korean native steers, 12-months-of- age, and weighing 516 kg±2.8 kg were used in this study.

A jugular vein catheter was inserted into each animal four days before the start of the experiment. Steers were fed 10 kg DM of basal diet (90% concentrate and 10% alfalfa hay) twice daily. The steers were randomly assigned to four treatment groups: (3 animals per treatment) i) basal control diet, ii) basal diet+250 g glucose, iii) treatment 2+13.5 g CrP and iv) treatment 3+2.52 g Vit C. Glucose and Vit C (ascorbic acid; Sigma, Germany) were infused for five days through an indwelling catheter fitted in the jugular vein and CrP was provided orally in encapsulated form for five days.

CrP was fed along with the basal diet at 07:00 h and infusion of glucose and Vit C was carried out at 10:00 h. Maximum efficiency of absorption of Cr from CrP was 2.8%. Since Cr is quickly lost through urine, its toxicity was not a problem in this study. However, direct infusion of Cr into the vascular system can cause toxicity, thus in this study, coated CrP was supplemented to reduce toxic effects and improve absorption.

Glucose and Vit C were infused through the jugular vein catheter within 45 min using a 50-ml syringe. At days 1 and 3 blood samples were collected and at day 5 muscle tissue was taken by biopsy.

All experimental procedures were in accordance with the "Guidelines for the Care and Use of Experimental Animals of Seoul National University", formulated according to the "Declaration of Helsinki" and "Guiding Principles in the Care and Use of Animals".

### Blood sample collection and analysis

Blood samples were collected for serum glucose and insulin assay at -30, -20, -10, 0, 15, 30, 45, 60, 90, 120, and 180 min from the administration of glucose, CrP, Vit C, or saline solution on days 1 and 6 of the experiment.

Blood samples were collected from an indwelling catheter fitted in the jugular vein using a 20-ml syringe and transferred to BD vacutainers (Becton Dickenson Co., Franklin Lakes, NJ, USA). The blood was centrifuged at 3,000 rpm for 15 min at 4°C to harvest serum, which was stored at -20°C for latter analysis. Serum glucose, triglyceride (TG), non-esterified fatty acids (NEFA), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were estimated using commercial kits from Wako Pure Chemical Industries (Osaka, Japan). The concentration of serum insulin was measured with specific bovine insulin enzyme-linked immunosorbent assay (ELISA) kits (Mercodia Co., Sweden).

### Longissimus muscle tissue sampling

Longissimus muscle tissue samples were collected to analyze the expression of genes related to intramuscular fat deposition. At day 5 skeletal muscle tissues samples were obtained from the longissimus dorsi at a 6-cm depth near the thirteenth rib by biopsy after local anesthesia using a spring-loaded biopsy instrument (Biotech, Republic of Slovakia) as described by Cheah et al. (1997). The collected muscle tissue was snap-frozen in liquid nitrogen and stored at -80°C until analyzed.

### Real-time polymerase chain reaction (PCR) analysis

Total RNA was isolated from muscle tissue using TRIzol® Reagent (Invitrogen Life Technologies, USA) in accordance with the manufacturer's instruction. First-strand complementary DNA (cDNA) was synthesized from total RNA (3 μg) using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) (Fermentas) with oligo (dT)15 (Promega) primer in a 20 μl reaction mixture. Real-time PCR was carried out in 20 μl of reaction solution containing 10 μl SYBR Green (Bio-Rad), 0.4 μl left primer, 0.4 μl right primer (each of the relevant bovine-specific primers; Table 1), 2.29 μg cDNA, others were double distilled water. Reactions were initiated at 95°C for 3 min, followed by 40 cycles of PCR at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The threshold cycles (Ct) for the internal control and genes of interest were determined and relative RNA levels were calculated by the ΔΔCt method where ΔΔCt is the ΔCt of the gene in treatment minus the ΔCt of the gene in control. ΔCt is the Ct of the gene of interest minus the Ct of internal control. The ΔΔCt values were used to calculate $2^{-[\Delta\Delta Ct]}$. β-Actin was used as an internal control. All results were obtained from at least three independent experiments.

### Statistical analysis

Statistical evaluation was made using analysis of variance (ANOVA) and the SAS program. Mean values among treatments were compared by S-N-K Test (Steel et al., 1980).

**Table 1.** Marker genes used in real-time polymerase chain reaction

| Gene | Primer nucleotide sequence | Product size (base pair) |
|---|---|---|
| β-actin | Forward 5' CGCACCACTGGCATTGTCAT3'<br>Reverse 5' TCCAAGGCGACGTAGCAGAG3' | 227 |
| PPAR $\gamma_2$ | Forward 5' CGCACTGGAATTAGATGACAG3'<br>Reverse 5' CACAATCTGTCTGAGGTCTGT3' | 214 |
| Glut 4 | Forward 5' TTTCTTCTATTCGCGGTCCT3'<br>Reverse 5' CCTGCTCCAGAAGAGAAGGT3' | 130 |
| SCD | Forward 5' CCTGGTGTCCTGTTGTTGT3'<br>Reverse 5' GGTAGTTGTGGAAGCCCTC3' | 244 |
| aP2 | Forward 5' CTGGCATGGCCAAACCCA3'<br>Reverse 5' GTACTTGTACCAGAGCACC3' | 186 |
| FAS | Forward 5' TGATGGCCTACACTCAGAGC3'<br>Reverse 5' GGGCCTCCAGCACTCTACTA3' | 129 |

PPAR-γ = Peroxisome proliferator-activated receptor-γ; Glut 4 = Glucose transporter type 4; SCD = Stearoyl-coenzyme A desaturase; aP2: adipocyte fatty-acid-binding protein; FAS = Fatty acid synthase.

## RESULTS

### Effects on serum glucose concentration

Serum glucose levels are presented in Table 2. On days 1 and 3, blood glucose concentrations were similar in control and treated steers. At day 1, jugular infusion significantly affected ($p<0.05$) the blood glucose concentration in steers at 30, 45, 60, and 90 min post-infusion. On day 3, blood glucose concentration was significantly different ($p<0.05$) among control and treated steers at 15, 30, 45, and 60 min post-infusion. On days 1 and 3, the steers in treated groups had higher ($p<0.05$) blood glucose than those in the control group (Table 2).

### Effects on serum insulin concentration

Serum insulin levels are presented in Table 3. On days 1 and 3, basal concentrations of serum insulin were similar in control and treated steers. Glucose infusion significantly increased ($p<0.01$) serum insulin at 15 min post-infusion. A similar pattern of serum insulin was observed after glucose infusion on day 3. On days 1 and 3, steers in treated groups had higher ($p<0.05$) insulin levels than those in the control group (Table 3). No difference in serum insulin levels was observed in steers in treatment groups 1, 2, and 3 although insulin levels in steers in treatment group 3 were numerically higher than in other treatment groups.

### Effects on serum HDL-C, LDL-C, TG, and NEFA concentrations

Serum HDL-C, LDL-C, TG, and NEFA levels are presented in Table 4. Serum basal and post-infusion (at 90 min) HDL-C, LDL-C, and TG concentrations in steers were not affected by treatments on days 1 and 3. Basal concentration of serum NEFA was similar in control and treated steers on day 1 and 3; however, the concentration was significantly different ($p<0.05$) in control and treated steers at 90 min post-infusion. Steers on treatment 3 had similar serum NEFA concentrations as controls and significantly higher levels than animals in treatment groups 1 and 2.

### Effects on gene expression related adipogenesis in longissimus muscle tissue

Longissimus muscle tissue gene expression related to

**Table 2.** Effects of glucose administration with vitamin C (Vit C) and chromium picolinate (CrP) supplementation on serum glucose levels at days 1 and 3 in Korean native steers (mg/dl)

| Items | | Treatments[1] | | | | SEM[2] | p value |
|---|---|---|---|---|---|---|---|
| | | Control | T1 | T2 | T3 | | |
| Day1 | Total AUC[3] | 12,125.50[b] | 25,057.07[a] | 23,434.43[a] | 26,461.30[a] | 1,865.46 | 0.0079 |
| | Incre AUC[4] | -1,887.73[b] | 11,545.73[a] | 8,950.33[a] | 14,613.13[a] | 2,044.17 | 0.0053 |
| Day3 | Total AUC[3] | 12,646.40[b] | 21,870.27[a] | 23,710.27[a] | 25,518.77[a] | 1,822.86 | 0.0187 |
| | Incre AUC[4] | -203.57[b] | 10,518.10[a] | 10,026.60[a] | 14,302.37[a] | 1,818.88 | 0.0081 |

[1] All values represent the mean of triplicates. [2] Standard error of the mean.
[3] Area was calculated for 180 min (between 0 and 180) periods. [4] Area was calculated for the basal AUC for 180 min.
[a, b] Means in the same row with different superscripts differ ($p<0.05$).
Control = Group treated with saline.
T1 = Group treated with glucose. T2 = Group treated with glucose and CrP. T3 = Group treated with glucose, CrP, and Vit C.
AUC = Area under the curve.

**Table 3.** Effects of glucose administration with Vit C and CrP supplementation on serum insulin levels on days 1 and 3 in Korean native steers (μg/L)

| Items | | Treatments[1] | | | | SEM[2] | p value |
|---|---|---|---|---|---|---|---|
| | | Control | T1 | T2 | T3 | | |
| Day 1 | AVG | 1.12[b] | 6.95[a] | 7.16[a] | 6.49[a] | 0.822 | 0.0032 |
| | Incre[3] | -0.54[b] | 4.16[a] | 4.71[a] | 5.11[a] | 0.702 | 0.0001 |
| | Total AUC[4] | 198.43[b] | 129.51[a] | 1,307.20[a] | 1,212.73[a] | 156.183 | 0.0051 |
| | Incre AUC[5] | -101.87[b] | 780.55[a] | 865.80[a] | 964.10[a] | 131.018 | 0.0001 |
| Day 3 | AVG | 1.08[b] | 6.03[a] | 6.19[a] | 5.65[a] | 0.662 | 0.0005 |
| | Incre[3] | -0.48[b] | 4.28[a] | 4.10[a] | 4.47[a] | 0.665 | 0.0006 |
| | Total AUC[4] | 185.57[b] | 1,061.57[a] | 1,180.10[a] | 1,123.03[a] | 1,126.992 | 0.0004 |
| | Incre AUC[5] | -95.33[b] | 746.77[a] | 811.87[a] | 911.37[a] | 127.599 | 0.0004 |

[1] All values represent the mean of triplicates. [2] Standard error of the mean.
[3] AVG was corrected for the basal average for 180 min. [4] Area was calculated for 180 min (between 0 and 180) periods.
[5] Area was corrected for basal AUC for 180 min.
[a, b] Means in the same row with different superscripts differ ($p<0.05$).
Control = Group treated with saline.
T1 = Group treated with glucose. T2 = Group treated with glucose and CrP. T3 = Group treated with glucose, CrP, and Vit C.

**Table 4.** Effects of glucose administration with Vit C and CrP supplementation on serum parameters at days 1 and 3 in Korean native steers (mg/dl)

| Items | | | Treatments[1] | | | | SEM[2] | p value |
|---|---|---|---|---|---|---|---|---|
| | | | Control | T1 | T2 | T3 | | |
| Day 1 | HDL | 0 min | 153.30 | 171.30 | 186.13 | 122.90 | 9.792 | 0.1632 |
| | | 90 min | 155.10 | 162.90 | 193.20 | 125.2 | 9.343 | 0.0939 |
| | LDL | 0 min | 34.67 | 36.00 | 37.67 | 24.67 | 2.434 | 0.3494 |
| | | 90 min | 34.67 | 33.67 | 39.00 | 25.00 | 2.258 | 0.2609 |
| | TG | 0 min | 14.67 | 18.67 | 18.67 | 13.33 | 1.263 | 0.3573 |
| | | 90 min | 14.67 | 13.33 | 12.67 | 11.67 | 0.883 | 0.7086 |
| | NEFA | 0 min | 0.31 | 0.19 | 0.16 | 0.38 | 0.053 | 0.0831 |
| | | 90 min | 0.25[a] | 0.09[b] | 0.06[b] | 0.19[a] | 0.028 | 0.0075 |
| Day 3 | HDL | 0 min | 145.40 | 153.10 | 175.83 | 119.47 | 9.156 | 0.2132 |
| | | 90 min | 148.60 | 162.47 | 174.03 | 122.90 | 9.065 | 0.2832 |
| | LDL | 0 min | 29.67 | 31.00 | 33.00 | 24.67 | 2.109 | 0.6611 |
| | | 90 min | 30.33 | 33.33 | 32.67 | 25.00 | 2.244 | 0.6767 |
| | TG | 0 min | 14.67 | 14.67 | 21.67 | 18.00 | 1.577 | 0.5002 |
| | | 90 min | 15.00 | 14.00 | 13.33 | 16.67 | 0.871 | 0.6030 |
| | NEFA | 0 min | 0.52 | 0.16 | 0.18 | 0.27 | 0.082 | 0.4271 |
| | | 90 min | 0.19[a] | 0.10[b] | 0.09[b] | 0.16[a] | 0.016 | 0.0135 |

[1] All values represent the mean of triplicates. [2] Standard error of the mean.
[a, b] Means in the same row with different superscripts differ ($p<0.05$).
Control = Group treated with saline.
T1 = Group treated with glucose. T2 = Group treated with glucose and CrP. T3 = Group treated with glucose, CrP, and Vit C.
HDL = High-density lipoprotein; LDL = Low-density lipoprotein; TG = Triglyceride; NEFA = Non-esterified fatty acid.

adipogenesis is presented in Table 5. The expression of peroxisome proliferator-activated receptor-γ (PPARγ)2, stearoyl-CoA desaturase-1 (SCD), and fatty acid synthase (FAS) in the longissimus muscle of steers on treatment 2 was higher than those on other treatments. The expression of adipocyte protein 2 (aP2) was similar in all experimental steers.

## DISCUSSION

Serum insulin concentration is related to serum glucose concentration in cattle. Under normal physiological conditions, the insulin concentration increases in serum with increasing levels of glucose that generally follows meal patterns. In the present study, a jugular infusion of glucose caused a surge in insulin secretion on day 1 of treatment. A similar pattern of serum insulin in steers was observed on infusion day 3. Although no significant differences were observed in serum insulin levels in steers on treatments 1, 2, and 3, the serum insulin level of steers on treatment 3 was numerically higher than that of the other treatments (Table 2). These results provide evidence that Vit C and CrP may be important factors that increase insulin secretion. The same results were reported by Sahin et al. (2003) in broiler chickens. The mechanism for glucose-induced insulin secretion from pancreatic β cells involves at least two signaling pathways, potassium ($K^+$)-ATP channel-dependent and independent. In the former, pancreatic β cells sense glucose concentration through its metabolism, which increases the ATP/ADP ratio. This increase closes the ATP-sensitive $K^+$-ATP channel causing plasma membrane depolarization and activation of voltage-dependent calcium ($Ca^{2+}$) channels thereby increasing $Ca^{2+}$ entry and stimulating insulin release. The $K^+$-ATP channel-independent pathway involves the opening of $K^+$-ATP channels with diazoxide and restores $Ca^{2+}$ influx by depolarizing the membrane with a high accumulation of extracellular $K^+$. Under these conditions glucose still caused a concentration-dependent increase of insulin release.

Previous literature has revealed that Vit C supports insulin secretion from pancreatic islets (Wells et al., 1995). The release of insulin from pancreatic islets in response to D-glucose is dependent on the normal availability of ascorbic acid which involves voltage-dependent $Ca^{2+}$ channels which increase cellular $Ca^{2+}$ entry (Parsey et al., 1993). However, the effect of CrP on insulin secretion is not clear and requires further experimentation. Insulin plays a pivotal role in the uptake of glucose by the cells (Stryer et al., 1995). Skeletal and muscle cells are the main consumers of glucose in the body (Pethick et al., 1984). Contrarily, adipose tissue in the ruminant accounts for only minor (1%) glucose disposal of the total amount of glucose utilization (Pethick et al., 1984). Smith and Crouse (1984) demonstrated that glucose provides 50-75% of the acetyl units for adipogenesis in intramuscular fat deposition but only 1 to 10% of the acetyl units for adipogenesis in subcutaneous fat. Thus, increased blood glucose could increase intramuscular fat deposition, without markedly affecting subcutaneous fat deposition.

Several transcription factors that are important players in adipogenesis were analyzed by real-time PCR using longissimus muscle tissues. PPARs are a subclass of the nuclear hormone receptor super family and are therefore ligand-activated transcription factors (Berger et al., 2002). PPARγ is expressed at a high level in adipose tissue and is considered a master regulator of adipogenesis (Tontonoz et al., 1994). PPARδ, also called PPARβ, is expressed in numerous tissues but more abundantly in lipid metabolizing tissues, such as muscle, intestine, or white adipose (Amri et al., 1995; Poirier et al., 2001). In such tissues, PPARδ regulates the expression of genes implicated in fatty acid uptake and metabolism (Bastie et al., 1999). A variety of substances have been suggested to be natural ligands for PPARγ, including fatty acids and eicosanoids, components of oxidized low-density lipoproteins, and oxidized alkyl phospholipids including lysophosphatidic acid and nitro-linoleic acid (Nagy et al., 1998; Desvergne et al., 1999; McIntyre et al., 2003). The prostaglandin J2 derivative, 15-deoxy-12,14-PGJ2, does not naturally exist at sufficient concentrations to activate PPARγ in mammalian cells and

**Table 5.** Relative fold induction of genes in muscle tissue (Mean fold change*)

| | Treatments[1] | | | |
|---|---|---|---|---|
| | Control | T1 | T2 | T3 |
| PPARγ2 | 1.02±0.209 | 0.60±0.177 | 1.30±0.412 | 0.60±0.062 |
| SCD | 1.00±0.000 | 0.57±0.347 | 1.40±0.176 | 0.88±0.348 |
| FAS | 1.00±0.000 | 1.04±0.898 | 1.50±0.189 | 0.76±0.385 |
| aP2 | 1.02±0.209 | 0.85±0.518 | 0.93±0.178 | 0.77±0.462 |

[1] All values represent the mean of triplicates.

T1 = Glucose, T2 = Glucose+CrP, T3 = Glucose+CrP+Vit C.

PPAR-γ2 = Peroxisome proliferator-activated receptor-γ; SCD = Stearoyl-coenzyme A desaturase; FAS = Fatty acid synthase; aP2 = adipocyte fatty-acid-binding protein.

* Relative fold induction was calculated using the $\Delta\Delta Ct$ method.

affects cellular pathways other than PPARγ (Straus et al., 2000; Bell-Parikh et al., 2003).

Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme in the cellular synthesis of unsaturated fatty acids from saturated fatty acids (Ntambi, 1999). In addition, the unsaturated fatty acids can function as ligands for PPARα and PPARγ (Kliewer et al., 1997). Therefore, SCD can increase cellular content of ligands for PPAR and induce its expression. Adipocyte fatty acid-binding protein or aP2, a-FABP or FABP4, is a marker of preadipocyte differentiation into adipocytes and is transcriptionally regulated by PPARγ (Bernlohr et al., 1985; MacDougald et al., 1995). FAS, which includes the enzymes of the fatty acid synthesis elongation cycle, is present in a single polypeptide chain, multifunctional enzyme complex in eukaryotes also involved in adipocyte differentiation at different stages. Those adipocyte specific genes regulate free fatty acid accumulation, thereby leading to the characteristic phenotype of the mature adipocyte.

In the present study, the expression of PPARγ2, SCD, and FAS in the longissimus muscle of steers receiving treatment 2 was higher than in other treatments, whereas the expression of aP2 in treatment 2 was not higher than control. The difference may not have appeared significant among treatments due to the fact that the study was conducted for only a few days. Low expression of aP2 in the treatment 2 group may be caused by insufficient natural ligands for PPARγ2 in the longissimus muscle. CrP also did not significantly induced SCD, and thus the synthesis ligands for PPARγ2 were of a smaller magnitude. As a result, only adipocyte number and size were increased; however, fatty acid deposition was not significantly increased.

In treatment 3, the addition of Vit C decreased PPARγ2, SCD, FAS, and aP2 gene expression in contrast to control, which implies that Vit C could not enhance the accumulation of intramuscular fat in Korean native steers. These findings are contradictory to the findings of Toriisin et al. (1995). In the present study, an increase in adipocyte differentiation with CrP supplementation probably reduced the serum NEFA level with no effect on serum HDL-C, LDL-C, and TG concentrations. A lower serum NEFA concentration is an established indicator of a lipogenic activity and/or reduced lipolysis.

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