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The effect of heat therapy and high-intensity interval training on fitness, blood pressure and osteoarthritis impact in patients with severe lower-limb osteoarthritis

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Osteoarthritis is a degenerative joint condition making traditional exercise painful and difficult to perform. Due to the lack of activity, osteoarthritis sufferers have lower fitness ($\dot{V}O_{2peak}$) and higher systolic (SBP) and diastolic (DBP) blood pressure, compared to those without osteoarthritis. The purpose of this study was to compare the effect of three low-/no-impact interventions on $\dot{V}O_{2peak}$, blood pressure and the subjective impact of osteoarthritis in patients with severe lower-limb osteoarthritis.

Ninety-three patients with severe knee or hip osteoarthritis awaiting total joint replacement were recruited from Dunedin Hospital. Participants were randomised to heat therapy (Heat; 20–30 min immersed in 40°C water followed by ~15 min light resistance exercise), upper-limb high-intensity interval training (HIIT; 6–8 x 60 s intervals on a cross-trainer or arm ergometer at 100% $\dot{V}O_{2peak}$, 60–90 s recovery) or home-based exercise (Home; 15–20 min light resistance exercise), for 36 sessions (3 sessions per week for 12 weeks).

Across the interventions, peak $\dot{V}O_2$ increased by 15% following HIIT (+2.9 [95%CI: +1.3, +4.4] $mL{\cdot}min^{-1}{\cdot}kg^{-1}$) but not Heat (+0.4 [-0.6, +1.3] $mL{\cdot}min^{-1}{\cdot}kg^{-1}$) or Home (-0.2 [-1.2, +0.8] $mL{\cdot}min^{-1}{\cdot}kg^{-1}$). The SBP and DBP had decreased following Heat (SBP -9 [-14, -3]; DBP -3 [-5, 0] mm Hg) and HIIT (SBP -6 [-9, -4]; DBP -2 [-5, 0] mm Hg), but not Home (SBP 0 [-3, +3;] DBP 0 [-2, +3] mm Hg). Osteoarthritis impact, as assessed by the Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index questionnaire (0 (no impact)–96 (most impact)), was unchanged with Heat (-1 [-7, +5]), HIIT (+2 [-4, +8]) or Home (-2 [-7, +4]).

Upper-limb HIIT was effective for improving $\dot{V}O_{2peak}$ in patients who have difficulty performing lower-limb exercise. Heat and HIIT had potent anti-hypertensive effects, of similar magnitude to some pharmaceuticals, but neither intervention improved patients' subjective impact of osteoarthritis.

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The preferences of people with rheumatoid arthritis for tapering biologic therapies

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Biologics are effective treatments for people with rheumatoid arthritis (RA) but are associated with adverse effects and are costly. Tapering of biologics is recommended when people with RA achieve remission but is not without risk of disease flare-up. Little is currently known about how the preferences of people with RA would influence their decisions to accept biologics tapering. This study aimed to assess the preference of people with RA for biologics tapering.

A total of 736 potential participants currently taking biologics or had stopped biologics due to medical reasons were recruited from Auckland, Wellington, Christchurch and Dunedin. Participants completed an online discrete choice experiment (DCE) survey, asking their preferences between three hypothetical treatment options with varying levels of four attributes: frequency of treatment, chances of known adverse effects, chances of regaining disease control and healthcare service-related features. DCE

data were analysed using a mixed logit model.

Of the 160 who responded, 142 complete responses were analysed. Frequency of biologic treatment was the most important treatment attribute influencing preference, followed by the chance of flare upon tapering. Time to see the rheumatology team after a flare was ranked the least important among the seven attributes. On average, people with RA were willing to accept between 25.3% to 50.2% increase in chance of disease flare in exchange for reducing the treatment frequency and chances of adverse effects (serious infection and skin cancer associated with biologic use).

This study shows that frequency of treatment and risk of disease flare was the most important determinant for people with RA when making hypothetical choices about tapering biologics. For these attributes, they were willing to accept a greater chance of flare in exchange for treatment benefits in the form of a reduction in dosing frequency and potential risk of adverse effects from continued use. These findings have implications for clinical practice and policy making about tapering.

Effects of wearing face masks on cognitive functioning and mood states: a randomised controlled trial in young adults

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Despite significant public concern that wearing face masks can adversely affect the brain, and self-report evidence in healthcare workers that it is associated with impaired cognition, few studies to date have measured the impact of wearing the types of face masks commonly worn during the COVID-19 pandemic (surgical or cloth face masks) on cognition. In the present study, we investigated the effects of wearing a surgical face mask for prolonged periods (at least 8 hours) on neuropsychological functioning, including objectively measured cognition (basic visuomotor performance, inhibitory control, mental flexibility, selective attention, short-term and working memory and self-reported current mood states).

We tested 42 younger adults (18–36 years old) using a controlled counterbalanced crossover design with a 1-week washout. Participants were given a surgical face mask to wear for at least 8 hours throughout the day of testing. Paired-sample *t* tests assessing differences in cognitive variables of interest between the control and mask sessions revealed a difference in cognitive performance between the two sessions for the more challenging condition of a selective attention task, $t(41) = -3.18$, $P = 0.003$, $g = 0.33$, reflecting 5.4% worse cognitive performance (as evidenced by slower reaction-times) during the mask session. Additionally, paired-sample *t* tests comparing mood scale ratings between the control and mask sessions showed that participants reported feeling less happy, $t(41) = -2.53$, $P = 0.015$, $g = 0.41$, and more tense, $t(41) = 2.12$, $P = 0.040$, $g = 0.30$, during the mask session compared to no-mask control.

In summary, the current study revealed small but significant adverse effects of wearing a surgical face mask on neuropsychological functioning. This evidence of adverse effects in a university population signals that future research is needed to investigate the effects of wearing surgical face masks in vulnerable populations (eg, people with asthma and older adults).

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Astrocyte-mediated trans-regional regulation of synaptic plasticity in the hippocampus

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The synaptic plasticity process of long-term potentiation (LTP) and long-term depression (LTD) is vital for memory formation and overall neural health. However, mechanisms must be in place to prevent pathologically excessive LTP and LTD. Such regulation comes partly through metaplasticity, whereby neural activity at one point in time influences later plasticity. We have discovered a unique trans-regional mode of metaplasticity in the hippocampus, whereby "priming" stimulation of inputs to the basilar dendrites of pyramidal cells in area CA1, inhibits later LTP at synapses in the middle molecular layer (MML) of the dentate gyrus, a neighbouring region ~800 microns away. As there are no known neuronal connections between these brain regions, we tested the hypothesis that the metaplasticity is in fact accomplished via astrocytic networks.

Intracellular astrocyte patch clamping and extracellular field potential recordings were conducted in the MML of acute hippocampal slices taken from young-adult male Sprague-Dawley rats and 2–7-month-old mice. The control and primed group sizes ranged from n = 6–9. In rat slices, Ca^{2+} was buffered in patched astrocytes by dialysing EGTA intracellularly while recording local synaptic potentials in MML. Priming stimulation (2x100 Hz trains) in CA1 was delivered 15 min prior to MML LTP induction (4x100 Hz trains). Priming inhibited MML LTP compared to non-primed control (*t*-test, $t_{(13)}=4.4$, $P = 0.0007$), while buffering astrocytic Ca^{2+} completely abolished this effect

(P = 0.73). The involvement of astrocytes was confirmed by a lack of LTP inhibition in $IP_3R2^{-/-}$ mice that do not display Ca^{2+} release from astrocyte-specific inositol triphosphate receptor-2 (IP_3R2)-dependent stores (P = 0.71, t-test). Further, we have shown that the glial cytokine tumour necrosis factor-alpha (TNFα), acting on TNFα receptor-1, is a critical signalling molecule.

Taken together, these data demonstrate a novel hippocampus-wide regulation of synaptic plasticity that is mediated by astrocyte-neuron communication. We propose that such metaplasticity plays an important role in hippocampal information processing while also homeostatically counteracting excitotoxicity under pathological conditions.

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Acute and chronic effects of nitric oxide exposure on cardiac arrhythmias

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Nitric oxide (NO) is a gaseous signalling molecule that regulates cardiac function by targeting calcium handling protein kinases such as calcium/calmodulin-dependent protein kinase II delta (CaMKIIδ). NO can alter CaMKIIδ activity in the heart and this leads to irregular heart rhythm (arrhythmias). Currently, it is unknown how the duration of NO exposure can affect cardiac function in the presence and absence of CaMKIIδ. This study was designed to determine how acute and chronic NO treatment affects cardiac arrhythmias in mice lacking CaMKIIδ.

To assess the effect of acute exogenous NO on arrhythmias, hearts from CaMKIIδ knock-out (KO) mice (n = 5) and wild-type (WT) C57BL/6 mice (n = 7), were isolated and perfused with S-nitrosoglutathione (GSNO), a NO donor for 10 minutes. In the chronic phase, WT (n = 5) and KO hearts (n = 5) were given GSNO-supplemented drinking water for 5 weeks. Thereafter, the hearts were isolated and perfused with GSNO.

Following acute GSNO treatment, there was no significant increase in arrhythmias in KO hearts compared to baseline (12.0 ± 4.8 vs 5.2 ± 3.2). However, the WT hearts developed arrhythmias (premature ventricular beats, bigeminy and trigeminy) with increasing GSNO concentrations compared to baseline (19.6 ± 6.8 vs 6.9 ± 2.3; $P < 0.05$). Chronic treatment of WT and KO hearts with GSNO led to an increasing trend in arrhythmias in both WT and KO isolated perfused hearts compared to baseline (WT = 25.2 ± 14.7 vs 8.4 ± 5.4; P = 0.05, KO = 30.4 ± 11.3 vs 15.2 ± 4.0; P = 0.08).

The responses of the mouse hearts to GSNO treatment depending on duration of treatment shows that WT hearts are more sensitive to acute NO and therefore increased arrhythmias. Additionally, KO mice are protected from arrhythmias during acute NO treatment. In the chronic phase of GSNO treatment, WT and KO mouse hearts are equally affected by GSNO treatment and the KO hearts showed increased susceptibility to arrhythmias. This suggests that CaMKII inhibition could attenuate cardiac arrhythmias only during acute NO exposure.

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Phosphorylation of RyR2 by CK2 is anti-arrhythmic

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Cardiovascular disease is one of the leading causes of mortality in New Zealand and is responsible for one third of all global deaths each year. Among cardiac diseases, arrythmias are one of the most prevalent forms. The main mechanism of arrhythmia are inappropriate releases of Ca^{2+} (termed Ca^{2+} sparks) through ryanodine receptor 2 (RyR2), a sarcoplasmic reticulum-located channel that plays an essential role in cardiac excitation-contraction coupling, releasing the bulk Ca^{2+} required for contraction. We have recently identified that RyR2 is phosphorylated by casein kinase 2 (CK2), and that *in vitro* loss of this phosphorylation increases Ca^{2+} sparks. This project aimed to determine the role of CK2 phosphorylation of RyR2 *in vivo*. This was achieved using phospho-specific mutant mice, which expressed a variant of RyR2 unable to be phosphorylated by CK2 ($S2692A/S2693A^{++}$).

To determine whether loss of phosphorylation increases Ca^{2+} sparks, line-scan imaging was performed on isolated cardiomyocytes from $S2692A/S2693A^{++}$ and wildtype controls. Cells isolated from $S2692A/S2693A^{++}$ animals exhibited 4.3 sparks/100 µm/s, which was significantly greater than in control animals' 2.9 sparks/100 µm/s (n = 45 cells, 8 animals per group respectively, one-way ANOVA, $P > 0.05$). Next, to determine whether this increase in Ca^{2+} spark frequency translated to an increased risk of arrhythmias, electrocardiograms were recorded before and after a pharmacological stress trigger, an intraperitoneal injection of caffeine (120 mg/kg) and epinephrine (1.6 mg/kg). In control animals this procedure increased the heart rate but had little effect on arrhythmogenicity, with brief changes in sinus rhythm occurring in only 2 out of 9 animals. In contrast, $S2692A/S2693A^{++}$ animals experienced a significant increase (7 out of 10 animal) in severe and prolonged non-sinus rhythm (one-way ANOVA, $P > 0.05$).

Combined, these data show that phosphorylation of RyR2 by CK2 is essential for normal channel function and Ca^{2+} release, and

that loss of phosphorylation increases Ca^{2+} leak and the susceptibility of arrhythmia. Clinically, understanding the regulation of RyR2 function may offer a novel target for treatment of cardiac arrhythmia.

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In vitro and *in vivo* evaluation of high-dose inhaled rifampicin powder formulations for tuberculosis treatment

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Despite the availability of oral and injectable drugs, tuberculosis (TB) treatment is hindered by suboptimal drug concentrations in the lungs and blood. Pulmonary delivery of anti-TB drugs may ensure sufficient drug concentration in the lungs and blood. This study reports on the *in vitro* and *in vivo* (rat) assessments of inhalable rifampicin formulations.

Four high-dose rifampicin powder formulations were prepared using spray drying and crystallization techniques. *In vitro* aerosolisation efficiency was evaluated using an artificial lung, the Next Generation Impactor. In *in vivo* assessments in Sprague-Dawley rats, repeated doses of formulations were administered by intra-tracheal insufflation or by oral gavage. Liver toxicity was evaluated by histopathology and alanine transaminase (ALT) assay in serum. Lung tissue was evaluated by histopathology. The pharmacokinetic study compared plasma concentration-time profiles of rifampicin after repeated intra-tracheal or oral administrations of powder formulations.

Amorphous and crystalline dihydrate powder formulations of rifampicin with high aerosolisation efficiency (lung dose ≥ 62.7%) were studied in rats (n = 6 per treatment group). Serum ALT levels were significantly lower after intra-tracheal administration compared to oral administration, suggesting lower hepatic effects from the pulmonary administration. Normal architecture of lung and liver histology after repeated intra-tracheal dosing indicated absence of toxicity. Intra-tracheal administration led to significantly higher peak plasma concentrations (C_{max}= 13.2 ± 1.7 µg/mL) and area under the plasma concentration-time curve (AUC = 193.1 ± 37.9 µg.h/mL) in a shorter time (T_{max} = 4.5 ± 2.9 h) compared to oral administration (C_{max} = 4.5 ± 2.3 µg/mL; AUC = 87.4 ± 64.7 µg.h/mL; T_{max} = 7.5 ± 5.0 h) at the same dose ($P < 0.05$), suggesting faster and higher absorption of rifampicin into the systemic circulation.

Intra-tracheal delivery of rifampicin powder formulations to rats was safe and resulted in higher bioavailability than oral rifampicin. This study is a foundation for future clinical studies on inhaled rifampicin and the development of other inhaled anti-TB drugs.

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Aryl hydrocarbon receptor ligands can modulate fructose-induced hepatic insulin resistance

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The increasing prevalence of hepatic insulin resistance, an underlying factor of non-alcoholic fatty liver disease and type 2 diabetes, necessitates the understanding of molecular pathways that could play a role in its development. The aryl hydrocarbon receptor (AhR), a transcription factor activated by a wide range of exogenous (environmental pollutants, phytochemicals) and endogenous (tryptophan derivatives) ligands, has been implicated in regulating insulin sensitivity by altering lipid metabolism, but research is conflicting regarding how different AhR ligands modulate this response, particularly in human cells.

Five AhR agonists, the tryptophan metabolite 6-formylindolo[3,2-b]carbazole (FICZ), synthetic agonist β-naphthoflavone (BNF), dietary phytochemical indole-3-carbinol (I3C), seaweed-derived 4,7-dibromo-2,3,-dichloroindole (4DBDCI) and prototypical AhR agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were tested in human HepG2 cells. Triglyceride levels were quantified via a kit and western blotting for the change in insulin-stimulated pAkt expression (normalised to total Akt) was used to measure insulin sensitivity. Data were analysed via one-way ANOVA with Bonferroni's post-hoc test (n = 3 per group).

No changes in triglyceride content in response to each compound were detected. However, in a hepatic insulin resistance model, FICZ prevented the fructose-mediated reduction of pAkt, producing 94.9 ± 10.5 (SEM) % of the vehicle control response, compared to fructose alone which produced 55.1 ± 5.6 (SEM) % of the vehicle control response ($P < 0.05$). BNF, I3C and 4DBDCI all increased the fructose-mediated insulin resistance, reducing pAkt expression to 20 – 25% ($P < 0.05$ vs fructose-only) of the vehicle control, while TCDD did not induce a change.

The protective effect of FICZ, as well as the compounding effect of BNF, I3C and 4DBDCI, against fructose-mediated insulin

resistance was independent of triglyceride accumulation and further investigation into the mechanism is therefore warranted. The contrasting effects on insulin sensitivity by these five compounds also warrants exploration into the complex activity of AhR agonists.

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