PULSE CENTERS

PEMF – Its Correlation to Enhanced Energy, Endurance and Performance

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Abstract

Pulsed electromagnetic fields (PEMFs) have been reported to affect a number of cellular and biological functions, but these accounts are generally not well documented with experimental evidence. Specifically, there are limited numbers of studies evaluating effects of PEMFs on blood flow and even fewer studies reporting effects on mitochondrial function. To address these deficiencies, we determined effects of PEMFs on rat skeletal muscle blood flow and respiratory function of mitochondria isolated from skeletal muscles that were treated or not treated with PEMFs. A treatment regimen of 3 treatments per week for three weeks demonstrated an increase in blood flow after each treatment. One to two weeks after this treatment protocol, mitochondria isolated from skeletal muscle treated with PEMFs consumed oxygen significantly better than mitochondria isolated from skeletal muscle not treated with PEMFs. In addition, skeletal muscles treated with PEMFs had more mitochondria than skeletal muscle not treated with PEMFs. Mitochondria isolated from skeletal muscle treated with PEMFs function better even with limited nutritional substrates, similar to hunger, and in the presence of high ADP levels, similar to tiredness or exhaustion. This study provides experimental evidence that PEMFs improve blood flow, enhance oxygen consumption and boost ATP production by facilitating electron transport through mitochondrial electron transport chain, thereby increasing skeletal muscle cellular energy potential.

Major Points:

- PEMF increases heat-independent blood flow to skeletal muscle after treatment. Increased blood flow, like that during exercise, enhances nutrient delivery and facilitates gas exchange in active or injured tissues. This is likely important in the overall advantages that PEMF provides for greater respiratory function.
- PEMF enhances mitochondrial respiratory function for as long as 2 weeks after treatment. This demonstrates long-term advantages for rapid and sustained respiration needed for ATP production upon demand. Similar long-term advantages are observed in interval sports training.
- PEMF increases mitochondrial function in each of 3 diverse mitochondrial respiration states, especially when nutrients are low, which is similar to hunger, and when ADP levels are high, which is similar to tiredness or exhaustion.
- PEMF also increases mitochondrial numbers or density as long as 2 weeks after treatment. This is advantageous because it spreads ATP production among more mitochondria, further enhancing respiration efficiency for optimal function. Similar long-term advantages are observed in interval sports training.
- PEMF boosts the potential for rapid energy production upon demand for healthy individuals and can provide needed energy reserves for individuals that are injured or sick and otherwise unable to exercise. In general, PEMF treatment mimics interval sports training by increasing blood flow, improving mitochondrial respiration efficiency and mitochondrial density in skeletal muscles.

Personal reports from individuals treated with pulsed electromagnetic fields (PEMFs) indicate a greater level of energy and performance in physical activities as well as less pain and faster healing following injuries. There are very few if any objective or well-controlled studies to support these claims. While there may be several PEMF effects that could account for these positive testimonials, we considered that PEMFs from Pulse Centers could have effects on blood flow to skeletal muscle as well as possible effects on the mitochondria that power them. Mitochondria are the powerhouse of the cell supplying muscles, brain and all tissues with ATP that is converted into energy for all body activities. Mitochondria also control cellular energy metabolism as well as cell death in senescent and injured cells. Your body converts the food and

nutrients you eat into substances that provide electrons for the mitochondria to consume oxygen and make ATP in processes called respiration. During exercise the mitochondria go into an "overdrive" mode, when efficient utilization of oxygen by mitochondria is required for maximum ATP production that power skeletal muscles and all tissues requiring oxygen and ATP. This requires increased blood flow to supply oxygen and efficient respiratory function of mitochondria for ATP and energy production.

In order to investigate PEMF effects on energy potential, we determined blood flow in skeletal muscle in rats after each of nine treatments, three times a week for three weeks. In addition, one to two weeks after nine treatments, we isolated mitochondria from the PEMF-exposed skeletal muscle and determined how well their mitochondria functioned, that is, how much oxygen they consumed compared to mitochondria from non-treated rats. We tested these PEMF-treated and non-treated muscle mitochondria in various states of respiration, which are determined when we do or do not give them substrates and when they do or do not need ATP (when we give them high ADP). These mitochondria were isolated from one of the four quadriceps muscles, which is in front of the thigh and extends the knee joint. We also determined the number or density of mitochondria in the gastrocnemius muscle in PEMF-treated and control rats. The gastrocnemius forms half of the calf muscle and functions to flex the foot at the ankle joint and flex the leg at the knee joint.

PEMFs Increase Blood Flow in Rat Skeletal Muscle

We measured blood flow using an instrument called a laser Doppler. It measures total local microcirculatory blood perfusion including flow in capillaries, arterioles, venules and shunting vessels. The laser Doppler signal is directly proportional to the velocity and number of blood cells moving in these vessels.

Figure 1 shows four typical laser Doppler blood flow images. The two images on top are from rats not treated with PEMFs and the two images on the bottom are from rats treated with PEMFs. The two images on the left are for a 15-minute treatment and the two images on the right are after a 60-minute treatment. Both treatments were with a 100 power setting and 10 pulses per second. As indicated by the bar at the bottom of each image, the increase in red color indicates an increase in blood flow through the muscle. The bottom images on the left (15-minute treatment) shows only a slight increase in red color compared to the top left image from a non-treated control muscle. So, the effects on blood flow after a 15-minute treatment were small. However, the image on the bottom right after a 60-minute treatment, shows a significant increase in red color compared to the top nontreated control rat. Therefore, there was a great increase in blood blow after a 60-minute treatment. To quantify blood flow, composite values from images like these are computed and blood flow is indicated in arbitrary units in figures 2 and 3 below

Figure 1: PEMF Impact on Blood Flow as Measured by Laser Doppler

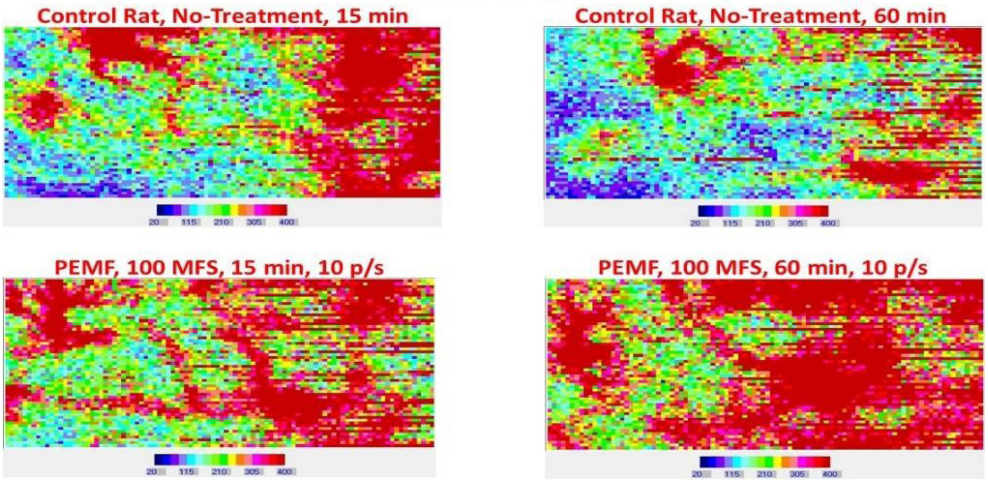
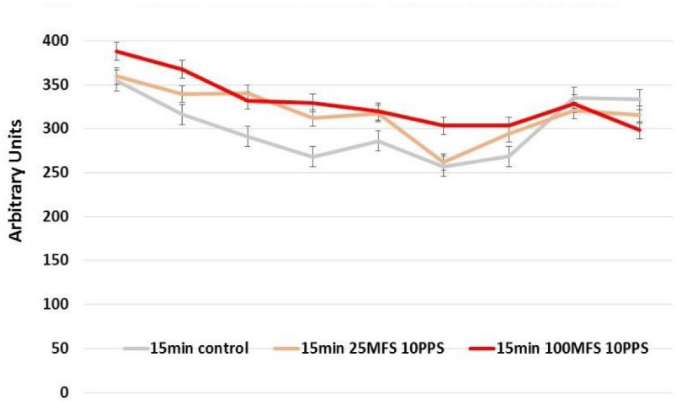


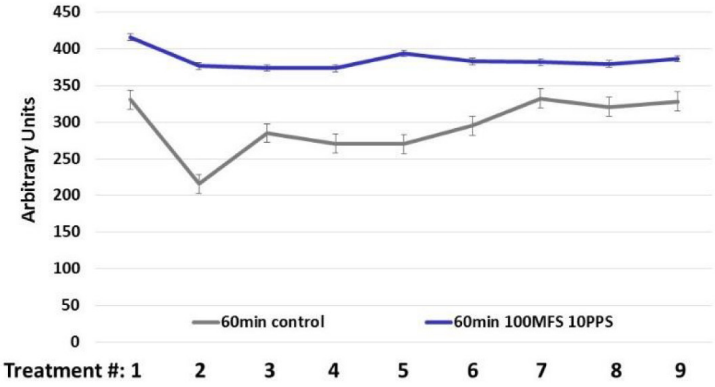
Figure 2 shows laser Doppler blood flow from rats not treated (gray), treated with PEMFs for 15 minutes with 10 pps and 25 power setting (pink) or treated with a 100 power setting (red). While the two PEMF treatments show small increases in blood flow, there were no statistical differences between treated and control muscles for either treatment condition. Thus, there were small increases in blood flow and there were some differences among the rats for increases in blood flow.

Figure 2:
15 Min PEMF Treatment - Mean Blood Flow Flux



However, ***Figure 3*** shows laser Doppler blood flow from rats not treated (gray) and treated with PEMFs at a 100 power setting for 60 minutes and 10 pulses per second. The PEMF treatment induced significant increases in blood flow at all 9 treatment times. The average increase in blood flow after each treatment was about 20% greater than blood flow in rats not treated with PEMFs.

Figure 3:
60 Min PEMF Treatment - Mean Blood Flow Flux



In general, these data indicate that PEMFs increase blood flow in rat gastrocnemius muscle when skeletal muscles are treated with PEMFs. While a 15-minute treatment slightly increased blood flow, a 60-minute treatment was clearly effective to increase circulation to these muscles and all muscles exposed to the same conditions. This study indicates that to get a significant effect from PEMF treatment some times between 15 and 60 minutes are needed to show a significant increase in blood flow.

What this study does not show is how long this increase in blood flow continues. These measurements were made 10-15 minutes after each treatment. We did not determine how long this effect lasted. It is most likely that this increase in blood flow was relatively short, at least not lasting all day or for hours. The most likely cause of this increase in blood flow is an increase in vessel dilation, which decreases the resistance as the vessels become larger and perfusion to the tissues is increased. This would also decrease the blood pressure in the vessels as the blood flows more easily. The cause of vasodilation is known to be due to relaxation of the smooth muscles in the vessel walls. Smooth muscle is mostly in arteries, smaller arterioles and larger veins. Since calcium is needed for muscle contraction, it is highly likely that the smooth muscles in these vessels have lower calcium levels around them, so they are less likely to contract. This smooth muscle relaxation is accompanied by mechanisms that decrease contraction. This increase in blood flow could also be due to relaxation of the muscle tissues themselves, such that pressure on the vessels in the muscles was less, allowing blood to flow more easily through them. Increase in blood flow due to vessel dilation could also be due to increased levels of hormones causing smooth muscle to relax. Perhaps one of the best known vasodilators is nitric oxide. Other vasodilators such as histamine and other inflammatory factors can cause blood vessels to dilate. However, PEMFs do not cause inflammation, and in fact produce anti-inflammatory factors, as will be discussed in the section concerning PEMF effects on macrophages.

PEMFs Enhance Rat Skeletal Muscle Mitochondrial Respiration

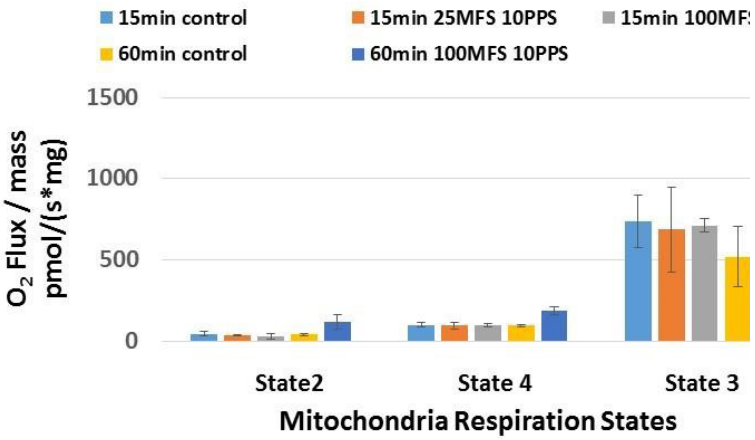
In order to determine effects of PEMFs on mitochondrial function, we isolated mitochondria from one of the thigh muscles, 1 to 2 weeks after the treatment regimens were completed. This is the same muscle that was used to determine blood flow. We then tested these mitochondria for their respiratory function. That is, we tested them for how effectively they could consume oxygen. We did this by putting mitochondria in different, so called, respiration states, by placing the isolated mitochondria in a closed chamber. Oxygen consumption (flux) was determined by the disappearance rate of oxygen as it was used by the mitochondria to make ATP. In order to test them in these different respiration states, we did or did not provide them with substrates or nutrients that give them the electrons they need to flow through the mitochondrial electron transport chain. We also did or did not give them ADP. ADP is a strong stimulus for mitochondria to consume oxygen and produce ATP. As a reminder, the movement of these electrons through the mitochondrial electron transport chain is needed to ultimately use oxygen and make ATP. Table 1 below indicates how we put mitochondria into these different states of respiration and then test how well they use oxygen as a measure of mitochondrial function.

Table 1: Definitions for States of Respiration

	Oxygen	ADP levels	Substrate Levels	Respiration Rate	Rate Limiting	ΔΨm
State 2	Present	High	Low	Slow (Basal)	Substrates	110 mV
State 4	Present	Low	High	Slow	Phosphate	180 mV
State 3	Present	High	High	Fast	Respiratory Chain	150 mV

Figure 4 shows effects on oxygen utilization (oxygen flux) by mitochondria isolated from rat muscle that were and were not treated with PEMFs in different respiration states. The graph is presented to represent all respiration states on the same graph, so the effects of PEMFs on states 2 and 4 are quite low because respiration in state 3 is quite high. It will be easier to see these effects when compared to untreated control respiration in Figure 5. Nevertheless, for Figure 4, in state 2, oxygen consumption is very low because substrate or nutrient levels are low, so there is not a good source of electrons. This is similar to muscle mitochondria from a person who is hungry or low on energy. Also, in state 2 ADP levels are high, which generally is a stimulus to increase respiration to use the ADP to make ATP. This is analogous to being completely worn out, exhausted or "decharged." Therefore, the low levels of substrates limit oxygen utilization and ATP production. However, notice that respiration is higher in state 2 when mitochondria were isolated from muscle treated with high PEMFs (100 power setting, 10 pulses per second, 60-minute treatment; dark blue bars) compare to mitochondria isolated from untreated muscles (yellow bars).

Figure 4: 0_2 Flux per Mass



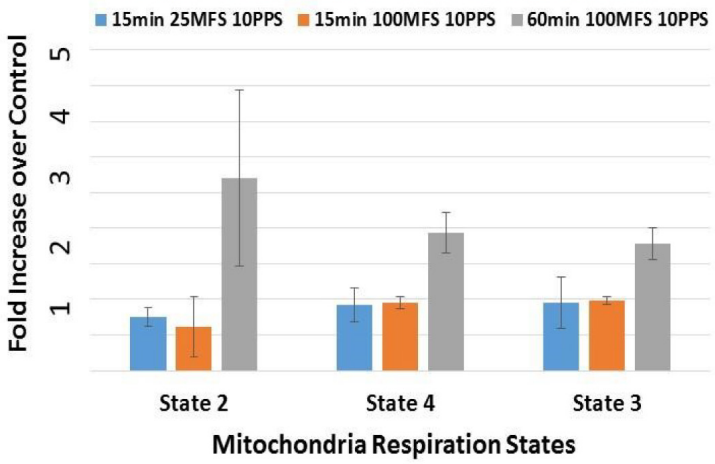
In *state 4*, respiration is slightly higher than in state 2, but still relatively low. Although substrates are available, ADP levels are low, suggesting ATP levels are high, so there is not a stimulus to increase respiration. Notice that respiration is also higher in state 4 when mitochondria were isolated from muscle treated with high PEMFs (100 power setting, 10 pulses for 60 minutes; dark blue bars) compared to mitochondria from untreated muscle (yellow bars).

In *state 3*, respiration is highest because substrates are available and ADP levels are high, so there is an

increased demand for ATP production. Note that respiration is also higher in state 3 when mitochondria were isolated from muscle treated with high PEMFs (100 power setting, 10 pulses per second for 60 minutes; dark blue bars) compared to mitochondria from untreated muscle (yellow bars).

Figure 5 shows this same data with respiration of PEMFs as a percent of respiration from muscle mitochondria not treated with PEMFs. PEMFs increased mitochondrial function in all states of respiration with the PEMF condition (100 power setting, 10 pulses per second for 60 minutes) (gray bars). The values are indicated as fold-increases above control.

Figure 5: 0_2 Flux per Mass as % of Control



The most dramatic effect is in state 2, or the basal state, when ADP levels are high, creating the greatest demand for ATP and oxygen utilization, even though the levels of substrates are limiting. The higher PEMF condition is also greater in state 4 when ADP levels are low and substrate levels are high as well as in state 3 when both ATP and substrate levels are high.

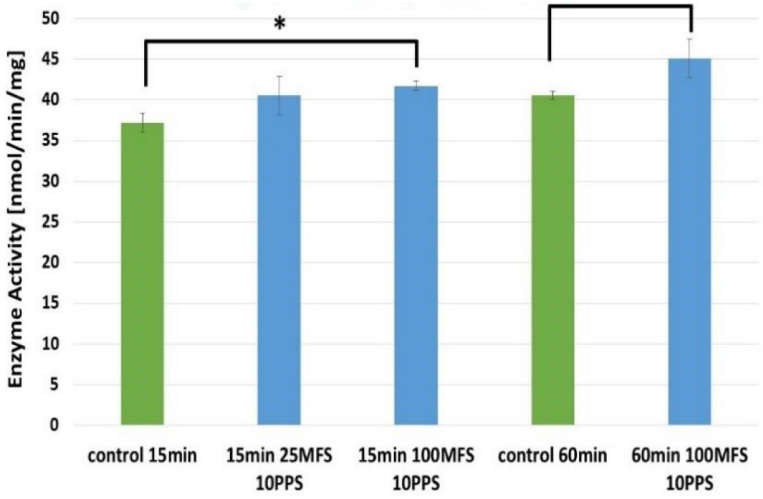
Notice that effects of PEMFs are the greatest when the demand for ATP is highest - when ADP levels are low and nutrients are not readily available. In other words, when you are hungry and exhausted. Again, a 15-minute treatment did not induce this effect. Therefore, some time between a PEMF treatment for 15 minutes and 60 minutes is needed to enhance mitochondrial function.

Pulsed Electromagnetic Fields Increase Numbers of Mitochondria in Rat Skeletal Muscle

In order to determine the effect of PEMFs on the number of mitochondria in muscles, we measured citrate synthase activity as determined one to two weeks after PEMF treatment from skeletal muscle after rats were treated 3 times a week for 3 weeks (or 9 treatments). Mitochondria from the gastrocnemius muscle or the calf muscle were used for these studies. Citrate synthase is commonly used to determine mitochondrial density. Citrate synthase activity is also used as a biochemical marker of the skeletal muscle oxidative adaptation to a training intervention. It has been demonstrated that training induced changes in whole body oxidative capacity is matched by changes in muscle citrate synthase activity [Vigelso et al., 2014].

Figure 6 shows that there were significant increases in the number (density) of mitochondria vs control for both PEMF conditions with 100 power setting and 10 pulses per second when rat muscle was treated for 15 or 60 minutes. Together with results from Figures 4, 5, and 6, this study demonstrates that PEMFs not only increase the respiratory efficiency of mitochondria, especially when ADP and substrate levels are low, but also increase the numbers or density of mitochondria in treated muscle. This increase in mitochondrial density or number is similar to that found with high-intensity interval training, which also shows an increase in mitochondrial density (increased citrate synthase activity) [Gillen et al., 2017; MacInnis et al., 2017].

Figure 6: Citrate Synthase Activity



Discussion

Pulsed electromagnetic fields have been touted to increase energy when individuals are treated with pulsed waves of intense magnetic fields. A lingering question remains concerning the mechanisms of PEMF action on these biological systems. Nevertheless, the PEMF effects presented here support these personal testimonials and the results speak for themselves.

Overall, these studies provide significant evidence to support testimonials of individuals who report having more energy after treatment with PEMFs. So how might PEMFs cause long-lasting effects on mitochondrial function and density? One possibility is that PEMF effects are related to increased blood flow that is seen 10-15 minutes after PEMF treatment. It is possible that the increased blood flow to muscles during this treatment regimen "trains" mitochondria to function more efficiently because they are receiving increased nutrients and oxygen during these treatments. This is similar to exercise or high-intensity interval sports training as suggested above. However, from these studies, it is not clear how long this increase in blood flow lasts. One might think that after the stimulus is removed, the effects would dissipate in some time-dependent manner. It is remarkable that effects of PEMFs on efficiency of muscle mitochondrial respiration were still present as long as two weeks after treatments ended. Such long-term changes are likely due to effects on proteins that carry out these mitochondrial functions. The idea that PEMFs can have long-term effects on proteins is supported by the finding that PEMF-treated skeletal muscle have greater numbers of mitochondria than untreated muscle.

The increase in muscle mitochondrial density suggests some further considerations concerning PEMF effects on skeletal muscle. It stands to reason that more mitochondria are better for energy production, yet there must be a reason for muscles to make more mitochondria. Mitochondria constitute a highly dynamic network of vibrant structures within cells that constantly undergo fusion and fission, which is important to maintain healthy functions and to respond to stress [Chan, 2012]. Like bacteria, from which they were derived, mitochondria carry out binary fission, whereby they duplicate forming two identical structures, thereby increasing their numbers or density [Scott and Youle, 2010]. This requires new gene transcription, increased protein synthesis and elevated metabolism among scores of other activities. In order to have an impact that increases mitochondrial numbers in skeletal muscle, PEMFs must provide a stimulus that gives cells a "reason" to perform more efficient respiratory function and ATP production. This increased energy allowance is established by increasing numbers of mitochondria and increasing their respiratory efficiency. The cellular PEMF sensors that produce these more resourceful conditions remain to be determined.

One common explanation of how these PEMFs might increase energy is to consider the cell and mitochondria as a battery that might need recharging due to stress, damage and/or aging. PEMFs are said to recharge your cells providing you with more energy to deal with de-charging mechanisms. The data presented here are generally in agreement with these claims. However, it is more correct to say that PEMF treatment provides you with the potential for more energy, because ATP is not stored in large amounts in skeletal muscle. Therefore, enhanced mitochondrial respiration from greater numbers of mitochondria are essential for prompt and prolonged ATP production. Mitochondria isolated from rat muscle treated with a 100 power setting at 10 pulses per second for one hour with the three week treatment protocol were significantly more efficient for oxygen utilization and consequently more effective for ATP production than mitochondria isolated from untreated muscles. These studies carried out under 3 different respiration states, indicate that PEMF-treated muscle would be expected to function more efficiently for longer periods of time than muscles from untreated animals. This occurs even when nutrients were limited, such as during hunger, and when ADP levels were elevated, such as during exhaustion.

It has been stated that PEMFs increase the flow of electrons through the electron transport chain. This is consistent with findings in this study that oxygen is utilized more efficiently from mitochondria isolated from PEMF-treated muscles. It is the flow of electrons through the electron transport chain resulting in more oxygen consumption that is a major determinant of how mitochondria function more efficiently for ATP production. As electrons flow more efficiently through the electron transport chain, fewer electrons are

lost as reactive oxygen species of ROS that cause inflammation and even cell death at higher levels. This is consistent with findings from another study showing that macrophages secrete lower levels of inflammatory substances (cytokines) and higher levels of anti-inflammatory cytokines after they have been treated with PEMFs. These macrophages are also better at pinocytosis, which is analogous to cleaning up debris as they would during wound healing. Like effects on skeletal muscle mitochondria, these effects on macrophages are relatively long lasting. These studies are presented in a separate report.

The finding that these effects on PEMF-treated muscle mitochondria are long lasting is intriguing. Such long lasting effects indicate that biological systems have been more permanently modified by these treatments. The findings that mitochondria are more efficient one to two weeks after PEMF treatment and that more mitochondria are present could be due to one or more of several effects. One possibility is related to PEMF effects on the structure of the electron transport chain. This is most reasonable because the function of biological systems is closely related to their structures. The electron transport chain is composed of four large complexes called complexes I - IV. Complex V is ATP synthase and the last step of the respiratory system where ATP is synthesized. Complexes I, III and IV can form a super-complex that functions much more efficiently as opposed to when they are individually or more randomly arranged in the inner membrane of the mitochondria. It is possible that PEMFs somehow facilitate the formation of these super-complexes after repeated PEMF exposure, as was done in this study. This suggests that PEMFs could have lasting effects on the structural arrangements of the electron transport chain complexes such that they function more effectively and efficiently in super-complexes.

Another possible effect that PEMFs could have on mitochondria is on the quality of the components of the electron transport chain and ATP synthase. For example, complex I is composed of 45-46 subunit proteins. The other complexes in the electron transport chain are also formed by multiple subunits, but complex I is the biggest. The subunits in these complexes must be arranged in a manner that facilitates the function of the complexes. Like other complexes, complex I is composed of core protein subunits that are essential for electron transport and others are accessory proteins that are involved in regulation or complex assembly. These subunits, like all proteins, are continually synthesized and degraded in all cells, a process called protein turnover. Basically, protein turnover is the balance between synthesis and degradation of proteins. These proteins are turned over at a regular rate depending on whether they have been modified or damaged during stress and/or during their performances. It is known that protein turnover is decreased with aging. It is possible that PEMFs increase protein turnover such that damaged subunits are replaced more often making the complexes function more efficiently. Effects of PEMFs could be analogous to aerobic exercise, which has been shown to increase skeletal muscle protein turnover in previously unfit individuals [Pikosky et al., 2006].

There are a number of analogies between effects of PEMFs and exercise. Like exercise, PEMFs increase blood flow to muscles [Figures 1 and 3]. In addition, during training there are changes in an individual's respiratory and oxidative capacity [Vigelso et al., 2014]. This is analogous to that seen in mitochondria isolated from skeletal muscle treated with PEMFs [Figures 4 and 5]. There are also increases in mitochondrial numbers during intense interval training [Gillen et al., 2017; MacInnis et al., 2017]. This is analogous to that seen in mitochondria isolated from skeletal muscle treated with PEMFs [Figure 6]. While it is not necessarily suggested that treatments with PEMFs are a preferable substitute for exercise, PEMF treatment does offer many of the advantages provided by exercise and can certainly be recommended to those individuals that are too old, sick, or injured to regular exercise.

Given that interval sports training and PEMF treatments of muscle both provide increased mitochondrial density and enhanced mitochondrial function, it is interesting to consider how much further these analogies extend. For example, it is clear that a higher demand for energy in muscles during sports training generates a mandate for more and better mitochondrial function. However, one would not necessarily expect that treatment with PEMFs would create the same demand for mitochondria to produce more energy. And yet, the periodic increase in blood flow during the PEMF treatment regimen provides more nutrients and better oxygen - carbon dioxide exchange in skeletal muscle and a potential for greater energy production. It would

be interesting to know if PEMF treatments may provide additional benefits for those who do train and/or exercise regularly. There could be additive or even synergistic benefits provided by exercise combined with PEMF treatment for healthy, exercising individuals. Alternatively, there could be no additional benefit of combining exercise with PEMF treatment. This latter result would at least indicate that PEMFs can substitute for exercise. From results presented here, it would be expected that for those individuals who do not have time to exercise on a regular basis, that PEMF treatment would be an advantageous healthy alternative. Both interval sports training and PEMF treatment increase respiratory function by increasing mitochondrial density and enhancing mitochondrial efficiency; however, the mechanisms that induce these responses appear to be different and remain to be determined.

References

- Chan, DC . Fusion and fission: interlinked processes critical for mitochondrial health. Annu Rev Genet. 2012;46:265–287.

- Gillen J, Martin BJ, MacInnis MJ, Skelly LE, Tarnopolsky MA, Gibala MJ. Twelve Weeks of Sprint Interval Training Improves Indices of Cardiometabolic Health Similar to Traditional Endurance Training despite a FiveFold Lower Exercise Volume and Time Commitment. PLoS One. 2016;11:e0154075.

- MacInnis MJ, Zacharewicz E, Martin BJ, Haikalis ME, Skelly LE, Tarnopolsky MA, Murphy RM, Gibala MJ. Superior mitochondrial adaptations in human skeletal muscle after interval compared to continuous single-leg cycling matched for total work. Physiol. 2017;595:2955-2968.

- Pikosky MA, Gaine PC, Martin WF, Grabarz KC, Ferrando AA, Wolfe RR, Rodriguez NR. Aerobic exercise training increases skeletal muscle protein turnover in healthy adults at rest. J Nutr. 2006;136:379-383.

- Scott I, Youle RJ. Mitochondrial fission and fusion. Essays Biochem. 2010;47:85-98.

- Vigelsø A, Andersen NB, Dela F. The relationship between skeletal muscle mitochondrial citrate synthase activity and whole body oxygen uptake adaptations in response to exercise training. Int J Physiol Pathophysiol Pharmacol. 2014;6:84-101.