Correlation between Keton Body Level in Selenium-Deficient Rats and Oxidative Damages

Ken-ichiro Matsumoto,[1] Aki Takuwa, Akemi Terashi, Iori Ui, Aya Okajo, and Kazutoyo Endo*

Department of Physical Chemistry, Showa Pharmaceutical University; 3–3165 Higashi-Tamagawagakuen, Machida, Tokyo 194–8543, Japan. Received November 27, 2004; accepted April 2, 2005; published online April 19, 2005

The age dependence of ketone body levels (KBLs) and oxidative damages in selenium-deficient (SeD) and normal rats were compared. The feeding SeD diets gave ketogenesis and higher KBLs especially in younger rats. However, KBLs in SeD rats seemed to decrease with their age. Feeding 0.1 mg/kg Se in water with SeD diet did not affect the KBLs in young (8 week old) rats, whereas the addition of Se reduced the KBLs in older (20 week old) rats. Blood KBLs showed some correlations with tissue damage. TBARSs showed no correlations with the tissue damages and KBLs when the values were compared between the same age, while better correlation was obtained between urinary KBLs of 6—20 week old normal rats and the liver TBARSs of 4—16 week old normal rats. The oxidative injury might induce liver damage with some delay. SeD rat kidney TBARS levels normalized by protein had some correlations with BUN and blood KBL. Kidney may be sensitive to the oxidative stresses and/or injuries. Tissue damages of SeD rats decreased with age. In contrast, oxidative injuries might be gradually accumulated in normal rat tissue. Oxidative stress can be visible by gradual accumulation of small damages during the aging, while large stress in young rats can be buffered and masked. The aging based accumulation of oxidative injuries might also be correlated with KBLs, while it might not give notable tissue damages.

Key words selenium deficiency; ketone body; oxidative stress; oxidative injury; liver; kidney

Selenium (Se) is known as the active center of glutathione peroxidase (GSH-Px). GSH-Px reduces hydrogen peroxide (H_2O_2) consuming a reduced form of glutathione (GSH) in the living body. Therefore, Se deficiency causes a malfunction of GSH-Px resulting in an increase of H_2O_2 in the body.[2] An increasing level of H_2O_2 may lead to the formation of hydroxyl radicals (·OH) through the Haber-Weiss and/or Fenton reactions. Hence, the intracellularly important molecular functions of lipids, sugars, proteins, and/or nucleic acids could be oxidatively damaged, which will finally result in some disorders. Selenium-deficient (SeD) rat model,[3—7] therefore, have been used to investigate the oxidative stress caused by H_2O_2.

The role of Se in the living body, however, is not limited to being the active center of the GSH-Px but is also related to other factors not associated with GSH-Px. For example, iodothyronin deiodinase produces active thyroid hormone from inactive precursors, hence Se should have an effect on the functions of the thyroid, since Se-dependent thioredoxin reductases are also involved in intracellular redox process. The effect of Se deficiency must result in a composite effect due to malfunctions of those proteins/enzymes. However, the individual roles of most selenoproteins/selenoenzymes are still unclear. In other words, the role of Se is multiple in organisms. Several important health effects of Se have been described, including immune response, cancer prevention, cardiovascular disease, reproduction, and mood.[8]

The increasing urinary KBL (KBL) in SeD rats has been shown in a numbers of earlier reports.[9—14] However, the mechanism of increasing KBLs as a result of Se-deficiency is still ambiguous. It has been reported that no significant differences were found between SeD and Se-adequate mice in urinary 3-hydroxybutyrate.[15] It has also been reported that the plasma KBL did not increase in Se-deficient rats.[11] Oxidative stress and the subscribed damages in SeD rat may be varied depending on age and feeding conditions, although the age dependence of oxidative tissue damage in SeD model rat was not clear.

It has been suggested that the organs/tissues of SeD rat are impaired by its oxidative stress. In addition, the tissue damages may be related to KBL. It has been proposed that the ketonuria of the SeD rat is a result of impaired renal function.[14] However, in the liver of SeD, high AST and ALT values are found only in young rats.[5] In contrast, oxidative damages of normal rat liver were gradually increased with their age.[5] In this paper, the age-dependence of KBLs and extent of oxidative injury levels are compared in liver and kidney of normal and SeD rats. The relationship among the KBL, tissue damage, and oxidative stress levels is described.

MATERIALS AND METHODS

Materials SeD diet was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). The Se content of the SeD was reported in our previous paper as 0.017 mg/kg by instrumental neutron activation analysis (INAA).[6] Deionized water prepared by Milli-Q system was used for all experiments and used for drinking water for rats. Other materials were in analytical grade.

Animals Wistar rats (15th day after pregnancy) were fed on a SeD diet and Milli-Q water. Newly born rats were kept with their mother for four weeks. They were then weaned and fed with SeD diets and Milli-Q water until experimentation (SeD group).[2—6] The control (SeC) group was prepared in the same manner as the SeD group except that Na_2SeO_4 (0.1 mg/kg as Se) was added to the drinking water. Healthy Wistar rats purchased from Japan Laboratory Animals, Inc. (Tokyo, Japan) were used as the normal group. The purchased normal rats had been fed on CLEA CE-2 diet (CLEA Japan, Inc., Tokyo) consistently before and after purchasing. The Se content of the CE-2 diet was reported in our previous paper as 0.86 mg/kg by INAA.[4] Rats were used for experiments at 6, 8, 12, 16, and 20 week old except that SeC rats were prepared for 8 and 20 week old groups. The body

* To whom correspondence should be addressed. e-mail: kazutoyo@ac.shoyaku.ac.jp

weight of each group is shown in Table 1. Se contents in the SeD rats liver were under detection limit by means of INAA throughout 6—20 weeks of age, while those in the kidney were 0.74—1.23 mg/kg of dry tissue weight.[16] Se contents in 6—20 week old normal rats liver and kidney were 4.65—5.76 and 7.42—9.50 mg/kg of dry tissue weight respectively.[16] GSH-Px activities in 6—20 week old SeD and normal rat livers were 4.5—22.2 and 354—707 U/mg protein, respectively.[5] Se contents in 8 week old SeC rats liver was 1.87 mg/kg of dry tissue weight.[7] GSH-Px activity in 8 week old SeC rats liver was 367 U/mg protein.[7] The animal experiments were carried out in compliance with the Guidelines for Animal Care and Use at Showa Pharmaceutical University (2001), and approved by the Ethical Committee for Animal Care and Use of Showa Pharmaceutical University.

Ketone Body in Urine and Blood Each rat was moved into a metabolic cage and fasted for 24 h before being sacrificed. Simultaneously, the urine of each rat was collected for 24 h. The urinary ketone body was preliminary tested using a test stick used clinically for humans (Pretest, Wako, Tokyo, Japan). The urine was centrifuged at 1000×***g*** for 10 min. The supernatant (0.25 ml) was added into 8.75 ml water and used for the analysis of ketone body. The rat was anesthetized with 0.05 mg/g b.w. intraperitoneal injection of pentobarbital (Nembutal, Dinabot, Osaka, Japan). Abdomens were opened and blood was collected from the abdominal aorta. 0.25 ml of blood was added into 8.75 ml water and used for the analysis of ketone body. The trinitrobenzene method[17] was employed for analysis of ketone body.

ALT, AST, and BUN in Plasma The collected blood was centrifuged at 1000×***g*** for 10 min. An aliquot of the plasma fraction was tested for ALT, AST, and BUN, performed by Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo, Japan).

TBARS in Liver and Kidney After blood collection, the whole body of the rat was perfused by ice-cold saline (0.9% NaCl) until the remaining blood was almost completely removed. The liver and kidney were removed. Each organ was homogenized with 10-fold volume of saline (1.15% KCl). The TBARS levels of the homogenates were determined according to the method of Ohkawa *et al*.[18]

H_2O_2 Level in Bile Rats are anesthetized with 0.05 mg/g b.w. intraperitoneal injection of pentobarbital. The bile duct was cannulated by PE-10 tubing. The bile was collected every 5 min after cannulation. The H_2O_2 level in the bile was measured by the EPR method.[2,7] The 0.025 mg/g b.w. pentobarbital are added in femoral muscle 40 min after first anesthetization. Mean H_2O_2 levels during 2 h measurement were compared with age.

Statistical Test The statistical differences were estimated with alternative Student's or Welch's *t*-test. The suitable test for the data was automatically selected according with distribution of the data. Grades of significance were estimated by $p<0.05$, $p<0.01$, and $p<0.001$.

RESULTS

Table 1 shows body weights of rats used in this paper. The body weights of SeD rats were 41—49% of the normal rats. Significances between SeD and normal groups were obtained all ages compared. Addition of 0.1 mg/kg Se in drinking water give no effect on rat growth at 8 week old, while the body weight was recovered to 67% of the normal rat weight. The 20 week old SeC rats were significantly heavier than the SeD rats in the same age.

Table 1. Body Weight of Rats Used in the Experiments

Age (week)	Normal groups (g)	SeC groups (g)	SeD groups (g)
6	162.3±26.0 (32)		75.6±7.9 (32)**
8	224.1±16.0 (37)	100.8±25.6 (4)**	110.3±25.5 (35)**
12	358.9±15.4 (16)		149.9±15.8 (13)**
16	424.1±15.9 (28)		194.0±38.4 (25)**
20	454.9±15.4 (10)	303.6±36.8 (5)**,##	190.2±19.7 (15)**

The body weights were measured after overnight fasting. Values are indicated as mean±S.D. Numbers indicated in parenthesis are the number of rats in each group. The number of rats includes rats not only for this paper but also for the other experiments in our laboratory. Significances were indicated as ** by means of $p<0.001$ when the value was compared with the normal group. Significances between SeD and SeC groups were indicated ## by means $p<0.001$.

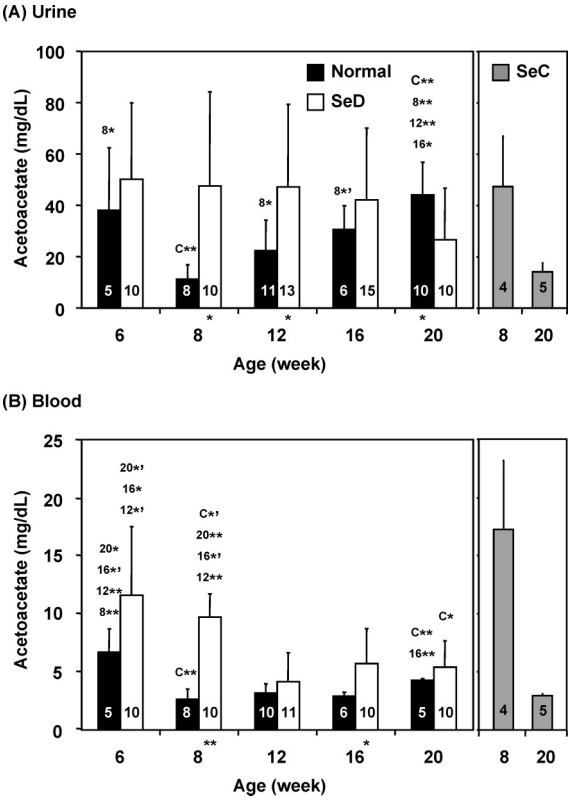

Fig. 1. Urinary and Blood KBLs of the Normal, SeD, and SeC Rats Measured by the Trinitrobenzene Method

(A) Urine and (B) blood ketone body were tested for several ages. The right panels show the results of SeC rat. Values are indicated as mean±S.D. Number indicated in each column shows the number of rats in the group. Significances are indicated as *, *′ and ** by means of $p<0.05$, $p<0.01$ and $p<0.001$ respectively. The marks below the horizontal axis show significances between the normal and SeD groups of the same age. The marks above each column with a number indicate the significances among other age groups and the number shows the age of compared group. C*, C*′ and C** means significance to the SeC group of the same age.

Figure 1 shows the time course of ketone body (acetoacetate) in urine and blood by means of the trinitrobenzene method. The urinary KBLs (Fig. 1A) were higher in SeD rats compared with normal rats at relatively young ages. However, no differences were obtained in the urinary KBLs of 16 week old rats, and the normal rat showed higher levels than the SeD group at 20 weeks of age. Urinary KBLs of normal

rats are gradually increasing through their age except that 6 week old rats showed higher values compared with other ages. The right panel showed the KBLs of SeC groups. The young SeC group showed no difference with SeD group of the same age. However, the old SeC group showed lower value than the SeD group of the same age, although no significance was obtained. Each SeC group has significance with the normal group of the same age.

The blood KBLs (Fig. 1B) showed different time course trends compared with those in the urine. The blood KBLs were lower compared with urinary KBLs. The blood KBLs of the SeD rat were always higher than those of normal group of the same age, and significance was obtained for 8 and 16 week old groups. The blood KBLs of normal rats looked also slightly increasing through their age except that 6 week old rats showed higher values compared with other ages. The 20 week old normal rats showed significantly higher values than the 16 week old group. In SeD rats, markedly higher values are shown in the youngest 2 groups compared with older group. The right panel showed the KBLs of SeC groups. The young SeC group showed significantly higher value than both normal and SeD groups of the same age. However, the old SeC group shows significantly lower value than both normal and SeD groups of the same age.

Figures 2A and B show time courses of TBARS (nmol/g tissue) in the liver and kidney respectively. The liver TBARS levels of SeD groups were highest at 8 weeks of age, while the 12 week old group showed the lowest value. The liver TBARS levels of SeD groups gradually seemed to increase after 12 weeks of age. In normal rats, liver TBARS levels gradually increased with age. The oldest three groups of normal rats showed significantly high TBARS values compared to the SeD groups. The kidney TBARS levels of both normal and SeD rat groups appeared to gradually increase with age (Fig. 2B). The 20 week old normal rats showed significantly higher values than the SeD rats.

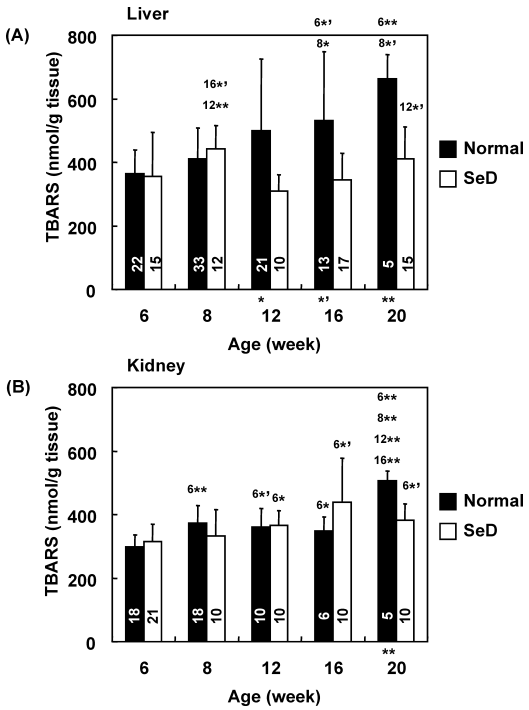

Fig. 2. Liver and Kidney TBARS Levels of the Normal and the SeD Rats

(A) Liver TBARS levels of and (B) kidney TBARS levels were obtained for several ages. Values are indicated by mean±S.D. Number indicated in each column shows the number of rats in the group. Significances are indicated as *, *′ and ** by means of $p<0.05$, $p<0.01$ and $p<0.001$ respectively. The marks below the horizontal axis show significances between the normal and SeD groups of same age. The marks above each column with a number indicate the significances among other age groups and the number shows the age of compared group.

Figure 3 shows time courses of AST, ALT, and BUN in plasma. The AST and ALT levels of SeD rats at a young age showed significantly higher values than normal rats. With increasing age, the AST and ALT level of SeD rats gradually decrease to the level of normal rats. No marked time dependence was obtained in the AST and ALT level of normal rats. The BUN showed a similar trend as shown in the AST and the ALT, while differences between normal and SeD groups in young rats were more notable. In addition, the patterns of time courses of BUN look similar to those shown in blood KBL levels.

Figure 4 shows the time course of the bile H_2O_2 levels of normal and SeD rats. The 8 week old SeD rat showed markedly high H_2O_2 level (nearly 150 μM) in the bile. Other SeD and normal rat groups showed the H_2O_2 levels close to the minimum detectable limit (10 μM), although younger rats below 6 weeks of age can not be subjected to the experiment due to difficulties in bile duct cannulation at this age.

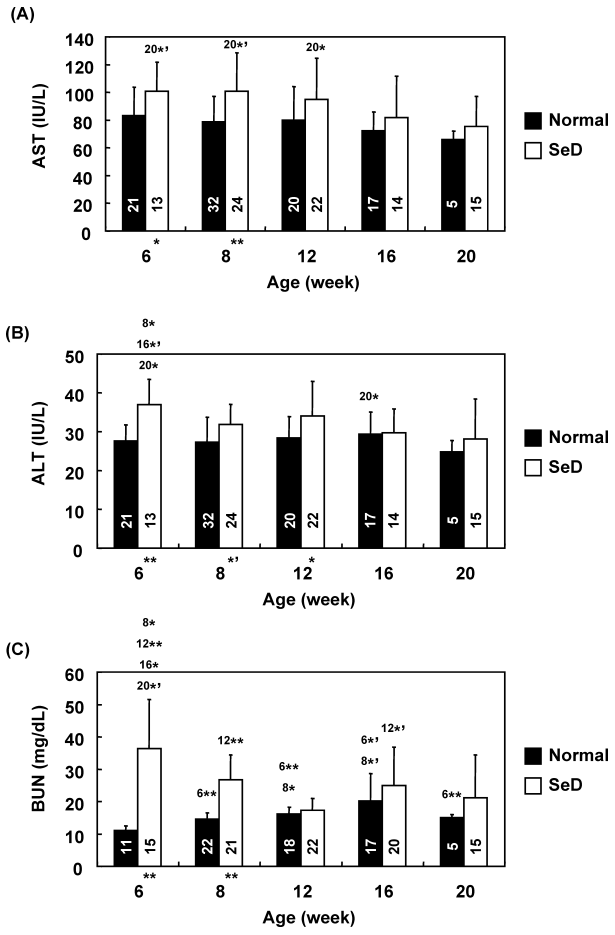

Fig. 3. Plasma AST, ALT, and BUN Levels of the Normal and the SeD Rats

(A) AST levels, (B) ALT levels, and (C) BUN levels were obtained for several ages. Values are indicated by mean±S.D. Number indicated in each column shows the number of rats in the group. Significances are indicated as *, *′ and ** by means of $p<0.05$, $p<0.01$ and $p<0.001$ respectively. The marks below the horizontal axis show significances between the normal and SeD groups of the same age. The marks above each column with a number indicate the significances among other age groups and the number shows the age of compared group.

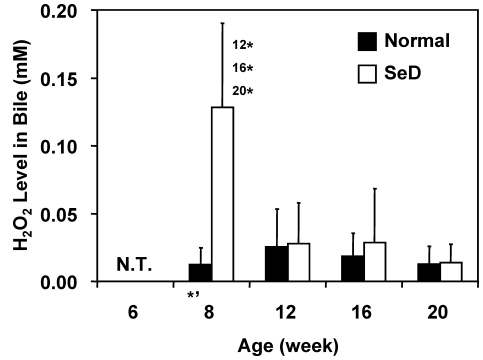

Fig. 4. Hydrogen Peroxide Levels in Bile of the Normal and SeD Rats at Several Ages

Values are indicated by mean±S.D. The number of rats in each group was 4, except that the number of the normal and the 20 week old SeD groups was 3. N.T. means "not tested." Significances between the normal and SeD groups are indicated below the horizontal axis as *' by means of $p<0.01$. The * with a number indicated above each column shows significances ($p<0.05$) among other age groups and the number is the age of compared group.

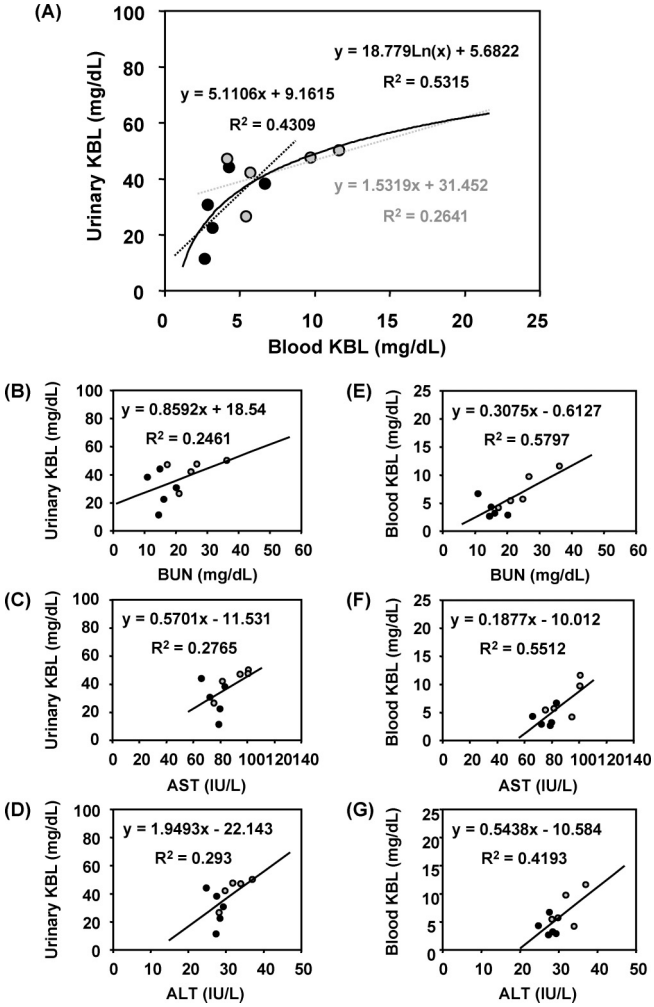

Fig. 5. Correlation between Blood and Urinary KBLs, and Those Correlations among Several Tissue Damage Indices

(A) Non-linear correlation can be found between blood and urinary KBLs. (B, C, and D) Correlations among urinary KBLs and several indices of tissue damage were small ($R^2>0.2$). (E, F, and G) Blood KBL have better correlations ($R^2>0.5$) with several tissue damages.

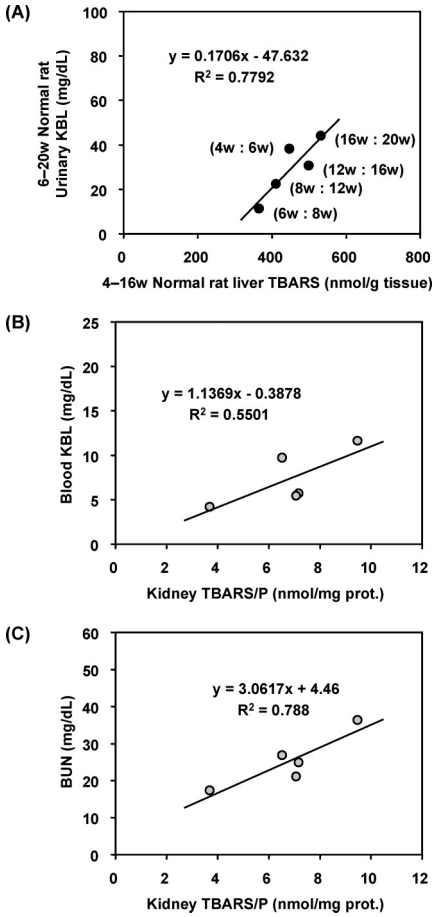

Fig. 6. Correlations Found in Part of Data

(A) Good correlation ($R^2>0.7$) was obtained between 6—20 week old normal rat urinary KBLs and 4—16 week old normal rat liver TBARS. Numbers in parenthesis indicate the ages of rats. The SeD rat kidney TBARS/P had some correlations ($R^2>0.5$) with (B) blood KBLs and (C) BUN.

Figure 5A shows a correlation of blood and urinary KBLs. Non-linear correlation can be found between blood and urinary KBLs. Urinary KBLs in SeD rat were relatively small when blood KBLs were high. Figure 5B—G shows correlations among KBLs and several indices of tissue damage. Blood KBL have weak correlations with several tissue damages, while urinary KBL have lesser correlations. Both blood and urinary KBL had no correlation with liver or kidney TBARS ($R^2<0.1$, data not shown).

Figure 6 shows correlations obtained for a part of the data. When 6—20 week old normal rat urinary KBLs were compared with 4—16 week old normal rat TBARS, a good correlation was found (Fig. 6A). TBARS of 4 week old normal rat was 448.2±53.1 nmol/g tissue ($n=5$). When the SeD rat kidney TBARS was normalized by the protein concentration, *i.e.* TBARS/P (nmol/mg protein), some correlations of TBARS/P with blood KBL and BUN were obtained.

DISCUSSION

Only a few cases of the blood KBLs have been reported so far,[11,14] although the blood and/or plasma KBLs may be important to elucidate the relationship between the ketone body and Se-deficiency. In this paper, the trinitrobenzene method[17] was employed to quantify KBLs in rat blood and

also urine. This method is sensitive to acetoacetate while 3-hydroxybutyrate is not detected by this method. The trinitrobenzene method was proposed as an easy and accurate method for ketone body (acetoacetate) quantification.

The feeding SeD diets reduce growth rate of rats and gave higher KBLs especially in younger rats. Feeding 0.1 mg/kg Se in water with a SeD diet did not affect growth rate nor KBLs in young (8 week old) rats, whereas the addition of Se recovered their body weight and reduced the KBLs in older (20 week old) rats. This fact suggests that the higher tissue damages and KBLs in young SeD rats may mainly be due to low nutritional statuses in the SeD diet rather than Se-deficiency. Addition of inorganic Se may give additional stress and accelerate the ketogenesis for young rats. However, the exogenous inorganic Se may act as an antioxidant in older rats, in addition to recovering the GSH-Px activity. Supplemental Se may be effective in older rats than younger ones. Se may be preventive to an accumulation of relatively mild tissue and/or cellular damages, such as aging, tumor genesis, etc, while it may not be effective in causing severe damages shown in young SeD rats.

A human volunteer study suggested that selenate showed grater absorption than selenite.[19] Intestinal absorption of selenate was mediated by active ileal selenate transport mechanism.[20,21] Selenate is supposed to be reduced to selenite then organic form of selenium in an utilization of selenium in the living body. In this study, the SeC group was administered selenate in the drinking water. The oxidized form of inorganic selenium, *i.e.* selenate, was expected to have a higher absorption when it was orally administered. However, it should be a mix dose of selenate, selenite, and organic selenium compounds in order to make a suitable control group considering the natural diet source of selenium. Differences in chemical form of the administered selenium may relate to the toxicity of side effects.

The result in this paper suggests that the urinary ketone bodies of the normal rats increased with rat age over 8 week old. However, the age dependent increases of urinary KBLs in normal rats also did not clearly show in the blood KBLs. It may be because that the ketone bodies excreted in the urine should be concentrated through the kidney. The higher blood KBLs in younger SeD (6 and 8 week old) rats are not reflected in the urinary KBLs. It can be considered that renal function of SeD rats may be impaired and urine concentration may not be sufficient. In other words, the blood KBL was magnified in the normal rat urine but not in the SeD rat urine. Although ketonuria in rats is believed to reflect the high blood KBL, it may not be possible beyond some limited level for urinary KBLs of SeD (Fig. 5A). The impaired renal function of SeD rats may relate to higher blood KBLs rather than the urinary KBLs. However, the higher blood KBLs in young SeD rats may be because of abnormal ketogenesis due to low nutritional level and fastening before the experiment, but may not be a result of oxidative injuries. The ketonuria in older normal rats (beyond 12 week old) may be a result of magnified blood KBL, which may reflect decrease of ketone body consumption in the liver and partly in the kidney due to accumulation of oxidative injuries in the tissues.

Oxidative injury appears to gradually accumulate in normal rat liver (Fig. 2A). However, the oxidative injury in SeD rat livers showed no marked change through their age and it looks smaller than that for normal rats. Age dependence of the oxidative injury in kidney looked similar between normal and SeD rats. Tissue TBARS levels showed no correlation with tissue damages and KBLs. While better correlation was obtained between urinary KBLs of 6—20 week old normal rats and the liver TBARSs of 4—16 week old normal rats (Fig. 6A). The oxidative injury might induce liver damage and raise AST, ALT levels after some delay.

TBARS/P has different age dependence from TBARS (data not shown). Liver TBARS/P of both rat groups shows no marked variation through their life. However, the liver TBARS/P of normal rats are almost double of SeD rats, because the protein level of SeD rats were 1.8—2.2 fold higher than normal rats. This may have some relations with the fact that the SeD rat liver had hypertrophicly swollen. Percentage of lipid decreased and in turn protein increased, which corresponds to the result that the percentage of cytosolic fraction increased in SeD rat liver.[4] No marked difference was obtained for kidney protein levels between normal and SeD rats. TBARS/P in liver did not show any correlations with other factors, while TBARS/P in SeD rat kidney showed some correlations with blood KBL and BUN (Figs. 6B, and C). In other words, the high oxidative stresses in young SeD rats may have some correlation with kidney damage and high blood KBL levels, while liver damage did not show any relations with oxidative stress or damage immediately.

The age dependence of H_2O_2 levels (Fig. 4) suggests that the oxidative stress in SeD rats actually decreased with age after 8 weeks. A possibility is an accumulation of GSH due to the malfunction of the GSH-Px activity. Our recent experiment showed that the GSH level of female 8 week old SeD rats was higher than that of normal rats.[22] It was our previous expectation that SeD rats would show some synergistically high oxidative damages. In contrast, the KBLs and tissue damages in SeD rats was not so high (Figs. 1, and 3), and again the TBARS levels looked same or lower in SeD rats compared with normal rats in our study (Fig. 2). Actually, the time courses of AST and ALT levels in this study (Figs. 3A and B) suggest that the liver damage observed in young SeD rats was gradually repaired with increasing age.

There is a report that shows variation of redox enzymes, such as catalase, SOD, GSH-Px, and mitochondrial P-450 become activated in the liver of rats, by administration of 1% acetone in drink water for 7 d.[23] However, TBARS level did not change significantly by acetone in that report. The acetone might make a metabolic change in liver and create some oxidative stresses, but damage would be expected to be weak.

There is another report that claims a combination of Se and high dose vitamin E administration protected liver, and kidney from cisplatin-induced oxidative damage.[24] Therefore, Se efficiently reduces severe oxidative stresses. The deficiency of Se, in contrast, does not exaggerate chronic oxidative stress level in the well buffered biological mechanism. Moreover, relatively long exposure to the Se-deficient condition may trigger another system to reduce weak chronic oxidative stress and damage. However, the substituted system may be fragile and may be easily damaged by additional oxidative stresses, such as exercise. In other words, the Se-deficiency is insignificant for relatively hypoxic tissues but can be very problematic for hyperoxic tissues, such as lung and heart. There are number of reports describing heart failure in

Se deficiency.[25,26]

The visible symptoms of SeD rats, such as hair loss, ateliosis, aggressiveness, etc., however, vary considerably with the feeding conditions and sometimes they can be difficult to distinguish compared with normal animals, especially in older rats. Those symptoms are not peculiar for Se-deficiency and are also observed in several experimental models for nutritional deficiency. Se-deficiency in rats is typically not lethal. Actually, our SeD rat model, which consists of rats fed a SeD diet while still in the womb of the mother, resulted in rats that were severely deficient in Se, but lived longer than 50 weeks. Therefore, oxidative stress in the SeD rat may, in total, decrease with age while oxidative stress in the normal rat may increase along with oxidative injury accumulation.

CONCLUSION

The feeding SeD diet may give tissue damage and higher KBLs in relatively young rats. Feeding Se in water with SeD diet showed no effect to the KBLs in young rats, while the Se reduced the KBLs in relatively older rats. Blood KBLs showed some correlations with tissue damage. Kidney may be sensitive to the oxidative stresses and/or injuries, but liver showed a delayed response. Tissue damages of SeD rats decreased with age. In contrast, oxidative injuries may gradually accumulate in normal rat tissue. Oxidative stress can be visible by gradual accumulation of small damages during the aging, while large stress in young rats can be buffered and masked. The aging based accumulation of oxidative injuries might also be correlating with KBLs, while it might not give notable tissue damages.

REFERENCES AND NOTES

1) Present Address: *Radiation Biology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892–1002, U.S.A.*
2) Ueda Y., Matsumoto K., Endo K., *Biochem. Biophys. Res. Commun.*, **271**, 699—702 (2000).
3) Matsumoto K., Endo K., Utsumi H., *Biol. Pharm. Bull.*, **23**, 641—644 (2000).
4) Matsumoto K., Inagaki T., Hirunuma R., Enomoto S., Endo K., *Anal. Sci.*, **17**, 587—591 (2001).
5) Matsumoto K., Ui I., Satoh K., Tobe T., Ushio F., Endo K., *Redox Rep.*, **7**, 351—354 (2002).
6) Matsumoto K., Yamazaki M., Satoh K., Ushio F., Endo K., *Yakugaku Zasshi*, **122**, 283—290 (2002).
7) Ui I., Okajo A., Endo K., Utsumi H., Matsumoto K., *Free Radic. Biol. Med.*, **37**, 2012—2017 (2004).
8) Rayman M. P., *Lancet*, **356**, 233—241 (2000).
9) Olsson U., *Possible Implications*. *Metabolism*, 3**4**, 993—998 (1985).
10) Olsson U., *Int. J. Vit. Nutr. Res.*, **60**, 89—94 (1989).
11) Yoshida M., *J. Nutr. Sci. Vitaminol.*, **37**, 425—434 (1991).
12) Yoshida M., *J. Trace Elem. Electrolytes Health Dis.*, **7**, 25—28 (1993).
13) Olsson U., *Prostaglandins Leukot. Essent. Fatty Acids*, **48**, 385—390 (1993).
14) Olsson U., Kallner A., *J. Trace Elements Med. Biol.*, **9**, 88—93 (1995).
15) Wendel A., Otter R., *Biochim. Biophys. Acta*, **925**, 94—100 (1987).
16) Matsumoto K., Terada S., Ariyoshi M., Okajo A., Hisamatsu A., Ui I., Endo K., (unpublished results).
17) Momose T., Ohkura Y., Kohashi K., Nagata R., *Chem. Pharm. Bull.*, **11**, 978—977 (1963).
18) Ohkawa H., Ohishi N., Yagi K., *Anal. Biochem.*, **95**, 351—358 (1979).
19) Thomson C. D., Stewart R. D., Robinson. M. F., *Br. J. Nutr.*, **33**, 45—54 (1975).
20) Wolffram S., Arduser F., Scharrer E., *J. Nutr.*, **115**, 454—459 (1985).
21) Arduser F., Wolffram S., Scharrer E., *J. Nutr.*, **115**, 1203-—1208 (1985).
22) Matsumoto K., Suzuki A., Washimi H., Hisamatsu A., Endo K., (unpublished results).
23) Orellana B. M., Guajardo V., Araya J., Thieleman L., Rodrigo R., *Comp. Biochem. Physiol. C*, **128**, 503—509 (2001).
24) Naziroglu M., Karaoglu A., Aksoy A. O., *Toxicology*, **195**, 221—230 (2004).
25) de Lorgeril M., Salen P., Accominotti M., Cadau M., Steghens J. P., Boucher F., de Leiris J., *Eur. J. Heart Fail.*, **3**, 661—669 (2001).
26) Witte K. K. A., Clark A. L., Cleland J. G. F., *J. Am. Coll. Cardiol.*, **37**, 1765—1774 (2001).