

## **Effect of Long-Term Treatment with C60 Fullerenes on the Lifespan and Health Status of CBA/Ca Mice**

### Abstract

Several studies claimed C60 fullerenes as a prospective geroprotector drug due to their ability to capture free radicals effectively and caused a profound interest in C60 in life extension communities. Multiple additives are already sold for human consumption despite a small body of evidence supporting the beneficial effects of fullerenes on the lifespan. To test the effect of C60 fullerenes on lifespan and healthspan, we administered C60 fullerenes dissolved in virgin olive oil orally to 10-12 months old CBA/Ca mice of both genders for 7 months and assessed their survival. To uncover C60 and virgin olive effects, we established two control groups: mice treated with virgin olive oil (vehicle) and mice treated with drinking water. To measure healthspan, we conducted daily monitoring of health condition and lethality and monthly bodyweight measurements. We also assessed physical activity, glucose metabolism, and hematological parameters every 3 months. We did not observe health deterioration in the animals treated with C60 compared with the control groups. Treatment of mice with C60 fullerenes resulted in an increased lifespan of males and females compared with the olive oil-treated animals. The lifespan of C60-treated mice was similar to the mice treated with water. These results suggest that the lifespan-extending effect in C60-treated mice appears due to the protective effect of fullerenes in opposition to the negative effect of olive oil in CBA/Ca mice.

Shytikov D, Shytikova I, Rohila D, Kulaga A, Dubiley T, Pishel I. Effect of Long-Term Treatment with C60 Fullerenes on the Lifespan and Health Status of CBA/Ca Mice. *Rejuvenation Res.* 2021 Oct;24(5):345-353. doi: 10.1089/rej.2020.2403. Epub 2021 May 19. PMID: 33849306.

## **C60 fullerene: a powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays**

### Abstract

Since the discovery of fullerenes in 1985, these carbon nanospheres have attracted attention regarding their physico/chemical properties. Despite little knowledge about their impact on the environment and human health, the production of fullerenes has already reached an industrial scale. However, the toxicity of C(60) is still controversially discussed. The aim of this study was to clarify the biological effects of tetrahydrofuran (THF) suspended C(60) fullerene in comparison to water stirred C(60) fullerene suspensions. Beyond that, we analyzed the effects on the Crustacea *Daphnia magna* an indicator for ecotoxicological effects and the human lung epithelial cell line A549 as a simplified model for the respiratory tract. We could demonstrate that water-soluble side products which were formed in THF nC(60) suspension were responsible for the observed acute toxic effects, whereas fullerenes themselves had no negative effect regardless of the preparative route on either A549 cell in vitro or *D. magna* in vivo.

Spohn P, Hirsch C, Hasler F, Bruinink A, Krug HF, Wick P. C60 fullerene: a powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays. *Environ Pollut.* 2009 Apr;157(4):1134-9. doi: 10.1016/j.envpol.2008.08.013. Epub 2008 Sep 27. PMID: 18824284.

## **Effects of fullerene C60 supplementation on gut microbiota and glucose and lipid homeostasis in rats**

### **Abstract**

The effects of twelve weeks of supplementation with fullerene C60 olive/coconut oil solution on a broad spectrum of parameters in rats were examined. The tissue bioaccumulation of C60 was shown to be tissue-specific, with the liver, heart, and adrenal glands being the organs of the greatest, and the kidney, brain, and spleen being the organs of the smallest accumulation. C60 did not change aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase serum activities level, nor the damage of liver cells DNA. There were no effects of fullerene on prooxidant-antioxidant balance in the liver, kidney, spleen, heart, and brain, nor any visible harmful effects on the liver, heart, aorta, spleen, kidney, and small intestine histology. Fullerene changed the gut microbiota structure towards the bacteria that ameliorate lipid homeostasis, causing a serum triglycerides concentration decrease. However, C60 significantly increased the insulin resistance, serum ascorbate oxidation, and brain malondialdehyde and advanced oxidation protein products level. The deteriorative effects of C60 on the brain and serum could be attributed to the specific physicochemical composition of these tissues, potentiating the C60 aggregation or biotransformation as the key element of its pro-oxidative action.

Đurašević S, Nikolić G, Todorović A, Drakulić D, Pejić S, Martinović V, Mitić-Ćulafić D, Milić D, Kop TJ, Jasnić N, Đorđević J, Todorović Z. Effects of fullerene C60 supplementation on gut microbiota and glucose and lipid homeostasis in rats. *Food Chem Toxicol.* 2020 Jun;140:111302. doi: 10.1016/j.fct.2020.111302. Epub 2020 Mar 28. PMID: 32234425.

## **C60 fullerene attenuates muscle force reduction in a rat during fatigue development**

### **Abstract**

C60 fullerene (C60) as a nanocarbon particle, compatible with biological structures, capable of penetrating through cell membranes and effectively scavenging free radicals, is widely used in biomedicine. A protective effect of C60 on the biomechanics of fast (m. gastrocnemius) and slow (m. soleus) muscle contraction in rats and the pro- and antioxidant balance of muscle tissue during the development of muscle fatigue was studied compared to the same effect of the known antioxidant N-acetylcysteine (NAC). C60 and NAC were administered intraperitoneally at

doses of 1 and 150 mg kg<sup>-1</sup>, respectively, daily for 5 days and 1 h before the start of the experiment. The following quantitative markers of muscle fatigue were used: the force of muscle contraction, the level of accumulation of secondary products of lipid peroxidation (TBARS) and the oxygen metabolite H<sub>2</sub>O<sub>2</sub>, the activity of first-line antioxidant defense enzymes (superoxide dismutase (SOD) and catalase (CAT)), and the condition of the glutathione system (reduced glutathione (GSH) content and the activity of the glutathione peroxidase (GPx) enzyme). The analysis of the muscle contraction force dynamics in rats against the background of induced muscle fatigue showed, that the effect of C60, 1 h after drug administration, was (15-17)% more effective on fast muscles than on slow muscles. A further slight increase in the effect of C60 was revealed after 2 h of drug injection, (7-9)% in the case of m. gastrocnemius and (5-6)% in the case of m. soleus. An increase in the effect of using C60 occurred within 4 days (the difference between 4 and 5 days did not exceed (3-5)%) and exceeded the effect of NAC by (32-34)%. The analysis of biochemical parameters in rat muscle tissues showed that long-term application of C60 contributed to their decrease by (10-30)% and (5-20)% in fast and slow muscles, respectively, on the 5th day of the experiment. At the same time, the protective effect of C60 was higher compared to NAC by (28-44)%. The obtained results indicate the prospect of using C60 as a potential protective nano agent to improve the efficiency of skeletal muscle function by modifying the reactive oxygen species-dependent mechanisms that play an important role in the processes of muscle fatigue development.

Prylutsky Y, Nozdrenko D, Gonchar O, Prylutska S, Bogutska K, Franskevych D, Hromovik B, Scharff P, Ritter U. C60 fullerene attenuates muscle force reduction in a rat during fatigue development. *Heliyon*. 2022 Dec 21;8(12):e12449. doi: 10.1016/j.heliyon.2022.e12449. PMID: 36590525; PMCID: PMC9801117.

## **C60 fullerene and its nanocomplexes with anticancer drugs modulate circulating phagocyte functions and dramatically increase ROS generation in transformed monocytes**

### **Abstract**

**Background:** C60 fullerene-based nanoformulations are proposed to have a direct toxic effect on tumor cells. Previous investigations demonstrated that C60 fullerene used alone or being conjugated with chemotherapeutic agents possesses a potent anticancer activity. The main aim of this study was to investigate the effect of C60 fullerene and its nanocomplexes with anticancer drugs on human phagocyte metabolic profile in vitro.

**Methods:** Analysis of the metabolic profile of phagocytes exposed to C60 fullerene in vitro revealed augmented phagocytic activity and down-regulated reactive nitrogen species generation in these cells. Additionally, cytofluorimetric analysis showed that C60 fullerene can exert direct cytotoxic effect on normal and transformed phagocytes through the vigorous induction of intracellular reactive oxygen species generation.

**Results:** Cytotoxic action as well as the pro-oxidant effect of C60 fullerene was more pronounced toward malignant phagocytes. At the same time, C60 fullerenes have the ability to down-regulate the pro-oxidant effect of cisplatin on normal cells. These results indicate that C60 fullerenes may influence phagocyte metabolism and have both pro-oxidant and antioxidant properties.

**Conclusions:** The antineoplastic effect of C60 fullerene has been observed by direct toxic effect on tumor cells, as well as through the modulation of the functions of effector cells of antitumor immunity.

Skivka LM, Prylutska SV, Rudyk MP, Khranovska NM, Opeida IV, Hurmach VV, Prylutsky YI, Sukhodub LF, Ritter U. C60 fullerene and its nanocomplexes with anticancer drugs modulate circulating phagocyte functions and dramatically increase ROS generation in transformed monocytes. *Cancer Nanotechnol.* 2018;9(1):8. doi: 10.1186/s12645-017-0034-0. Epub 2018 Oct 31. PMID: 30416604; PMCID: PMC6208740.