

研究论文

极低频电磁辐射增强秀丽线虫能量代谢并诱导氧化应激反应

孙永彦^{1,2,3,4}, 王亚红^{1,2,3}, 李志辉^{1,2,3}, 石振华^{2,5}, 廖艳艳^{1,2,3}, 唐超^{1,3}, 蔡澎^{1,3,6,*}

¹中国科学院城市环境研究所, 厦门 361021; ²中国科学院大学, 北京 100049; ³厦门市物理环境重点实验室, 厦门 361021; ⁴天津理工大学环境科学与安全工程学院, 天津 300384; ⁵福建农林大学资源与环境学院, 福州 350002; ⁶中国科学院上海生命科学研究院营养与健康研究所, 上海 200031

摘要: 本文旨在研究极低频电磁场(extremely low frequency electromagnetic field, ELF-EMF)暴露对秀丽隐杆线虫(以下简称线虫)能量代谢和氧化应激反应的影响。50 Hz、3 mT ELF-EMF暴露, 20 °C条件下分别培养线虫56 h(成虫初期)、72 h(产卵期)和90 h(产卵高峰期), 检测三个时期的成虫体内ATP水平、ATP合成酶活性、活性氧(reactive oxygen species, ROS)含量以及线虫总抗氧化能力(total antioxidant capacity, TAC)的变化情况, 并评估ELF-EMF暴露对线虫氧化应激(ROS-TAC分数)的影响。结果表明, 经ELF-EMF暴露的线虫与对照组比较, 体内ATP含量上升, ATP合成酶活性上调; 在成虫初期, 与对照组比较, 线虫ROS水平经ELF-EMF暴露后显著增加, 同时TAC增强, 主成分分析法比较二者变化程度提示ROS-TAC分数显著降低, 表明ELF-EMF引起成虫初期氧化应激反应。产卵期与产卵高峰期成虫氧化应激反应较对照组无显著差异。以上结果提示, 50 Hz极低频电磁辐射可致成虫能量代谢增强并引起成虫初期氧化应激反应。

关键词: 能量代谢; 氧化应激; 电磁辐射; 秀丽线虫

中图分类号: R730.2; Q493.8

Extremely low frequency electromagnetic radiation enhanced energy metabolism and induced oxidative stress in *Caenorhabditis elegans*SUN Yong-Yan^{1,2,3,4}, WANG Ya-Hong^{1,2,3}, LI Zhi-Hui^{1,2,3}, SHI Zhen-Hua^{2,5}, LIAO Yan-Yan^{1,2,3}, TANG Chao^{1,3}, CAI Peng^{1,3,6,*}

¹Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China; ²University of Chinese Academy of Sciences, Beijing 100049, China; ³Xiamen Key Laboratory of Physical Environment, Xiamen 361021, China; ⁴College of Environmental Science and Safety Engineering, Tianjin University of Technology, Tianjin 300384, China; ⁵College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002, China; ⁶Shanghai Institute of Nutrition and Health, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

Abstract: The aim of this study was to determine the effects of extremely low frequency electromagnetic field (ELF-EMF) on energy metabolism and oxidative stress in *Caenorhabditis elegans* (*C. elegans*). Worms in three adult stages (young adult stage, egg-laying stage and peak egg-laying stage) were investigated under 50 Hz, 3 mT ELF-EMF exposure. ATP levels, ATP synthase activity *in vivo*, reactive oxygen species (ROS) content, and changes of total antioxidant capacity (TAC) were detected, and worms' oxidative stress responses were also evaluated under ELF-EMF exposure. The results showed that ATP levels were significantly increased under this ELF-EMF exposure, and mitochondrial ATP synthase activity was upregulated simultaneously. In young adult stage, worms' ROS level was significantly elevated, together with upregulated TAC but with a decreased ROS-TAC score indicated by principal component analysis. ROS level and TAC of worms had no significant changes in egg-laying and peak egg-laying stages. Based on these results,

Received 2018-08-23 Accepted 2019-01-19

This work was supported by grants from the Science and Technology Project of State Grid Corporation of China (GY71-16-008), the Focus Deploy Project of Chinese Academy of Sciences (KGFZD-135-160-024).

*Corresponding author. Tel: +86-592-6190920; +86-21-54920003; E-mail: pcai@iue.ac.cn; pcai@sibs.ac.cn

we concluded that ELF-EMF can enhance worm energy metabolism and elicit oxidative stress, mainly manifesting as ATP and ROS level elevation together with ATP synthase upregulation and ROS-TAC score decrease in young adult *C. elegans*.

Key words: energy metabolism; oxidative stress; electromagnetic field; *C. elegans*

代谢性疾病 (metabolic disease, MD) 在全球范围内发病率逐年上升^[1]。能量代谢异常是 MD 发生的重要病理基础^[2]。能量代谢紊乱通常表现为氧化应激损伤与线粒体能量代谢异常^[3-5]。当生物体内重要供能物质 ATP 的代谢发生失调, 可引起细胞活动广泛性改变, 特别是一些新陈代谢旺盛的细胞如神经细胞, 能量代谢失调造成其功能显著异常, 可引发阿尔茨海默症、亨丁顿舞蹈症、帕金森病等神经退行性疾病^[6], 神经退行性疾病与线粒体能量代谢障碍密切相关, 目前能量代谢障碍机制研究是 MD 的研究热点。

极低频电磁辐射 (extremely low frequency electromagnetic field, ELF-EMF) 作为国际癌症研究机构 (International Agency for Research on Cancer, IARC) 定义的一种 2B 级致癌因子^[7], 被报道与癌症和其他 MD 密切相关。流行病学研究结果显示, 长期暴露于 ELF-EMF 条件下的人员罹患白血病^[8]及一些神经系统损伤性疾病如阿尔茨海默症、癫痫^[9]等的风险增加。分子机制研究表明 ELF-EMF 暴露可引起不同生物、组织和细胞氧化损伤, 损伤程度受暴露时间、强度、生物样品等因素影响^[10]。我们的前期工作证实, ELF-EMF 暴露能够引起能量代谢异常, 如酵母三羧酸循环与葡萄糖转运蛋白基因表达变化, 还可引起果蝇氧化应激反应增强, 改变线虫糖酵解代谢水平^[11-13]。目前 ELF-EMF 暴露影响能量代谢的作用机制仍不明确。本文以秀丽隐杆线虫 (*Caenorhabditis elegans*) 模式生物为研究对象, 探讨 50 Hz、3 mT ELF-EMF 暴露条件下成虫期线虫 ATP 和活性氧 (reactive oxygen species, ROS) 的含量变化, 并检测 ATP 合成酶活性以及成虫总抗氧化能力 (total antioxidant capacity, TAC) 的变化情况, 综合评估线虫 ELF-EMF 暴露后的氧化应激反应, 以期进一步阐明 ELF-EMF 暴露作为一种潜在致癌风险因子对能量代谢与氧化损伤的影响。

1 材料和方法

1.1 主要试剂 5-氟尿嘧啶 (5-Fluorouracil) 试剂购自国药集团化学试剂有限公司; ATP 检测试剂盒、

ROS 检测试剂 DCFH-DA 购自上海碧云天生物技术有限公司; ATP 合成酶检测试剂盒、TAC 检测试剂盒和总蛋白测定试剂盒 (考马斯亮蓝法) 购自南京建成生物工程研究所; 野生型秀丽隐杆线虫 (strain N2) 购自美国线虫遗传中心 (Caenorhabditis Genetic Center)。

1.2 线虫培养与实验分组 所用培养基为线虫生长培养基 [nematodes growth media, NGM, 含 1.7% 琼脂、25 mmol/L 氯化钠、2.5 g/L 蛋白胨、5 mg/L 胆固醇、pH 6.0 的 25 mmol/L KPO₄ 缓冲液 (108.3 g KH₂PO₄、35.6 g K₂HPO₄, 定容至 1 L)、1 mmol/L MgSO₄、1 mmol/L CaCl₂]。线虫以大肠杆菌 *Escherichia coli* OP50 饲喂, 采用标准程序^[14]在 20 °C 下培养。将线虫按照标准方法同期化后的卵随机分为对照 (control) 组和暴露 (ELF-EMF) 组, 每皿放置线虫卵 5 000 个左右。对照组即常规培养线虫由卵至成虫初期 (56 h)、产卵期 (72 h)、产卵高峰期 (90 h); 暴露组为温度、培养时间等一切条件不变情况下 ELF-EMF 持续暴露至合适虫期。暴露时开启变频电源, 线虫样品放置于一对亥姆霍兹线圈组中心位置, 持续放在均匀分布的强度为 3 mT 的 50 Hz 交变电磁场内。为抑制产卵期子代对实验的干扰, 期间将培养至 L4 时期的线虫取出转移到含有终浓度 50 μg/mL 5-氟尿嘧啶的 NGM 培养基中, 继续暴露或对照培养至检测时间。分别收集对照和暴露组线虫样品, 加入 M9 缓冲液 (3 g KH₂PO₄、6 g Na₂HPO₄、5 g NaCl、1 mol/L MgSO₄ 1 mL, 定容至 1 L) 清洗 3 次后, 300 g 离心 2 min, 保留虫液沉淀约 150 μL, 用液氮速冻后置于 -80 °C 留存备用。

1.3 电磁暴露装置 本实验选用 50 Hz, 3 mT ELF-EMF 为暴露条件。自制电磁暴露实验舱^[15]由一个频率可调电源 (艾普斯电源, 美国), 一台生化培养箱 (上海精宏, SHP-250), 一对亥姆霍兹线圈 (每个线圈分别高 15 cm, 直径 40 cm, 每线圈上缠绕 260 圈铜线), 一台大功率低温冷却液循环器 (宁波天恒公司, THD-0520) 和一只温度记录仪 (杭州美控公司, MIK-214B) 组成。亥姆霍兹线圈组外周紧贴铜线密集缠绕注入冷却液的硅胶管, 与外部冷却液循环器相连, 保证暴露系统内温度恒定。温度记

录仪探头放置在两个线圈的中心位置, 即样品放置区。对照组培养条件除不含亥姆霍兹线圈外, 其他与暴露组一致。

1.4 检测 ATP 含量 取 3 板线虫约 15 000 条, 清洗、破碎, 4 °C, 15 000 g 离心后取上清 20 μ L, 加入到 96 孔板内与 ATP 检测工作液混合 (每孔 100 μ L ATP 检测工作液, 室温反应 3~5 min 消耗本底 ATP), 迅速混匀后至少间隔 2 s, 利用荧光光度计测定相对光单位 (relative light unit, RLU) 值, 同时按照说明书方法测定标准曲线, 根据标准曲线计算样品中 ATP 浓度 (单位: nmol/L/mg protein 表示)。

1.5 ATP 合成酶活性测定 线虫经收集破碎处理后, 收集上清, 15 000 g 离心 10 min, 按照试剂盒说明书操作检测上清液中 ATP 合成酶活性, 以单位时间每毫克蛋白含量提取液中还原型烟酰胺腺嘌呤二核苷酸 (NADH) 被氧化的含量 (μ mol NADH/min/mg protein) 来表示。

1.6 DCFH-DA 染色法检测 ROS 含量 分别收集对照组与暴露组线虫各 300 条, 置于含有 1 mL, 浓度为 20 μ mol/L 的 2,7-dichlorodihydrofluorescein diacetate (H₂DCF-DA) 工作液中, 20 °C 避光孵育 2 h 后, M9 缓冲液清洗 3 次, 破碎、离心后取上清。应用多功能酶标仪 (SpectraMax M3, 上海美谷分子仪器公司), 使用 488 nm 激发波长, 525 nm 发射波长检测荧光强度。ROS 含量以各组荧光强度值占线虫提取总蛋白含量的比值表示, 总蛋白含量通过考马斯亮蓝染色法进行检测。

1.7 TAC 检测与氧化应激反应分析 取 3 板线虫约 15 000 条, 清洗、破碎, 4 °C, 15 000 g 离心, 收集上清液, 按照试剂盒说明书以铁还原法测定 TAC。氧化应激反应分析即将各时期线虫 ROS 值与 TAC 值采用 z-score 法标准化处理, 以主要成分值表示, 计算过程为:

$$\text{标准化 ROS} = [\log(\text{ROS} + 1) - 1.3885]/0.7271$$

$$\text{标准化 TAC} = (\text{TAC} - 1650.93)/532.22$$

主成分 (principal component) = $(-0.707 \times \text{标准化 ROS}) + (0.707 \times \text{标准化 TAC})$; ROS-TAC 分数 = $50 + (\text{主成分} \times 10.629)$ 。

1.8 统计学分析 结果用 mean \pm SD 来表示。应用 SPSS 16.0 统计软件进行单因素方差分析 (ANOVA), 以 LSD 法进行组间两两比较。P < 0.05 为差异有统计学意义。

2 结果

2.1 ELF-EMF 暴露引起线虫体内 ATP 水平上升

ATP 水平检测结果 (图 1) 显示, 与对照组相比, ELF-EMF 暴露组成虫 ATP 水平显著上升 (P < 0.05)。成虫初期线虫 ELF-EMF 暴露后, ATP 水平较对照组升高 0.28 倍 (P = 0.039), 产卵期成虫 ATP 水平上升最为显著 (P = 0.043), 较正常对照组升高 1.53 倍, 产卵高峰期成虫 ATP 水平上升 0.28 倍 (P = 0.034)。

2.2 ELF-EMF 暴露诱导线虫 ATP 合成酶活性增强

为了进一步证实 ELF-EMF 暴露对线虫 ATP 合成具有增强作用, 我们检测了线粒体复合物 V (即 ATP 合成酶) 活性变化情况, 结果如图 2 所示, 与对照组比较, ELF-EMF 暴露组线虫成虫期 ATP 合成酶活性明显增强 (P < 0.05)。ELF-EMF 暴露分别使成虫初期、产卵期和产卵高峰期的线虫体内 ATP 合成酶活性增加 1.63 倍 (P = 0.002)、0.50 倍 (P = 0.024) 和 0.41 倍 (P = 0.045)。

2.3 ELF-EMF 暴露诱导 ROS 产生

采用 DCFH-DA 染色法进行 ROS 含量分析, 以 300 条线虫染色后破碎提取的上清液的荧光强度值经过上清液总蛋白含量校准后来表示 ROS 含量。结果显示 (图 3): 经 ELF-EMF 暴露处理的成虫 ROS 水平与正常对照组比较, 成虫初期线虫 ROS

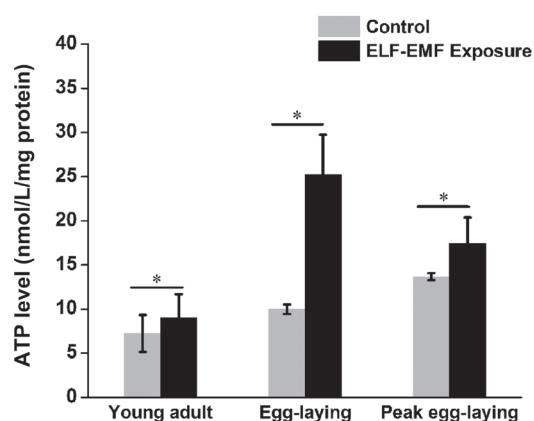


图 1. 极低频电磁辐射暴露诱导成虫期线虫体内 ATP 水平升高
Fig. 1. Extremely low frequency electromagnetic field (ELF-EMF) exposure elevated worms' ATP level *in vivo*. Worm pellets from different adulthood stages (approximately 15 000 worms) were collected in M9 buffer, and samples' homogenates were prepared after grinding, and centrifugation. Luciferase enzyme reaction was used as a method for ATP level detection. The experiment was repeated thrice. Mean \pm SD. *P < 0.05 vs control group.

增多 ($P = 0.039$), 产卵期与产卵高峰期 ROS 含量未显著变化 ($P > 0.05$)。

2.4 ELF-EMF暴露提高成虫TAC, 引起氧化应激反应

为进一步证实 ELF-EMF 暴露在影响能量代谢的同时是否引起氧化应激反应, 我们检测了线虫 ELF-EMF 暴露后体内 TAC 的变化情况, 并以 ROS 与 TAC 的变化程度 ROS-TAC 分数综合判断氧化应激反应效应。TAC 分析结果 (图 4) 显示, ELF-EMF 可明显增强成虫初期线虫 TAC, 与对照组比较有统计学差异 ($P = 0.022$)。而产卵期与产卵高峰期成虫体内 TAC 于 ELF-EMF 暴露后无显著变化 ($P > 0.05$)。为综合评价成虫氧化应激反应, 采用主成分分析计算成虫初期 ROS-TAC 分数 (计算步骤见材料与方法 1.7)。结果显示 (表 1), ELF-EMF 暴露引起成

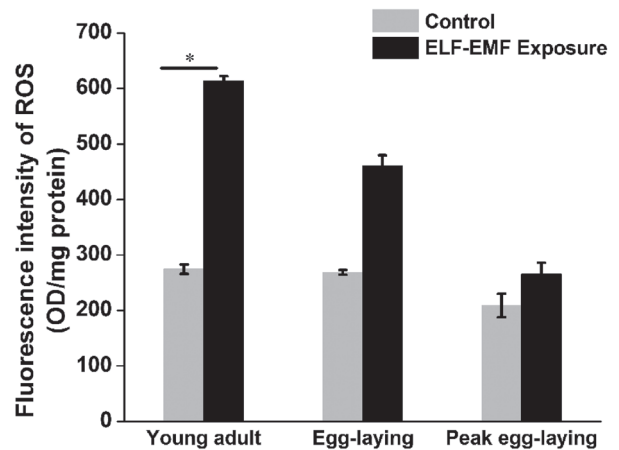


图 3. 极低频电磁辐射暴露对成虫ROS水平的影响
Fig. 3. Effect of extremely low frequency electromagnetic field (ELF-EMF) exposure on adult worms' ROS level. Worms were treated by DCFH-DA staining for 2 h and detected with fluorescence intensity normalized by total proteins. Mean \pm SD. The experiment was repeated thrice. * $P < 0.05$ vs control group.

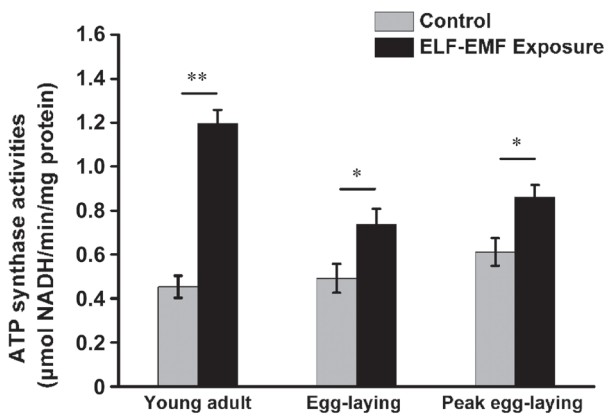


图 2. 极低频电磁辐射暴露诱导成虫期线虫ATP合成酶活性增强

Fig. 2. Extremely low frequency electromagnetic field (ELF-EMF) exposure induced enhancement of ATP synthase activity. Worms were treated, and homogenate was prepared as described in Fig. 1. ATP synthase activity was measured by the reaction of absorptive peak value reduction after oxidation of the reduced nicotinamide adenine dinucleotide (NADH) and expressed as the OD value per total protein content. The experiment was repeated thrice. Mean \pm SD. ** $P < 0.01$, * $P < 0.05$ vs control group.

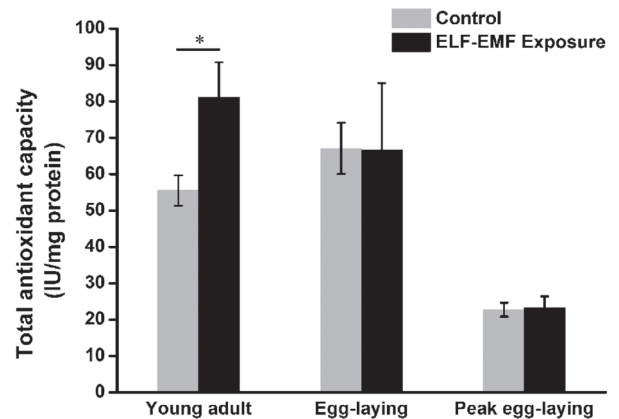


图 4. 极低频电磁辐射暴露对成虫期总抗氧化能力的影响
Fig. 4. Effect of extremely low frequency electromagnetic field (ELF-EMF) exposure on adults' total antioxidant capacity (TAC). TAC was determined by a kit following the manufacturer's protocol. Mean \pm SD. The experiment was repeated thrice. * $P < 0.05$ vs control group.

表 1. 成虫初期(56 h)线虫氧化应激反应

Table 1. Comparison of measures of oxidative stress between extremely low frequency electromagnetic field (ELF-EMF) exposed worms and the normal controls at young adult stage (56 h)

Variables	Control	ELF-EMF exposure	P-value
log(ROS + 1)	0.10 \pm 0.01	0.18 \pm 0.04	0.039*
TAC	55.50 \pm 4.16	81.08 \pm 9.67	0.022*
ROS-TAC score	25.04 \pm 0.62	23.80 \pm 0.39	0.008**

ROS: reactive oxygen species; TAC: total antioxidant capacity. All values were expressed as mean \pm SD. The experiment was repeated thrice. * $P < 0.05$, ** $P < 0.01$ vs control group.

虫初期 ROS-TAC 分数显著下降 ($P = 0.008$), 表明 ELF-EMF 引起的 ROS 变化比 TAC 变化更显著, ELF-EMF 暴露可引起成虫初期氧化应激响应。

3 讨论

能量代谢异常是 MD 的重要诱因, ATP 作为能量代谢中的主要物质为细胞供给能源, ATP 代谢失常与癌症、神经退行性疾病等紧密相关^[16]。文献报道非电离辐射与 MD 相关可能与氧化损伤导致的线粒体功能异常、能量代谢紊乱密不可分^[17]。本实验以秀丽线虫为模式生物研究 ELF-EMF 对能量代谢的影响。结果显示, 50 Hz、3 mT ELF-EMF 暴露可促进线虫的 ATP 合成。已有文献报道, 经过 ELF-EMF 暴露后, 其他生物体内也出现类似 ATP 水平上升的现象。如 50 Hz、5 mT ELF-EMF 暴露致人类的精子细胞内 ATP 升高^[18]。另外, 本实验中 ATP 合成酶活性在 ELF-EMF 暴露下增强, 提示上述 ATP 水平升高可通过提高 ATP 合成酶活性实现。ATP 合成酶即线粒体复合物 V, 属于 F_0F_1 -ATPase, 位于线粒体内膜, 通过质子梯度驱动实现 ATP 合成。ATP 合成酶活性已被报道受磁场影响, 并与暴露时间、暴露强度相关, 例如 0.5 mT、24 Hz ELF-EMF 暴露可抑制 F_0F_1 -ATPase 的水解活性, 而 0.5 mT、60 Hz ELF-EMF 暴露对其水解活性具有增强作用^[19]。还有研究表明, 胞外 ATP 具有压力信号传递的功能^[20]。ELF-EMF 暴露下 ATP 升高可能参与应激信号转导。本研究检测到 ATP 水平升高的同时, 经 ELF-EMF 暴露处理的初期成虫出现了氧化应激反应。ELF-EMF 暴露可能通过促进线虫体内 ATP 合成从而提高线虫能量代谢, 并与氧化应激反应有关。本研究中 ELF-EMF 暴露后成虫 ATP 合成增加, 对线虫而言可被认为是一种适应性响应, 利于线虫成虫期内适应 ELF-EMF 暴露环境。成虫通过增加 ATP 合成促进能量代谢活跃, 一方面利于保证成虫期产卵过程消耗大量 ATP, 另一方面也可能是 ELF-EMF 能量与生物能量形式联结的一种表现。已知生物体剧烈运动、应激刺激等条件下, ATP 合成增加, 新陈代谢水平升高, 被广泛认为是一种应激响应^[21]。ELF-EMF 暴露对于生物而言可能产生促进代谢的效应。已有文献报道称 ELF-EMF 暴露有利于促进角化细胞增殖, 加快伤口愈合^[22]。本研究中线虫卵细胞的产生可看作细胞增殖过程, 增殖需要消耗大量能量, 一定条件下 ELF-EMF 暴露具

有正面的生物学意义。

氧化应激已被广泛证实参与不同生物体不同强度、频率 ELF-EMF 暴露处理后的响应, 通常伴有 ROS 增多与抗氧化防御系统负荷加重^[23]。临床应用方面, 通过利用一定强度范围的 ELF-EMF 暴露可以刺激抗氧化应激反应发生, 从而达到治疗效果^[24, 25]。已有报道表明低频电磁辐射主要以场的形式对生物体产生影响, 且一定条件低频电磁场暴露能引起生物体抗氧化酶如超氧化物歧化酶 (superoxide dismutase, SOD)、谷胱甘肽过氧化物酶 (glutathione peroxidase, GSH-PX) 活性上升^[26]。50 Hz ELF-EMF 暴露诱发大鼠焦虑行为并使下丘脑氧自由基浓度上升^[27]。人慢性髓白血病 K562 细胞暴露于 50 Hz、1 mT ELF-EMF 后胞内 ROS 增加^[28]; 60 Hz、1 mT ELF-EMF 暴露条件下前列腺癌细胞系 DU145、PC3 和 LNCaP 内 ROS 水平上升^[29]。本研究中, 成虫初期 ROS 增多, 提示线虫体内氧化应激参与了 ELF-EMF 诱导的响应机制。同时, 初期成虫体内抗氧化能力 TAC 上调。分析原因, 一定程度的 ROS 增多可通过短暂提高抗氧化能力缓解, 从而达到维持线虫在 ELF-EMF 胁迫下生存的目的。但过强或过久的 ROS 增多和抗氧化防御系统负荷则会造成线虫自身正常功能的损伤^[30]。本研究结果中, 成虫产卵期和产卵高峰期线虫 ROS 水平和 TAC 较对照组未检测出显著性差异。一方面由于产卵期内线虫 DNA 大量复制、细胞增殖活跃, 高水平 ROS 和强氧化应激反应引起 DNA 损伤^[31], 为保证产卵活动正常, ELF-EMF 暴露产卵期成虫未出现显著氧化应激响应。另一方面可能由于线虫对 ELF-EMF 暴露胁迫产生了一定适应性, 通过 ROS 及抗氧化防御系统动态调节使得产卵期线虫体内氧化应激反应下降, 适应 ELF-EMF 暴露环境。分析成虫初期 ROS 增多的来源, 文献报道提示, ATP 合成增强与 ROS 水平升高密不可分^[32]。线粒体在合成 ATP 过程中有部分电子会从电子传递链 (electron transport chain, ETC) 上逃逸, 与细胞质中 O_2 、 H_2O 及 NO 等结合产生 ROS, 这是细胞内 ROS 的主要来源。ELF-EMF 诱导的 ATP 合成上升提示 ELF-EMF 可能导致电子传递增多, 从而引起电子逃逸增强, ROS 增多。在今后的实验中我们将采用抑制剂抑制呼吸链功能的方法, 观察其是否可拮抗 ELF-EMF 对 ATP 合成的诱导作用, 以进一步证实上述结论。此外我们还将探讨 ELF-EMF 暴露的生物遗传效应问题。在本研究中

我们只探讨了 ELF-EMF 对一代线虫 ATP 合成的影响，对于后代能量代谢的影响未进行阐述。因此需要我们进一步开展长期暴露 ELF-EMF 后线虫能量变化和机制研究，以及 ELF-EMF 对能量代谢的影响是否存在累积效应的研究。如探讨多代暴露后线虫 ATP 合成酶的变化以及利用表观遗传学分析方法探讨 ELF-EMF 对线虫氧化防御系统及 ATP 合成代

谢途径的影响。

综上所述，ELF-EMF 暴露诱导线虫能量代谢增强，具体表现为 ATP 合成增加，其机制可能与 ATP 合成酶活性增强有关，同时 ELF-EMF 暴露诱导成虫初期 ROS 水平增加，并引发氧化应激反应，随暴露时间延长，成虫发生适应，氧化应激效应与对照组比较无显著差异（图 5）。

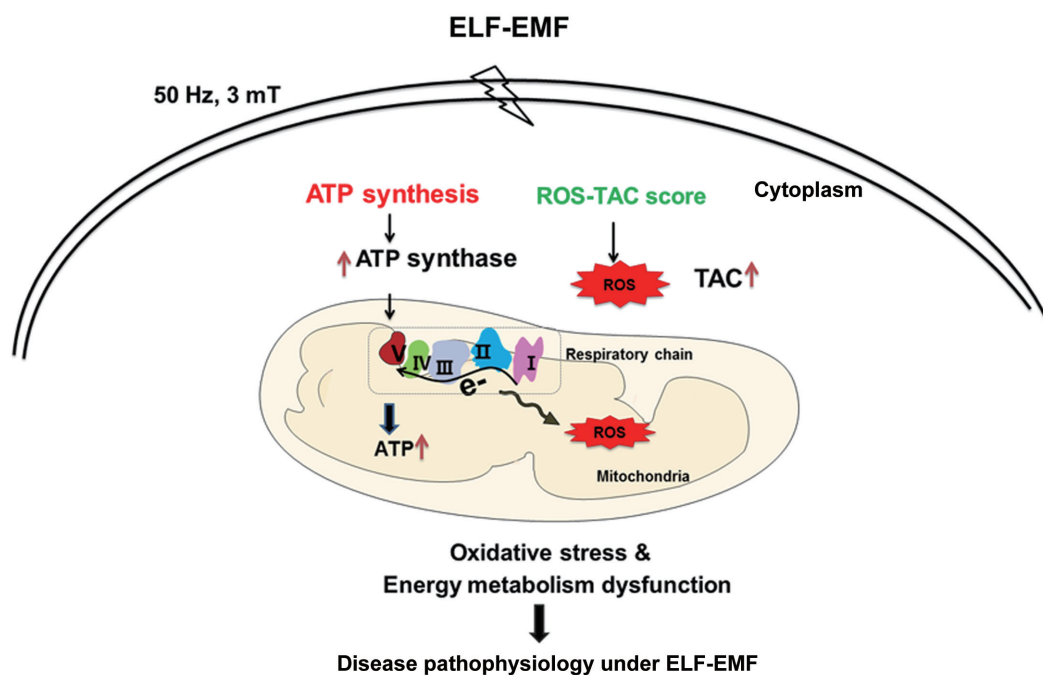


图 5. 极低频电磁辐射暴露诱导氧化应激相关的线虫能量代谢增强

Fig. 5. Extremely low frequency electromagnetic field (ELF-EMF) exposure enhanced energy metabolism accompanied with oxidative damage in worm adulthood. ATP level showed a significant elevation, and increased ATP synthase activity was apparent during the adulthood under ELF-EMF exposure. ROS level and total antioxidant capacity (TAC) were elevated under ELF-EMF stress in early adults and then with no significant changes in following adulthood. Declined ROS-TAC score indicated ROS elevation was more than TAC alteration which implied worms' oxidative stress response was induced by ELF-EMF exposure. Terms enhanced or elevated were marked in red, and inhibited or decreased terms were marked in green.

参考文献

- 1 Kaur J. A comprehensive review on metabolic syndrome. *Cardiol Res Pract* 2014; 2014: 943162.
- 2 Atamna H, Frey WH 2nd. Mechanisms of mitochondrial dysfunction and energy deficiency in Alzheimer's disease. *Mitochondrion* 2007; 7(5): 297–310.
- 3 Burt JJ, Thompson PA, Lafrenie RM. Non-targeted effects and radiation-induced carcinogenesis: a review. *J Radiol Prot* 2016; 36(1): 23–25.
- 4 Zuo JJ (左晶晶), Chen C, Zeng ML, Li M, Hu ZW, Tao ZZ. Advances of studies on the occurrence, development and prognosis of tumors correlative with gene-polymorphisms and cigarette smoking. *Prog Modern Biomed (现代生物医学进展)* 2007; 17(16): 3180–3183 (in English with Chinese abstract).
- 5 Muscat JE, Wynder EL. Tobacco, alcohol, asbestos, and occupational risk factors for laryngeal cancer. *Cancer* 2015; 69(9): 2244–2251.
- 6 Cai H, Cong W, Ji S, Rothman S, Maudsley S, Martin B. Metabolic dysfunction in Alzheimer's disease and related neurodegenerative disorders. *Curr Alzheimer Res* 2012; 9(1): 5–17.
- 7 Abbate N. Motivation and significance of IARC classification for mobile phone. *G Ital Med Lav Ergon* 2011; 33(3): 384–389 (in Italian with English abstract).

- 8 Kheifets L, Shimkhada R. Childhood leukemia and EMF: review of the epidemiologic evidence. *Bioelectromagnetics* 2010; 26(S7): S51–S59.
- 9 Pedersen C, Poulsen AH, Rod NH, Frei P, Hansen J, Grell K, Nielsen RO, Schüz J, Johansen C. Occupational exposure to extremely low-frequency magnetic fields and risk for central nervous system disease: an update of a Danish cohort study among utility workers. *Int Arch Occ Env Hea* 2017; 2017(5): 1–10.
- 10 Dutta SK, Das K, Ghosh B, Blackman CF. Dose dependence of acetylcholinesterase activity in neuroblastoma cells exposed to modulated radio-frequency electromagnetic radiation. *Bioelectromagnetics* 2010; 13(4): 317–322.
- 11 Lin KW, Yang CJ, Lian HY, Cai P. Exposure of ELF-EMF and RF-EMF increase the rate of glucose transport and tea cycle in budding yeast. *Front Microbiol* 2016; 7: 1378.
- 12 Zhang ZY, Zhang J, Yang CJ, Lian HY, Yu H, Huang XM, Cai P. Coupling mechanism of electromagnetic field and thermal stress on *Drosophila melanogaster*. *PLoS One* 2016; 11(9): e0162675.
- 13 Shi ZH, Yu H, Sun YY, Yang CJ, Lian HY, Cai P. The energy metabolism in *Caenorhabditis elegans* under the extremely low-frequency electromagnetic field exposure. *Sci Rep* 2015; 5: 8471.
- 14 Stiernagle T. Maintenance of *C. elegans*. *Wormbook* 2005: 1–11.
- 15 Sun YY, Shi ZH, Wang YH, Tang C, Liao YY, Yang CJ, Cai P. Coupling of oxidative stress responses to tricarboxylic acid cycle and prostaglandin E2 alterations in *Caenorhabditis elegans* under extremely low-frequency electromagnetic field. *Int J Radiat Biol* 2018: 1–8. DOI: 10.1080/09553002.2019.1524943.
- 16 Huo HY (霍宏宇), Hu SQ, Sun Y, Ruan JF, Sun ZY, Liao GY, Yu M. Tumor microenvironment and energy metabolic reprogramming. *Chin Bull Life Sci (生命科学)* 2017; 29(1): 70–76 (in Chinese with English abstract).
- 17 Havas M. When theory and observation collide: Can non-ionizing radiation cause cancer? *Environ Pollut* 2017; 221: 501–505.
- 18 Iorio R, Delle Monache S, Bennato F, Di Bartolomeo C, Scrimaglio R, Cinque B, Colonna RC. Involvement of mitochondrial activity in mediating ELF-EMF stimulatory effect on human sperm motility. *Bioelectromagnetics* 2011; 32(1): 15–27.
- 19 Penefsky HS, Cross RL. Structure and mechanism of FoF1-type ATP synthases and ATPases. *Adv Enzymol Relat Areas Mol Biol* 1991; 64: 173–214.
- 20 Roux D, Faure C, Bonnet P, Girard S, Ledoigt G, Davies E, Gendraud M, Paladian F, Vian A. A possible role for extra-cellular ATP in plant responses to high frequency, low amplitude electromagnetic field. *Plant Signal Behav* 2008; 3(6): 383–385.
- 21 Cruz CM, Rinna A, Forman HJ, Ventura ALM, Persechini PM, Ojcius DM. ATP activates an ROS-dependent oxidative stress response and secretion of pro-inflammatory cytokines in macrophages. *J Biol Chem* 2016; 282(5): 2871–2879.
- 22 Patruno A, Ferrone A, Costantini E, Franceschelli S, Pesce M, Speranza L, Amerio P, D'Angelo C, Felaco M, Grilli A, Reale M. Extremely low-frequency electromagnetic fields accelerates wound healing modulating MMP-9 and inflammatory cytokines. *Cell Prolif* 2018; 51(2): e12432.
- 23 Wang H, Zhang X. Magnetic fields and reactive oxygen species. *Int J Mol Sci* 2017; 18(10). pii: E2175. doi: 10.3390/ijms18102175.
- 24 Cichoń N, Bijak M, Miller E, Saluk J. Extremely low frequency electromagnetic field (ELF-EMF) reduces oxidative stress and improves functional and psychological status in ischemic stroke patients. *Bioelectromagnetics* 2017; 38(5): 386–396.
- 25 Cichoń N, Rzeźnicka P, Bijak M, Miller E, Miller S, Saluk J. Extremely low frequency electromagnetic field reduces oxidative stress during the rehabilitation of post-acute stroke patients. *Adv Clin Exp Med* 2018; 27(9): 1285–1293.
- 26 Zhang QJ (张清俊), Wu K. Biological oxidative stress induced by electromagnetic irradiation. *Space Med Med Eng (航天医学与医学工程)* 2004; 17(2): 152–156 (in Chinese with English abstract).
- 27 Djordjevic NZ, Paunović MG, Peulić AS. Anxiety-like behavioural effects of extremely low-frequency electromagnetic field in rats. *Environ Sci Pollut Res Int* 2017; 24(27): 21693–21699.
- 28 Garip AI, Akan Z. Effect of ELF-EMF on number of apoptotic cells; correlation with reactive oxygen species and HSP. *Acta Biol Hung* 2010; 61(2): 158–167.
- 29 Koh EK, Ryu BK, Jeong DY, Bang IS, Nam MH, Chae KS. A 60-Hz sinusoidal magnetic field induces apoptosis of prostate cancer cells through reactive oxygen species. *Int J Radiat Biol* 2008; 84(11): 945–955.
- 30 Geng JW (耿军伟), Yu H, Lin Z, Xue L, Guan MX. Formation and metabolism of reactive oxygen species in cell. *Chin Bull Life Sci (生命科学)* 2015; 27(5): 609–617 (in Chinese with English abstract).
- 31 Mandavilli BS, Santos JH, Van Houten B. Mitochondrial DNA repair and aging. *Mutat Res* 2002; 509(1–2): 127–151.
- 32 Li LD (李良德), Wang DF, Wu GY. Progresses on producing sites of reactive oxygen species in mitochondria. *Life Sci Res (生命科学研究)* 2015; 19(6): 530–535 (in Chinese with English abstract).