Thiol supplementation in aged animals alters antioxidant enzyme activity after heat stress

Joanna P. Morrison,1 Mitchell C. Coleman,2 Elizabeth S. Aunan,1 Susan A. Walsh, Douglas R. Spitz,2 and Kevin C. Kregel1,2

1 Integrative Physiology Laboratory, Department of Exercise Science, and 2 Free Radical and Radiation Biology Program, Department of Radiation Oncology, Holden Comprehensive Cancer Center, The University of Iowa, Iowa City, Iowa

Submitted 13 April 2005; accepted in final form 11 August 2005

Morrison, Joanna P., Mitchell C. Coleman, Elizabeth S. Aunan, Susan A. Walsh, Douglas R. Spitz, and Kevin C. Kregel. Thiol supplementation in aged animals alters antioxidant enzyme activity after heat stress. J Appl Physiol 99: 2271–2277, 2005. First published August 11, 2005; doi:10.1152/japplphysiol.00412.2005.—Declines in oxidative and thermal stress tolerance are well documented in aging systems. It is thought that these alterations are due in part to reductions in antioxidant defenses. Although intracellular thiols are major redox buffers, their role in maintaining redox homeostasis is not completely understood, particularly during aging, where the reliance on antioxidant enzymes and proteins may be altered. To determine whether thiol supplementation improved the antioxidant enzyme profile of aged animals after heat stress, young and old Fischer 344 rats were treated with N-acetylcysteine (NAC; 4 mmol/kg ip) 2 h before heat stress. Liver tissue was collected before and after, and following a 60 min heat stress. Aging was associated with a significant decline in tissue cysteine and glutathione (GSH) levels. There was also an age-related decrease in copper-zinc superoxide dismutase activity. Heat stress did not alter liver GSH, glutathione disulfide, or antioxidant enzyme activity. With NAC treatment, old animals took up more cysteine than young animals as reflected in an increase in liver GSH and a corresponding decrease in glutamate cysteine ligase activity. Catalase activity increased after NAC treatment in both age groups. Copper-zinc superoxide dismutase activity did not change with heat stress or drug treatment, whereas manganese superoxide dismutase activity was increased in old animals only. These data indicate that GSH synthesis is substrate limited in old animals. Furthermore, aged animals were characterized by large fluctuations in antioxidant enzyme balance after NAC treatment, suggesting a lack of fine control over these enzymes that may leave aged animals susceptible to subsequent stress.

Address for reprint requests and other correspondence: K. C. Kregel, Integrative Physiology Laboratory, 532 FH, The Univ. of Iowa, Iowa City, IA 52242 (e-mail: kevin-kregel@uiowa.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

normal aging is characterized by a reduction in stress tolerance. For instance, aged animals are less able to tolerate challenges such as heat stress (11, 12, 35, 37, 38) or oxidative stress (4, 13). Moreover, heat stress, particularly in older organisms, is known to generate reactive oxygen species (ROS), oxidative damage, and alterations in intracellular signal transduction (7). Factors such as these are likely to contribute to the cellular dysfunction and loss in homeostatic control that are hallmarks of aging (23).

Generation of ROS due to heat stress can be blocked by administering superoxide (O2−) scavengers (37, 40). If excess O2− is not removed efficiently, it can spontaneously dismutate to form hydrogen peroxide (H2O2). The toxic effects of O2− in vivo are buffered by the enzymatic activities of superoxide dismutase (SOD) enzymes, most notably cytosolic copper- and zinc-containing SOD (CuZnSOD), and mitochondrial manganese SOD (MnSOD), whereas removal of H2O2 is accomplished largely by the actions of the catalase enzyme or the thiol antioxidant system among many (31). However, the benefits of antioxidant overexpression or supplementation during physiological stress, in pathological conditions, or with aging are equivocal. For instance, catalase alone, given exogenously, has been shown to potentiate the lethality of heat shock by blunting heat shock protein 70 induction, presumably by inhibiting H2O2-mediated activation of heat shock factor-1 transcription factor binding. Heat shock factor-1 binding turns on the heat shock response (30). Overexpression of CuZnSOD, rather than providing further protection against oxidative damage, is associated with the increased lipid peroxidation and oxidative DNA damage observed in trisomy 21 (Down syndrome). Additionally, there is an imbalance in glutathione (GSH) homeostasis in this patient population (27). In Drosophila melanogaster, it has been determined that mitochondrial targeting of manganese superoxide dismutase (MnSOD) results in an acceleration of aging (22). These investigators concluded that MnSOD is maintained at an optimal level, and this level was related to the other antioxidant enzymes and proteins in the cell. These studies demonstrate that an overall fine balance between cellular antioxidants is essential for maintenance of cellular homeostasis.

Small-molecular-weight thiol-containing compounds are also important in maintaining cellular redox homeostasis because they are easily oxidized and reduced (8, 21). The peroxide-removing actions of the GSH system is one of the most important redox buffers within a cell because of its overwhelming abundance (8). Thiol-mediated, particularly GSH-mediated, metabolism of ROS is also believed to be important in aging. It is difficult to make general statements about thiol homeostasis, as there are clear age-related and species differences, although it has been suggested that GSH depletion occurs naturally with aging (14). There are conflicting reports regarding the effect of age on liver GSH in Fischer 344 (F344) rats. For example, it has been reported that an age-related reduction in mRNA and protein of glutamate cysteine ligase (GCL), the rate-limiting enzyme in GSH synthesis, reduces liver GSH with aging but has no effect on cellular cysteine levels (19). There is also evidence that aging has little effect on
Conscious and unrestrained animals were heated to a core temperature
liver glutathione disulfide (GSSG), the product of GSH oxidation, is elevated (18), reflecting an age-associated increase in oxidative stress. Finally, it has also been reported that both myocardial (18) and hepatic (4) GSH content is augmented with aging; however, there also appears to be an age-related increase in GSSG in these tissues (4), suggestive of age-associated oxidative stress.

The role of the GSH system in heat stress is not clear. It has been shown that GSH oxidation prevents expression of protective heat shock proteins at the level of transcription factor activation (39). Given the relationship between the GSH system and heat shock proteins, it is unknown whether age- or heat stress-related GSH depletion may be helpful or harmful. Administration of N-acetylcysteine (NAC) is believed to protect against acetalminophen-induced GSH depletion and liver toxicity by increasing the cellular pool of cysteine available for GSH synthesis (17). Under these conditions, the reduction in cellular GSH concentration removes the feedback inhibition of GSH on GCL and, as a result, cysteine availability becomes rate limiting for GSH synthesis (17). Although NAC itself protects against ROS damage by scavenging the hydroxyl radical (HO·1), NAC has also been shown to be a poor scavenger of O2· (2). To augment cellular GSH levels, some investigators have suggested that a prior reduction in cellular GSH concentrations is required for NAC to stimulate GSH synthesis (1). However, an existing shortage of GSH may already be present with aging; therefore, aged animals may have more robust responses to thiol supplementation with NAC.

The impact of aging on thiol metabolism during and after heat stress and the contribution from other antioxidant enzyme systems is still unknown. Although the effects of aging on heat-induced thermostolerance are well characterized, the impact of old age on maintenance of redox homeostasis during and after a physiologically relevant level of heat stress has not been characterized. Therefore, the purpose of this study was to test the hypothesis that supplementation of cellular thiol pools with NAC treatment would ameliorate the damaging effects of heat stress in aged animals by aiding in the maintenance of antioxidant enzyme balance in the liver.

MATERIALS AND METHODS

Animals. Young (6 mo) and old (24 mo) male F344 rats (n = 3 per age and treatment group at each time point) were obtained from the National Institute on Aging. Body weights were similar between age groups, ranging from 341 to 410 g in the young cohort and 368 to 473 g in the old cohort. Animals were housed in The University of Iowa Animal Care Facility, and all protocols were approved by the Institutional Animal Care and Use Committee of The University of Iowa. All rats received food (standard rat chow) and water ad libitum.

Heat stress, drug treatments, and tissue preparation. Animals received an intraperitoneal injection of 4 mmol/kg NAC (Sigma Aldrich, St. Louis, MO) or 1 ml saline (control). Two hours after injection, a colonic thermistor probe (YSI, Yellow Springs, OH) was placed ~8 cm beyond the anal sphincter, and heating was then initiated. The 2-h delay was determined to be optimal for augmenting liver NAC levels in vivo in a preliminary study (data not shown). Conscious and unrestrained animals were heated to a core temperature of 41°C over 60 min at a rate of ~1°C per 15-min period via a heat lamp. The rate of heating was controlled by raising or lowering the lamp as necessary. Core temperature was clamped at 41°C for a further 30 min. Animals were euthanized with an overdose of pentobarbital sodium (80 mg/kg ip) at three time points: immediately after heat stress (0 min) and 30 and 60 min after heat stress. Control animals from each treatment were euthanized without heating 2 h after injection. Livers were removed, rinsed in ice-cold PBS, and trimmed of any adherent tissue. Liver samples were immediately frozen in liquid nitrogen for later analysis.

Spectrophotometric analysis of GSH and GSSG. Liver samples were stored in liquid nitrogen and did not undergo any freeze-thaw cycles until thiol analysis was completed. Protein was measured using the method of Lowry et al. (20). GSH and GSSG were measured according to the method of Griffith (10).

HPLC measurement of cysteine. Liver cysteine was measured by HPLC using a modification of a previously established protocol (29, 36). This protocol was followed with the exception of the use of 0.5 mM ThioGlo 3 (Covalent Associates, Woburn, MA) in acetonitrile instead of N-(1-pyrenyl)maleimide (NPM). Similar to NPM, ThioGlo fluoresces after reaction with thiol groups in proteins, enzymes, and simple peptides. Samples were run on a 15-cm C18 HPLC column (Column Engineering, Ontario, CA) with a pore size of 5 μm at a flow rate of 0.5 ml/min for 30 min. The retention time of cysteine was determined from standard curves. Tissue samples were stored in liquid nitrogen until analyzed. Samples were prepared simultaneously for spectrophotometric and HPLC analysis of thiols on the same day, to avoid repeated freeze-thaw cycles.

Glutamate cysteine ligase activity. Protein concentration was measured by the Bradford method (Bio-Rad). Activity of GCL was determined by HPLC using a modification of a method originally described by Nardi et al. (25, 29). A known amount of protein was added to a reaction buffer containing 0.1 M Tris, 20 mM MgCl2, 0.75 mM L-glutamic acid, 6 mM ATP, 50 mM KCl, 6 mM DTT, and 0.75 mM cysteine. A sample was removed immediately and added to 0.5 mM ThioGlo 3 in acetonitrile for HPLC analysis. The reaction was incubated at 37°C. A sample was removed every 5 min up to 20 min and added to 0.5 mM ThioGlo 3 for HPLC analysis. After HPLC analysis a linear regression curve was fitted to the five time points for each sample (i.e., 0, 5, 10, 15, and 20 min), and the rate of γ-glutamylcysteine (γ-GC) appearance was calculated from the slope of this curve. GCL activity is presented in units of femtomoles of γ-GC produced per milligram of protein per minute.

Antioxidant enzyme activity. Catalase activity of cell lysates was measured spectrophotometrically by a modification of a method originally described by Beers and Sizer (3, 32). Superoxide dismutase activity was determined spectrophotometrically using the method of Spitz and Oberley (33).

Statistical analyses. Data were analyzed with a three-way ANOVA. Pairwise contrasts to determine differences between age groups, drug treatment, and changes from control were performed. Bonferroni-adjusted P values were calculated for each variable to control for multiple comparisons. A P value of <0.05 was taken as significant for the main effects and each pairwise comparison. Data are presented as means ± SE.

RESULTS

Thiol metabolism. Old saline-treated animals had significantly (P < 0.05) less liver cysteine before heat stress compared with their young saline-treated counterparts (Fig. 1). However, after heat stress, young and old saline-treated animals displayed paradoxical changes in liver cysteine. Old saline-treated animals had a progressive increase in liver cysteine, whereas a decline was observed in young saline-treated animals. In both age groups these changes became significantly different from control by 30 min after heat stress. Liver cysteine was significantly increased with NAC treatment in old animals, but this increase was not maintained and liver cysteine declined precipitously immediately after heat stress in this group and remained significantly less than in old saline-treated.
animals at 30 and 60 min after heat stress. It is also interesting to note that NAC treatment resulted in a reduction in liver cysteine in young control NAC-treated animals. Heat stress had little effect and liver cysteine remained reduced in young NAC-treated compared with young saline-treated animals after heat stress.

Saline-treated old rats had significantly less liver GSH than their younger saline-treated counterparts before heat stress (Fig. 2A). There were no significant heat stress-associated changes in liver GSH in either age group. Contrary to expectations, there was no age-related difference in liver GSSG until 60 min after heat stress, when liver GSSG was significantly decreased in old saline-treated animals compared with young saline-treated animals (Fig. 2B). Moreover, liver GSSG did not change with heat stress in either age group. Liver GSH was significantly elevated in old NAC-treated control rats; however, this elevation in GSH, which was similar to the levels attained in young saline-treated control animals, was not maintained, and liver GSH significantly decreased immediately after heat stress such that it was comparable to that of old saline-treated animals. There was a corresponding significant increase in liver GSSG in old NAC-treated animals at 30 and 60 min after heat stress (Fig. 2B). Young NAC-treated animals experienced a significant decrease in liver GSH. This decrease was further exacerbated by heat stress. There was little change in liver GSSG in young NAC-treated animals either before or after heat stress (Fig. 2B). The differential response of liver GSH in young and old rats was reflected in a significant three-way interaction between age, drug, and time. When the effect of age was split out in the statistical model, it was found that the interaction between drug and time was significant for old animals, indicating that the drug effect varied with time in old NAC-treated but not young NAC-treated animals.

Old saline-treated control animals displayed a slight decrease in GCL activity compared with young saline-treated control animals (Fig. 3); however, this was not a statistically significant difference. Although GCL activity remained stable after heat stress in young saline-treated animals, there was an increase in GCL activity in old saline-treated animals that did not reach statistical significance. Treatment with NAC resulted in a slight reduction in GCL activity in young control animals that was not statistically significant. GCL activity in young NAC-treated animals did not change with heat stress. In contrast, there was no change in GCL activity in old NAC-treated control animals, followed by a significant decline in liver GCL activity immediately after heat stress. GCL activity remained significantly decreased in old NAC-treated animals for the time period after heat stress.

Antioxidant balance. Catalase activity in the saline-treated groups was unchanged with aging or heat stress (Fig. 4). With NAC treatment, catalase activity was significantly increased in old animals at all time points examined. Young NAC-treated animals experienced a larger significant increase in catalase activity, but these increases were not evident until 30 and 60 min after heat stress, whereas the control time point was unaffected by NAC treatment in this group. Although old...
NAC-treated animals displayed no variation in catalase activity as a result of heat stress, young NAC-treated animals had a progressive significant increase in catalase activity. These relationships were emphasized by the significant statistical interactions between age, drug, and time for both catalase protein and activity. When age was separated from the analysis, young animals had a significant drug × time interaction, suggesting that the effect of NAC varied over time in young NAC-treated but not old NAC-treated animals.

Old saline-treated control animals had significantly lower CuZnSOD activity than their younger counterparts (Fig. 5). The response of CuZnSOD to heat stress was opposite in young and old saline-treated animals. Immediately after heat stress there was a trend for CuZnSOD activity to decline in young saline-treated animals, followed by a progressive increase back to control levels, although none of these changes were statistically significant. CuZnSOD activity changed little in old saline-treated animals until 60 min after heat stress when CuZnSOD activity was significantly lower than that observed in young saline-treated animals. These changes were not statistically different from control values within each group. After treatment with NAC there was a significant reduction in CuZnSOD activity in young control NAC-treated animals, but this activity was rapidly recovered after heat stress. Treatment with NAC did not affect CuZnSOD activity in old animals before or after heat stress.

There was no difference in MnSOD activity between young and old saline-treated groups, and MnSOD activity was unaffected by heat stress in these groups (Fig. 6). There was a significant age-related increase in MnSOD activity in old...
NAC-treated animals. The striking feature of these data is the lack of change in MnSOD in young NAC-treated animals that is starkly contrasted with a significant increase in MnSOD activity at all time points examined in old animals receiving NAC.

**DISCUSSION**

We have previously demonstrated that heat stress causes increased oxidative damage in the liver of both young and old animals (11, 12, 16, 37, 38). In the present study, we show that the hepatic GSH reduct system is robustly maintained in the face of a heat-induced oxidative stress regardless of the age of the animal. In agreement with previous work (9, 19), we observed an age-associated reduction in GCL activity in saline-treated animals in control conditions. However, this decrease in activity was not due to differences in protein content, as determined by Western blots for the heavy (catalytic) subunit of the GCL enzyme, between age or treatment groups (data not shown) as has been found in other work (19).

Although heat stress caused a marked increase in liver GCL activity in old saline-treated animals only, this increase in enzyme activity was not accompanied by an increase in GSH content of the tissue, suggesting an age-related substrate limitation (28) or rapid consumption of GSH. It is possible that the age-associated reduction in liver GSH is due to a slight, nonsignificant reduction in liver cysteine observed in old control animals. After heat stress, liver cysteine was significantly increased in old animals, a result that may have contributed to the small increases in liver GSH observed after heat stress. In contrast, whereas liver GSH remained unchanged after heat stress in young animals, liver cysteine progressively and significantly declined, indicating the possibility of GSH resynthesis to replenish the GSH levels.

Old NAC-treated control animals displayed a promiscuous increase in liver cysteine and GSH before heat stress that was not associated with an increase in GCL activity but instead may be due to supplementation of cellular thiols with the NAC treatment. The marked reduction in GCL activity in old animals after heat stress is likely due to feedback inhibition of GCL by this, perhaps inappropriate, excess tissue GSH (15, 19). There was also a slight reduction in GCL activity in young NAC-treated animals, suggesting that some of the large decrease in GCL activity in old animals may be due to a negative effect of NAC on GCL activity; however, there is little evidence currently available to support this hypothesis. Of note, the apparent uncontrolled effect of NAC on liver GSH in old animals suggests that NAC is vigorously taken up by many tissues in the aged animal, deacetylated, and then synthesized into GSH with what appears to be a lack of “fine control” of the GSH synthetic pathway.

Although other work has shown that a hyperthermic challenge increases oxidative stress (7, 38, 40), we saw little change in liver GSSG, and it is thought that a decline in GSH/GSSG is indicative of increased ROS generation as a result of a stress. These data may suggest that H$_2$O$_2$ is not a major by-product of heat stress, that the recycling of GSSG back to GSH via glutathione disulfide reductase is robustly maintained with aging, or that GSSG is transported out of the liver after heat stress. It is also possible that thiols are lost from the measurement system via conjugation of thiols to the aldehyde by-products of lipid peroxidation (34), or the formation of mixed protein disulfides, or the export of GSSG from the tissue to the plasma compartment. Observations of reduction in ROS formation after treatment with scavengers for O$_2^\cdot$ suggest that O$_2^\cdot$ is the primary reactive species produced during heat stress (40), in which case cellular GSH would not be the primary defense.

Similar to other reports, there was no age-related reduction in catalase activity (5), and catalase activity remained unchanged after heat stress. This fact, combined with the resulting decrease in liver GSH from treatment with NAC, suggest a reduction in the capacity of young animals to remove liver H$_2$O$_2$. Initially, old NAC-treated animals had both an increase in liver GSH and an increase in catalase activity. However, it must be noted that the increased liver GSH was accompanied by a marked decline in GCL activity followed by a severe decline in liver GSH, thereby likely increasing the reliance on catalase for removing H$_2$O$_2$ from the system. Care must be taken when interpreting the catalase activity data. The method employed to measure catalase activity in fact measures H$_2$O$_2$ removal from a cell lysate. Although it is likely that catalase is largely responsible for this H$_2$O$_2$ removal, the contribution of other enzyme systems cannot be ruled out.

Although total SOD activity has been observed to decline in aged skeletal muscle, MnSOD activity was observed to increase, presumably to defend against the age-associated increase in ROS generation (26). In contrast, the present study demonstrated that hepatic CuZnSOD and MnSOD activity are unchanged by aging or heat stress in vivo. However, treatment with NAC significantly increased MnSOD activity in old animals. This observation was coupled with a significant increase in thiol-based oxidative stress in this group. Previous work has demonstrated increased MnSOD gene expression resulting from NAC treatment (6). Increased MnSOD gene expression was mediated by a NAC-stimulated increase in O$_2^\cdot$ production causing an increase in NF-kB activation. Given the presence of both SOD and H$_2$O$_2$ in an in vivo model, it is likely that some oxidation of NAC occurs and may be partly responsible for the observed increase in MnSOD activity (6). That the increase occurred in old animals may be a reflection of age-associated increases in oxidative stress that were present before experimental intervention or may have facilitated additional oxidation of NAC beyond that which may have occurred in young animals.

In summary, the rapid increase in GSH in old NAC-treated groups suggests that an age-related substrate limitation was responsible for the significant reduction in liver GSH in aged animals. Negative feedback regulation of GCL by GSH does occur in vivo, and this was illustrated in the present study by the sharp decline in GCL activity in old NAC-treated animals that was observed after the rapid and significant increase in liver GSH that occurred within 2 h after NAC injection. GCL activity was also reduced in young NAC-treated animals, which may indicate further regulation of GCL activity by other small-molecular-weight thiols. This is a hypothesis that deserves further investigation. The extreme responses to NAC treatment seen in old animals indicate a lack of fine control over GSH synthesis. It is possible that NAC autooxidized in vivo and generated ROS, as evidenced by increased MnSOD activity. That this increase was only seen in old animals may indicate that the already oxidizing environment of aged ani-
mals potentiated this autooxidation, necessitating the increase in redox-controlled MnSOD. This may also be further evidence of a lack of fine control over antioxidant defense mechanisms in aged animals. A concomitant increase in catalase activity observed in both age groups would remove excess H$_2$O$_2$ from the system and limit any potential inhibitory effects of H$_2$O$_2$ on CuZnSOD activity that remained unchanged after NAC treatment and heat stress. Given the alterations in antioxidant enzyme activity after NAC treatment, NAC appeared to behave more as a prooxidant in old animals, disrupting redox balance, and an antioxidant in young animals. Further work to determine the levels of tissue ROS formation after NAC treatment is necessary to investigate this hypothesis.

ACKNOWLEDGMENTS

The authors acknowledge the administrative assistance of Joan Seye.

GRANTS

This work was supported by National Institutes of Health Grants RO1-AG-12350 (K. C. Kregel), RO1-HL51469 (D. R. Spitz), O1-CA100045 (D. R. Spitz), and P01-CA66081 (D. R. Spitz).

REFERENCES

4. Bejma J, Ramires P, and Ji LL. Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and heat stress. Given the alterations in antioxidant enzyme activity after NAC treatment, NAC appeared to behave more as a prooxidant in old animals, disrupting redox balance, and an antioxidant in young animals. Further work to determine the levels of tissue ROS formation after NAC treatment is necessary to investigate this hypothesis.

ACKNOWLEDGMENTS

The authors acknowledge the administrative assistance of Joan Seye.

GRANTS

This work was supported by National Institutes of Health Grants RO1-AG-12350 (K. C. Kregel), RO1-HL51469 (D. R. Spitz), O1-CA100045 (D. R. Spitz), and P01-CA66081 (D. R. Spitz).

REFERENCES

4. Bejma J, Ramires P, and Ji LL. Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and heat stress. Given the alterations in antioxidant enzyme activity after NAC treatment, NAC appeared to behave more as a prooxidant in old animals, disrupting redox balance, and an antioxidant in young animals. Further work to determine the levels of tissue ROS formation after NAC treatment is necessary to investigate this hypothesis.

ACKNOWLEDGMENTS

The authors acknowledge the administrative assistance of Joan Seye.

GRANTS

This work was supported by National Institutes of Health Grants RO1-AG-12350 (K. C. Kregel), RO1-HL51469 (D. R. Spitz), O1-CA100045 (D. R. Spitz), and P01-CA66081 (D. R. Spitz).

REFERENCES

4. Bejma J, Ramires P, and Ji LL. Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and heat stress. Given the alterations in antioxidant enzyme activity after NAC treatment, NAC appeared to behave more as a prooxidant in old animals, disrupting redox balance, and an antioxidant in young animals. Further work to determine the levels of tissue ROS formation after NAC treatment is necessary to investigate this hypothesis.

ACKNOWLEDGMENTS

The authors acknowledge the administrative assistance of Joan Seye.

GRANTS

This work was supported by National Institutes of Health Grants RO1-AG-12350 (K. C. Kregel), RO1-HL51469 (D. R. Spitz), O1-CA100045 (D. R. Spitz), and P01-CA66081 (D. R. Spitz).

REFERENCES

4. Bejma J, Ramires P, and Ji LL. Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and heat stress. Given the alterations in antioxidant enzyme activity after NAC treatment, NAC appeared to behave more as a prooxidant in old animals, disrupting redox balance, and an antioxidant in young animals. Further work to determine the levels of tissue ROS formation after NAC treatment is necessary to investigate this hypothesis.

ACKNOWLEDGMENTS

The authors acknowledge the administrative assistance of Joan Seye.

GRANTS

This work was supported by National Institutes of Health Grants RO1-AG-12350 (K. C. Kregel), RO1-HL51469 (D. R. Spitz), O1-CA100045 (D. R. Spitz), and P01-CA66081 (D. R. Spitz).

