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GPx-1: An important primary antioxidant enzyme.

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What is GPx-1?

- GPx-1 (glutathione peroxidase-1), the first identified and the most abundant Se (selenium)-dependent protein in mammals.
- Chromosomal Location: 3p21.3.
- Se (selenium) is an essential antioxidant nutrient because it is essential for the activate site of GPx and related antioxidant enzyme.

The GPx Family

- **GPx-1: cellular GPx**
- Gpx-2: gastrointestinal GPx
- GPx-3: plasma or extracellular GPx
- GPx-4: phospholipid hydroperoxide GPx
- GPx-5: secretory GPx

Antioxidant enzyme system

- GPx-1 is considered as the major enzyme responsible for removing H_2O_2

  $2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$

  $2GSH + ROOH \xrightarrow{GPx} GSSG + ROH + H_2O$

- GPx-1 can metabolize a range of organic peroxides, including cholesterol and long–chain fatty acid peroxide.
- The ranking order of tissue-specific stabilities of GPx-1 is:

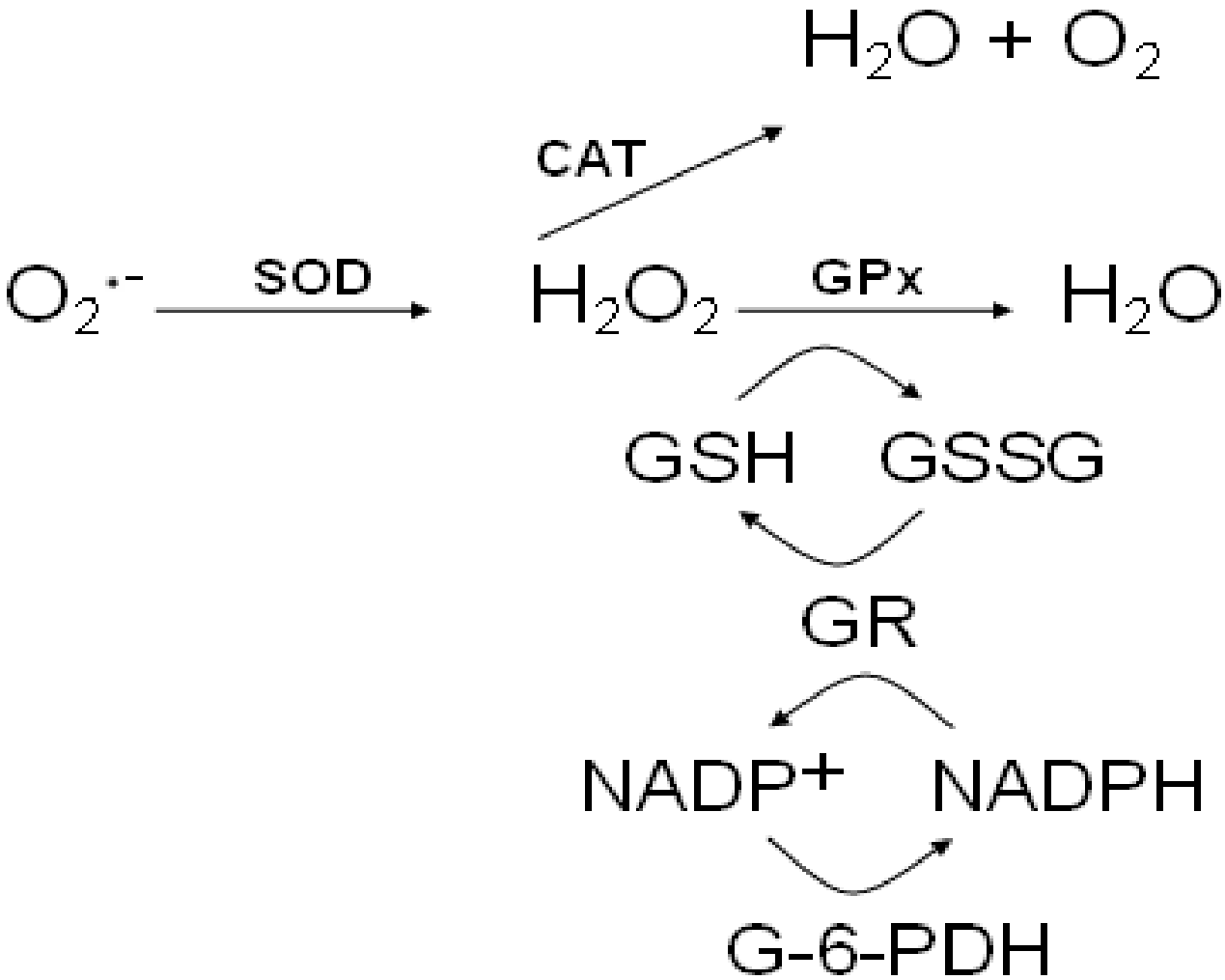
  Brain> Thymus> Thyroid> Heart> Liver, Kidney, Lung

GPx-1 reacts faster than PhGPx

Substrate	GPx-1 k ($M^{-1}S^{-1}$)	PhGPx k ($M^{-1}S^{-1}$)
Hydrogen peroxide	3.9×10^7	3.2×10^6
Cumene hydroperoxide	3.9×10^7	3.2×10^6
Linoleic acid hydroperoxide	3.1×10^7	3.9×10^7

Giffith OW *et al*. (1985) Origin and turnover of mitochondria glutathione Proc. Natl. Acad. Sci. USA **82**: 4668-4672.

GPx as part of the antioxidant enzyme system



Some properties of GPx-1

- **Mills** first describe GPx-1 activity in 1957. GPx-1 was hypothesized to protect red blood cells against haemolysis by oxidation. Mills G. C. (1957) Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J. Biol. Chem. **229**: 189-197.
- GPx-1 is one of the best characterized selenoproteins. When replace selenocysteine with cysteine at the active site of GPx will cause a large decrease in enzyme activity. This is because selenocysteine has more efficient redox catalyst than cysteine at physiological pH.

Some properties of GPx-1

- GPx-1 is a homotetramer of ~22 KDa subunits and located in the cytosol, and motochondria.
- GPx-1 may serve as a selenium storage or selenium buffer protein.
- Within appropriate selenium intake, GPx-1 activity in cells is very closely regulated.

(Vadim N, et al 1999 Biomed. Sci.**6**:151-160; and J. R. Arthur 2000 CMLS. **57**:1825-1835)

What will happen when GPx-1 is overexpressed?

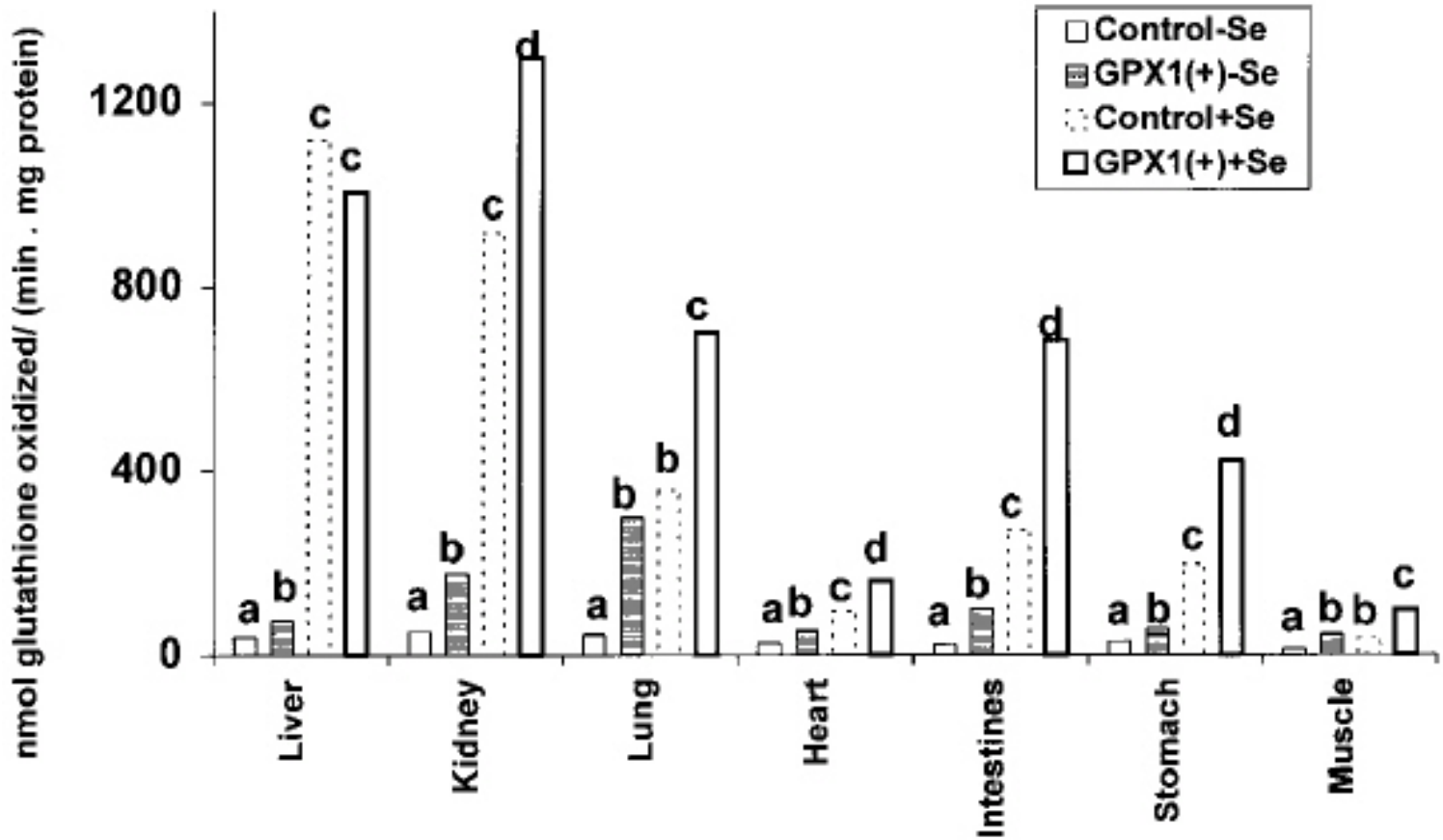
- Many studies show that overexpression of GPx-1 can against hydroperoxide. Taylor S. D. *et al.* (1993) Glutathione peroxidase protects cultured mammalian cells from the toxicity of adriamycin and paraquat. Arch. Biochem. Biophys. **305**: 600-605.
- Overexpression GPx-1 can also inhibit hydrogen peroxide-induced apoptosis in cell lines.
- However, overexpression of GPx-1 and GPx-3 in transgenic mice are less able to produce heat shock protein 70. Therefore, overexpression of GPx-1 in animals can not be said to be beneficial.

Will selenium intake affect GPx-1 expression?

- Mice overexpressing GPx-1 have been fed diets containing different levels of selenium. The mice expressed more GPx-1 activity than control animals at high or low dietary selenium intakes. The difference in GPx-1 activity was only apparent in selenium-deficient animals.

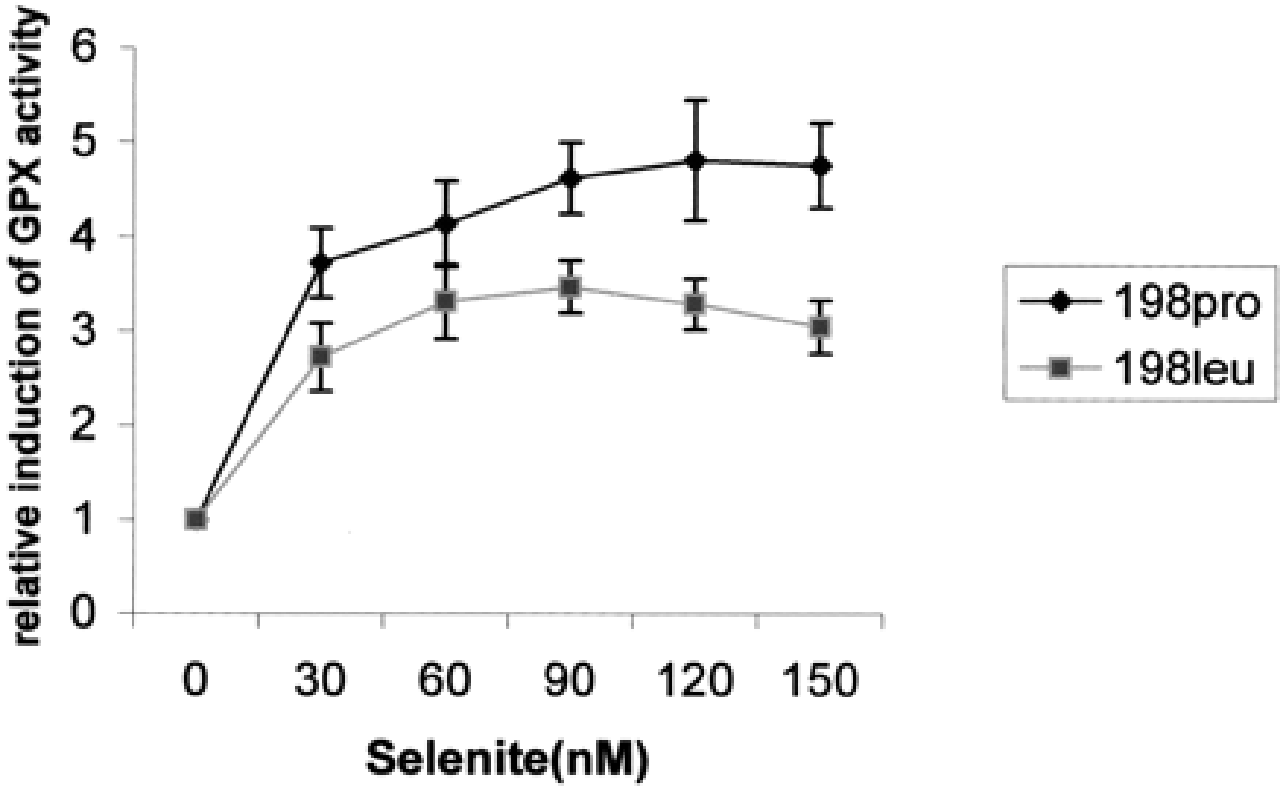
Cheng WH *et al*. (1997) Over-expression of cellular glutathione peroxidase does not affect expression of plasma glutathione peroxidase or phospholipid hydroperoxide glutathione peroxidase in mice offered diets adequate or deficient in selenium. J. Nutr. **127**: 675-680.

Effects of dietary Se concentrations and overexpression of GPx-1 (Cheng *et al.* 1997 ACNS **127**:675-680.)



+Se: with Selenium; –Se: without selenium.
GPX1(+): Overexpressing GPx-1 gene.

Selenium induction of GPx activity in MCF-7 cells Hu YJ *et al*. (2003) Cancer Res. **63**(12):3347-51.



GPx activity was measured after supplementation of the culture media with the indicated concentration of selenium in the form of sodium selenite for 5 days. **198pro: proline-containing allele 198leu: leucine-containing allel**

Loss of GPx-1 activity

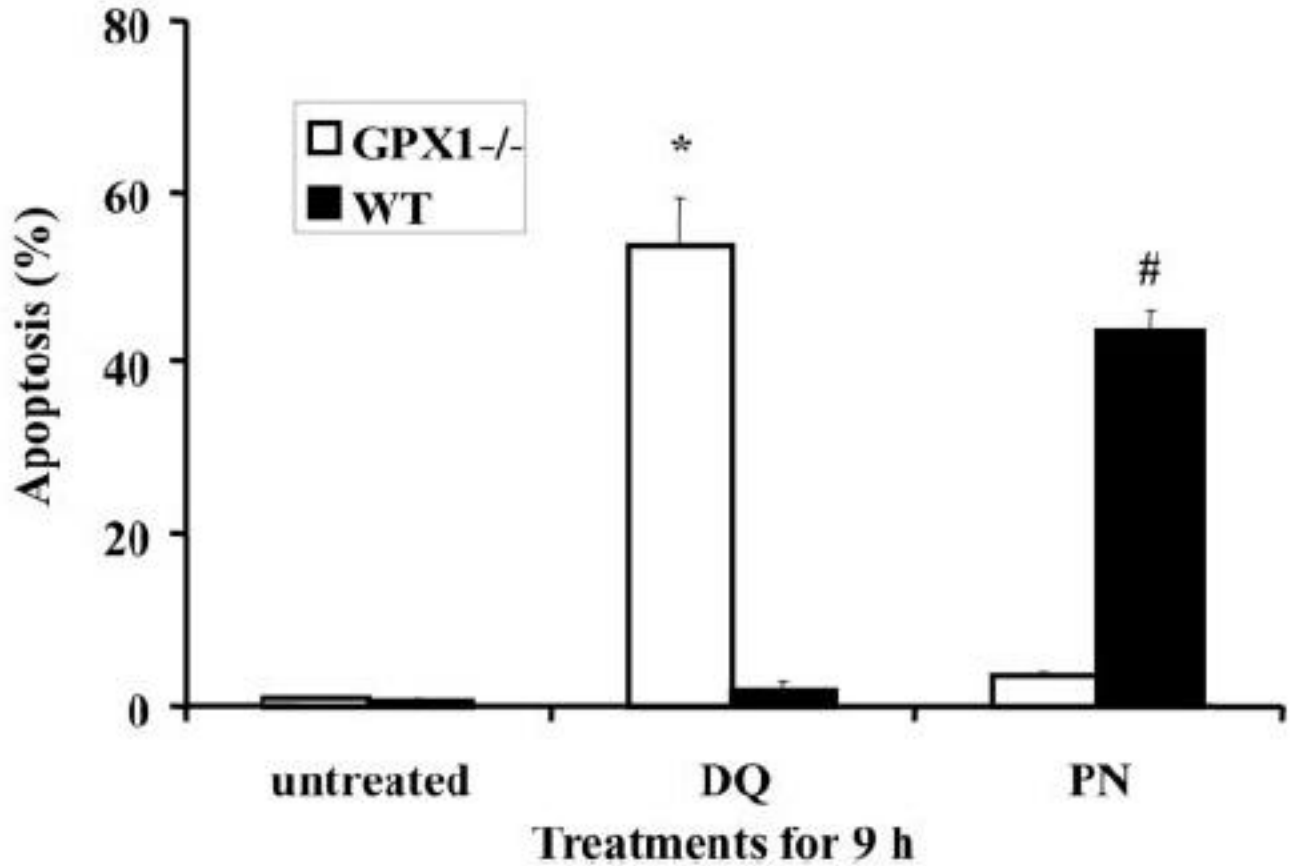
- Model: The gene knockout (KO) technique has been used to explore the effects of loss of GPx-1 activity.
- In the KO animal, other GPx is may be able to compensate for the loss of GPx-1.
- The GPx-1 KO mice had no obvious deterious phenotype. However, KO mice have increased susceptibility to different oxidative stress when compared with normal mice.

Yoshida T *et al*. (1997) Glutathione peroxidase knockout mice are susceptible to myocardial ischaemia reperfusion injury. Circulation 96: 216-220.

Loss of GPx-1 activity

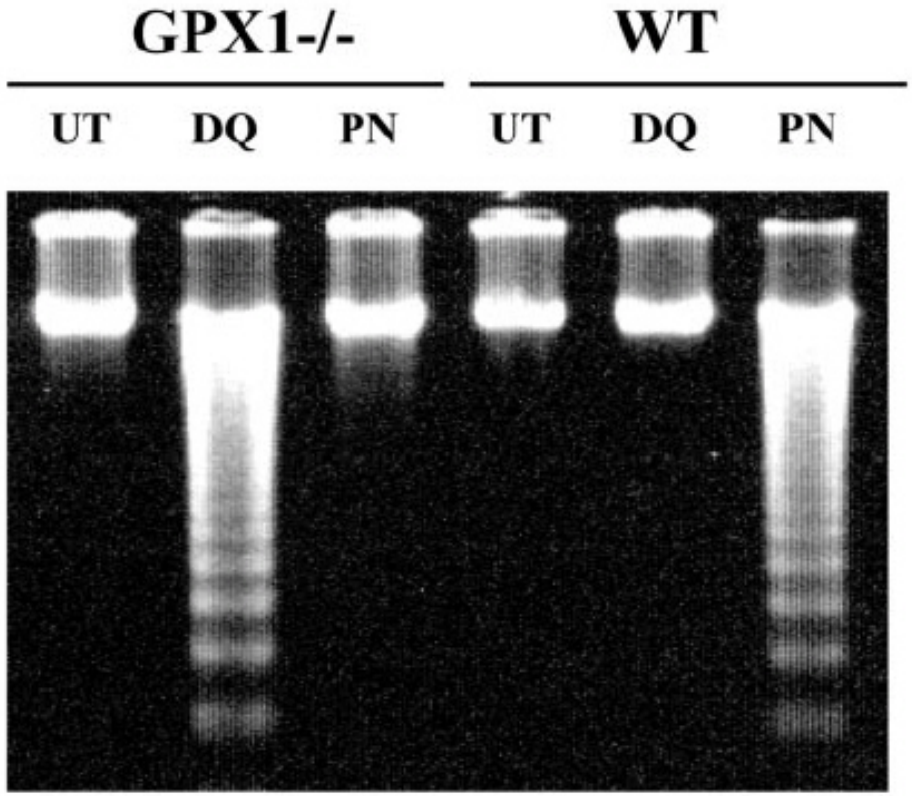
- It has been reported that homocysteine inhibited the expression of GPx-1 and lead to an increase in ROS that inactivated nitric oxide and promoted endothelial dysfunction. Upchurch GR Jr. *et al*. (1997) Homocysteine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. J Biol Chem **272**:17012-17017.
- Furthermore, heterozygous GPx-1-deficient mice show endothelial dysfunction, and an increase in the plasma and aortic level of isoprostane iPF2α-III, a marker of oxidant stress. Forgione MA *et al*. (2002) Cellular glutathione peroxidase deficiency and endothelial dysfunction. Am J Physiol Heart Circ Physiol 282: H1225-H1261.

GPx-1 in superoxide generator-induced apoptosis and signaling (Fu *at el.* 2001 JBC **276**: 43004-43009.)



DQ: Diaqut, a ROS generator. PN: Peroxynitrite, a potent RNS.

GPx-1 in superoxide generator-induced apoptosis and signaling (Fu *at el.* 2001 JBC **276**: 43004-43009.)



DNA fragmentation induced by 0.5mM DQ and 0.4 nM PN.
UT: untreated

GPx-1 vs. Bcl-2

- Apoptosis is efficiently inhibited by the anti-apoptosis Bcl-2 gene product.
- Overexpression Bcl-2 enhances cell viability after exposure cytotoxic agents and favored HIV infection in cell culture by facilitating cell-to-cell transmission and spreading of the virus.

DL Laux *et al*. (1998) Nature **335**: 440-442.

PA Sandstrom *et al*. (1995) FEBS Lett **365**: 66-70

GPx-1 vs. Bcl-2

- GPx-1 and Bcl-2 display analogous effects on an oxidative event in the signaling cascade leading to apoptosis.
- Although GPx-1 and Bcl-2 have different mechanisms:

  GPx-1: directly reduces hydroperoxide

  Bcl-2: prevents hydroperoxide formation

  PA Sandstrom *et al*. (1995) FEBS Lett **365**: 66-70.

Summary (1)

- GPx-1 is a kind of Se-dependent enzyme.
- GPx-1 is also an important enzyme to get rid of H_2O_2 or ROOH.
- The manipulation of GPx activities by changing selenium levels in diet and by overexpression or knockout mice techiques indicate GPx have more subtle functions.

Summary (2)

- Many studies believe that overexpression of GPx-1 can protect cells against ROS.
- The effects of overexpression of GPx-1 are reversed by Se deficiency.
- Se plays a critical role in GPx-1 avtivities.
- Furthermore, the effects of GPx-1 is specific and cannot be replaced by general antioxidants.