Arch. Tierz., Dummerstorf **49** (2006) Special Issue, 41-51

[1]Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, 02-776 Warsaw, Nowoursynowska 159
[2]Department of Physical-Chemical Analyses, Faculty of Animal Sciences, Warsaw Agricultural University, 02-786 Warsaw, Ciszewskiego 8

MAŁGORZATA ŁOKOCIEJEWSKA[1], JOLANTA WAGNER[2], JOANNA ZARZYŃSKA[1], MICHAŁ JANK[1], PIOTR OSTASZEWSKI[1], ANNA BURDZIŃSKA[1], TOMASZ SADKOWSKI[1], JUSTYNA OLCZAK[1], ANNA MRÓWCZYŃSKA[1] and ARKADIUSZ ORZECHOWSKI[1]

Sodium ascorbate (ASC) and ascorbic acid phosphate (ASC-P) differently modulate glucocorticoid-dependent metabolic effects in growing rats

Abstract

It is well known that dexamethasone treatment in certain doses induces oxidative stress, insulin resistance, diabetes, and muscle cachexia. This survey was carried out to investigate the effect of ascorbate derivatives on dexamethasone-induced metabolic disturbances. Experiment was performed on 6 weeks old male rats. Oral dose of sodium ascorbate (600 mg/kg b.w., ASC) or ascorbic acid phosphate (785 mg/kg b.w., ASC-P) was given separately (BID) or as co-treatment with dexamethasone phosphate (daily dose of 2 mg/kg b.w., DEX). Rats were randomly divided into control and experimental groups and the effect of 5-day treatment without (CTRL) or with ASC, ASC-P, DEX, or DEX combined with ASC or with ASC-P (Treatment) was compared with respect to indices of animal growth, somatic indices and results of glucose tolerance test. The effects of 5-day treatment and 5-day recovery period (when none of the experimental factors was used) were compared. Administration of DEX caused significant decline of serum ascorbate and dehydroascorbate (-87%, $P<0.001$). This was in part corrected by ASC or ASC-P co-treatment with DEX (-68%, $P<0.001$). ASC and ASC-P were of minor importance to limit DEX-induced growth retardation although they enhanced sensitivity to insulin at least at the level of glucose utilization ($P<0.001$). ASC-P was superior to ASC in sensitization to insulin ($P<0.05$). DEX significantly raised somatic indices (SI) of liver (+52%), kidneys (+44%), heart (+52%) and soleus muscles (+44%) but not gastrocnemius muscles ($P>0.05$). In contrast, spleen SI dropped significantly upon DEX treatment (-57%). After 5-day recovery period DEX-altered SI-s did not return to control values ($P<0.05$). Neither ASC nor ASC-P affected SI-s nor they could reverse DEX-induced changes in SI-s except ASC-P which confined the rise of renal SI ($P<0.05$). ASC in contrast to ASC-P even augmented DEX-dependent hepatomegaly ($P<0.05$). Interestingly, both ascorbate derivatives efficiently inhibited DEX-induced muscle cachexia at least with respect to gastrocnemius muscle ($P<0.05$). Summing up, these results suggest differences between the fast and slow effects evoked by ascorbate in the experimental model of growth retardation and muscle cachexia induced by DEX and accompanied by glucocorticoid-dependent diabetes.

Key Words: ascorbate, ascorbic acid phosphate, glucocorticoids, growth, muscle cachexia, insulin resistance

Zusammenfassung

Titel der Arbeit: **Natriumascorbat (ASC) and Ascorbinsäurephosphat (ASC-P) beeinflussen Glukokortikoid-abhängige metabolische Prozesse bei wachsenden Ratten unterschiedlich**

Es ist etabliert, dass Dexamethasonbehandlung Dosis-abhängig oxidativen Stress, Insulinresistenz, Diabetes und Muskelkachexie verursacht. Hier wurde der Effekt von Ascorbatderivaten auf Dexamethason-ausgelöste Stoffwechselauslenkungen bei 6 Wochen alten männlichen Ratten untersucht. Natriumascorbat (600 mg/kg, ASC) and Ascorbinsäurephosphat (785 mg/kg, ASC-P) wurden oral einzeln oder in Kombination mit Dexamethasonphosphat (2 mg/kg, DEX) verabreicht. Ratten wurden zufällig der Kontroll- bzw. den Versuchsgruppen zugewiesen und hinsichtlich Wachstum, Glukosetoleranz und somatischen Indizes nach fünftägiger Behandlung und nach weiteren fünf Tagen Rekonsolidierung verglichen. DEX verursachte eine signifikante Abnahme des Serumascorbats und des Dehydroascorbats (-87%, $P<0.001$). Die Wirkung wurde teilweise durch Ko-Behandlung mit ASC oder ASC-P (-68%, $P<0.001$) behoben. ASC und ASC-P begrenzten die DEX-verursachte Wachstumsverlangsamung nur geringfügig, obgleich sie die Empfindlichkeit zum Insulin mindestens auf dem Niveau der Glukoseanwendung ($P<0.001$) erhöhten. ASC-P war ASC in der Sensibilisierung für Insulin ($P<0.05$) überlegen. DEX erhöht signifikant somatische Indizes (SI) der Leber (+52%), der Nieren (+44%), des Herzens (+52%) und des Soleusmuskels (+44%) aber nicht des Gastrocnemiusmuskels ($P>0.05$). Demgegenüber fiel der Milz SI erheblich nach DEX-Behandlung (-57%). Nach 5tägiger Rekonsolidierung gingen DEX-geänderte SI-s nicht auf die Ausgangswerte zurück ($P<0.05$). Weder ASC noch ASC-P beeinflussten SI-s, noch

konnten sie DEX-verursachte Änderungen der SI-s ausgleichen, ausgenommen dem Anstieg von Nieren-SI durch ASC-P($P<0.05$) begrenzte. ASC, im Gegensatz zu ASC-P, vergrößerte sogar DEX-abhängige Hepatomegalie ($P<0.05$). Interessanterweise hemmten beide Ascorbatderivate DEX-verursachte Muskelkachexie, zumindestens in Bezug auf den Gastrocnemiusmuskel ($P<0.05$). Zusammenfassend zeigen die Ergebnisse Unterschiede zwischen den schnellen und langsamen Effekten in den experimentellen Modellen der DEX-induzierten Wachstumsverlangsamung, Muskelkachexie begleitet von Glukocorticoid-abhängiger Diabetes.

Schlüsselwörter: Ascorbat, Ascorbinsäurephosphat, Glucokortikoid, Wachstum, Muskelkachexie, Insulinresistenz

Introduction

It is well documented that excess of glucocorticoids leads to growth retardation and muscle cachexia (DARDEVET et al., 1995; HASSELGREN, 1999). Dexamethasone, a synthetic glucocorticoid is thus frequently used to attenuate growth and to trigger muscle wasting (SAVARY et al., 1998; ORZECHOWSKI et al., 2000; ORZECHOWSKI, 2002; MA et al., 2003). The molecular basis for glucocorticoid-dependent muscle atrophy is complex and includes oxidative stress (ORZECHOWSKI et al., 2000), resistance to insulin (DARDEVET et al., 1998), activation of proteasomal system (ATTAIX et al., 1998) and higher activity of myostatin (MA et al., 2001; 2003). In contrast, insulin plays important role in the maintenance of whole-body anabolism. Previously, we reported positive effect of sodium ascorbate on insulin-mediated mitogenicity and cell viability in mononuclear L6 muscle cells (ORZECHOWSKI et al., 2002). Similarly ascorbate derivative, namely ascorbic acid 2-phosphate was shown to accelerate molecular mechanism of terminal differentiation of L6 myoblasts and muscle formation in cell culture model (MITSUMOTO et al., 1994). It was also demonstrated that sodium ascorbate raised phosphorylation of Akt and c-Jun in the same undifferentiated muscle cells (ORZECHOWSKI et al., 2005).

On one hand, several studies indicate that either glucocorticoids excess, or lack of insulin (insulin dependent diabetes mellitus, IDDM) or insulin resistance (non-insulin dependent diabetes mellitus, NIDDM) all trigger oxidative stress (ERIKSSON and KOHVAKKA, 1995; BAKER et al., 1996; PEREIRA et al., 1999; ORZECHOWSKI et al., 2000; CHOI et al., 2003). On the other hand, vitamin C supplementation often results in strenghthening the antioxidant defenses (ARRIGONI and TULLIO, 2002; EGUCHI et al., 2003). Therefore, the purpose of this study was to establish if ascorbate derivatives could prevent disturbances induced experimentally by a high dose of dexamethasone in growing rats. We assumed that at least some of dexamethasone-induced effects (growth retardation, insulin resistance or muscle cachexia) were in causal relationship with oxidative stress. Furthermore, our own observations and available literature data indicate that ascorbate modulates regulatory processes where signals from insulin and insulin-like growth factors (IGF-s) are activated and transduced to target genes (PETERKOFSKY et al., 1991; GOSIEWSKA et al., 1994; MAHMOODIAN and PETERKOFSKY, 1999; ORZECHOWSKI et al., 2005). IGF-binding proteins (IGFBP-s) are often indicated to antagonize IGF-s-dependent growth promoting effects. There is evidence that glucocorticoids upregulate *igfbp-1* gene, whereas insulin acts in opposite way to stop dexamethasone-dependent stimulation of *igfbp-1* gene promoter activity (SUH et al., 1994; SUH and RECHLER, 1997). Thus, in theory insulin action could be augmented by ascorbate so that it could inhibit glucocorticoids in negative control of animal growth. It seems necessary to determine the way of action, effective concentrations and whether vitamin C can

shelter insulin activity impaired by glucocorticoids (secondary glucocorticoid-dependent diabetes). As above-mentioned it was assumed, that insulin plays considerable anabolic function, therefore with regard to developmental processes the positive effect of vitamin C might be associated with the positive relationship between vitamin C and biological effects of insulin.

Various derivatives of ascorbic acid are commercially used as dietary supplements to prevent foods oxidation. Ascorbic acid phosphate (ASC-P) is preferred to fortify feeds since ascorbate (ASC) is released from ASC-P in a relatively slow and constant rate by the action of alkaline phosphatase (AP) present in gut mucosa. In contrast, ascorbate is absorbed almost instantly from the GI tract and within minutes is excreted with urine. By the individual use of equimollar quantities of ASC and ASC-P as feed supplements or intragastric boli it is easy to differentiate between long-term versus short-term effects of ascorbate. In this experiment, we decided to find out how some vitamin C derivatives (sodium ascorbate and ascorbic acid phosphate) would slow down the catabolic effect of dexamethasone and how they can amplify insulin action in a dexamethasone-induced insulin-resistant state.

Material and methods

Animals

Polish Ministry of Agriculture rules for animal welfare were followed during these experiments. All experimental procedures on animals were approved by the Local Ethic and Animal Welfare Commission of the Warsaw Agricultural University. Four-week of age (young) Wistar male rats (n=120) were purchased from Institute of Animal Physiology and Animal Feeding, Polish Academy of Sciences (Jabłonna near Warsaw, Poland). Standard laboratory rodent chow (Wytwórnia Pasz, Andrzej Morawski, Kcynia, Poland) containing 13 MJ kg^{-1} metabolizable energy and 21.2% w/w crude protein was provided twice a day. Any remaining uneaten food was weighed and feed intake was calculated daily. Water was provided *ad libitum*. Each animal was housed individually in controlled environmental conditions (22^{o}C, 75% humidity, 12:12-h light-dark cycle period started 8:00 AM). After a 2-week acclimatization period, the Dex treatment was begun. At this point the rats were 6 weeks of age (180-200 g). Because Dex alters food intake, control animals were pair-fed the average daily amount consumed by the corresponding DEX-treated group. Two 5 days experimental periods - Treatment and Recovery were investigated. From our preliminary experiments (ORZECHOWSKI et al., 2000, 2002) we found that dexamethasone treatment led to muscle cachexia and insulin resistant state in growing rats. To achieve these, DEX-treated rats received 1 mL of dexamethasone disodium phosphate (Sigma, St. Louis, MO, USA) dissolved in saline (0.85% w/v NaCl) given twice a day at 8.00 AM and 4.00 PM (1.0 mL) by intragastric tube in a daily dose of 2 mg/kg b.w. • day^{-1} during 5 consecutive days. After 5 days of treatment one set of rats (Treatment, DEX) was killed and dissected, and another set of rats (Recovery, DEX/REC) was killed after 5 days of the recovery period. Afterwards, control animals (Treatment, CTRL plus Recovery, CTRL/REC) receiving 1 mL of saline (vehicle, 0.85% NaCl) were fed the same amount of food as was consumed by its dexamethasone-treated pair mate during previous day. Similarly, animals from other groups were given the average daily amount of food being eaten by DEX-treated animals. Sodium ascorbate (ASC) and ascorbic acid phosphate (ASC-P) were

purchased from Sigma (St. Louis, MO, USA). ASC and ASC-P were given individually or as co-treatment with DEX at dose of 600 mg/kg b.w • day^{-1} and 785 mg/kg b.w • day^{-1}, respectively. Either factor was dissolved in distilled-deionized water (Aqua pro injectione, Polpharma S.A., Poland) and introduced to rats by the intragastric tube in a volume of 1 mL. Time-schedule was the same as for DEX. Twelve randomized groups of animals (CTRL, CTRL/REC, DEX, DEX/REC, ASC, ASC/REC, P-ASC, P-ASC/REC, ASC-DEX, ASC-DEX/REC, P-ASC-DEX, P-ASC-DEX/REC) were formed (n=10).
Food intake, body weight and wet organ weight data were used to calculate somatic indices: body weight gain [final body weight - initial body weight, BWG in grams], relative body weight gain [(BWG/initial body weight)•100, RBWG in percentage], specific growth rate [(ln final body weight – ln initial body weight)•100, SGR in percentage], protein efficiency ratio [daily protein intake/daily BWG, PER], instantaneous food intake [(daily dry food intake/body weight)•100, IFI in percentage] and somatic indices [(organ wet weight•100/body weight), SI in percentage]. Water intake was also calculated and confronted with PCV to check for dehydration hazard.

Biochemical analyses
Animals were anaesthetised by intraperitoneal injection of 0.3 mL pentobarbital sodium salt (Pentobarbitalum 26.7 mg/ml; Pentobarbitalum Natrium 133/ml, Morbital, Biowet, Puławy, Poland). Abdominal cavity was opened and blood samples were collected into heparinized syringes directly from the abdominal aorta of each animal. Immediately afterwards, selected organs were dissected, washed with ice-cold saline, blotted on absorbent paper, weighed, covered with aluminium foil and placed into liquid nitrogen. The blood samples kept on ice were divided into separate tubes and whole blood, blood plasma and blood cells (after centrifugation at 800 ***g***, 10 min) were each frozen at -80^{o}C. Packed Cell Volume (PCV) was determined routinely with a microcapillar method. Ascorbate and oxidized form of ascorbate (dehydroascorbate) were assayed in blood plasma by HPLC method described by WANG et al. (1995) and RUMELIN et al. (1999).

Determination of glucosuria
To check whether animals are diabetic (*diabetes mellitus*) every morning urine samples from each animal (from treatment and recovery periods) treated individually with DEX or DEX-co-treated were collected on reagent strips (Keto-Diastix, Bayer B. V. Division Diagnostics, Brussel, Belgium) and glucose presence was easily recognized by color indicator.

Glucose tolerance test
One day prior to the end of Treatment or Recovery period all animals (12 groups, 120 animals in total) were individually tested for insulin resistance by the use of glucose tolerance test. Initially, fasting glucose concentration in the whole blood was determined. Five minutes later 1 mL of 40% (w/v) glucose water solution (Pliva, Poland) was given intragastrically („0" time) and 15, 30, 60, 90 and 120 minutes later glucose level was determined in the microliter volumes of blood collected from tail vein. The measurement was performed with Glucocard II GT-1620 apparatus (KDU Corporation, Kyoto, Japan).

Statistical evaluation

Results were statistically evaluated using one way ANOVA and Tukey's multiple range test or two-way ANOVA with Benferroni post test by GraphPad Prism™ version 3.03 software (GraphPad Software Inc., San Diego, CA, USA). Results are expressed as mean ± SEM and a value of $P<0.05$ was determined to be significant, $P<0.01$ as highly significant and $P<0.001$ as very highly significant.

Results

Effect of DEX treatment on glucosuria.

It should be pointed out that glucose was detected in urine of growing rats 2 days after the initiation of DEX treatment (data not shown). When DEX was administered with ASC or ASC-P glucosuria was diagnosed one day later (data not shown). Glucose disappeared from urine one day following DEX treatment (data not shown). Anyway, urine tests confirmed that DEX at the dose of 2 mg/kg b.w. • day^{-1} causes secondary diabetes. Diabetic state recesses immediately after DEX withdrawal from the treatment.

Effect of 5-day DEX and ASC or ASC-P co-treatments on growth indices.

Growth indices decreased markedly in animals, particularly in the 3rd day of dexamethasone treatment. IFI dropped markedly upon DEX action from 13.4% ± 1.14 at day "0" to 10.67% ± 1.0 at day 3 of treatment (-20%, $P<0.05$). At the same time points (day 1 vs. day 3 of DEX-treatment) other dynamic growth indices were impaired as follows: SGR dropped by 210% ($P<0.01$), RWBG by 168% ($P<0.01$), while PER by 358% ($P<0.001$). In contrast to SI-s, after additional 5-day recovery period IFI, SGR, RWBG and PER returned to control levels ($P>0.05$, data not shown) pointing toward transient character of DEX-induced growth retardation. Neither ASC, nor ASC-P influenced feed intake confined by DEX ($P>0.05$, data not shown). In contrast to ASC, however, ASC-P was shown to diminish IFI in average by 10% in comparison to CTRL during recovery period ($P<0.01$, data not shown). DEX administration led to the progressive loss of body weight illustrated by decreased RBWG. The latter became negative (-4.68% ± 0.59, $P<0.05$) starting from the 3rd day of DEX treatment but almost returned to control value (3.02% ± 1.2, $P>0.05$) the day after the end of DEX treatment (1st day of recovery period). Again, neither of ASC derivatives could affect DEX-induced negative effect on RBWG ($P>0.05$, data not shown). When data were plotted on XY graphs the slopes of RBWG and SGR curves fit almost identical. Again, similarly to RWBG SGR became negative (-4.81% ± 0.61, $P<0.05$) at 3rd day and became positive at 1st day of recovery (2.91% ± 1.17, $P>0.05$). These findings were additionally reflected by the significant fall of protein efficiency ratio (from +2.18 ± 1.67 at day "0" to -2.33 ± 0.4 at 3rd day of DEX treatment, $P<0.05$). Again, next day after DEX removal PER raised to +1.06 ± 0.62. There was absolute lack of ASC or ASC-P effect on DEX-induced lower PER ($P>0.05$). From these studies it is clear that in growing rats ASC and ASC-P were of minor importance to limit DEX-induced growth retardation. In contrast to ASC-P which significantly elevated PER at the end of recovery period, ASC significantly diminished PER all along the recovery period ($P<0.05$). These outcomes occur irrespective to the enhanced sensitivity to insulin caused by either of the ascorbate derivative (see glucose tolerance test).

Effect of 5-day DEX and ASC or ASC-P co-treatments on metabolic indices. At 5th day of treatment, total endogenous plasma ascorbate and dehydroascorbate dropped significantly from 65.54 μmol/L found in control to 8.19 μmol/L in DEX-treated rats (-87%, Fig. 1C, P<0.001). Anyway, we did not observe any increase in dehydroascorbate even though we assumed that ascorbate underwent oxidation during DEX-induced insulin resistant state (Fig. 1B). More likely ascorbate/dehydroascorbate were degraded and straight away excreted with urine. Addition of ASC or ASC-P incompletely but significantly made higher serum levels of ascorbate/dehydroascorbate at 5th day of DEX co-treatment (-68%, Fig. 1C, P<0.05).

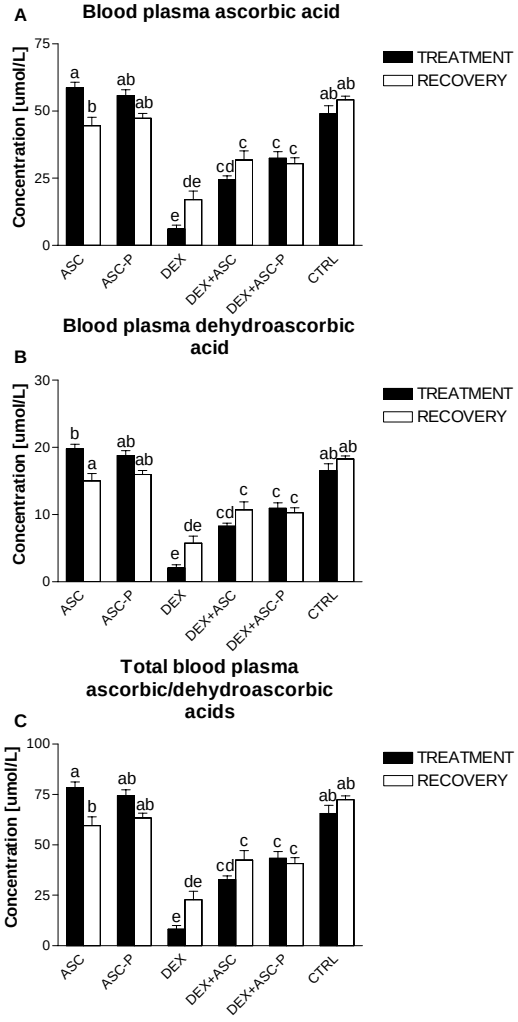

Fig. 1: Bar charts illustrating changes in blood plasma concentrations of (A) ascorbic acid; (B) dehydro-ascorbic acid; (C) total ascorbic/dehydroascorbic acids. Bars represent average concentration ± SEM determined in blood plasma collected after treatment (black) or after recovery period (white). Different letters indicate significant differences (P<0.05). Treatments indicated with abbreviation under abscissa. (Balkendiagramme zu Blutplasmakonzentrationen von (A) Ascorbinsäure, (B) Dehydroascorbinsäure und (C) Gesamtascorbin-/dehydroascorbinsäuren. Balken geben die durchschnittliche Konzentration ± SEM im Blutplasma nach Behandlung (schwarz) oder nach Rekonsolidierung (weiß) an. Unterschiedliche Buchstaben kennzeichnen signifikante Differenzen (P<0.05). Behandlungen sind an der Abzisse mit Abkürzungen spezifiziert)

Whenever given individually, either ASC or ASC-P increased ascorbate/ dehydroascorbate serum concentrations but these effects were statistically insignificant (Fig. 1A). Interestingly, although ASC and ASC-P were of minor importance to limit DEX-induced growth retardation they enhanced sensitivity to insulin (Fig. 2). It was shown that at 90th and 120th min of test the blood glucose concentration descended significantly after combined ASC and DEX co-treatment (Fig. 2A, P<0.001). Apparently, in this regard ASC-P was capable to reduce significantly blood glucose level - starting at 15th min, and was maintained through the test (Fig. 2B, P<0.05; P<0.01; P<0.001). Without DEX co-treatment hypoglycemic effects of either ASC or ASC-P were less evident although remained statistically highly significant (Fig. 2C, Fig. 2D, P<0.01, P<0.001).

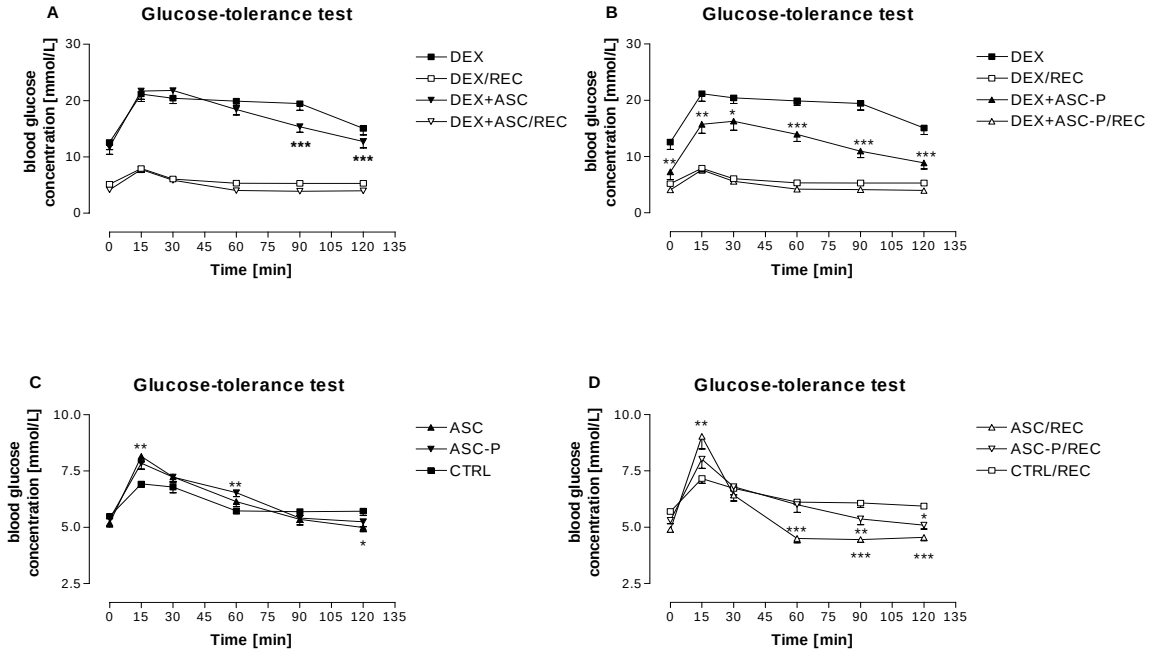

Fig. 2: Figures illustrating time course of changes in whole-blood glucose during glucose tolerance tests performed in rats during treatment and recovery periods that have been given (A) DEX or DEX plus ASC; (B) DEX or DEX plus ASC-P; (C and D) ASC or ASC-P. Average values ± SEM marked with asterisks differ significantly (*, $P<0.05$), highly significantly (**, $P<0.01$), or very highly significantly (***, $P<0.001$) from the respective reference values different for A (DEX), B (DEX), C (CTRL) and D (CTRL) (Verlauf der Blutglukosekonzentration im Glukosetoleranztest bei Ratten nach Behandlung und Rekonstitution mit (A) DEX oder DEX plus ASC; (B) DEX oder DEX plus Asc-p-p; (C und D) Asc oder Asc-p-p. Durchschnittswerte ± SEM. Mit Sternchen gekennzeichnet signifikante Unterschiede zu DEX in A und B sowie zur Kontrolle in C und D)

Effect of 5-day DEX and ASC or ASC-P co-treatments on metabolic indices. Similarly to dynamic indices of growth, where ASC and ASC-P could hardly attenuate DEX-induced alterations, some somatic indices were efficiently modulated by ascorbate derivatives. At the 5th day of treatment, DEX significantly raised SI-s of liver (+52%, $P<0.001$), kidneys (+44%, $P<0.001$)), heart (+52%, $P<0.001$)) and soleus muscles (+44%, $P<0.01$) but not gastrocnemius muscles ($P>0.05$). In contrast, spleen-somatic index dropped significantly upon DEX treatment (-57%, $P<0.001$). After 5-day recovery period DEX-mediated changes in SI-s did not come back to control values ($P<0.05$). Overall, when given individually, neither ASC nor ASC-P affected SI-s, nor they could reverse DEX-induced changes in SI-s with the exception of ASC that deepened DEX-induced hepatomegaly ($P<0.05$). This was not the case for ASC-P, which locked up the rise in renal-somatic index ($P<0.05$). Particularly distinct effect was evoked by both ASC and ASC-P on DEX-induced muscle cachexia at least with respect to gastrocnemius muscle (Fig. 3E). Interestingly, in contrast to soleus muscle, both ascorbate derivatives efficiently inhibited DEX-induced fall in relative weight of gastrocnemius muscles ($P<0.05$). In conclusion, we would like to emphasize that dissociation exists between the fast and slow effects of ascorbate on dynamic indices of growth, somatic indices, and DEX-induced insulin desensitization associated with the secondary diabetes in growing rats.

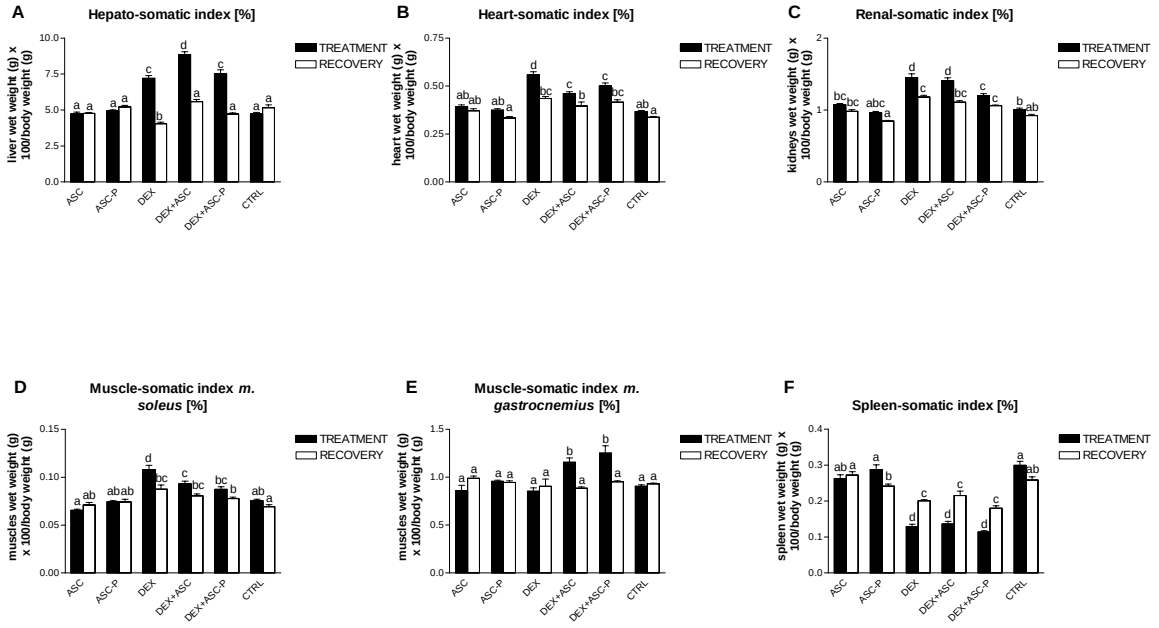

Fig. 3: Bar charts illustrating changes in somatic indices calculated according to the formula described in Material and methods. Bars represent average values ± SEM obtained from *post mortem* measurements after treatment (filled black) or after recovery period (empty white), respectively. Bars marked with different lower case letters differ at least significantly ($P<0.05$). Each treatment is indicated with abbreviation located under abscissa. (Balkendiagramme zur Änderungen metabolischer Indizes, errechnet entsprechend der Formel in Material und Methoden. Balken geben die Durchschnittswerte ± SEM der postmortem Messungen nach Behandlung (schwarz) oder nach Rekonsolidierung (weiß). Unterschiedliche Buchstaben kennzeichnen signifikante Differenzen ($P<0.05$). Behandlungen sind an der Abzisse mit Abkürzungen spezifiziert)

Discussion

5-day administration of DEX led to a progressive significant and severe fall of growth indices in 6-weeks old rats. These changes were caused by both loss of apetite and most likely accelerated whole-body catabolism. Growth retarded by DEX was reversible, since the day after the withdrawal of DEX the dynamic indices of growth increased steadily up to 5th day of recovery period. This was not the case when changes in somatic indices were monitored at 5th day of treatment and recovery period. Relative weights of liver, heart, kidneys and soleus muscles rose during DEX treatment whereas they declined when DEX was removed from treatment. Even then, however, in the 5th day of recovery period the somatic indices were statistically different from control, untreated rats (Fig. 3). One should bear in mind that DEX evoked secondary diabetes and water balance ought to be seriously disturbed. To check dehydration hazard we manage PCV and found no indications that animals were dehydrated as we assume due to the compensatory water intake that raised considerably (data not shown). The losses in IFI, RBWG, SGR and PER found in growing rats were accompanied by a deep fall in the average concentrations of plasma ascorbate and dehydroascorbate (Fig. 3). The latter confirms indirectly that DEX induces oxidative stress. The reducing size of spleen upon DEX treatment is consistent with the general awareness how efficiently glucocorticoids suppress limphoid system. Anyway, the most intriguing observation is linked to gastrocnemius muscle. ASC and ASC-P apparently inhibited DEX-induced changes in the relative weight of the

muscle. It is not clear, why we could not noticed similar effect in soleus muscle. To comprehend the consequences of DEX administration one has to know, that a bulk of data indicates that glucocorticoids in abundance slow down prenatal and postnatal growth (AIN et al., 2005; ORZECHOWSKI et al., 2002). Glucocorticoids and catecholamines are released from adrenals in response to environmental stress that often accompany animals kept indoors in high producing farms. The side-effect of excess glucocorticoids and catecholamines is the developing oxidative stress. The molecular mechanisms of glucocorticoid/catecholamines-associated oxidative stress result from both the impaired antioxidant defenses (PEREIRA et al., 1999; ORZECHOWSKI et al., 2000) and upregulated oxidative metabolism (MANOLI et al., 2005). In our studies we did not measure the indices of oxidative stress (it is currently determined) but we did similar evaluation in the past with similar results (ORZECHOWSKI et al. 2000, 2002). Moreover, short- and long-term administration of exogenous corticosterone caused oxidative stress in broiler chickens (LIN et al., 2004a, 2004b). Interestingly, in broilers and lying hens dietary ascorbate decreased endogenous plasma corticosterone and heat shock protein 70 elevated by cyclic heat stress (MAHMOUD et al., 2003, 2004). Some of the dexamethasone-induced negative affects on growth are associated with the elevated IGFBP-s (SUH et al., 1994) and impaired action of IGF-s whereas other are believed to be a consequence of higher level of myostatin protein (MA et al., 2003). Regardless of the underlying mechanism of DEX action ascorbate derivatives were of minor importance to correct DEX-induced growth restriction. The crucial factor for improvement was apparently the recovery period ($P<0.05$). However, ASC and ASC-P moderately modulated some of the somatic indices; particularly they lowered DEX-induced resistance to insulin.

In conclusion, we demonstrated that oral DEX loading induced metabolic changes such as growth retardation, peripheral blood hyperglycemia, and relative increase in liver, heart, kidneys and soleus muscles wet weight. The above-mentioned alterations in growth indices resulted from the progressive loss of body weight and muscle cachexia. This was not the case for tigh muscles (particularly soleus muscle was not affected). To explain the observed fluctuations in organ wet weight in relation to body weight it is important to stress that other muscles have to be severely affected by DEX. Total ascorbate plasma concentration markedly dropped in DEX-treated animals (Fig. 2, $P<0.001$). Efforts to correct DEX-mediated catabolic effect by the co-treatment with ASC or ASC-P led to slight but not spectacular improvement. This could be a consequence of a high, sub-toxic dose of DEX and/or other not oxidant dependent mechanisms of DEX action in growing rats. Anyway, we observed that ASC and ASC-P significantly advanced sensitivity to insulin, while ASC-P additionally has an advantage to lower DEX-induced average blood glucose level. The latter and other beneficial effects of ASC-P as we suppose were consequence of slow and relatively continuous release of this water soluble antioxidant from GI, since released ascorbate was reported to additionally modulate the activity of phosphatases (EGUCHI et al., 2003). Further studies are needed to evaluate whether other antioxidants (vitamin E, α-lipoic acid, taurine etc.) posses anticatabolic properties to control or prevent DEX- or stress-induced growth constraints. Otherwise, we admit that at least a part of the catabolic activity of DEX originated from alternative reasons other than oxidative stress.

Acknowledgements

This work was partially supported by a grant No 3 PO6T 013 25 and grant No 117/E-385/SPB/COST/P-06/DWM from the State Committee for Scientific Research in Poland. This study was performed in the frame of COST 925 Action on "The importance of prenatal events for postnatal muscle growth in relation to the quality of muscle based foods".

References

AIN, R.; CANHAM, L. N.; SOARES, M. J.:
Dexamethasone-induced intrauterine growth restriction impacts the placental prolactin family, insulin-like growth factor-II and the Akt signaling pathway. J. Endocrinol. **185** (2005), 253-263

ARRIGONI, O.; DE TULLIO, C.:
Ascorbic acid: much more than just an antioxidant. Biochim. Biophys. Acta **1569** (2002), 1-9

ATTAIX, D.:
The critical role of the ubiquitin-proteasome pathway in muscle wasting in comparison to lysosomal and Ca^{2+}-dependent systems: In: Advances in Molecular and Cell Biology, edited by Rivett AJ. Greenwich, CT: JAI, (1998) pp 235-266

BAKER, A. F.; BRIEHL, M. M.; DORR, R.; POWIS, P.:
Decreased antioxidant defence and increased oxidant stress during dexamethasone-induced apoptosis: bcl-2 prevents the loss of antioxidant enzyme activity. Cell Death and Diff. **3** (1996), 207-213

CHOI, H. J. ; JE, H. D. ; JEONG, J. H. ; MIN, Y, S. ; CHOI, T, S. ; PARK, J. H. ; SHIN, C. Y. ; SOHN, U. D.:
The role of ascorbic acid on the redox status and the concentration of malondialdehyde in streptozocin-induced diabetic rats. Arch. Pharm. Res. **26** (2003), 237-243

DARDEVET, D. ; SORNET, C. ; ATTAIX, D. ; BARACOS, V. E. ; GRIZARD, J.:
Insulin-like growth factor-1 and insulin resistance in skeletal muscles of adult and old rats. Endocrinol. **134** (1994), 1475-1484

DARDEVET, D.; SORNET, C.; SAVARY, I.; DEBRAS, E.; PATUREAU MIRAND, P.; GRIZARD, J.:
Glucocorticoid effects on insulin- and IGF-I- regulated muscle protein metabolism during ageing. J. Endocrinol. **156** (1998), 83-89

DARDEVET, D. ; SORNET, C. ; TAILLANDIER, D. ; SAVARY, I. ; ATTAIX, D. ; GRIZARD, J.:
Sensitivity and protein turnover response to glucocorticoids are different in skeletal muscle from adult and old rats. J. Clin. Invest. **96** (1995), 2113-2119

EGUCHI, M.; MIYAZAKI, T.; MASATSUJI-KATO, E.; TSUZUKI, T.; ORIBE, T.; MIWA, N.:
Cytoprotection against ichemia-induced DNA cleavages and cell injuries in the rat liver by pro-vitamin C via hydrolytic conversion into ascorbate. Mol. Cell. Biochem. **252** (2003), 17-23

ERIKSSON, J.; KOHVAKKA, A.:
Magnesium and ascorbic acid supplementation in diabetes mellitus. Ann. Nutr. Metab. **39** (1995), 217-223

GOSIEWSKA, A.; WILSON, S.; KWON, D.; PETERKOFSKY, B.:
Evidence for an *in vivo* of insulin-like growth factor-binding protein-1 and -2 as inhibitors of collagen gene expression in vitamin C-deficient and fasted guinea pigs. Endocrinol. **134** (1999), 1329-1339

HASSELGREN, P. O.:
Glucocorticoids and muscle catabolism. Curr. Opin. Clin. Nutr. Metab. Care **2** (1999), 201-205

LIN, H.; DECUYPERE, E.; BUYSE, J.:
Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*) I. Chronic exposure. Comp. Biochem. Physiol. B **139** (2004a), 737-744

LIN, H.; DECUYPERE, E.; BUYSE, J.:
Oxidative stress induced by corticosterone administration in broiler chickens (*Gallus gallus domesticus*) 2. Short-term effect. Comp. Biochem. Physiol. B **139** (2004b), 745-751

MAHMOUD, K. Z., EDENS, F. W., EISEN, E. J., HAVENSTEIN, G. B.:
Effect of ascorbic acid and acute heat exposure on heat shock protein 70 expression by young white Leghorn chickens. Comp. Biochem. Physiol. C **136** (2003), 329-335

MAHMOUD, K. Z.; EDENS, F. W.; EISEN, E. J.; HAVENSTEIN, G. B.:
Ascorbic acid decreases heat shock protein 70 and plasma corticosterone response in broilers (*Gallus gallus domesticus*) subjected to cyclic heat stress. Comp. Biochem. Physiol. B **137** (2004), 35-42

MANOLI, I.; LE, H.; ALESCI, S.; MCFANN, K. K.; SU, Y. A.; KINO, T.; CHROUSOS, G. P.; BLACKMAN, M. R.:
Monoamine oxidase-A is a major target gene for glucocorticoids in human skeletal muscle cells. FASEB J. **19** (2005), 1359-1361

MITSUMOTO, Y.; LIU, Z.; KLIP, A.:
A long-lasting vitamin C derivative, ascorbic acid 2-phosphate, increases myogenin gene expression and promotes differentiation in L6 muscle cells. Biochem. Biophys. Res. Comm. **199** (1994), 394-402

ORZECHOWSKI, A.; ŁOKOCIEJEWSKA, M.; MURAS, P.; HOCQUETTE, J-.F.:
Preconditioning with millimolar concentrations of vitamin C or *N*-acetylcysteine protects L6 muscle cells insulin-stimulated viability and DNA synthesis under oxidative stress. Life Sci. **71** (2002), 1793-1808

ORZECHOWSKI, A.; ŁOKOCIEJEWSKA, M.; PAWLIKOWSKA, P.; KRUSZEWSKI, M.:
Preincubation with sodium ascorbate potentiates insulin-dependent PKB/Akt and c-Jun phosphorylation in L6 rat myoblasts challenged with reactive oxygen/nitrogen species. Life Sci. **77** (2005), 496-511

ORZECHOWSKI, A.; OSTASZEWSKI, P.; BRODNICKA, A.; WILCZAK, J.; JANK, M.; BAŁASIŃSKA, B.; GRZELKOWSKA, K.; PŁOSZAJ, T.; OLCZAK, J.; MRÓWCZYŃSKA, A.:
Excess of glucocorticoids impairs whole body antioxidant status in young rats. Relation to the effect of dexamethasone in soleus muscle and spleen. Horm. Metab. Res., **32** (2000) 174-180

ORZECHOWSKI, A.; OSTASZEWSKI, P.; WILCZAK, J.; JANK, M.; BAŁASIŃSKA, B.; WARĘSKI, P.; FULLER, J. JR.:
Glucocorticoid-induced catabolic rats show symptoms of oxidative stress and spleen atrophy. The effect of age and recovery. J. Vet. Med. A **49** (2002), 256-263

PEREIRA, B.; BECHARA, E.J.H.; MENDONCA, R.; CURI, R.:
Superoxide dismutase, catalase and glutathione peroxidase activities in the lymphoid organs and skeletal muscles of rats treated with dexamethasone. Cell Biochem. Funct. **17** (1999), 15-19

PETERKOFSKY, B.; PALKA, J.; WILSON, S.; TAKEDA, K.; SHAH, V.:
Elevated activity of low molecular weight insulin-like growth factor-binding proteins in sera of vitamin C-deficient and fasted guinea pigs. Endocrinology **128** (1991), 1769-1779

RUMELIN, A.; FAUTH, U.; HALMAGYI, M.:
Determination of ascorbic acid in plasma and urine by high performance liquid chromatography with ultraviolet detection. Clin. Chem. Lab. Med. **37** (1999), 533-536

SAVARY, I.; DEBRAS, E.; DARDEVET, D.; SORNET, C.; CAPITAN, P.; PRUGNAUD, J.; PATUREAU MIRAND, P.; GRIZARD, J.:
Effect of glucocorticoid excess on skeletal muscle and heart protein synthesis in adult and old rats. Br. J. Nutr. **79** (1998), 297-304

SUH, D. S.; OOI, G. T.; RECHLER, M. N.:
Identification of cis-elements mediating the stimulation of rat insulin-like growth factor-binding protein-1 promoter activity by dexamethasone, cyclic adenosine 3'-5'-monophosphate, and phorbol esters, and inhibition by insulin. Mol. Endocrinol. **8** (1994), 794-805

SUH, D. S.; RECHLER, M. N.:
Hepatocyte nuclear factor 1 and the glucocorticoid receptor synergistically activate transcription of the rat insulin-like growth factor protein-1 gene. Mol. Endocrinol. **11** (1997), 1822-1831

WANG, S.; SCHRAM, I. M.; SUND, R. B.:
Determination of plasma ascorbic acid by HPLC: method and stability studies. Eur. J. Pharmacol. Sci. **3** (1995), 231-239

Corresponding Author
Dr. ARKADIUSZ ORZECHOWSKI
Department of Physiological Sciences
Warsaw Agricultural University
Nowoursynowska 159
02-776 Warsaw
Poland