

EFFECT OF AGE ON THE LEVELS OF KIDNEY TISSUE GLUTATHIONE  
IN THE ROYAL COLLEGE OF SURGEONS STRAIN OF RAT

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## ABSTRACT

**THESIS:** EFFECT OF AGE ON THE LEVELS OF KIDNEY TISSUE  
GLUTATHIONE IN THE ROYAL COLLEGE OF SURGEONS STRAIN OF RAT

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The focus of this study was to investigate differences in the levels of glutathione in the cytosolic and mitochondrial portions of the kidneys in young (2 months of age) and old (24 months of age) male Royal College of Surgeons (RCS) strain of rat. A previous study in the rat suggested that changes in kidney tissue glutathione in the mitochondria occurs with age. Kidneys were harvested from young and old RCS rats, homogenized and separated into cytosolic and mitochondrial portions by differential centrifugation. Levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were determined by chemical assay and compared using a Student's t-test. Body weight and kidney weight were significantly higher in older rats. A significant increase in mitochondrial GSH levels, when expressed in  $\mu$ moles per gram of kidney weight, was observed. The levels of total mitochondrial glutathione (GSH + GSSG) were also significantly higher in older rats. There was no difference in cytosolic levels of GSH or total glutathione between kidneys from young and old RCS rats. This study suggests that mitochondrial levels of GSH and total glutathione increase with age in the RCS rat.

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Thank you to all my friends, who expressed compassion and advice when I worried that I was taking on more than I could handle. I appreciate Ken Gee for taking an uncanny interest in glutathione and providing stimulating, thought-provoking conversations. Thanks also to those friends who asked every time they saw me if I had finished yet – providing even more motivation to finish this.

I owe the largest debt of gratitude to my parents. Even though you didn't know anything about glutathione or understand what I was going through, you expressed your undying faith that I was capable of handling this project and that "everything will be alright".

Thank you, everyone, for your faith in my abilities even when I felt you believed in something that did not exist. I see now that you were right after all.

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### **RESULTS**

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## INTRODUCTION

The purpose of the present study was to determine whether kidney tissue glutathione levels decrease with age in the Royal College of Surgeons (RCS) strain of rat. The incidence of organ disease and dysfunction increases with age in both humans and animals (Timiras, 1988). It has been suggested that the aging cell has lost its ability to protect itself against toxic molecules, such as free radicals, that are generated in oxidative metabolism (Gonzalez-Flecha et al, 1993). The cell has natural antioxidants, such as the reduced form of glutathione (GSH), that protect it against free radicals. With age, the level of glutathione within cells may decrease, leading to cell damage by free radicals. Evidence that glutathione levels in the kidney decrease with age has been reported in mice (Hazelton and Lang, 1980).

This study determined the levels of both reduced and oxidized (GSSG) glutathione in the cytosolic and mitochondrial compartments of rat kidney tissue in young (two months of age) and old (22 months of age) RCS rats. Kidneys were harvested from the rats after being perfused with cold saline solution to flush blood out of the kidney. Kidneys were homogenized and then separated into cytosolic and mitochondrial portions by differential centrifugation. Glutathione levels in each compartment were then determined.

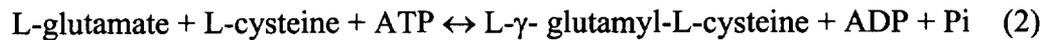
## BACKGROUND

### Role of Glutathione in the Cell

Glutathione is the major antioxidant produced by cells to protect DNA, organelles and membranes from being destroyed by toxins and free radicals (Campos et al, 1993). Glutathione exists mainly in two forms: the reduced form (GSH) and the oxidized form

(GSSG). As a result of the chemical reaction between GSH and a free radical, GSH changes into its oxidized form. Approximately 93-95% of the total glutathione in tissue is found in the reduced form (Meister, 1988). This is desirable since a large pool of GSH is readily available to neutralize free radicals.

Glutathione is a molecule made up of three amino acids. Its two-step formation is shown below:



To neutralize the free radical hydrogen peroxide, two GSH molecules and the enzyme glutathione peroxidase are needed:



Glutathione reductase and an electron-carrying molecule, NADPH, regenerate GSSG back to its oxidant-fighting form (see below).



The largest amounts of glutathione are found in the cytosol, but it is believed that the presence of glutathione in the mitochondria is most crucial because this is where the majority of free radicals are produced. Mitochondrial glutathione only comprises 15-30% of total tissue glutathione (Lash, 1995), but it has the very important task of chemically deactivating free radicals primarily produced in the mitochondria. Because the enzymes necessary for glutathione production are not present in the mitochondria, glutathione is first produced in the cytosol, then transported into the mitochondria when needed (McKernan et al., 1991). The mitochondrial pool of glutathione is not readily depleted, suggesting that mitochondrial glutathione is relied upon more heavily for

cellular stability (Deneke et al., 1989). Studies of kidney and liver tissues have shown that mitochondrial function is affected by oxidant stress more than cytosolic function. Studies by McKernan et al. (1991), Cadenas et al. (1995), and Meredith et al. (1982) found that chemical-induced depletion of the mitochondrial pools of glutathione (GSH) - rather than cytosolic depletion - was most likely to lead to irreversible cellular damage. Also, the ratio between GSH and its inactivated, oxidized form (GSSG) is more protected from fluctuation in the mitochondrial pool than in the cytosolic pool. This indicates that the mitochondrial pool of GSH is indeed important for protection against oxidative stress.

### Oxidative Stress

Exposure to agents that generate production – either directly or indirectly – of free radicals is known as oxidative stress. Sources of oxidative stress are numerous: hyperoxia, exposure to xenobiotics, ozone, nitrite and/or other environmental pollutants, from internal reactions initiated by radiation or activated lymphocytes...and the list goes on. It comes as no surprise that depletion of intracellular GSH has been seen with various sources of oxidative stress. When GSSG is generated faster than it is converted back to two molecules of GSH, GSH depletion can occur. To put further strain on intracellular stores of GSH, GSSG can be transported from the cell if its concentration becomes too high, or it can form compounds with nearby proteins and be transported out of the cell (Forman et al, 1990).

Low GSH levels in cells, induced by administration of oxidizing chemicals or low-protein diets, can make cells even more sensitive to oxidative stress. A low-protein diet weakens cellular resistance to oxidizing agents by failing to supply the amino acids

necessary for glutathione production (Forman et al, 1990). It has been shown that GSH depletion compromises cellular defenses against radiation and other oxidizing agents (Deneke and Fanburg, 1989). This makes the cell more vulnerable to pathology and/or disease.

Toxin and oxidant stresses have been implicated as causative agents of many ailments such as cancers and kidney dysfunctions (Campos, 1993; Zamlauski-Tucker, 1999; Halliwell, 1984), including kidney failure (Canavese and Vercellone, 1988). Because a decrease in glutathione concentrations is considered an index of oxidant stress, it is beneficial to determine the normal levels of glutathione in tissues so that pathological levels can be identified. It is also of great interest to see how glutathione levels change with age in an animal. If kidney tissue glutathione levels decrease with age, this would contribute to decreased ability of the kidney to respond to oxidative stress.

There are several ways of evaluating the magnitude of oxidant stress. One is to express levels of GSH and GSSG in a ratio, known as the redox ratio. This ratio can be calculated by dividing the GSSG levels by GSH levels and multiplying by 100 (Asuncion, 1996). This ratio shows how much GSH has been oxidized in the process of neutralizing free radicals. A larger redox ratio percentage indicates a greater amount of oxidant stress, since more GSH has been converted to GSSG. Another way to express the redox ratio is by dividing GSH by total glutathione (ie, GSH divided by GSH + GSSG) (Zamlauski-Tucker, 1999). This ratio shows how much of the total glutathione pool is still present as GSH. A decrease of this ratio indicates increased oxidative stress.

Although the mitochondrial GSH pool is resistant to depletion, it is not untouchable. A chemical oxidizing agent, buthionine sulfoximine, gives a biphasic

decline in total levels of total tissue glutathione. It was found that the secondary decline in GSH represented depletion of the mitochondrial pool (Griffith and Meister, 1985; Romero and Galaris, 1990). The buthionine sulfoximine cannot directly enter the mitochondria, so mitochondrial GSH depletion in this case is accomplished by first depleting the cytosolic pool. Since GSH is not available in the cytosol to be transported into the mitochondria and the mitochondria cannot synthesize its own GSH, the mitochondrial pool remains depleted until the oxidative stress is over and GSH can be synthesized in the cytosol again.

#### Cell Damage Caused by Free Radicals

Free radicals are intermediates of oxygen reduction, produced by electron-transfer processes in the mitochondrial membrane. Two common free radicals are the superoxide anion ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ). Actually, neither of these molecules poses an imminent threat. They exist in small concentrations as natural by-products of cellular oxidative metabolism and are relatively unreactive. They become dangerous after exposure to iron or copper ions, which can be present in the fluid of the cell (Canavese et al, 1988). After this, they react with each other to form the highly toxic hydroxyl ( $OH\bullet$ ) free radical (Equation 1).



Because of its unstable highly reactive energy state, the hydroxyl free radical is one of the most dangerous free radicals. Once it is created, it immediately reacts with surrounding molecules in attempt to restore a natural energy state. This leads to the generation of more free radicals and/or damaged cellular constituents that happen to be nearby.

Unfortunately, free radicals generated in the mitochondria are in close proximity of mitochondrial DNA, and can cause genetic mutations in the mitochondria. To make matters worse, DNA repair mechanisms designed to protect against mutations is not as competent in the mitochondria (Halliwell, 1994).

Free radicals can also go on to initiate chain reactions that ultimately create polyunsaturated fatty acids. Since saturated fatty acids are essential for the integrity of membranes, rendering these fatty acids to a polyunsaturated form leads to membrane instability and alters the membrane structure to the point where the cell may lyse (Canavese et al., 1987; Gonzalez-Flecha et al., 1993). The process of causing membrane lipids to become polyunsaturated is known as lipid peroxidation. Lipid peroxidation is simply the process of stripping a membrane lipid of a hydrogen atom. Free radicals steal hydrogen atoms to achieve a stable energy state. This causes a conformational change in the lipid structure and challenges the integrity of the membrane. If this continues unchecked, the membrane will break down, expel its contents into the surrounding tissue or fluid, and die. This allows the free radicals to spread and continue to wreak havoc. Lysis of the mitochondrial membrane is especially undesirable because mitochondria are necessary for a large majority of the energy produced in an organism (Halliwell et al., 1994).

### Glutathione Levels in the Kidney

Glutathione levels have been determined in various tissues, including the brain, liver, kidney, testes, and small intestine (Boyd et al, 1979; Beatrice, 1984; Asuncion et al, 1996). Glutathione levels are much higher in the kidney than in the liver and most other organs (Xia et al., 1985). Surprisingly, the levels of glutathione in kidney tissue have not

been studied to the same extent as they have in the liver and brain. Most studies that have been performed on rat kidney focused on finding the glutathione content of the whole kidney tissue (Nissim et al., 1996; Roberts et al., 1993; Hazelton et al., 1980; Mohandas, 1984). Hazelton et al (1980) found that the older mice (age 31 months) had 34% lower total glutathione and GSH concentrations in the kidney when compared with younger mature animals (6 months of age). They also noted that GSSG did not change much at all with age, and constituted less than 3% of total glutathione. A study done by Asuncion et al (1996) reported a significant increase in GSSG in the kidney tissue mitochondria of older rats (24 months of age) compared to younger rats (3 months of age). They also found that the redox ratio (expressed as GSSG/GSH) increased with age in kidney tissue mitochondria.

## **MATERIALS AND METHODS**

### **Kidney Harvest**

Kidneys were obtained from male RCS rats (Ball State University, Muncie, IN). Young rats were approximately two months of age; old rats were approximately 24 months old. A typical diet of grain pellets and water was available up to the time of kidney harvest. Rats were anesthetized with Inactin (100 mg per kg of body weight, ip injection) and a midline incision was made to access the kidneys. The inferior aorta was isolated and a cannula was inserted so that it was above the right and left renal arteries. The aorta was tied off above the cannula and 30 mL of cold isotonic saline solution was infused into the kidneys. Perfusing the kidneys with saline solution pushes out all the blood. After this, the kidneys were cut out, decapsulated, and accurately weighed. The

kidneys were then finely chopped, placed in 3 mL of cold 5% metaphosphoric acid in isotonic saline, and homogenized.

#### Analysis of Glutathione in Different Tissue Compartments

The kidney tissue samples were centrifuged at 900 rpm (rotations per minute) for ten minutes to separate out junk and debris. Then the cytosolic and mitochondrial portions were separated by centrifugation at 12,000 rpm for 15 minutes (the mitochondria sink to the bottom of the tube as a pellet). The cytosol was decanted from the tube accurately weighed and frozen for later analysis of glutathione. The mitochondrial pellet was accurately weighed, then resuspended in 600 uL of 5% metaphosphoric acid (MPA) in distilled water to lyse the mitochondria and free the glutathione. The suspension was then centrifuged for 10 minutes at 10,000 rpm to remove remaining protein. The supernatant was accurately weighed and saved for later analysis of glutathione; the protein pellet was also accurately weighed and saved for future determination of the amount (in mg) of protein in mitochondria per gram of whole kidney tissue weight.

#### Glutathione Assay

All samples were assayed for GSH and total GSH using a spectrophotometric glutathione assay kit from Calbiochem (San Diego, CA). Cytosolic samples were diluted 1:45 and mitochondrial samples were diluted ~1:5 with the kit buffer. This kit uses a two-step chemical reaction. The first step is a reaction between a reagent (chromagen) and glutathione in standards and samples of unknown concentrations. Then the absorbance of the standards and the samples of unknown concentrations were read by

a spectrophotometer set at a wavelength of 356 nanometers. The second step is the reaction of sodium hydroxide with standards and samples of unknown concentrations. The sodium hydroxide converts GSSH to GSH, then GSH to a substance called a chromophoric thione. This chromophoric thione represents total glutathione (GSH + GSSG) in the sample. Standard concentrations and samples were read by the spectrophotometer at a wavelength of 400 nm. The concentration of GSH and total glutathione in the samples were determined from the standard curve. Thus, the GSSG concentration can be indirectly determined by subtracting the total glutathione concentration from the GSH concentration found in the first step and dividing this value by two.

#### Determination of Mitochondrial Protein

The protein content of the mitochondrial pellet was measured using Peterson's modification of the micro-Lowry procedure and a Lowry Protein Assay Kit from Sigma Diagnostics. The mitochondrial pellet was dissolved in 1.6 mL of 0.1M NaOH and diluted 1:6 to run the assay. The Lowry method uses an alkaline cupric tartrate reagent that complexes with peptide bonds of proteins to form a purple color after addition of a phenol reagent. Peterson's modification precipitates the protein with deoxycholate and trichloroacetic acid to prevent interference from other chemicals. The absorbance was read at 600 nm. Protein content is determined from a calibration curve obtained from samples of known protein concentrations (ie., by extrapolating from a standard curve).

### Statistical Analysis

All data are expressed as the mean  $\pm$  standard error of the mean (SEM).

Statistical tests were performed with MINITAB software for Macintosh. A two-tailed Student's t-test (Hogg and Tanis, 2000) was used to compare the cytosolic and mitochondrial levels. A p value of  $<0.05$  was considered statistically significant. Sample calculations can be seen in Appendix 1: Calculations.

## **RESULTS**

### Body Weight and Kidney Weight (see Table 1)

There was a significant increase in body weight of old rats compared to young rats. Kidney weight of old rats was also significantly higher. These findings are expected, due to the fact that an organism's body weight typically increases with age. It follows that kidney weight would also increase as the kidney enlarges in proportion with the increased body mass and metabolic demand. However, increases in body weight and kidney weight did not affect evaluation of glutathione content, since glutathione levels were expressed in units per gram of kidney weight.

### Cytosolic Glutathione Levels (see Figure 1)

The slight increase we found of cytosolic GSH in older rats was not significant. Levels of GSSG were approximately half the levels of GSH. Total cytosolic glutathione was slightly higher in old rats than in young rats due to the contribution of reduced GSH, but the difference was not significant.

TABLE 1: Body and Kidney Weight of Young and Old Rats

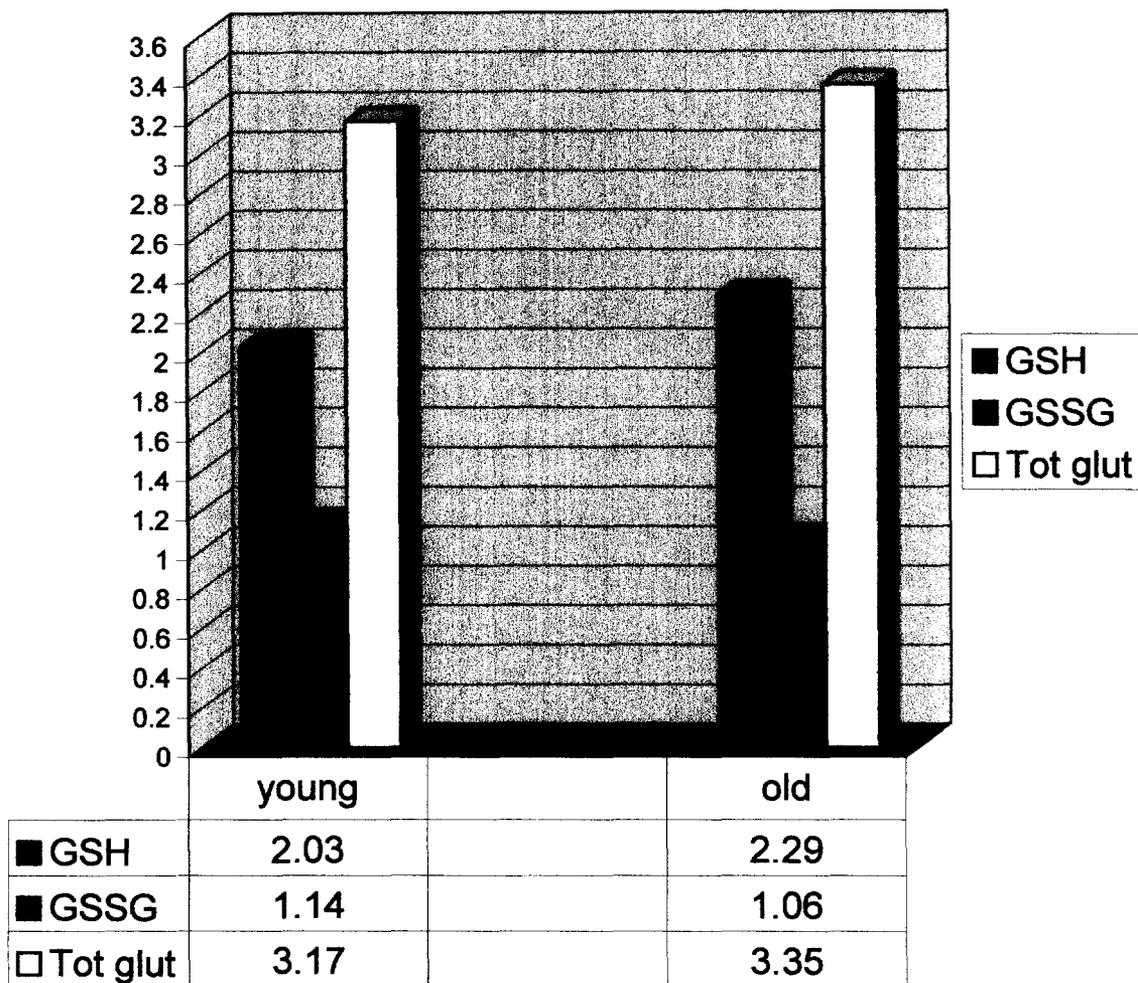
	<u>2 months</u>	<u>24 months</u>	
Body Wt. (g)	241+/- 12	301+/- 16	*
Kid. Wt. (g)	1.765 +/- 0.085	2.335 +/- 0.087	*
Kid. Wt./Body Wt.	0.73% +/- 0.053	0.79% +/- 0.061	
Mg Mit. Prot./g Kid. Wet Wt.	0.667 +/- 0.056	0.461 +/- 0.14	

TABLE 1: Body and Kidney Weight of Young and Old Rats

Body weight was measured from the live animal. Kidney weight represents wet tissue weight before homogenization. Significant increases found in older rats using the Student's t-test is designated by an \*. Abbreviations: Wt. = weight, Kid. = kidney, Mit. = mitochondria, Prot. = protein.

Fig. 1. Mean levels of cytosolic glutathione in young and old rats. Values are given in  $\mu\text{mol/g}$  of kidney wet weight. Bars drawn indicate range for SEM. Abbreviations: GSH = reduced glutathione, GSSG = oxidized glutathione, Tot. Glut. = total glutathione (ie, GSH + GSSG).

**Fig. 1. Mean levels of cytosolic glutathione in young and old rats. Values are given in  $\mu\text{mol/g}$  of kidney wet weight.**



### Mitochondrial Glutathione Levels (see Figure 2 and Figure 3)

There was a significant difference in the levels of mitochondrial GSH expressed in  $\mu\text{mol}$  per gram of kidney wet weight. Surprisingly, higher levels of GSH were found in the older rats. The significantly greater levels of mitochondrial GSH in old rats resulted in significantly greater levels of total glutathione. These higher levels can not be accredited to significantly greater body weight or kidney weight, since GSH levels were expressed per gram of kidney weight. When mitochondrial levels of GSH were expressed in  $\text{nmol}/\text{mg}$  mitochondrial protein, the levels in older rats approached significance ( $p=0.13$ ), but were not quite statistically significant. Total glutathione levels in the mitochondria were also almost significantly higher in older rats ( $p = 0.12$ ). Mitochondrial GSSG levels of young and old rats were not significantly different.

### Redox Ratio's (see Table 2)

The redox ratio's of kidneys from older rats indicated that these kidneys were exhibiting less oxidant stress than the kidneys from younger rats. The ratio of GSSG to GSH decreased with age in the cytosol, approaching significance with a  $p$  value of 0.096. This redox ratio also decreased with age in the mitochondria when expressed in  $\mu\text{mol}/\text{g}$

Fig. 2. Mitochondrial Glutathione Levels in Young and Old Rats Expressed in  $\mu\text{mol/g}$  of kidney wet weight. Bars drawn indicate range for SEM. Data marked with an \* show significance ( $p < 0.05$ ). Abbreviations: GSH = reduced glutathione, GSSG = oxidized glutathione, Tot. Glut. = total glutathione (ie, GSH + GSSG).

**Fig. 2. Mitochondrial Glutathione Levels in Young and Old Rats Expressed in  $\mu\text{mol/g}$  of Kidney Wet Weight.**

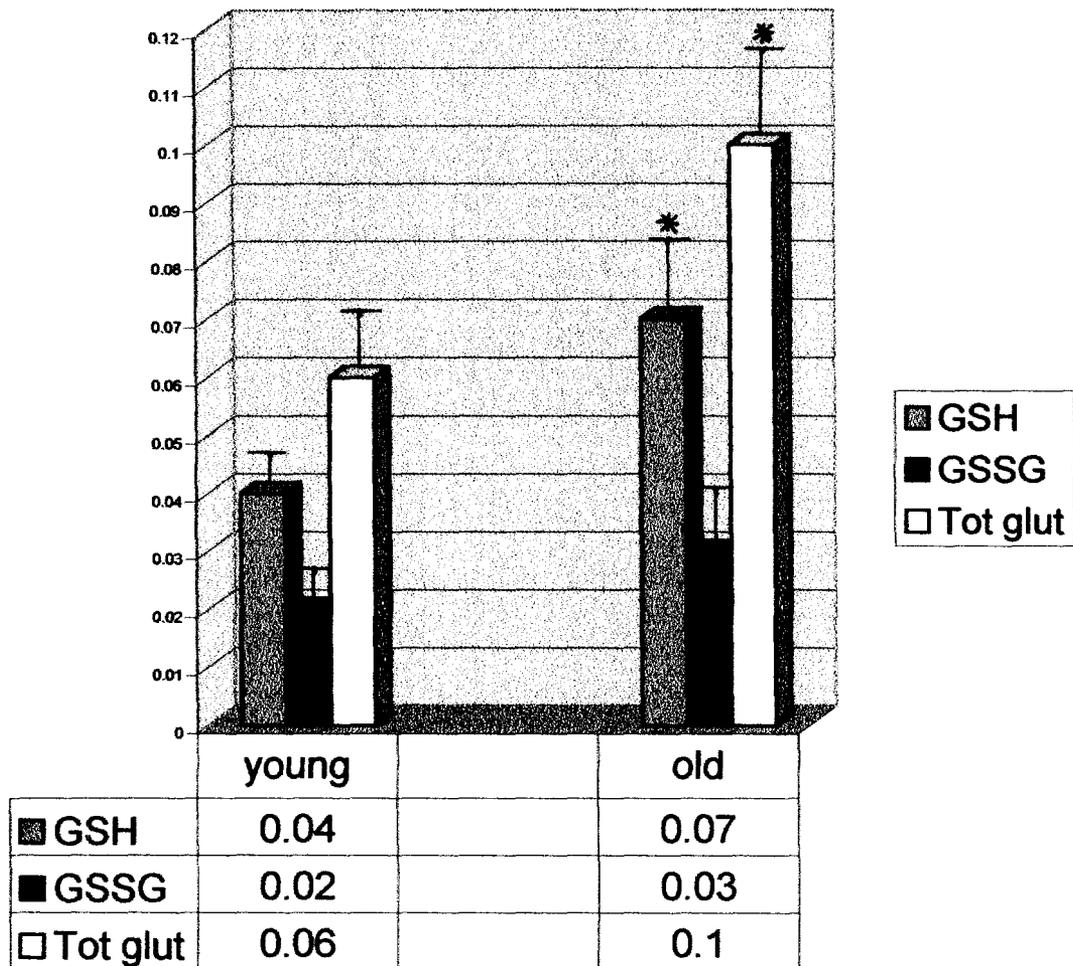


Fig. 3. Mitochondrial Glutathione Levels in Young and Old Rats Expressed in nmol/mg Mitochondrial Protein. Bars drawn indicate range for SEM. @ denotes near-significance ( $p = 0.13$ ); # denotes near-significance ( $p = 0.12$ ). Abbreviations: GSH = reduced glutathione, GSSG = oxidized glutathione, Tot glut = total glutathione (ie, GSH + GSSG).

**Fig. 3. Mitochondrial Glutathione Levels in Young and Old Rats Expressed in nmol/mg of Mitochondrial Protein.**

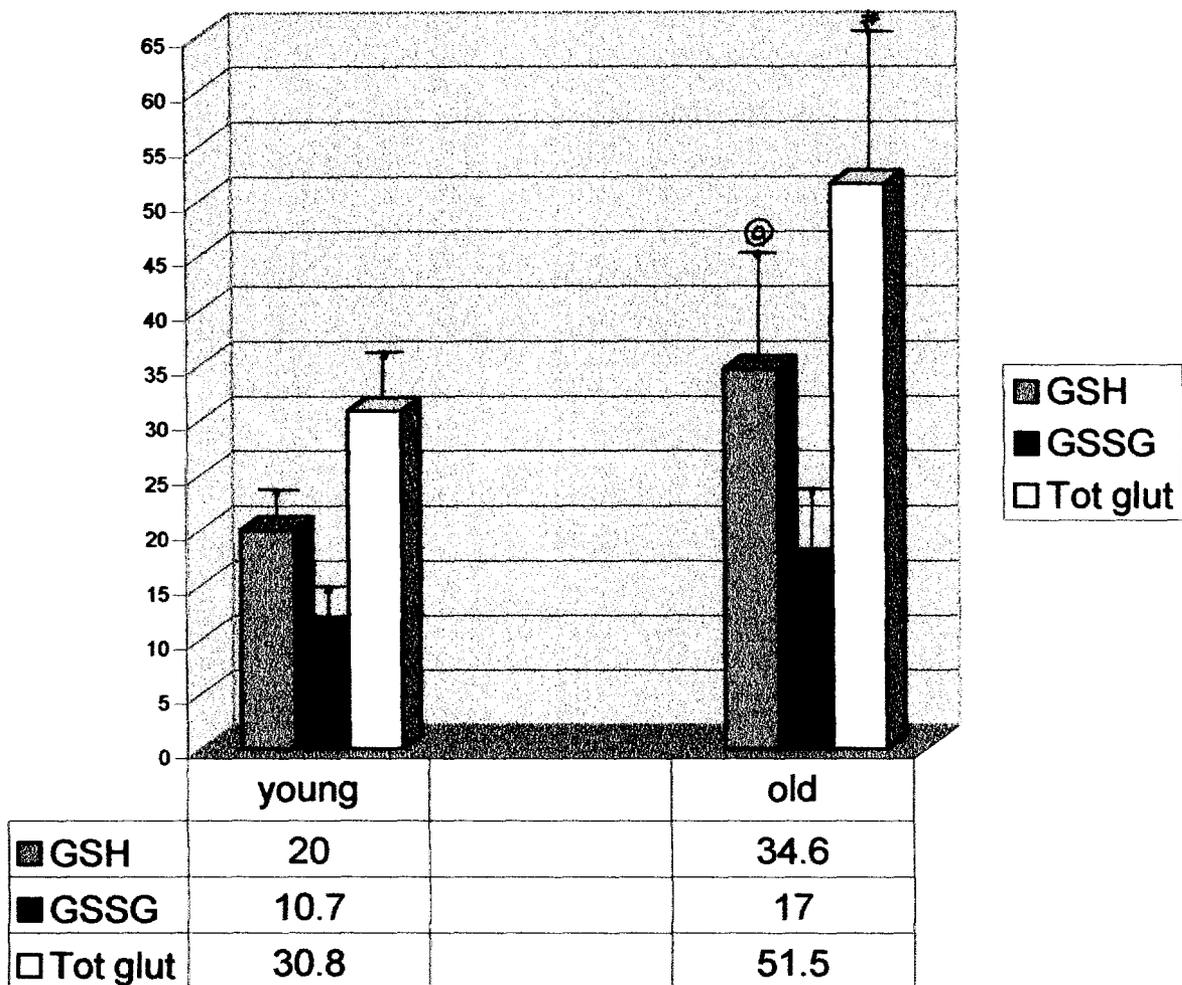


TABLE 2: Redox Ratio's in Cytosol and Mitochondria from Young and Old Rats

	Young Rats	Old Rats
<u>Cytosolic Redox Ratio</u>		
( $\mu\text{mol/g}$ kidney wet weight)		
GSSG/GSH X 100	59.2 +/- 4.8	46.8 +/- 4.6 $\ominus$
GSH/Total GSH X 100	63.6 +/- 1.8	68.4 +/- 2.1 $\text{@}$
<u>Mitochondrial Redox Ratio</u>		
( $\mu\text{mol/g}$ kidney wet weight)		
GSSG/GSH X 100	54.4 +/- 8.8	50.3 +/- 12.5
GSH/Total GSH X 100	65.6 +/- 3.3	68.4 +/- 4.7
<u>Mitochondrial Redox Ratio</u>		
( $\text{nmol/mg}$ mitochondrial protein)		
GSSG/GSH X 100	54.3 +/- 8.7	53.6 +/- 14.0
GSH/Total GSH X 100	65.6 +/- 3.3	67.2 +/- 5.0

TABLE 2: Redox Ratio's in Cytosol and Mitochondria from Young and Old Rats

The Redox Ratio was calculated as GSSG/GSH X100. The redox ratio referred to as the Glutathione Ratio was calculated as (GSH/GSSG + GSH) X 100. All values expressed as mean +/- SEM. Significance was  $p < 0.05$  using the Student's t-test. Abbreviations: GSG = reduced glutathione, GSSG = oxidized glutathione, Total GSH = total glutathione (ie, GSH + GSSG).

$\ominus$  This ratio approached significance with a p value of 0.096

$\text{@}$  This ratio approached significance with a p value of 0.11

of wet kidney weight and in nmol/mg of mitochondrial protein. The redox ratio expressed as  $GSH/GSSG + GSH$  increased in the cytosolic and mitochondrial compartments, also indicating less oxidant stress in older animals. This ratio approached significance in the cytosolic compartment with a p value of 0.11. Although the redox ratio's were not significantly different between young and old rats, they consistently indicate that the older rats were experiencing less oxidative stress.

## DISCUSSION

This is the first study to investigate changes in kidney tissue glutathione with age in the RCS rat and differentiate between cytosolic and mitochondrial compartments. The findings of this study correlate with those of Bishayee and Chatterjee (1994), who reported slight increases of GSH with age in the cytosolic compartment of rat kidney tissue ( $\mu\text{mol/g}$  wet tissue) among groups of rats aged 4, 8 and 12 weeks. However, rats that are 12 weeks old are not considered mature animals, so to date ours is the only study that compares cytosolic glutathione levels in mature young and old adult rats.

Muthukumar and Selvam (1998) measured glutathione levels in mitochondria in rat kidney tissue, but they expressed glutathione levels in units of  $\mu\text{g/mg}$  of mitochondrial protein, making their data difficult to compare to ours. Also, they investigated the glutathione status of the mitochondrial tissue with respect to administration of agents that create kidney stones, not age. The glutathione content of rat kidney mitochondria reported by Asuncion et al (1996) was elevated in the older rats, though not significantly. They found significant increases in GSSG levels in the older rats; the increases found in

GSSG in older rats were not significant. The current study is the first to evaluate glutathione content of rat kidney tissue between young and old rats and find a significant increase in mitochondrial GSH of older rats. Asuncion et al (1996) also found that the mitochondrial redox ratio (GSSG/GSH) increased with age in rat kidney tissue, while the current study saw the same redox ratio decrease with age.

It is reasonable to hypothesize from this study that the older rat kidney tissue experienced less oxidative stress than that of younger rats because older rats have a slower metabolism (Hagan et al, 1999). A decreased metabolism would undergo fewer oxidative reactions, produce fewer free radicals, convert less GSH to GSSG, and put less strain on the mitochondrial glutathione pool. This theory is supported by the fact that the mitochondrial glutathione pool – where cellular oxidative processes may first show effect on glutathione content – was significantly greater in the older rats. Also, the finding that the cytosolic redox ratio's ( $p < 0.14$ ) were higher in older rats seems to support that kidneys from older rats seem to experience less oxidative stress.

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## APPENDIX 1: CALCULATIONS

### Calculations:

#### 1. Sample Calculation used to Determine the Levels of GSH and GSSG in the Cytosol

$$\text{Cytosolic GSH} = \text{GSH } \frac{\mu\text{mol}}{\text{L}} \times \text{Dilution Factor} \times \frac{\text{L homogenate}}{\text{g kidney wet wt.}} = \frac{\mu\text{mol GSH}}{\text{g kidney wet wt.}}$$

$$= 16.5 \frac{\mu\text{mol}}{\text{L}} \times 60 \times \frac{0.0053 \text{ L}}{2.0807 \text{ g}} = 2.5218 \frac{\mu\text{mol GSH}}{\text{g kid. wet wt.}}$$

$$\text{Cytosolic GSSG} = \frac{([\text{GSH}] \text{ at } 400 \text{ nm} - [\text{GSH}] \text{ at } 356 \text{ nm}) \mu\text{mol/L}}{2} \times \frac{\text{L homog.}}{\text{g kid. wet wt.}} = \frac{\mu\text{mol GSSG}}{\text{g kid. wet wt.}}$$

$$\frac{\mu\text{mol GSSG}}{\text{g kid. wet wt.}}$$

$$= \frac{(1920 - 990) \mu\text{mol}}{2 \text{ L}} \times \frac{0.0053 \text{ L}}{2.0807 \text{ g}} = 1.1845 \frac{\mu\text{mol GSSG}}{\text{g kidney wt.}}$$

#### 2. Sample Calculation used to Determine the Levels of GSH and GSSG in the Mitochondria:

$$\text{Mit. GSH} = \frac{([\text{GSH}] \text{ at } 356 \text{ nm } \mu\text{mol/L} \times \text{vol. pellet in mL})}{\text{Vol. of supernatant 1 (from 900 X g spin)}} \times \frac{\text{L homog.}}{\text{g kidney}} = \frac{\mu\text{mol GSH}}{\text{g kid. wt.}}$$

$$= \frac{56.25 \mu\text{mol/L} \times 0.5148 \text{ mL}}{0.6852} \times \frac{0.0053 \text{ L}}{2.0807 \text{ g}} = 0.1077 \frac{\mu\text{mol GSH}}{\text{g kid. wt.}}$$

$$\text{Mit. GSSG} = \frac{([\text{GSH}] \text{ at } 400 \text{ nm} - [\text{GSH}] \text{ at } 356 \text{ nm}) \mu\text{mol/L}}{2} \times \frac{\text{vol. pellet in mL}}{\text{Vol. of supernatant 1}}$$

$$\times \frac{\text{L homog.}}{\text{g kidney}} = \frac{\mu\text{mol GSSG}}{\text{g kidney}}$$

$$= \frac{(103.5 - 56.25) \mu\text{mol/L}}{2} \times \frac{0.5148}{0.6852} \times \frac{0.0053 \text{ L}}{2.0807 \text{ g}} = 0.0452 \frac{\mu\text{mol GSSG}}{\text{g kidney}}$$

