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# Does menaquinone participate in brain astrocyte electron transport?

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ABSTRACT

Quinone compounds act as membrane resident carriers of electrons between components of the electron transport chain in the periplasmic space of prokaryotes and in the mitochondria of eukaryotes. Vitamin K is a quinone compound in the human body in a storage form as menaquinone (MK); distribution includes regulated amounts in mitochondrial membranes. The human brain, which has low amounts of typical vitamin K dependent function (e.g., gamma carboxylase) has relatively high levels of MK, and different regions of brain have different amounts. Coenzyme Q (Q), is a quinone synthesized *de novo*, and the levels of synthesis decline with age. The levels of MK are dependent on dietary intake and generally increase with age. MK has a characterized role in the transfer of electrons to fumarate in prokaryotes. A newly recognized fumarate cycle has been identified in brain astrocytes. The MK precursor menadione has been shown to donate electrons directly to mitochondrial complex III.
*Hypothesis:* Vitamin K compounds function in the electron transport chain of human brain astrocytes.


*Menaquinone in the electron transport chain*

## Introduction

The classic studies on the utility of menaquione (MK) in electron transport have been done in the prokaryote *Escherichia coli*, which is capable of the biosynthesis of both coenzyme Q (Q) and MK. The synthesis of the naphthoquinone structure (menadione) or "head" of MK is restricted to mainly prokaryotes (MK) and plants (phylloquinone) [1,2] while human nutrition involves replacement of the alkyl tail of phylloquinone for association with tissues, becoming MK [3]. Save for a recent startling exception [4] investigations of electron transport function in higher eukaryotes with alternate quinones describe the nitrogen containing quinone, rhodoquinone [5]; this alternative quinone participates in electron transport when the organism is in low oxygen environments [6]. The lack of biosynthetic capability for MK in higher eukaryotes [6]; [7] is interpreted as MK does not have a role in electron transport. However, this argument suggests that the source (diet, via phylloquinone or bacterial production), alters the role of a quinone (MK) hypothesized to be a supporting player in electron transport, because MK is not made *de novo*. As a comparison, most higher eukaryotes can synthesize their own vitamin C, yet humans and all higher primates have lost this capacity. Primates consumed enough plant material to readily survive the loss of vitamin C and vitamin K biosynthetic capability, although lack of these biochemical cofactors leads to loss of life. The question posed here is whether the role of the tightly controlled structural form and level of MK in human brain may include an unexplored role in electron transport.

Importantly, there are other experimental reasons why a role for MK in electron transport may have been overlooked. The amounts of MK or menadione added to studies of electron transport have not been targeted to supply physiological amounts. High levels would predictably inhibit normal Q binding. Q is in vast excess compared to MK, making contributions to electron transport difficult to detect. Few vitamin K studies have used the newer sensitive methods of quantitative mass spectrometry [8]. Vitamin K has multiple forms in human beings, but the quinone/electron transporting function is done by the naphthoquinone "head", while membrane association is given by the alkyl four isoprene unit prenyl "tail". A significant source of MK for human nutrition is the intestine. MK and menadione (naphthoquinone nucleus) are made both in the human liver from phylloquinone (the plant form of vitamin K), but significant amounts of MK and menadione are also created by colonic bacteria [1,3]. Therefore it is difficult to make an animal deficient – and once they are deficient the death of the animal from decreased clotting factors would have to be addressed. However, the studies of biosynthesis, organ storage sites and intra-cellular distribution of vitamin K (phylloquinone) and active metabolic form MK, support the view of a carefully conserved compound with patterns of regulated partitioning between organs and

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intra-cellular organelles [9]. Of particular interest to the present hypothesis, the human body and especially the brain serves as a reservoir for MK [10].

Another reason why the contribution of MK in the electron transport of a eukaryotic organism is not studied is because anoxic (no oxygen) environments cause death of the organism, therefore, the utility of MK in electron transport cannot be studied *in vivo* as a factor for survival. Clearly Q does the major work of electron transport in eukaryotic organisms, but MK is present as well. In prokaryotes and some lower eukaryotes, MK is a preferred mobile carrier progressing electron transport in a low or no oxygen environment [7]. However, even in the presence of a steady state oxygen environment, menaquinone maintains and supplements electron transport flow in prokaryotic periplasmic membranes along with Q. There is synergistic activity from the presence of both quinone compounds, as seen in the results of an excellent comparison of ubiquinone and menaquinone mutants versus controls in an isogenetic background [11,12]. It is also possible that a metabolic relationship exists between the vast quantities of Q and the much lesser MK. For example, it has been described that the likely precursor for the biosynthesis of rhodoquinone is Q [13]. Likewise a possible donor compound for long chain forms of MK could be Q [14]. This hypothesis is further reinforced by the recent recognition that the UBIAD gene, encoding the prenyl transferase for the synthesis of MK also synthesizes Q in the Golgi, outside the mitochondria [15].

Lipoid mobile electron carriers like Q and MK, are embedded inside membranes to carry reducing equivalents, but natural selection tailors predominant forms of preferred quinones based on their biochemical reaction environments and chemical compounds to be reduced. The midpoint oxidation and reduction potentials [$E_{m,7}$] [16] of these two compounds ($MK/MKH_2$: −0.08 mV; $Q/QH_2$: +11 mV) – configures MK for reduction of "lower potential" electron acceptor compounds like fumarate. Fumarate has been discovered recently to accumulate in human brain astrocytes [17]. The astrocytes in human brain perform vital functions for closely adjacent neurons. For example pyruvate carboxylase is an enzyme located in astrocytes (and not neurons), where this function provides about 6% of the critical neuronal transmitter glutamine [18]. Pyruvate carboxylase makes oxaloacetate, a tricarboxylic acid intermediate important in typical mitochondrial metabolism, but recent careful measurements using positional labeled $^{13}C$ glucose isomers has demonstrated that an oxaloacetate to fumarate cycle also exists, revealing an astrocyte and brain region dependent reliance on this "backward" or "backflux" in normal human brain [17], Fig. 1.

How could a reduced form of MK (MKred) contribute to human electron transport? The reducing function possibly provided by Menadione, MK and short chain quinones involves a bypass of mitochondrial complex I and direct reduction of mitochondrial complex III [19,20]; (Fig. 1: *Mechanism* I). This activity is linked to ATP generation through a direct donation of reducing equivalents carried on quinones from NAD(P)H to complex III, and has been observed to be reliant on prenyl chain structure [21], where suggested levels of 1–3.3 μM were effective [21]. The reduction of short chain quinones is linked to the activity of a vitamin K reductase, aka NAD(P)H quinone oxidoreductase.

## The vitamin K reductase, NQO1 and glutamate

Menadione is a metabolic intermediate in MK metabolism (Fig. 1A) and can participate in two electron reduction mechanisms involving electron transport [22] via NAD(P)H quinone oxidoreductase (DT-diaphorase; NQO1) [23]. NQO1, as the vitamin K reductase, was discovered because of a unique sensitivity to dicoumarol, the compound which caused the "vitamin-identifying" deficiency symptoms to develop in cattle. Using this specific inhibition, the role of NQO1 to reduce vitamin K in mammalian electron transport with *in vitro* experiments was well explored in a series of important papers by Lars Ernster and colleagues [24–28]. These works describe the contribution of MK reducing equivalents to mitochondrial electron transport in many papers detailing *in vitro* analyses of electron transport with the purified NQO1, mitochondrial preparations and the skilled use of electron transport inhibitors and different quinone compounds. They describe the electron transport specificity for MK/menadione (and not other quinones) and suggest this NQO1 reduction of MK/menadione contributes to electron transport functions in the mitochondrial oxidation of glutamate [25]. In further work [28], the Ernster group showed that adding MK/menadione to tumor cells with glucose allowed the cells to bypass the respiratory chain inhibitor amytal (inhibiting at complex III). Their prediction for the acceptor within the electron transport of mitochondria is via cytochrome$_b$ [26].

MK can accept electrons from complex I (NADH reductases) [16]. The specificity of the role of the nicotinamide donor compound (NADH or NADPH) is important – since when Q is added to electron transport experiments with NAD(H) it stimulates the "downstream reduction" of cytochrome$_c$, whereas NAD(P)H added to the NQO1 enzyme cannot stimulate this transfer well with Q, but the transfer proceeds specifically with menadione [25]. Also of interest, the rate of this electron transfer was directly correlated with the amount of reduced menadione/MK [29]. In humans NQO1 has a high distribution in nervous tissue, eye lens and in capillary endothelial cells; it is over-expressed in solid tumors. Importantly about ten percent of this enzyme co-purifies with microsomes and mitochondria [30] – and this membrane associated fraction of NQO1 has differences in distribution within different organs. For instance the heart and liver appear to have higher levels of the mitochondrial form of the enzyme, while the brain and solid tumors have predominantly the microsomal form [30]. Of interest, NQO1 expression in brain appears to be highly induced in Parkinson's tissues [31].

It is compelling that the original scientific investigations by Ernster's group targeted a role for glutamate oxidation and contribution to electron transport via the role of NQO1. Both astrocytes and neurons can take up glucose and glutamate; glutamate uptake from synapses comprises an essential feature of neuronal signaling, but this metabolic interplay has vast consequences for energy supply especially during periods when glucose levels fall, and acidity rises during lactate production [32]. Recent evidence suggests that when glucose, and glucose derived pyruvate is limiting, the glutamate – glutamine shuttle becomes essential for survival [33]. Glutamate is a co-transmitter in ventral midbrain dopamine neurons [34]. The brain has highly regional energy demands, and metabolic adaptations to compensate for changing energy demands [35]. The author speculates that glutamate metabolism may be especially important in the regions of brain where the highest levels of MK are found [9]. MK status has been reported to be associated with other brain important lipids like sulfatide [36] and sphingolipids [10].

## Electron transport by menaquinone (MK) in a Drosphila Parkinson's model

A recent paper in Science by Vos et al. [4] describes the isolation of a ubiquitious prenyltransferase gene product (*E. coli UbiA:* drosphilia *UBIAD*), found in a genetic screen for defects that accentuate a deficiency in a mitochondrial kinase PINK. The UBIAD1 protein functions to prenylate the naphthoquinone