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BIOW ION INDUCTOR ENHANCES BRAIN AND MUSCLE ENERGY EFFICIENCY DURING AGEING WITH OXIDATIVE IMPROVEMENT: A MOUSE STUDY

The new BioW equipment incorporates a previously studied system for removing environmental nanoparticles, which had shown significant results in reducing oxidative stress and increasing energy capacity in peripheral blood mononuclear cells, as detailed in our first published paper on the topic (Antuña et al., 2022). This is combined with a novel ion induction process based on laminar flow, achieved through its levitation motor and internal geometry, generating bioavailable anions for possible absorption. This advancement in BioW equipment requires in-depth study to understand its multi-organ effects. We conducted experiments using 13-week-old male C57BL/6 mice, kept with food and water ad libitum under standardised light and dark conditions for 7 weeks in the same room (all in a nanoparticle-free atmosphere due to the equipment's removal capability). The control mice were kept in a clean atmosphere but sufficiently distanced from the necessary ion induction. In contrast, experimental mice (BioW mice) were placed with no separation from the BioW device, ensuring constant anion reception. Older mice (19 months old) were kept under the same conditions, referred to as Aging CON and Aging BioW respectively. We, therefore, studied four groups: young controls (CON), young BioW (BIOW), aged controls (AG CON), and aged BioW (AG BIOW). After 7 weeks, the mice were euthanised by exsanguination and cervical dislocation. Brains and livers were collected from the young mice (CON and BIOW), and muscles were added for the older mice (Aging CON and Aging BioW) for oxidative stress and energy production capacity characterisation.

The measurement of oxidative stress was conducted through antagonistic studies that allow us to understand, on one hand, the variations in antioxidant response produced by the individual in each of the organs exposed (Total Antioxidant Activity - TAA) along with oxidative damage to lipids, measured as lipid peroxidation (LPO) in response to generated free radicals. Alongside these studies, the measurement of total energy generation capacity, measured in the form of ATP (adenosine triphosphate, a fundamental nucleotide in cellular energy storage) in all organs of the individuals studied was also carried out.

Statistical analysis and the resulting graphs were created using GraphPad Prism 6 software. Data were presented as mean ± SEM. Normality was determined by the Shapiro–Wilk normality test. Data sets following a normal distribution were analysed using Student's t-test, while those not following a normal distribution were analysed using the non-parametric Mann-Whitney test. The significance level was set at $P< 0.05$.

The results obtained in each case are shown below:

LIVER

OXIDATIVE CHARACTERISATION

The liver is a fundamentally detoxifying organ, making it particularly exposed to free radicals resulting from toxicity. At the same time, it requires a high level of energy to perform its activities, which primarily comes from the mitochondria and consequently increases the production of free radicals. Therefore, a significant reduction in oxidative damage could protect the organ from the numerous diseases that can result from the accumulation of free radicals in the liver. This situation becomes even more dangerous with ageing, where the gradual and constant build-up of free radicals in all the body's organs has a particularly harmful effect on the liver. Hepatic alterations directly induced by the accumulation of free radicals have been observed (Tomas-Zapico et al., 2006).

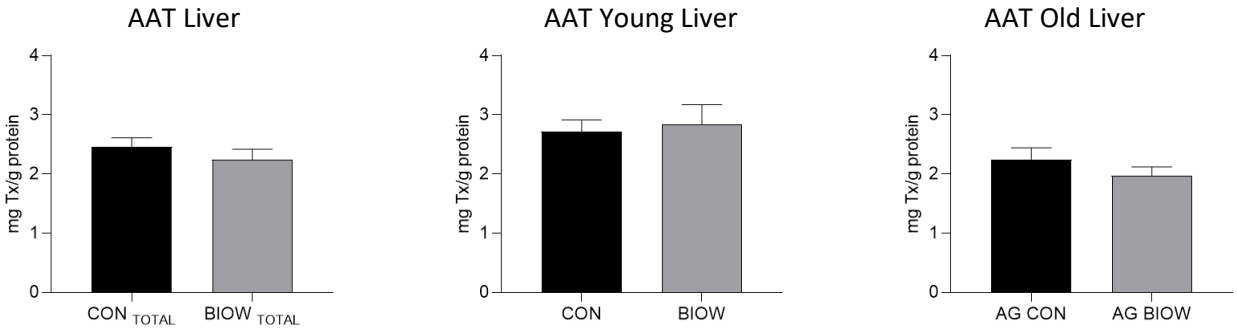

Figure 1. Total Antioxidant Activity (TAA expressed in mg of Trolox/g protein) in homogenised liver tissue of young control mice (CON), young mice with BioW (BIOW), aged control mice (AG CON), and aged mice with BioW (AG BIOW). Data are presented as mean ± SEM. The number of asterisks indicates the level of significance: one for $p < 0.05$, two for $p < 0.01$, and three for $p < 0.001$.

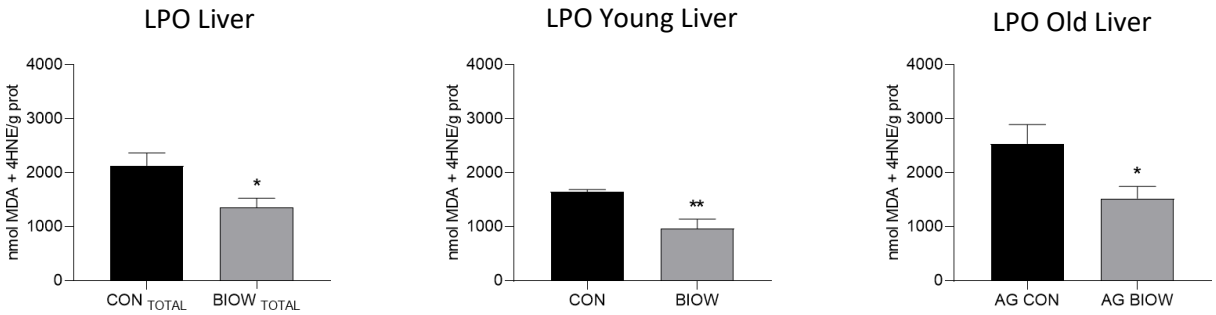

Figure 2. Lipid Peroxidation (LPO expressed in nmol MDA + 4HNE/g protein) in homogenised liver tissue of young control mice (CON), young mice with BioW (BIOW), aged control mice (AG CON), and aged mice with BioW (AG BIOW). Data are presented as mean ± SEM. The number of asterisks indicates the level of significance: one for $p < 0.05$, two for $p < 0.01$, and three for $p < 0.001$.

The results show that lipid damage caused by free radicals is significantly reduced in both young and aged individuals treated with BioW (CON vs BIOW and AG vs AG BIOW). Consequently, the overall study maintains the significant differences observed (CONtotal vs BIOWtotal). This indicates that ion induction has specifically reduced free radical damage in the liver, the organ responsible for the individual's detoxification. While this isolated result already demonstrates a beneficial role of the treatment, it could be due to either an increase in the individual's antioxidant defence or direct action. The results observed in the liver's antioxidant capacity indicate that this capacity remained unchanged throughout the study, so the observed reduction in LPO must be attributed exclusively to the direct action of the equipment used. It is important to note that in this type of experiment, the effect of BioW ion induction is exclusively detected, as mentioned earlier, the effect of nanoparticle reduction is generalised throughout the room, benefiting all participating groups. This implies that, considering the effects shown in our first article (Antuña et al., 2022), the LPO reduction effect might be underestimated.

Thus, based on the results obtained, the liver could be, at least partially, protected during ageing thanks to the ionic induction generated by BioW.

TOTAL ENERGY CAPACITY

The liver is a highly energy-demanding organ due to its role in multiple detoxification and homeostasis activities within the body. However, in this case, ionic induction did not produce any significant change in energy production in either young or elderly individuals, leaving the total effect on ATP production unchanged.

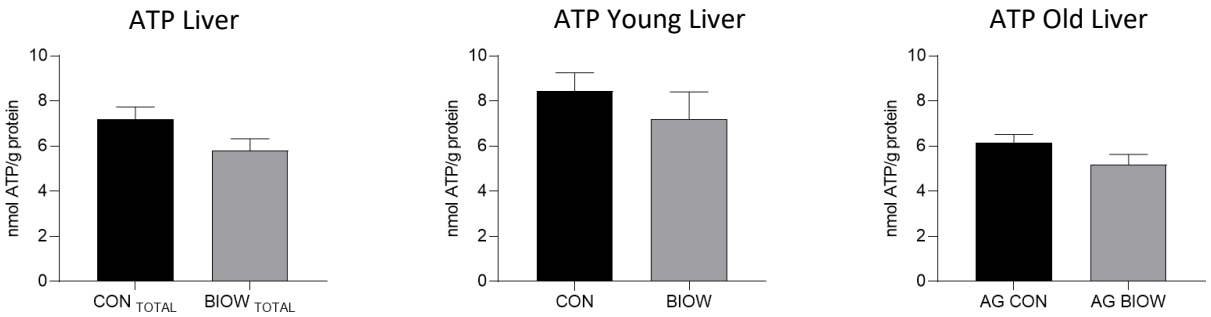

Figure 3. ATP production (expressed in nmol ATP/g protein) in homogenised liver tissue of young control mice (CON), young mice with BioW (BIOW), elderly control mice (AG CON), and elderly mice with BioW (AG BIOW). Data are represented as mean ± SEM. The number of symbols "*" indicates the level of significance: one for $p < 0.05$, two for $p < 0.01$, and three for $p < 0.001$.

BRAIN

OXIDATIVE CHARACTERISATION

The brain is particularly sensitive to damage caused by free radicals due to multiple reasons: its preference for glucose-based nutrition, which demands greater mitochondrial effort and thus higher production of free radicals; a high concentration of lipids due to their essential role in protecting and isolating neuronal extensions, making it particularly susceptible to damage by free radicals as oxidative damage cascades from the first altered lipid; a high level of transition metals that promote the development and maturation of these free radicals and their evolution into more toxic elements, and finally, an unusually low antioxidant environment. All of this makes the brain an exposed and vulnerable target for free radicals, both in young individuals and in ageing ones. However, we have not found, in the individuals studied, significant differences in either total antioxidant defence (AAT) or lipid damage (LPO). Given the detoxifying role of this organ, it would be of great interest to assess the effect of reducing environmental nanoparticles, which in this case is not being evaluated as it affects all individuals in the study.

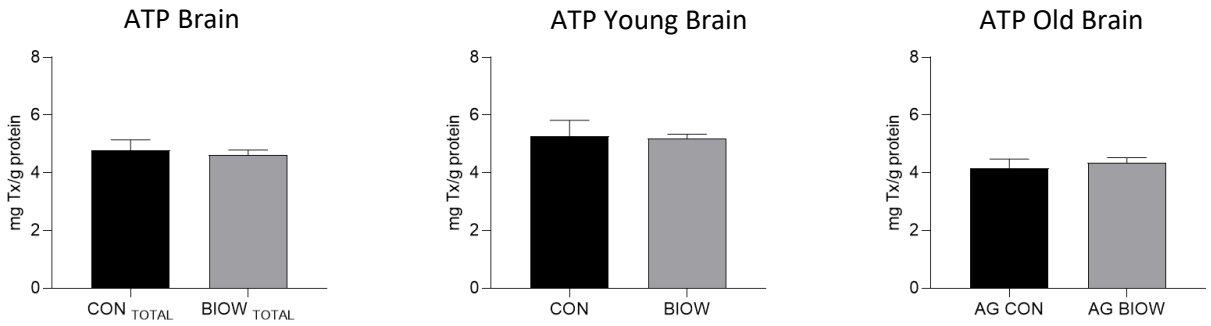

Figure 4. Total antioxidant activity (AAT expressed in mg of Trolox/g protein) of homogenised brain tissue from young control mice (CON), young mice with BioW (BIOW), aged control mice (AG CON), and aged mice with BioW (AG BIOW). Data are represented as mean ± SEM. The number of symbols "*" indicates the level of significance: one for $p < 0.05$, two for $p < 0.01$, and three for $p < 0.001$.

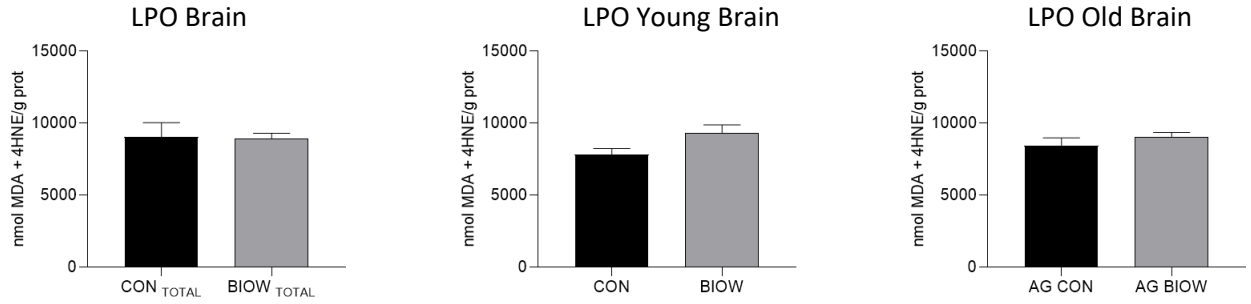

Figure 5. Lipid peroxidation (LPO expressed in nmol MDA + 4HNE/g prot) in homogenised brain tissue from young control mice (CON), young mice with BioW (BIOW), aged control mice (AG CON), and aged mice with BioW (AG BIOW). Data are represented as mean ± SEM. The number of symbols "*" indicates the level of significance: one for $p < 0.05$, two for $p < 0.01$, and three for $p < 0.001$.

TOTAL ENERGY CAPACITY

ATP production is essential in all organs of the body, but even more so in the case of the brain, due to its essential role in information transmission via synaptic impulse. Good ATP production implies good mitochondrial function, which in turn requires adequate cellular status. A reduction in energy capacity is present, without exception, in all neurodegenerative diseases and is a determining factor in the onset of senile dementia associated with unhealthy ageing. Therefore, the finding of a clearly significant increase in ATP production in the brains of ageing individuals is of great importance because facilitating an increase in energy production capacity in weakened cells is essential to strengthen their action capacity and protect them from possible ageing-associated alterations. In light of these results, the lack of increase at the level of non-ageing animals may simply be due to correct ATP production by these individuals, making its increase unnecessary and therefore maintaining levels within normal ranges.

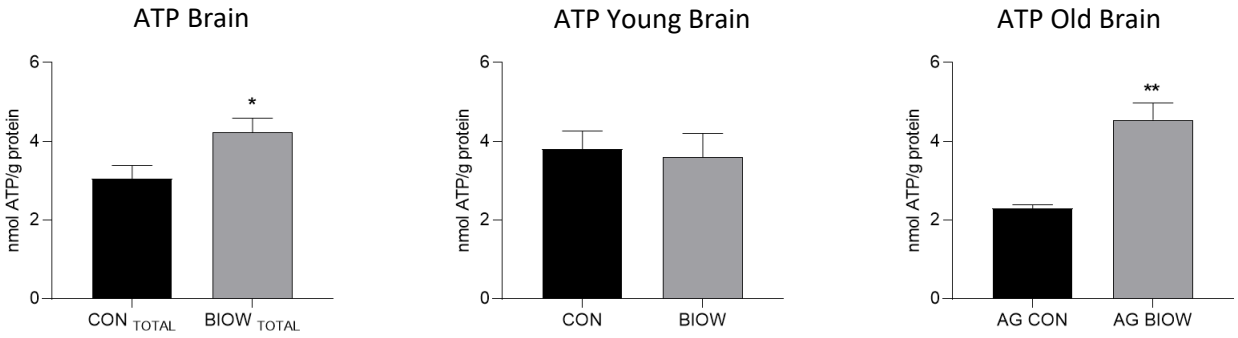

Figure 6. ATP production (expressed in nmol ATP/g protein) in homogenised brain tissue from young control mice (CON), young mice with BioW (BIOW), aged control mice (AG CON), and aged mice with BioW (AG BIOW). Data are represented as mean ± SEM. The number of symbols "*" indicates the level of significance: one for $p < 0.05$, two for $p < 0.01$, and three for $p < 0.001$.

MUSCLE

OXIDATIVE CHARACTERISATION

Skeletal muscle, as voluntary striated muscle, is one of the most abundant tissues in mammals, responsible for carrying out the contraction movements necessary to produce movement. Due to its practically continuous function, muscle is one of the most energy-dependent organs. Proof of this is the constant presence of mitochondria permanently arranged between the sarcomeres and therefore lacking the cellular diffusion capacity found in cells of other tissues. Additionally, along with the liver, muscle cells store glycogen as a reserve substance, as glucose is essential for total glycolysis, of which its aerobic phase and main energy producer occurs in the mitochondria. If its cells are so energy-dependent, this organ must be highly vulnerable to free radical production. In our study, we have only been able to assess total antioxidant capacity (AAT), which shows no significant differences with ionizing treatment. However, in view of the results obtained in the liver and as shown previously, it would be of great interest to know the levels of lipid damage by free radicals present in muscle, as a reduction in oxidative damage at this organ level could have a significant impact on individual quality of life. Likewise, we must not forget that all animals participating in the study have benefited from a practically total reduction in levels of environmental nanoparticles, so the role of environmental pollution has not been evaluated in the study. This purified environment is especially important at the muscular level since multiple studies have shown the devastating effect that pollution, including nanoparticle pollution, has on muscle and physical exercise (McGrath, 2000). Therefore, even though the data indicate no variations in AAT, future studies would be necessary to further investigate the role of total BioW treatment on muscle capacity and protection against oxidative stress from the new BioW equipment.

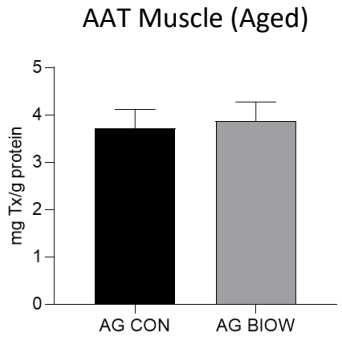

Figure 7. Total antioxidant activity (AAT expressed in mg of Trolox/g protein) of homogenised muscle tissue from aged control mice (AG CON) and aged mice with BioW (AG BIOW). Data are represented as mean ± SEM. The number of symbols "*" indicates the level of significance: one for $p < 0.05$, two for $p < 0.01$, and three for $p < 0.001$.

TOTAL ENERGY CAPACITY

One of the main causes of dependence in ageing is sarcopenia. Sarcopenia is defined as the loss of quality and muscle capacity that occurs with ageing, which does not necessarily parallel muscle element degradation but ultimately progressively hinders movement and gradually induces the characteristic immobility of elderly dependence. Therefore, combating sarcopenia is one of the main battles of active and healthy ageing, aiming to slow down the progression of sarcopenia by maintaining muscle contractile capacity intact for as long as possible. The accumulation of free radicals, typical of ageing, along with deficient mitochondrial function, has been defined as the main factors inducing sarcopenia (Jackson et al., 2022), and many research efforts are currently underway to find mechanisms that delay the onset of this condition. The data obtained in the study of ATP production capacity in the muscle of aged mice have shown that the ion-inducer developed within the BioW equipment is sufficient to significantly increase energy capacity in the elderly.

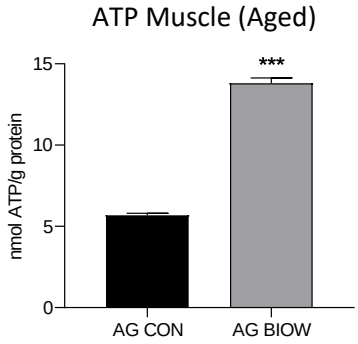

Figure 8. PATP production (expressed in nmol ATP/g protein) in homogenised muscle tissue from aged control mice (AG CON) and aged mice with BioW (AG BIOW). Data are represented as mean ± SEM. The number of symbols "*" indicates the level of significance: one for $p < 0.05$, two for $p < 0.01$, and three for $p < 0.001$.

Several factors must be taken into account to correctly assess the significant discovery that this data implies. On one hand, the recovery of mitochondrial capacity would be highly relevant in preserving muscle activity, which is crucial for delaying dependency, with an overwhelming percentage directly related to the lower limbs studied in this research. Moreover, we cannot overlook that this equipment does not have a targeted but rather comprehensive effect.

The fatigue experienced by the elderly, dependent on a reduction in pulmonary and cardiac capacity, has a direct effect on the decrease in oxygen uptake at the muscular level and the onset of dependency. Most likely, this significant increase in ATP production observed in the muscles of aged mice also depends, albeit partially, on an improvement at a systemic level and therefore also pulmonary and cardiac, which facilitates mitochondrial function in the muscle. This improvement, which will be directly influenced not only by ion induction but also by the reduction of contaminating pollution in the form of nanoparticles, must also be thoroughly studied due to the importance that this result would imply, as it could be used as a preserver of healthy ageing, providing not only a reduction in fatigue in the elderly but also muscle preservation, as these organs are the main ones involved in elderly dependency. We must always bear in mind that ageing is not the properly devastating factor in this period of life, but rather the dependency that, sometimes but not necessarily, accompanies this ageing. The development of a system that allows, even just significantly reducing, this dependency would

have very important health, social, and economic repercussions in developed and highly aged countries.

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