Evaluating the health benefits of fruits for physical fitness: A research platform

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Abstract. To evaluate the health promoting attributes of fruits and their compounds the New Zealand Institute for Plant & Food Research Ltd (PFR) is using exercise as a model for oxidative stress and immune depression. Regular exercise has health benefits believed to be derived from adaptive responses to moderate oxidative stress. However, following exhaustive or unaccustomed exercise, excessive and prolonged oxidative stress and inflammation can be detrimental and the right balance of modulation from nutritional support via fruit phytochemicals (and vitamins) may prevent damage, aid recovery, and/or enhance muscular and immune function. We have developed a research platform to evaluate physical health, performance and recovery to position new fruit varieties in this area. Utilising compositional analysis of fruit extracts, *in vitro* screening of muscle cells, electrically stimulated muscle *ex vivo*, and animal and human intervention and exercise trials, we are evaluating the physical health-promoting effects of polyphenolic phytochemicals derived from fruit, particularly berry fruits. Our research demonstrates that certain fruits may complement the benefits of regular exercise through appropriate modulation of excessive oxidative stress and inflammation.

Keywords: Berry fruit, kiwifruit, fruit, polyphenolics, phytochemicals, anthocyanins, oxidative stress, inflammation, exercise, skeletal muscle

1. Introduction

The New Zealand Institute for Plant & Food Research Ltd (PFR) provides research and innovation for New Zealand's horticultural sector. PFR has developed a research platform to evaluate physical performance and recovery. Regular exercise has health benefits derived from moderate oxidative stress. However, depending on the mode, intensity and duration, exercise can inflict high levels of mechanical and metabolic stress on muscle [28]. Dietary supplementation is an attractive choice for health-conscious individuals. However, the right balance of modulation from nutritional support needs to be established to prevent damage/injury, maximise recovery for enhanced muscle function, but also to optimise immune function.

Utilising fruit varieties from breeding programmes at PFR with well-defined compositional data combined with a range of cell-based screening, *ex vivo* muscle experiments, and animal/human trials, we are evaluating the physical health-promoting effects of phytochemicals derived from some new fruit varieties, including berry fruits. The determination of biochemical markers of oxidative stress, inflammation and immune function in cell and tissue assays, and animal and human trials enables us to evaluate the effects of fruit consumption and to ultimately guide the breeding of health promoting fruit.

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2. Materials and methods

2.1. Fruit extract preparation and composition

The ZESPRI® GOLD kiwifruit (*Actinidia chinensis* 'Hort16A') extract was prepared by homogenising freeze-dried fruit (20 g) with methanol (50 mL), followed by 30 min in an ultrasonic bath. Solvent was recovered by filtration and the residue extracted twice more in methanol. The extract was rotary evaporated, dissolved in the minimum volume of water and applied to a 10 g C-18 solid-phase extraction cartridge (Strata, Phenomenex, Torrance, CA). The cartridge was flushed with deionised water (30 mL) and eluted with methanol (40 mL). Evaporation of the methanol eluate yielded 0.24 g of extract which was prepared in buffer for the analysis of *ex vivo* muscle performance.

The polyphenolic extract of blueberry fruit (*Vaccinium corymbosum*, 'Reka') was prepared from approximately 800 g of frozen fruit homogenised with acetone and the residue recovered by filtration. Following a further homogenisation and filtration with acetone/water (70:30), acetone extracts were combined and concentrated by rotary evaporation. Lipids were removed by partitioning the aqueous extract with heptane and the residual heptane in the aqueous layer removed by rotary evaporation. The polyphenolics were isolated from this extract by absorption, recovery, and elution (methanol) of XAD-7 (Sigma, Sydney, Australia) and dried by rotary evaporation to yield a friable powder. In all experiments, the blueberry fruit extracts were prepared from a stock in DMSO. DMSO concentrations in the final experimental conditions were below 1% and had no observable effect.

Juices of various cultivars of blackcurrant (*Ribes nigrum*) were prepared by blending fruit (180 g) with a domestic hand blender, then treating with Pectinex (0.18 mL; Novozymes, Bagsvaerd, Denmark) at 48°C for 4 h. After centrifugation (4000 rpm, 10 min) the yield of juice was typically ∼50%. Blackcurrant juice samples were analysed by liquid chromatography-mass spectrometry (LC-MS) using a Shimadzu (Auckland, New Zealand) 20-Series UFLC system including an autosampler, column oven and photodiode-array detector, linked to an LCMS 2020 single-quadrupole mass spectrometer. The LC system was fitted with a 150×2 mm, 4μ Synergi Fusion RP column (Phenomenex). For analysis, blackcurrant samples were diluted $\times 10$ into 1% aqueous formic acid and kiwifruit extracts dissolved in water at ∼2 mg/mL. Samples of 10 μL were injected and eluted by the following solvent programme. Flow rate 0.6 mL/min, Solvent A: 2% formic acid, solvent B: methanol. The initial conditions were 8% B and ramped linearly to 10% at 2.5 min, 18% at 5 min, 38% at 8.5 min, 63% at 10.5 min, held at 70% between 11.5 and 12.5 min, then back to the starting conditions between 13 and 15 min. Column oven temperature was 50°C. The mass spectrometer was operated under standard tuning conditions, as specified by the manufacturer and half the LC flow was diverted to waste through a split valve. Identification for compounds of interest was achieved by comparison of a combination of UV-visible and mass spectra with standard compounds.

The commercially available juices GHO "Natural Quenchers" (Good Health Organisation, Nelson, New Zealand) contained 65% of either ZESPRI® GOLD kiwifruit or blackcurrant juice.

2.2. Determination of muscle cell intracellular free radical levels

The rat skeletal muscle cell line L6 (American Type Culture Collection, Manassas, USA) was cultured and differentiated as described elsewhere [13]. Muscle cells were plated at 5000 cells/well in 96 well plates and incubated with 2′,7′-dichlorohydrofluorescein diacetate (DCFDA) (10 μM) for 60 min at room temperature. DCFDA is cleaved intracellularly into DCF, which produces an increase in fluorescence in the presence of reactive free radicals. Following a wash in D-PBS ($2 \times$), cells were equilibrated at room temperature for 10 min prior to incubation (simultaneously [time zero] and at the times indicated) with the blueberry fruit extract (50 μg/mL), followed by exposure to H_2O_2 (0.5 mM). Fluorescence intensity was determined over time using a fluorescence platereader (BMG FluorStar Optima, Alphatech Systems Ltd) with excitation and emission wavelengths of 485 and 520 nm respectively.

2.3. Evaluation of muscle cell total glutathione (GSH) levels and glutathione peroxidase (GPx) activity

The total amount of muscle cell GSH and GPx was determined colourimetrically using Bioxytech® assay kits (GSH-420™ and GPx-340™, Sapphire Bioscience Pty. Ltd, Auckland). Differentiated L6 myotubes were incubated with the blueberry fruit extract both simultaneously and for 24 h before GSH and GPx determination. For GSH

analysis, cells were plated at a density of 1×10^6 cells/well in 6 well plates and grown until confluent. Following extract exposure, cells were harvested using Tryple^{TM}, washed in D-PBS, and permeabilised by sonication in the precipitation reagent provided by the kit. All other analysis steps were then as recommended by the supplier. To determine muscle cell GPx levels, cells were grown in T75 flasks until confluent. Following extract exposure, cells were harvested using a cell scraper and prepared into 3×10^6 cell aliquots before sonication in D-PBS containing 1 mM mercaptoethanol. All other analysis steps were as recommended by the supplier.

2.4. Animals

Male Swiss mice, 8–12 weeks old, were used for the muscle bath and animal exercise experiments. Animals were cared for in accordance with the Code of Ethical Conduct, Regulations Act, 1987 (New Zealand) and all procedures were approved by our local Animal Ethics Committee.

2.5. Ex vivo model of muscle performance

To investigate the effect of fruit extracts on muscle tissue, a model bath system was established in which isolated fresh muscle (Soleus) was exposed to extracts and subsequently electrically stimulated to examine the effects on contractile activity (both maximum force produced and fatigue profiles).

Animals were killed by CO_2 asphyxiation. Soleus muscles were isolated from both hindlimbs, leaving tendons attached. Muscles were placed in an organ bath in Krebs-Ringer solution (in mM: 118 NaCl, 4.75 KCl, 1.18 $MgSO_4$, 2.54 $CaCl_2$, 24.8 $NaHCO_3$, 1.18 KH_2PO_4, 10 glucose), bubbled continuously with 95% O_2 – 5% CO_2 to maintain a pH of 7.5 at 25°C. Muscles were attached to a force transducer (World Precision Instruments, Sarasota, USA) and positioned between electrodes. Using a micrometer, muscles were adjusted to their optimum length to yield maximal force at 120 Hz stimulation. The output of the force transducer was monitored using Data-Trax software (World Precision Instruments, Sarasota, USA).

Baseline tetanic force production (3 millisecond pulses at 20 Hz over 14 s) in each individual muscle was measured in the absence of fruit extract exposure. After a recovery period of 10 min, the buffer was replaced with fresh Krebs-Ringer solution for control muscles, or Krebs-Ringer solution containing 5 μg/mL ZESPRI® GOLD kiwifruit extract or 2% GHO "Natural Quenchers" containing 65% of either ZESPRI® GOLD kiwifruit or blackcurrant juice. Muscles were incubated for 15 min before stimulating another tetanus response. Muscles were then removed and length and weight measured; the cross sectional area was estimated according to Mendez and Keys [19] and force expressed as mN/mm^2. Force measured during the fatigue protocols was assessed in relation to the peak force generated in each individual muscle's baseline stimulation.

2.6. Animal exercise

Mice were randomly assigned to the following five groups: no-exercise controls, exercising mice sacrificed at 0, 1, 3, or 24 h after fatiguing uphill running. All mice were acclimated to exercise conditions on the motorized treadmill (Muromachi Kikai, Tokyo, Japan) for five days, during which they walked/ran for 15 min at a speed of 0–15 metres/min and a maximum incline of +2°. The running adaptation was followed by at least five days of rest. During the fatiguing exercise, mice ran on the treadmill with a maximum speed of 36 metres/min and a maximum incline of +8° until the point of fatigue, at which they were no longer able to maintain pace despite gentle prodding and discharges of compressed air. Control mice were kept in the room with the treadmill to expose them to the same noise and handling as the exercising mice. After completion of the exercise, mice in the control and 0 hour group were sacrificed immediately, all other mice were sacrificed after 1, 3, or 24 h of recovery. Animals were killed by CO_2 asphyxiation and blood was collected by heart puncture. Blood was centrifuged (9000g, 10 min) and plasma was stored at –80°C until further analysis. Plasma creatine kinase (CK) activity was analysed by Gribbles Veterinary Laboratory (Hamilton, New Zealand). Results are expressed as international units per litre of plasma (IU/L). Plasma with a haemolysis index greater than 60 was excluded from the analysis. Interleukin-6 (IL-6) concentration in plasma was analysed using the Quantikine Mouse IL-6 ELISA from R&D Systems (Minneapolis,

USA) and was performed according to the manufacturer's instructions. Results are expressed as pg IL-6 per mL plasma (pg/mL).

2.7. Statistical analysis

Results are expressed as means ± standard errors of the mean (SEM) for at least four observations in each case. Statistical significance for the comparison of two groups was assessed by the Student's *t*-test. A probability value (*P*) of less than 0.05 was considered significant.

3. Results and discussion

3.1. Anthocyanin content of fruit juices and effects of fruit on muscle cell oxidative stress

Many fruits, and especially berries, contain bioactive compounds whose health-promoting properties are widely recognised [1, 27]. Utilising fruit varieties from breeding programmes at PFR with compositional analysis we are evaluating the physical health-promoting effects of phytochemicals derived from some new fruit varieties, including berry fruits. Breeding programmes at PFR have targeted high anthocyanin content as one of the possible desirable characteristics of new fruit cultivars for health benefits. A compositional survey of juices prepared from commercially grown non-New Zealand as well as New Zealand blackcurrant cultivars and new selections from the PFR breeding programmes demonstrates that the anthocyanin content of the New Zealand fruit juice is approximately 1.5 times that of the non-New Zealand and one new cultivar is particularly high in anthocyanins (Table 1). The fruits for the juices were grown under New Zealand conditions and the data suggest that New Zealand cultivars perform well when compared with those grown in North America [20]. The exceptionally high anthocyanin content makes some New Zealand blackcurrants cultivars unique and might provide potential strategies to counter/modulate the stress and damage associated with over-exercise, as well as to improve overall body wellness.

Cellular studies can be used to determine the underlying mechanisms of action of active fruit extracts/compounds. We report here insights into the protective mechanism of action of a blueberry fruit extract. We analysed the antioxidant ability of skeletal muscle myotubes (differentiated from muscle myoblast cell lines) following exposure to the extract. Using DCFDA, which accumulates intracellularly and is cleaved to produce the radical sensitive product DCF, enabled the determination of intracellular free radical levels by monitoring of relative fluorescence intensities after exposure to H_2O_2. Figure 1 displays the antioxidant ability of muscle cells following different lengths of incubation exposure

Table 1

Anthocyanin content of blackcurrant juice from New Zealand and non-New Zealand cultivars

Non-New Zealand Cultivars[*]	Anthocyanins (mg/100 mL)	New Zealand Cultivars	Anthocyanins (mg/100 mL)
'Baldwin'	310	'Ben Ard'	463
'Ben Dorain'	297	'Ben Rua'	477
'Ben Lomond'	249	'Blackadder'	446
'Ojebyn'	179	'Magnus'	336
		New Selection 1	471
		New Selection 2	850

Juices were made from fruit harvested in the 2009 season from bushes on research plots in Canterbury, New Zealand.
*A comparable range of anthocyanin content was obtained from the large study carried out on 32, mostly European, blackcurrant fruit cultivars grown in North America [20].
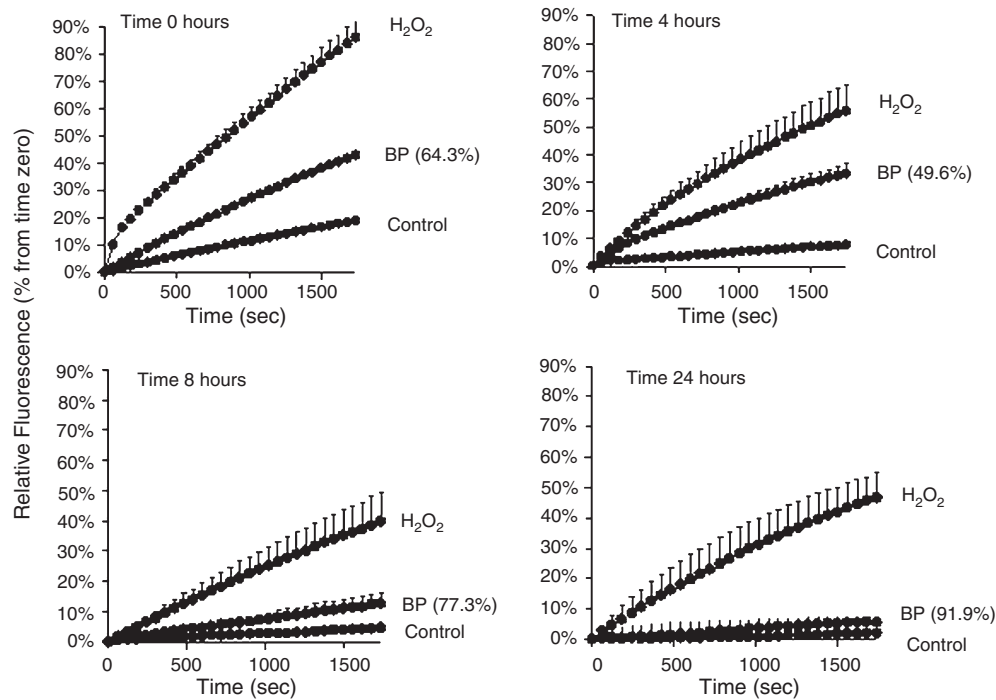
Fig. 1. Effect of incubation time on the intracellular free radical scavenging ability of muscle cells following exposure to a blueberry polyphenolic extract. Muscle cells were loaded with the reactive oxygen species indicator DCF as described in Materials and Methods and incubated (simultaneously [time zero] and at the times indicated on the figures) with a blueberry fruit (*Vaccinium corymbosum* 'Reka') polyphenolics extract (BP, 50 μg/mL) prior to challenge with H_2O_2 and fluorescence intensity determination. Values are means ± SEM from at least four experiments and are expressed as relative fluorescence intensity (% from time zero). The percentage inhibition of H_2O_2-induced fluorescence by the blueberry extract is shown in parentheses.

(simultaneously [time zero] to 24 h) with the blueberry extract (50 μg/ml) before oxidative challenge. H_2O_2 elicited a sustained, progressive, and large increase in muscle intracellular free radical levels. The magnitude of this response was reduced from 4 h onwards and was probably due to the leakage of the DCF from the cells over time, but this did not affect the determination of the intracellular antioxidant ability. With the simultaneous incubation of the muscle cells with the blueberry fruit extract, a marked increase in cellular antioxidant ability was observed, which was reflected by a 64.3% inhibition of fluorescence signal elicited by H_2O_2. This immediate antioxidant ability was further enhanced by longer extract pre-incubations (Fig. 1). After a 24-h extract pre-incubation, the antioxidant scavenging ability of the muscle cells had increased and was reflected by a 91.9% inhibition of the fluorescence signal elicited by H_2O_2. We further evaluated whether washing the blueberry extract from the cells prior to challenge with H_2O_2 prevented the immediate and longer-term antioxidant effects. There was no significant difference between washed and non-washed cells (data not shown).

In vitro models of oxidative stress using skeletal muscle cells have been used successfully in the past to monitor, under controlled conditions, the responses of muscle to oxidative stress that cannot be easily evaluated in human trials, and to provide a cost-effective method for the screening of beneficial substances. A useful and simple system is the use of skeletal muscle myotubes (differentiated from muscle myoblast cell lines) exposed to calcium ionophores [17].

We have previously demonstrated that the blueberry fruit (*Vaccinium corymbosum* 'Reka') polyphenolic extract mediated protection against muscle cell oxidative stress and damage [13]. The data presented here give insights into the potential process of action. We compared the functional activity of anthocyanin rich sub-extracts derived from the total extract with individual anthocyanin components and demonstrated that the most likely actives responsible

for the protection against the oxidative stress were malvidin glycosides, particularly malvidin galactoside, and/or malvidin glucoside [13].

3.2. Effects on muscle cell GSH and GPx activity

The data in Fig. 1 suggest that while there is an immediate antioxidant ability of the fruit extract, the increase in antioxidant ability over time is not associated with the extract itself. Tissues and cells are equipped with efficient enzymatic and non-enzymatic antioxidant defence systems, which serve to counter the damaging action of oxidative stress [10]. Figure 2 shows the determination of muscle cell GSH and GPx activity following blueberry fruit extract exposure. While there were no changes in GPx activity, GSH levels after a 24-h exposure were significantly enhanced and suggest the induction of endogenous antioxidant pathways. These data support other reports that highlight that polyphenolics can modulate the levels of antioxidant defence enzymes such as superoxide dismutase, catalase, GSH and GPx [29]. Evidence suggests that these adaptive actions to ensure cell and tissue survival following oxidative stress may be mediated by the induction of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Nrf2 is a transcription factor that binds to the antioxidant response element (ARE) in the upstream promoter region of many antioxidative genes, where it initiates the transcription of many cytoprotective proteins including endogenous antioxidant enzymes [15, 22]. More research is warranted to elucidate the significance of fruit polyphenolic-mediated induction of endogenous antioxidant pathways for modulation of unregulated oxidative stress and the potential long-term effects for muscle performance.

3.3. Effect of fruit on muscle function

Exposure of isolated muscle tissue to some fruit extracts also modulates muscle tissue contractile activity and performance. Assessing force production and fatigue profile in electrically stimulated murine soleus muscles, we have previously reported [26] that a ZESPRI® GOLD kiwifruit extract mediated an increased maximum tetanic force in a similar manner to the antioxidant superoxide dismutase (1000 IU/mL).

Here we report (Fig. 3) the contractile performance of soleus muscle following exposure to a ZESPRI® GOLD Kiwifruit extract and two commercially prepared ZESPRI® GOLD kiwifruit and blackcurrant juices (2% GHO
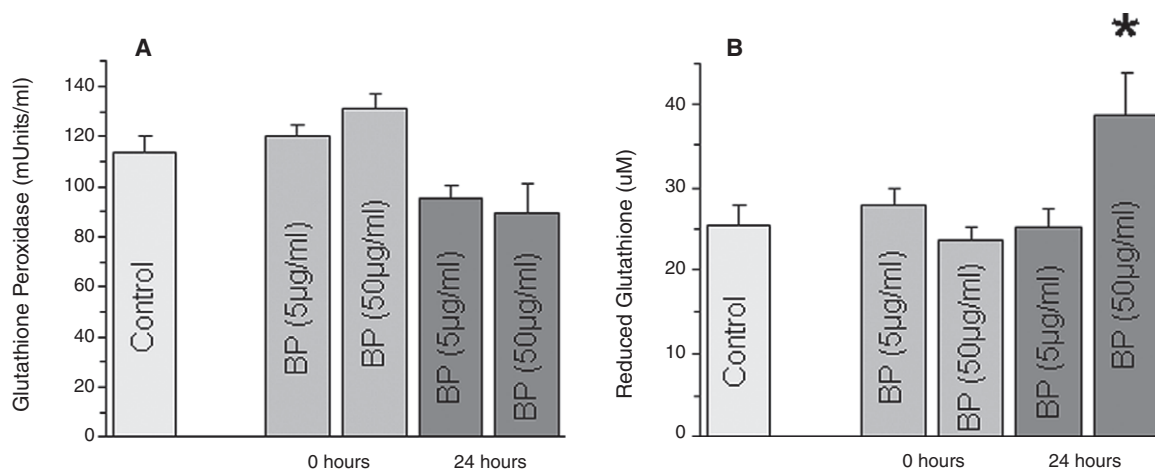

Fig. 2. Muscle cell reduced glutathione levels and glutathione peroxidase activity following incubation with a blueberry fruit polyphenolic extract. The effect of a blueberry (*Vaccinium corymbosum* 'Reka') polyphenolics extract (BP, 5 and 50 μg/mL) on muscle cell glutathione peroxidase (GPx) activity and reduced glutathione (GSH) levels was evaluated. Muscle cell GPx (A) and GSH levels (B) were determined ▨ immediately and ■ 24 h after incubation with the blueberry extract (BP) as described in Materials and Methods. Values are means ± SEM from at least four experiments and are expressed as mU/mL and μM (GPx and GSH respectively). *Represents $p < 0.05$ statistical significance (paired Student t-test) compared with the control.
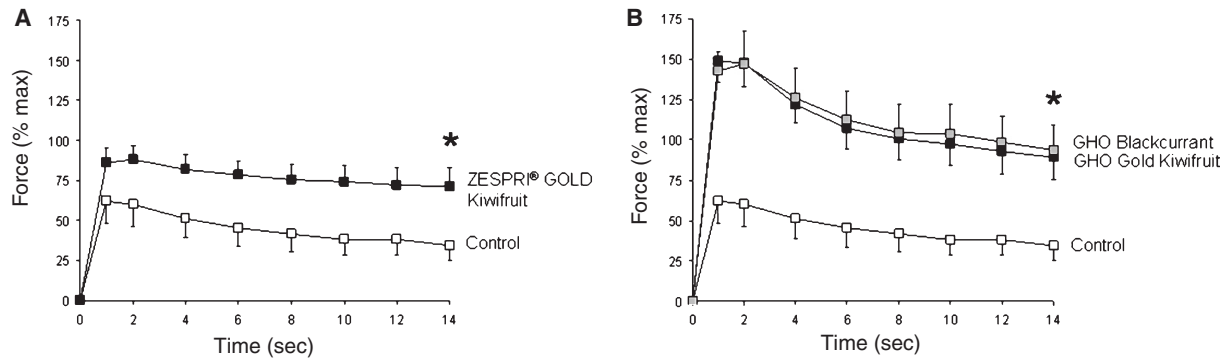
Fig. 3. Effect of fruit extract and juices on fatigue in mouse soleus muscles induced by electrical stimulation. Muscles incubated with (A) ZESPRI® GOLD Kiwifruit (*Actinidia chinensis* 'Hort16A') phenolic extract (5 μg/ml, □ control, ■ kiwifruit extract), or (B) GHO ZESPRI® GOLD Kiwifruit and blackcurrant juices (2%, □ control, ▧ kiwifruit, ■ blackcurrant) were compared with buffer-only controls. The recorded force was divided by the cross sectional area of the muscle and normalized to the maximum force generated during baseline stimulation. A minimum of five muscles were analysed for each group. Values are means ± SEM. *Represents $p < 0.05$ statistical significance (paired Student t-test) between the area under the curve for muscles incubated with buffer-only and fruit extract.

"Natural Quenchers"). Mouse soleus muscles were electrically stimulated *ex vivo* after 15 min of incubation with the ZESPRI® GOLD Kiwifruit extract (5 μg/ml), or control buffer and tetanic force production was measured over 14 sec (Fig. 3A). Mean peak force in fruit extract exposed muscles was 86.1 ± 9.7%, 23.8% higher than the peak force in the controls (62.3 ± 14.2%). After the 14-sec tetanus stimulation the muscles demonstrated fatigue – a decline in muscle performance due to muscle activity. With kiwifruit extract exposure, muscle fatigue was reduced, with the mean force retaining 71.4% of the peak baseline force, whereas force in the controls dropped to 34.4%. Comparison of the area under the curves highlighted that the ZESPRI® GOLD Kiwifruit mediated protection against fatigue was statistically significant (kiwifruit 1108.5 ± 147.3, control 615.2 ± 151, $p < 0.05$). The commercially prepared ZESPRI® GOLD Kiwifruit and blackcurrant juices mediated a greater enhancement in muscle performance and protection against fatigue (Fig. 3B). Mean peak force in blackcurrant and kiwifruit juice-exposed muscles was 147.1 ± 20.6% and 148.9 ± 13.8% respectively, 85% higher than the peak force in controls. After the 14 sec tetanus stimulation, muscle fatigue was reduced by the blackcurrant and kiwifruit juice extracts with the mean force retaining 93.6% (blackcurrant) and 89.2% (kiwifruit) of the peak baseline force. Protection against fatigue, analysed as area under the curve, was statistically significant for both blackcurrant (1546.5 ± 225.8) and kiwifruit juices (1498.4 ± 170.1, $p < 0.05$).

These data demonstrate that ZESPRI® GOLD Kiwifruit and blackcurrant fruit extracts and juices have an effect on muscle performance, which could be mediated by antioxidant scavenging of exercise-mediated reactive oxygen species (ROS). Active muscles produce excess ROS and it is widely accepted that they play a role in fatigue. During intensive exercise, muscle oxygen consumption increases up to 100-fold, and in parallel, superoxide anion production in mitochondria increases to a level where endogenous ROS scavengers are overwhelmed and hydrogen peroxide diffuses into the muscle cell cytoplasm. Some exogenous antioxidants like N-acetylcysteine (NAC), vitamins C and E, have been reported to reduce fatigue in isolated muscles, but only NAC has so far been shown to improve fatigue performance *in vivo* [23].

The fruit compound responsible for the enhanced force production and reduced fatigue in our isolated mouse soleus muscles remains to be elucidated, but it is possible that vitamin C and/or a variety of polyphenolics present in the fruits and extracts play a role. Indeed, Dorchies and colleagues showed that five weeks of feeding green tea extract and (−)-epigallocatechin gallate (EGCG) to muscular dystrophic mice protected the animals from fatigue and improved muscle force generation [11]. However, while many antioxidants have been shown to provide protection against oxidative stress and fatigue *ex vivo*, this has not always translated into the *in vivo* situation. With this in mind we have recently initiated the establishment of animal models of exercise at PFR. Figure 4 shows biochemical data in blood samples taken at various times from mice undertaking an exhaustive uphill running exercise. While not statistically significant, there was a trend towards plasma CK activity (an indicator of muscle tissue damage),
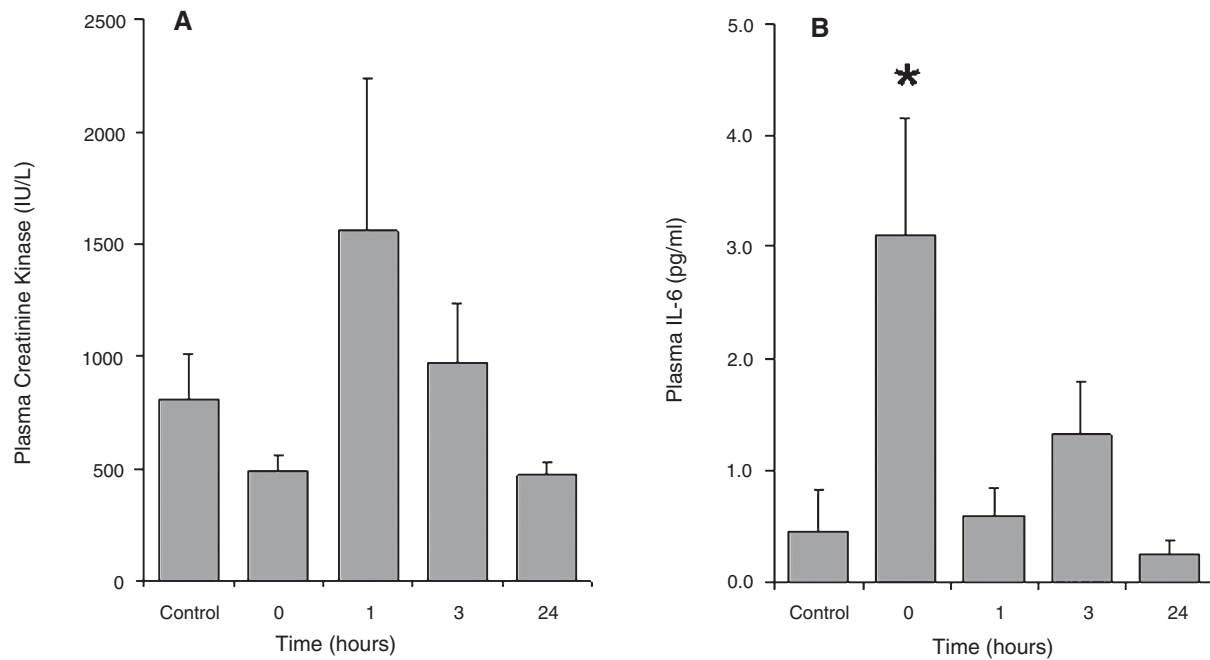
Fig. 4. Markers of stress and inflammation in plasma of exercising mice. Mice performed uphill running on a treadmill until exhaustion and were sacrificed at 0, 1, 3 or 24 h after the exercise. Resting mice functioned as a control group. Blood samples were taken at the time of sacrifice and plasma was isolated. (A) CK activity was expressed as IU/L and (B) IL-6 concentration was expressed as pg/mL. Each group consisted of 12 mice. Values are means $\pm$ SEM. *Represents $p < 0.05$ statistical significance (paired Student t-test) between control and exercise group.

slightly higher one hour after the exercise, compared with the activity in the control (non-exercised mice Fig. 4A). The release of IL-6 from muscle post exercise is known to be dependent upon both exercise intensity and duration [6, 12]. Figure 4B shows the plasma levels of IL-6 in exercising mice following our exercise regime. Immediately after the exercise (0 h), the mean plasma IL-6 concentration significantly increased (7-fold) from 0.45 ± 0.37 pg/mL in resting control mice to 3.11 ± 1.04 pg/mL in exercised mice ($p < 0.05$). After only 1 hour of recovery, plasma IL-6 concentrations returned to pre-exercise levels. While the exact role of the release of muscle tissue IL-6 is not clear, it is probably released in response to metabolic changes and to oxidative stress and/or tissue damage. It is suspected that IL-6 may be involved in activating aspects of the immune system to assist with tissue repair and recovery. These data also suggests that our uphill running protocol was inducing IL-6 response in exercising mice, without being strenuous or damaging enough to significantly increase CK levels. This is consistent with the findings of others in which no CK increase was observed with an uphill run, while downhill running, which mediates more tissue damage because of its eccentric nature, consistently increased CK in plasma [4, 18]. Others have utilised exercise routines in animals to evaluate the effects of nutrient supplementation on exercise-induced stress and performance. For example, curcumin [9], green tea extract [21], and Ginseng extract [3] have all been shown to decrease markers of damage, oxidative stress, and inflammation in exercising animals. Our preliminary data are therefore encouraging and in the near future we hope to be able to evaluate the benefits of fruits on stress and inflammation robustly using animal models of exercise.

In humans, exercise can have benefits on the immune system including an ability to positively modulate the acute inflammatory response to a simulated bacterial infection [14]. Furthermore, appropriate fruit extract consumption may assist these health benefits. In a recent study we reported the effect of supplementation with a blackcurrant fruit extract on exercise-induced health benefits [16]. Consumption of the anthocyanin-rich blackcurrant extract prior to a moderate rowing exercise mediated an amelioration of plasma markers of oxidative stress and micro muscle damage, but also complemented the ability of exercise to stimulate an acute inflammatory response to a simulated bacterial infection in *ex vivo* experiments. These findings could be significant in the augmented activation (by fruit polyphenolics) of

appropriate adaptive immune responses and of associated positive health benefits derived from moderate and regular exercise. Other studies have evaluated the potential of fruits and/or polyphenolic compound supplementation as antioxidants and reported potential protective effects and benefits to muscle and exercise performance [2, 7, 25]. In contrast however, some studies show that antioxidant supplementation may in fact neutralize the health benefits of regular exercise [5, 24] and that in some individuals undergoing long-term strenuous exercise regimes immune suppression can be observed [8].

Further studies are being undertaken at PFR in this area to elucidate the health potential of fruit polyphenolics as appropriate modulators of oxidative stress and inflammation.

4. Summary

Our emerging evidence suggests that some fruits (and derived phenolic compounds) offer significant health and wellness potential. Our aim is to utilise unique New Zealand germplasm to unleash opportunities for the creation of premium functional foods (backed by science), to prevent muscle damage/injury, aid recovery, and/or enhance muscular and immune function.

Acknowledgements

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