

Supporting Online Information for

Distribution of Polysulfide in Human Biological Fluids and Their Association with
Amylase and Sperm Activities

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Supporting methods

Methods

Determination of thiol contents in plasma

Twenty microliter of plasma was mixed into 100 μ L of 5 mM DTNB in 100 mM of potassium phosphate buffer/ 1 mM DTPA (pH 7.0). After incubation for 60 min at room temperature, absorbance at 412 nm was measured by a plate reader. GSH (31.3 to 1000 μ M) was used for constructing a standard curve.

Measuring anti-oxidative activity against AAPH radical [1]

Sixteen millimolar of linoleic acid solution was prepared by mixing 5 mL of borate buffer (50 mM, pH 9.0), 250 μ L of linoleic acid, 1 mL of sodium hydroxide and 250 mL of tween20 and diluting in measuring cylinder to 50 mL by borate buffer (50 mM, pH 9.0). AAPH was dissolved in cold water on ice. Nine hundred twenty microliter of phosphate buffer saline (PBS) preheated at 37 °C and 20 μ L of plasma were mixed and 10 μ L of linoleic acid solution (16mM) was added. Fifty microliter of AAPH solution (50 mM) was added and incubated for 1 hr at 37°C. After the reaction, the sample solution was dispensed into 96 well of ultraviolet plate and absorbance read at 234 nm. Radical elimination activity was calculated as follows:

Eliminated radical (%) = $(\text{Abs. at 234 nm of sample with AAPH} - \text{Abs. at 234 nm of sample without AAPH}) \times 100 / (\text{Abs. at 234 nm of PBS with AAPH} - \text{Abs. at 234 nm of PBS without AAPH})$

Detection of sulfane sulfur by a fluorescence probe [2]

Plasma was diluted in 1 mL of 1 mM CTAB/PBS. 2 mL of 1 mM SSP4 in DMSO was added and incubated for 10 min at room temperature. Fluorescence intensity was measured at ex/em = 457 nm/514 nm.

Statistical analysis

Differences between the groups were evaluated by Welch's t-test. P < 0.05 was regarded as statistically significant.

References

- [1] Kontush, A.; Reich, A.; Baum, K.; Spranger, T.; Finckh, B.; Kohlschütter, A.; Beisiegel, U., Plasma ubiquinol-10 is decreased in patients with hyperlipidaemia. *Atherosclerosis* **1997**, 129, (1), 119-126.
- [2] Chen, W.; Liu, C.; Peng, B.; Zhao, Y.; Pacheco, A.; Xian, M., New fluorescent probes for sulfane sulfurs and the application in bioimaging. *Chem Sci* **2013**, 4, (7), 2892-2896.