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# Effect of molecular hydrogen on coenzyme Q in plasma, myocardial tissue and mitochondria of rats

**Abstract**

Beneficial effects of molecular hydrogen ($H_2$) in various experimental models of human diseases and in many clinical studies was documented. $H_2$ can be administered by various ways, as a gas inhalation, drinking of $H_2$-enriched water, or taking a $H_2$-dissolved bath as well as in saline infusions. As antioxidant selectively scavenges hydroxyl and peroxynitrite radicals, decreases oxidative stress. However, the$H_2$ effect on antioxidant–coenzyme Q information is lacking. This pilot study found protective effects of $H_2$ on coenzyme $Q_9$ in plasma, myocardial tissues and mitochondria of rats. Our results can contribute to the explanation of a new beneficial mechanism of $H_2$ on a part of antioxidant protection in organism.

**Keywords:** molecular hydrogen, myocardium, coenzyme $Q_9$, rat

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## Introduction

In the last decade positive the effect of $H_2$was documented in several experimental and clinical studies. In the in preventive and therapeutic treatment of various diseases as cancer, cardiovascular system, central nervous system, metabolic syndrome, respiratory system diseases beneficial effects of $H_2$ have been observed.[1,2] $H_2$ can be administrated by various ways, as a gas inhalation, by saline injection, by drinking hydrogen rich water, by injection or eye-dropping of $H_2$-dissolved saline or taking $H_2$-rich water bath.[3,4] Due to the low molecular weight $H_2$ rapidly diffuses into tissues and cells.[5,6] As an antioxidant $H_2$selectively scavenges hydroxyl radical ($^{\cdot}OH$) and peroxynitrite radical ($ONOO^-$) to reduce oxidative stress. Further identified $H_2$modes of action are anti-inflammatory and anti-apoptotic effects, regulation of gene expression,[7-10] protection of antioxidant enzyme (superoxide dismutase) and regulation of antioxidant defence.[11] The effect of $H_2$ on other antioxidantin organism - coenzyme Q(a key component for mitochondrial bioenergetics) - remains unknown. We hypothesized that the oral intake of molecular hydrogen rich water may protectcoenzyme $Q_9$($CoQ_9$) concentration in rats.

## Material and methods

In the study male Wistar rats were included, 3 months aged, with body weight 200g. Number of rats in each group was 5. *Three control groups* were fed with pure water. *Three $H_2$ groups* were fed with $H_2$ rich water by gastric tube 3x3ml daily for 2 days ($CH_2$-2); for 2 weeks gavage and 4 weeks without $H_2$ ($CH_2$-45/2); 45 days with $H_2$ gavage ($CH_2$-45). The animal experiments were in compliance with the Ethics Commitee of the Institute for Heart Research, Slovak Academy of Sciences and protocols approved by the State Veterinary and Food Administration of the Slovak Republic (permit No. 4091/16-221). Male Wistar rats used in this study were purchased from the Department of Toxicology and Laboratory Animals Breeding, Slovak Academy of Sciences, housed and bred under standard environmental conditions (12h light/dark cycle, ambient temperature 22 – 24 °C and 45 – 65% humidity) in the Institute for Heart Research, Slovak Academy of Sciences. Food and water were available during the whole experiment *ad libitum*.

### Isolation of mitochondria

Mitochondria from heart muscle were isolated by differential centrifugation.[12] Isolated solution contained 180mmol/l KCl, 4mmol/l EDTA, 20mmol TRIS and 0.1% of albumin with the addition of Nagarse 2.5mg/g of the tissue. Sedimented mitochondria were washed twice without albumin. Mitochondrial proteins were estimated spectrofotometrically.[13]

### Determination of coenzyme $Q_9$

$CoQ_{9\text{-TOTAL}}$ (ubiquinone – oxidized form + ubiquinol – reduced form) was determined in plasma, $CoQ_{9\text{-OX}}$ (oxidized form) in myocardial tissue and mitochondria by HPLC method with UV detection. For $CoQ_9$ extraction hexane/ethanol (5/2 v/v) was used, organic phase was collected, evaporated under nitrogen, the residue dissolved in ethanol and injected into column SGX C18 7µm (Tessek). The mobile phase consisted of methanol/acetonitrile/ethanol (6/2/2 v/v/v, Merck). Coenzyme Q was detected at 275nm using external standards (Sigma).[14,15] The results were evaluated using unpaired Student´s t-test, $p<0.05$ were considered statistically significant.

## Results

### Effect of molecular hydrogen on coenzyme Q

$CoQ_{9\text{-TOTAL}}$ concentrations in plasma increased after 2-days of $H_2$ application by 23.3%, after 45 days ($CH_2$-45/2) increased by 12.3%, after 45 days ($CH_2$-45) increased by 31.3%, ($p= 0.088$) in comparison with control groups. In the tissue and mitochondria of the heart concentration of $CoQ_{9\text{-OX}}$ was evaluated. Two days of $H_2$ application in myocardial tissue had no effect on $CoQ_{9\text{-OX}}$ concentration. Long term ($CH_2$-45/2) and ($CH_2$-45) effect of $H_2$ application stimulated $CoQ_{9\text{-}}$

$_{OX}$ concentration by 16.6% and by 31.79% respectively, significantly ($p<0.035$). In isolated mitochondriathe positive effect of $H_2$ was found after 2 days. $CoQ_{9\text{-}OX}$ concentration increased by 44.4%, in group ($CH_2$-45/2) by 42.8%. When $H_2$ was applicated for 45 days ($CH_2$-45), its effect was lower. $CoQ_{9\text{-}OX}$ concentration increased by 17.1% in comparison with control group (CC-45). $CoQ_{9\text{-}OX}$ concentration in isolated mitochondria were evaluated in %, while some samples were collected due to small quantities of tissues for the mitochondrial isolation (Table 1).

**Table 1** Effect of $H_2$ on $CoQ_{9\text{-}TOTAL}$ in plasma and $CoQ_{9\text{-}OX}$ in tissue and myocardial mitochondria of rats

| | | CC-2 | $CH_2$-2 | CC-45/2 | $CH_2$-45/2 | CC-45 | $CH_2$-45 |
|---|---|---|---|---|---|---|---|
| **Plasma** | **mean** | **0.155** | **0.191** | **0.122** | **0.137** | **0.115** | **0.151** |
| $CoQ_{9\text{-}TOTAL}$ | sem | 0.006 | 0.022 | 0.012 | 0.017 | 0.012 | 0.009 |
| (μmol/L) | p vs CC | | NS | | NS | | p=0.088 |
| | % vs CC | | ↑23.2% | | ↑12.3% | | ↑31.3% |
| **Tissue** | **mean** | **164.1** | **161.8** | **187.3** | **218.4** | **187.5** | **247.1** |
| $CoQ_{9\text{-}OX}$ | sem | 5.61 | 4.35 | 15.7 | 13.1 | 4.75 | 17.7 |
| (nmol/g ww) | p vs CC | | NS | | NS | | P<0.035 |
| | % vs CC | | ↓1.4% | | ↑16.6% | | ↑31.8% |
| **Mitochondria** | **mean** | **1.87** | **2.70** | **1.66** | **2.37** | **1.99** | **2.33** |
| $CoQ_{9\text{-}OX}$ | | | | | | | |
| (nmol/mg prot) | % vs CC | | ↑44.4% | | ↑42.8% | | ↑17.1% |

## Discussion

Molecular hydrogen is a colorless and odorless gas, which selectively scavenges hydroxyl and peroxynitrite radicals, but not the same applies for hydrogen peroxide and nitric oxide in cells and tissues.[16] $H_2$ successful effect in animal models of human disease was documented. The role of $H_2$ was found in hypoxic post-conditioning, radiation-induced heart injury, mediastinal irradiation in rats, acute cardiac injury, radiation-induced heart disease and changes in microRNA-1, -15b and -21 levels in irradiated rat hearts.[17-22] Beneficial effect of $H_2$ in clinical medicine was found in various diseases, such as cardiovascular diseases, type 2 diabetes, dyslipidemia, obesity and metabolic syndrome, in vascular health.[23-25] Molecular hydrogen water improved the progression of Parkinson´s disease.[26] Molecular hydrogen is a short-live small molecule which is able to diffuse through membranes upon theconcentration gradient.[21] Afterthe $H_2$ inhalation by rats, its level immediately increases in the myocardial tissue and probably diffusesinto mitochondria.[27] $H_2$ protects antioxidant enzyme – superoxide dismutase.[22] Molecular hydrogen effect on other antioxidant – coenzyme Q (CoQ) – is not known up to now.

Coenzyme Q was discovered by Frederick Loring Crane in 1957. Human's dominant form is $CoQ_{10}$, rats dominant form is $CoQ_9$. $CoQ_{10}$ as a crucial mobile component of the mitochondrial respiratory chain acts in three forms in the„ Q-CYCLE". CoQ –oxidized form (ubiquinone), $CoQH_2$ – reduced form (ubiquinol) and CoQ – radical from (ubisemiquinone). The central role of $CoQ_{10}$ is electrons and protons transfer from Complex I and Complex II to Complex III ofthe mitochondrial respiratory chain. $CoQ_{10}$ as antioxidant scavenges free oxygen radicals, decreases oxidative stress. Its concentration is changed during semicircadian cycles, every twelve hours has maximum (PEAKS) and minimum (NADIRS) concentration. CoQ has its own biological clock – $Q_{10}$-CLOCK.[28,29] $CoQ_{10}$ was found in all the tissues of the body, and its higher concentrations were found in tissues with very active metabolism and energy demands, as heart, brain, kidney and skeletal muscle. Ubiquinol is a lipophilic antioxidant and is capable to recycle and regenerate other antioxidants, as alpha-tocopherol and vitamin C.[30] In this study the stimulation of $CoQ_{9\text{-}TOTAL}$ concentrations in plasma and $CoQ_{9\text{-}OX}$ concentration in myocardial tissues and mitochondria after $H_2$ application in rats were found (Table 1).

## Conclusion

To the best of our knowledge, we present the first data showing short and prolonged effect of $H_2$ on coenzyme $Q_9$ in plasma, myocardial tissues and mitochondria of rats.[31] We suppose, that the$H_2$ application could protect the mitochondrial concentration of Co Q by reducing the concentration of ·OH. Our results can contribute to the explanation of a new beneficial mechanism of $H_2$ on a part of the antioxidant protection in organism. Next studies of the effect of molecular hydrogen effect on human mitochondrial function using a non-invasive method in isolated platelets could confirm our pilot results in rats.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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