The Journal of Experimental Biology 214, 3542-3548
© 2011. Published by The Company of Biologists Ltd
doi:10.1242/jeb.058305

RESEARCH ARTICLE

Variation in expression of calcium-handling proteins is associated with inter-individual differences in mechanical performance of rat (*Rattus norvegicus*) skeletal muscle

Rob S. James[1,*], Isabel Walter[2] and Frank Seebacher[2]

[1]Department of Biomolecular and Sport Sciences, Coventry University, Coventry CV1 5FB, UK and [2]Integrative Physiology, School of Biological Sciences, University of Sydney, NSW 2006, Australia

*Author for correspondence (r.james@coventry.ac.uk)

Accepted 26 July 2011

SUMMARY

An important constraint on locomotor performance is the trade-off between sprint and endurance performance. One intuitive explanation for this trade-off is that an individual muscle cannot excel at generating both maximal force/power and high fatigue resistance. The underlying reasons for this muscle trade-off are poorly defined. The aim of this study was to test the hypothesis that inter-individual variation in muscle mechanics is associated with inter-individual differences in metabolic capacities and expression of calcium-handling proteins. Lateral gastrocnemius muscles were isolated from 20 rats (*Rattus norvegicus*) and analysed to determine metabolic capacity, sarco/endoplasmic reticulum calcium ATPase (SERCA)1 protein concentration, total SERCA activity, and mRNA concentrations of SERCA1, SERCA2, troponin I and ryanodine receptors. Isometric studies of lateral gastrocnemius muscles at 30°C showed that muscles with higher sprint performance had lower fatigue resistance. More rapid muscle contraction was correlated with higher lactate dehydrogenase activity and increased expression of ryanodine receptor 1. More rapid muscle relaxation was correlated with increased expression of troponin I type 2 (fast) isoform and decreased expression of SERCA2 (slow) isoform. Treating muscles with dantrolene confirmed that ryanodine receptor activity is important in determining tetanus force and muscle contraction rates, but has no effect on fatigue resistance. Thapsigargin treatment revealed that SERCA activity determines fatigue resistance but does not affect maximal muscle force or contraction rates. We conclude that the opposing roles of SERCA activity and expression of ryanodine receptors in determining fatigue resistance and force production, respectively, at least partly explain differences in sprint and endurance performance in isolated rat gastrocnemius muscle.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/214/21/3542/DC1

Key words: muscle mechanics, metabolic capacity, gene expression.

INTRODUCTION

Skeletal muscle performance and locomotion underlie the non-cognitive dimension of animal behaviour and are therefore major determinants of organismal fitness (Dickinson et al., 2000). The specialization of species for long distance locomotion, such as for migration (Piersma, 1998), or for short burst activity (Vanhooydonck et al., 2001) will partly determine their biogeography and ecological relationships (Irschick and Losos, 1998). At an individual level, better sprint or endurance performance will modulate sporting success in humans (Van Damme et al., 2002), and will affect fitness in natural systems by influencing predator escape and prey capture (Jayne and Bennett, 1990; Le Galliard et al., 2004). There can be pronounced differences in locomotor performance between individuals (Wilson et al., 2002; Rupert, 2003). For example, in human athletes that excel in sprint performance, endurance performance is compromised, and *vice versa* (Van Damme et al., 2002). A similar trade-off exists between species of vertebrates at the level of locomotion in the whole animal (Vanhooydonck et al., 2001) or within species at the level of isolated skeletal muscle performance (Wilson et al., 2002; Wilson and James, 2004; Wilson et al., 2004). Such sprint–endurance trade-offs may reflect a typical response of the vertebrate locomotory system to conflicting demands. The implication is that, although animal fitness often relies on both sprint and endurance performance, the two cannot be maximized simultaneously.

The mechanistic basis for a sprint–endurance trade-off remains largely unexplained despite its far-reaching importance for understanding physiological constraints in animal ecology, sporting performance and medicine. Most evidence documenting a trade-off is correlational at the level of the whole animal or muscle. Our aim was to advance knowledge by investigating the potential roles of specific molecular mediators of muscle contractile performance in the rat (*Rattus norvegicus*) lateral gastrocnemius muscle. Our approach was to represent both Ca^{2+} handling, which underlies muscle contraction and relaxation, and metabolic capacity with multiple parameters to determine their relative importance and possible interaction. We correlated muscle kinetics with: (1) mRNA expression of candidate proteins to determine possible changes at the genome level, and (2) protein concentration and enzyme activity to determine responses at the protein level. Following correlational analyses, we determined cause and effect between molecular mechanisms and muscle performance by specific pharmacological manipulation of Ca^{2+}-handling proteins.

The morphology of the skeletal system poses only a limited constraint on either sprint or endurance performance (Vanhooydonck et al., 2001), such that a major limitation to locomotion is likely to reside in muscle physiology. The most likely processes that constrain muscle function are excitation–contraction coupling *via* Ca^{2+} cycling (Berchtold et al., 2000), and the capacity of the muscle to produce the necessary energy (ATP) to power contraction. Ca^{2+} release into the myocyte and subsequent binding with troponin controls the interaction between actin and myosin and thereby the rate of muscle force generation and, conversely, re-sequestration of Ca^{2+} into the sarcoplasmic reticulum determines relaxation rates (Farah and Reinach, 1995; Berchtold et al., 2000). Two of the key elements of this calcium-handling process are the ryanodine receptor and sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA). The ryanodine receptor interacts with the dihydropyridine receptor during the excitation–contraction coupling process, and acts as the sarcoplasmic reticulum calcium release channel. An increased density of dihydropyridine and ryanodine receptors in muscle is correlated with increased shortening velocity and force in isolated muscle (Kandarian et al., 1992; Golden et al., 2003; Mänttäri and Järvilehto, 2005), and their density is increased following endurance training in trout (Anttila et al., 2008). SERCA is responsible for calcium re-uptake from the cytoplasm to the sarcoplasmic reticulum, and its activity can therefore determine muscle relaxation rate (Fleming et al., 1990; Wilson et al., 1998). SERCA activity is regulated by phosphorylation of phospholamban, which in its unphosphorylated form inhibits SERCA activity (Verboomen et al., 1992; Periasamy and Kalyanasundaram, 2007). Phospholamban phosphorylation is under sympathetic control *via* β2-adrenoreceptor-mediated release of cAMP (McCormick et al., 2010). ATP is used during myosin ATPase activity, which powers the interaction between actin and myosin leading to muscle contraction, and by SERCA-mediated Ca^{2+} transport into the sarcoplasmic reticulum during muscle relaxation. Hence, ATP supply can be an important constraint for muscle function. Ca^{2+} transport and cellular energy metabolism may therefore be mechanisms underlying the functional sprint–endurance trade-off.

Characteristics of Ca^{2+} transport and energy metabolism are commonly used for muscle fibre typing, which is variously based on the relative proportions of protein isoforms of myosin heavy chain (Bottinelli and Reggiani, 2000; Plomgaard et al., 2006), SERCA and troponin (Bottinelli and Reggiani, 2000; Berchtold et al., 2000). Differences in glycolytic and oxidative metabolic capacities are also used as the basis of muscle fibre classification, where slow, endurance fibres are more oxidatively poised and fast fibres have greater glycolytic capacity (Bottinelli and Reggiani, 2000). However, the molecular traits used to categorize muscle fibre types often do not correspond to the expected patterns of muscle performance and sprint–endurance trade-offs (Gibb and Dickson, 2002; James et al., 2005; Plomgaard et al., 2006; Seebacher and James, 2008). This may be because actual muscle contractile performance is determined by interactions between multiple molecular traits, so that deducing a direct relationship between fibre type categories and muscle performance is difficult. We therefore investigated individual traits separately without any preconceived definitions of fibre types.

We tested the following hypotheses. (1) There is a trade-off between maximal force generated and fatigue resistance of force generation in isolated lateral gastrocnemius. (2) Muscle oxidative metabolic capacity is associated with fatigue resistance and muscle glycolytic metabolic capacity is associated with maximal muscle activity. Specifically, muscles with a shorter contraction time will have higher lactate dehydrogenase activity, while muscle fatigue resistance will be positively related to citrate synthase and cytochrome *c* oxidase activities. (3) Ca^{2+}-handling dynamics are associated with muscle performance during sustained and maximal activities. Specifically, muscles with shorter contraction times will have higher ryanodine receptor mRNA concentrations, and variations in SERCA isoform mRNA concentration, SERCA protein concentration and total SERCA activity are associated with muscle relaxation times; additionally, the relative proportion of troponin I isoforms will be associated with variation in maximal (sprint) and sustained muscle performance.

MATERIALS AND METHODS

Animal housing and dissection

All experimental and animal-handling procedures were approved by the University of Sydney Animal Ethics Committee (approval number L04/6-2008/1/4865). We obtained rats [*Rattus norvegicus* (Berkenhout); N=20, body mass 130±9 g] from three different breeding backgrounds to increase inter-individual variation. Rats were kept in standard rat cages at 22°C with a 14 h light:10 h dark cycle, and with access to *ad libitum* food and water. Rats were killed by an injection of dilute sodium pentabarbitone (150 mg kg^{-1} body mass) immediately before experimentation. Body mass was measured to the nearest 0.1 g using an electronic balance. The skin was removed from the hindlimbs, then the hindlimbs were removed and placed in aerated Krebs solution (composition, in mmol l^{-1}: 118 NaCl, 4.75 KCl, 1.18 $MgSO_4$, 24.8 $NaHCO_3$, 1.18 KH_2PO_4, 10 glucose, 2.54 $CaCl_2$; pH 7.5 at room temperature prior to oxygenation) kept on ice. Muscle tissue samples (approximately 0.05 g) were removed from one lateral gastrocnemius head in each leg and either placed in RNAlater for real-time PCR assays or immediately frozen in liquid nitrogen for enzyme activity assays. The other lateral gastrocnemius muscle head in each leg was removed, leaving tendons attached to the muscle and a small section of bone, for use in mechanical measurements of muscle performance. This approach makes the assumption that the gastrocnemius muscle taken from the left leg will be structurally, mechanically and functionally similar to that taken from the right leg of the same individual.

Enzyme assays

We measured the activities of the mitochondrial enzymes cytochrome *c* oxidase (COX) and citrate synthase (CS) as indicators of aerobic metabolic capacity, and the activity of lactate dehydrogenase (LDH) as an indicator of anaerobic metabolic capacity (St-Pierre et al., 1998). Frozen muscle samples (0.05–0.1 g) were homogenized immediately in 9 volumes of ice-cold extraction buffer (50 mmol l^{-1} imidazole, 2 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} EDTA, 0.1% Triton and 1 mmol l^{-1} glutathione, pH 7.5 at 0°C) using a rotor-stator homogenizer (Pro200, Pro Scientific, Oxford, CT, USA). All assays were conducted according to published protocols (Seebacher et al., 2003), and activities were expressed as μmol of substrate converted min^{-1} g^{-1} tissue.

Total SERCA activity was determined according to a previously described ATPase assay (Walter and Seebacher, 2009), except that the assay medium consisted of (mmol l^{-1}): imidazole 25, $CaCl_2$ 0.2, KCl 0.6, $MgCl_2$ 0.048 (Ramnanan and Storey, 2008). Inorganic phosphate concentration was quantified by the ammonium molybdate assay (Bonting et al., 1961). Total SERCA activity was calculated as the difference in inorganic phosphate liberated in the presence and absence of 1 μmol l^{-1} thaspsigargin (Ramnanan and Storey, 2008). All enzyme assays were conducted in duplicate at 30°C in a spectrophotometer with a temperature-controlled cuvette

holder (Ultrospec 2100 pro UV; Amersham Pharmacia, Sydney, Australia).

RNA isolation and quantitative real-time PCR

RNA was extracted from 40–100 mg of muscle samples using TRIreagent (Molecular Research Centre, Cincinnati, OH, USA), following the manufacturer's instructions. RNA quality and concentration were verified using a Bioanalyzer (Agilent Biotechnologies, Santa Clara, CA, USA). Total RNA (1 μg) was treated with DNase I (Sigma-Aldrich, Castle Hill, NSW, Australia) and reverse-transcribed using RNase $H^{–}$ MMLV reverse transcriptase (Bioscript, Bioline, Taunton, MA, USA) and random hexamer primers (Bioline). Pre-validated Taqman probes (Applied Biosystems, Scoresby, Victoria, Australia) were used to quantify troponin I type 1 (TNNI1; catalogue number Rn00567843_m1), troponin I type 2 (TNNI2; Rn00437157_g1), Ca^{2+}-transporting ATPase 1 (SERCA1; Rn00589545_m1), Ca^{2+}-transporting ATPase 2 (SERCA2; Rn00568762_m1) and ryanodine receptor 1 (RYR1; Rn01545082_g1) expression and values were normalized to expression of eukaryotic translation elongation factor 1 α2 (Eef1a2; Rn00561973_m1). Quantitative real-time PCR runs were performed on an Applied Biosystems 7500 qRT-PCR machine using Taqman Gene Expression Mastermix with the standard PCR protocol as recommended by the manufacturer. Relative gene expression of the five target genes was calculated according to Pfaffl (Pfaffl, 2001), and normalized to that of Eef1a2. The grand mean of all tissues was used as a control.

Immunoblotting

Tissue lysates were prepared in homogenization buffer (65 $mmol\,l^{-1}$ Tris HCl, 150 $mmol\,l^{-1}$ NaCl, 5 $mmol\,l^{-1}$ EDTA pH 7.4, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 10% glycerol) with PhosStop phoshatase inhibitors and complete protease inhibitors (Roche Diagnostics, Basel, Switzerland), and centrifuged for 15 min at 12,000 ***g***. Protein content of supernatants was quantified by the bicinchoninic acid assay (BCA, Sigma-Aldrich) according to the manufacturer's instructions. Aliquots, diluted 1:1 in 2× Laemmli buffer and denatured at 95°C, were resolved on 10% precast SDS-PAGE gels (BioRad, Gladesville, NSW, Australia) (Laemmli, 1970). Proteins were transferred to a nitrocellulose membrane (BioRad) and stained with Ponceau red dye to ensure accurate protein loading and transfer. Membranes were blocked for 1 h with 5% skimmed milk powder in Tris-buffered saline with 0.1% Tween-20. Blots were probed with anti-SERCA1 ATPase (MA3-912; Affinity Bioreagents, Golden, CO, USA) at 1:2500 and with anti-α-tubulin (T5168; Sigma-Aldrich) at 1:8000 in blocking buffer as a loading control. Secondary anti-mouse horseradish peroxidase (Abnova, Waterloo, NSW, Australia) was used at 1:10,000. Horseradish peroxidase localization was detected with Amersham ECL Plus (GE Healthcare, Rydalmere, NSW, Australia) according to the manufacturer's instructions and visualized by the G:Box gel documentation system. Relative band intensity was quantified using Alpha Innotech Software (Alpha Innotech Corp., Santa Clara, CA, USA) and normalized with α-tubulin.

Isometric studies of mechanical performance of isolated muscle

Muscle preparations were transferred to a bath of aerated circulating Krebs solution maintained at 30.0±0.5°C. The bone at one end of the lateral gastrocnemius was attached *via* a crocodile clip to a strain gauge (model UF1, Pioden Controls Ltd, Canterbury, Kent, UK) and the bone at the other end was attached *via* a crocodile clip to a servomotor (V201, Ling Dynamic Systems, Royston, Hertfordshire, UK). Electrical stimulation was delivered to the muscle in 1.5 ms pulses *via* parallel platinum electrodes. Muscle stimulation was controlled using custom-written software (CEC Testpoint version 7, Measurement Computing, Norton, MA, USA) *via* a D/A board (KPCI3108, Keithley Instruments, Cleveland, OH, USA). Force data were sampled at a rate of 2 kHz. A series of isometric twitches were used to optimize stimulation amplitude and muscle length to yield the maximum isometric twitch force. An isometric tetanic response was elicited by subjecting the muscle to a 200 ms train of stimulation delivered at 160 Hz stimulation frequency. Maximal force generated, time to half-peak tetanic force (defined as 'contraction time') and time from the last stimulus to 50% tetanus relaxation (defined as 'relaxation time') were measured. A 5 min recovery period was allowed between each tetanic response.

Additionally, to test for the importance of ryanodine receptors in determining muscle force production and fatigue resistance, we incubated one muscle preparation from nine of the rats in 10 $\mu mol\,l^{-1}$ dantrolene (Sigma-Aldrich) for 10 min (Van Winkle, 1976; Fruen et al., 1997) while subjecting the contralateral preparation from each of these rats to control Krebs solution for the same time duration. Similarly, to test the role of SERCA, we incubated one muscle preparation from nine of the rats in 10 $\mu mol\,l^{-1}$ thapsigargin (Sigma-Aldrich) for 5 min (Kurebayashi and Ogawa, 2001; Galli et al., 2006), using the contralateral preparation from each of these rats as a control.

To measure fatigue resistance of muscle force generation (endurance), we subjected each control muscle preparation to a fatigue run consisting of a series of 25 tetani, where a 200 ms train of stimulation was delivered once per second. Peak tetanic force was measured in the 1st and the 25th tetanus of the fatigue run. Therefore, every muscle that was subjected to a fatigue resistance test was also tested for maximal force generation.

Lateral gastrocnemius muscle mass was determined to the nearest 1.0 mg at the end of the experiments using an electronic balance, after blotting the muscle on absorbent paper to remove excess Krebs solution. Mean muscle cross-sectional area was calculated from mean muscle fibre length, muscle mass and an assumed muscle density of 1060 $kg\,m^{-3}$ (Méndez and Keys, 1960). Muscle stress was calculated as force divided by mean muscle cross-sectional area. The muscle mechanics variables indicative of maximal (sprint) muscle performance were considered to be all twitch and tetanus times, and twitch and tetanus stress measurements from the maximal twitch and tetanus for each individual. A measure of fatigue resistance was determined by calculating peak tetanic force in the 25th tetanus of the fatigue run as a percentage of the peak tetanic force in the 1st tetanus of the fatigue run. For each of the 20 rats the mechanics data from the lateral gastrocnemius muscle sample from the left leg were used for analysis of the inter-individual relationship between maximal (sprint) and sustained (endurance) muscle performance.

The effects of incubation in drug or control treatments on force production were analysed by calculating peak tetanus force, activation and relaxation times in the first tetanus of the fatigue run (during drug incubation) as a percentage of peak tetanus force prior to incubation.

Statistics

All statistical analyses were performed using SPSS version 17. Pearson's product moment correlation was used to analyse correlations (Table 1) between the variables. Student's paired *t*-tests were performed to compare the effect of dantrolene and thapsigargin

Table 1. Pearson product moment correlation matrix for rat lateral gastrocnemius muscle mechanics, metabolic capacity and gene expression of calcium-handling proteins

	LDH activity	COX activity	CS activity	SERCA activity	SERCA1 protein	SERCA1 RNA	SERCA2 RNA	RyR RNA	Trop1 RNA	Trop2 RNA
CT	**–0.487**	–0.024	0.108	–0.183	0.216	0.081	0.100	**–0.452**	**0.213**	**–0.007**
	(*0.015*)	(0.460)	(0.325)	(0.221)	(0.181)	(0.367)	(0.338)	**(*0.023*)**	**(0.183)**	**(0.488)**
RT	–0.261	0.115	0.031	**0.030**	**0.241**	**–0.013**	**0.507**	0.087	**0.272**	**–0.413**
	(0.133)	(0.315)	(0.448)	**(0.449)**	**(0.153)**	**(0.479)**	**(*0.011*)**	(0.357)	**(0.123)**	**(*0.035*)**
FR	0.291	**–0.036**	**–0.221**	0.376	–0.296	–0.179	–0.231	0.086	**–0.068**	**–0.211**
	(0.106)	**(0.440)**	**(0.174)**	(0.051)	(0.103)	(0.225)	(0.163)	(0.359)	**(0.389)**	**(0.186)**

Data are *r*-values, with corresponding *P*-values underneath in parentheses, $N=20$ in each case. *P*-values in italics are significant ($P<0.05$). Values in bold relate to specific hypotheses stated in the Introduction. (See supplementary material Table S1 for raw data.)

CT, contraction time (time to half-peak tetanus); RT, relaxation time (last stimulus to half relaxation); FR, fatigue resistance; LDH, lactate dehydrogenase; COX, cytochrome *c* oxidase; CS, citrate synthase; SERCA, sarco/endoplasmic reticulum calcium ATPase; RyR, ryanodine receptor; Trop1, troponin I type 1.

treatment with their respective controls. The truncated product method (Zaykin et al., 2002) was used to combine all the *P*-values in this study to determine whether there was a bias from multiple hypothesis testing. The truncated product method *P*-value was <0.001, showing that the results were not biased. Significance was taken at the level of $P<0.05$.

RESULTS

Correlation between maximum muscle stress and fatigue resistance

Maximum force produced (maximum tetanus stress) was negatively correlated with fatigue resistance in lateral gastrocnemius muscle ($r=-0.496$, $P=0.013$). Those muscles that produced high maximal tetanic stress were more likely to have a poor fatigue resistance, and *vice versa*, indicating a trade-off between maximal (sprint) and sustained (endurance) performance within skeletal muscle, consistent with hypothesis 1.

Correlation between metabolic capacity and muscle mechanics

Contraction time (time to half-peak tetanus) was significantly negatively correlated with lactate dehydrogenase activity (Fig. 1; Table 1; $r=-0.487$, $P=0.015$). This finding demonstrates that muscles with more rapid tetanus force production have higher anaerobic capacity. This is in agreement with hypothesis 2, where we postulated that the rate of force generation would be related to glycolytic capacity. In contrast muscle fatigue resistance was not significantly correlated with any measures of metabolic capacity (Table 1). Therefore, contrary to hypothesis 2, oxidative capacity was not correlated with muscle endurance mechanics.

Correlation between calcium handling and muscle mechanics

Contraction time was significantly negatively correlated with ryanodine receptor 1 mRNA concentration (Fig. 2; Table 1; $r=-0.452$, $P=0.023$). This finding demonstrates that faster lateral gastrocnemius muscles had greater expression of ryanodine receptor 1, in agreement with hypothesis 3.

Muscle relaxation time (time from last stimulus to half tetanus relaxation) was significantly positively correlated with SERCA2 (the slow isoform of SERCA) mRNA concentration (Fig. 3; Table 1; $r=0.507$, $P=0.011$) and significantly negatively correlated with troponin I type 2 (the fast isoform of troponin I) mRNA concentration (Fig. 4; Table 1; $r=-0.413$, $P=0.035$). These findings are in agreement with hypothesis 3. Maximum force produced (peak tetanic force) decreased between the 1st and 25th tetanus (Tables 2 and 3) in each experimental treatment. Inhibition of SERCA with $10\,\mu mol\,l^{-1}$ thapsigargin significantly decreased sustained muscle performance (fatigue resistance) compared with control treatments

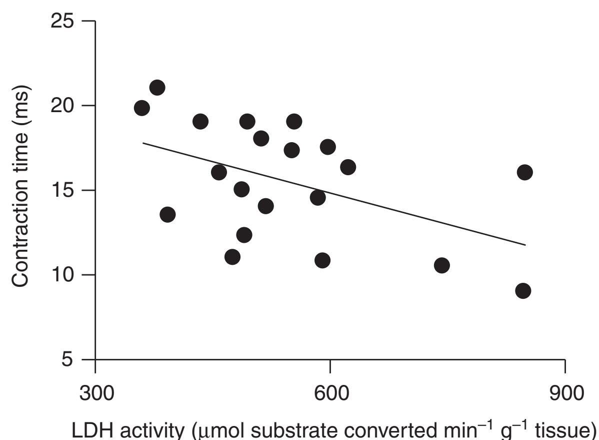

Fig. 1. Negative correlation between contraction time and lactate dehydrogenase (LDH) activity in rat lateral gastrocnemius muscle. Contraction time was defined as the time taken to reach half-peak tetanus force. $r=-0.487$, $P=0.015$, $N=20$ individuals.

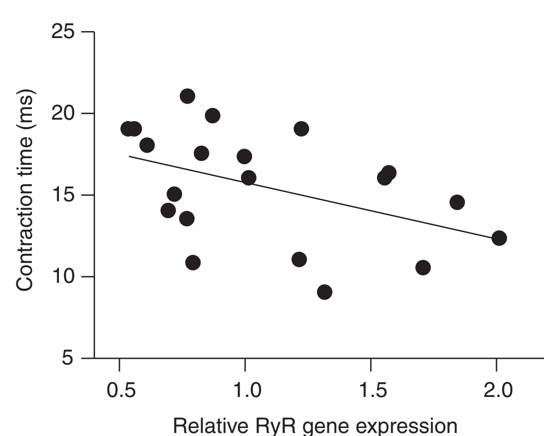

Fig. 2. Negative correlation between contraction time and ryanodine receptor 1 (RyR) gene expression in rat lateral gastrocnemius muscle. Contraction time was defined as the time taken to reach half-peak tetanus force. $r=-0.452$, $P=0.023$, $N=20$ individuals.

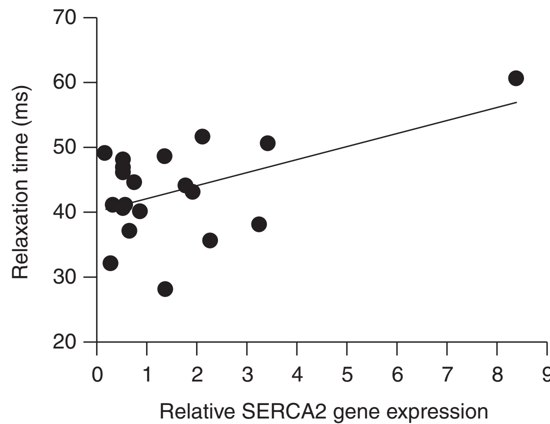

Fig. 3. Positive correlation between relaxation time and SERCA2 gene expression in rat lateral gastrocnemius muscle. Relaxation time was defined as time from the last stimulus to 50% tetanus relaxation. r=0.507, P=0.011, N=20 individuals.

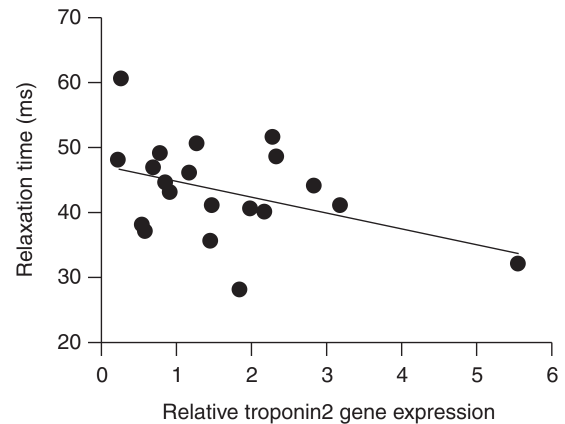

Fig. 4. Negative correlation between relaxation time and troponin I type 2 (troponin2) gene expression in rat lateral gastrocnemius muscle. Relaxation time was defined as time from the last stimulus to 50% tetanus relaxation. r=–0.413, P=0.035, N=20 individuals.

(P<0.001; Table 2). This result is linked to the finding that there was a tendency for fatigue resistance to be positively correlated with total SERCA activity (Table 1; r=0.376, P=0.051). However, thapsigargin treatment did not affect acute peak tetanic force production significantly (P=0.92; Table 2), rate of tetanus force (stress) production in the first tetanus after incubation (P=0.21; Table 2), rate of tetanus force (stress) production in the 25th tetanus of the fatigue run (P=0.78) or rate of tetanus force (stress) relaxation in the 1st tetanus after incubation (P=0.61; Table 2). These findings support hypothesis 3.

Fatigue resistance was not affected significantly by incubation in 10 $\mu mol\,l^{-1}$ of the ryanodine receptor blocker dantrolene (P=0.12; Table 3) when compared with control muscles. However, dantrolene treatment significantly decreased acute peak tetanus force production (P<0.001), and the rate of tetanus force production (P<0.001) and tetanus force relaxation (P<0.001) compared with controls (Table 3). These findings support hypothesis 3, that the ryanodine receptor is associated with determining rates of tetanus force (stress) production and relaxation.

DISCUSSION

Here, we show that lateral gastrocnemius muscles with relatively high maximal (sprint) performance have lower endurance performance (fatigue resistance), and that this trade-off between sprint and endurance performance is associated with a combination of variation in glycolytic metabolic capacity and variation in Ca^{2+} handling in the muscle. Interestingly, inter-individual variation in the capacity of mitochondria to produce ATP was not associated with inter-individual variation in fatigue resistance. Specifically, maximal (sprint) lateral gastrocnemius muscle performance was positively associated with both high anaerobic ATP production capacity and rapid Ca^{2+} release from the sarcoplasmic reticulum stores by ryanodine receptors. Conversely, increased SERCA2 mRNA concentration was associated with longer muscle relaxation times, and inhibition of total SERCA activity significantly decreased fatigue resistance.

In agreement with our first hypothesis, there was a trade-off between sprint and endurance performance. Previous studies have demonstrated a similar trade-off in skeletal muscle isolated from frog (*Xenopus laevis*) peroneus muscle (Wilson et al., 2002), toad (*Bufo viridis*) gastrocnemius muscle (Wilson et al., 2004) and mouse (*Mus musculus*) extensor digitorum longus muscle (Wilson and James, 2004). These previous studies have used the work loop technique to demonstrate that individuals with lower muscle fatigue resistance (measured as maintenance of work loop force or power) had higher work loop maximum force and power output. The consequence of such a skeletal muscle sprint–endurance trade-off is that vertebrate skeletal muscle cannot be optimized to produce both high force (or power) and high fatigue resistance. Therefore, in different species, specific muscle groups are likely to be tuned to greater sprint or endurance performance (different points on a sprint–endurance continuum) depending on the locomotory demands of the ecological niche of the species (Vanhooydonck et al., 2001). For example, species that rely on brief sprints to capture prey and to escape from predation are more likely to have muscle better suited for sprint performance in the relevant muscle groups. In contrast, species that rely on endurance performance, for example for migration, are more likely to have muscle better suited to endurance in the relevant muscle groups.

Metabolic capacity and muscle mechanics

We hypothesized that variation in metabolic capacity between muscles would be associated with both variation in endurance muscle performance and variation in maximal force production. However, the associations between metabolic capacity and muscle mechanics were less clear than stated in our original hypotheses. Although variation in lactate dehydrogenase activity between muscles was significantly associated with variation in maximal force generation, variation in oxidative capacity was not correlated with variation in sustained muscle performance (fatigue resistance). The most parsimonious explanation is that oxidative ATP production was not limiting in our experimental conditions. It is possible, however, that oxidative ATP production can become limiting under natural conditions. In particular, failure to meet nutritional requirements and changes in thermal conditions could compromise mitochondrial function and thereby muscle performance (Seebacher et al., 2010). An experimental test of these relationships involving dietary and thermal treatments in live animals followed by analysis of muscle mechanics and biochemistry would significantly advance understanding of the relationship between energy metabolism and

Table 2. Effect of 5 min incubation in 10 μmol l^{-1} thapsigargin on mechanical properties of rat lateral gastrocnemius muscle

	Thapsigargin	Control	*P*
Acute peak tetanus force production (%)	87.6±4.0	87.3±3.7	0.92
Fatigue resistance (%)	56.3±5.3	68.1±4.9	*0.006*
Rate of tetanus stress production 1 (kN m^{-2} ms^{-1})	4.40±0.54	3.56±0.48	0.21
Rate of tetanus stress relaxation 1 (kN m^{-2} ms^{-1})	1.23±0.14	1.12±0.15	0.61

Values are means ± s.e.; *P*, Student's paired *t*-test. *N*=9. *P*-values in italics are significant ($P<0.05$).

Acute force production represents peak tetanus force of the 1st tetanus in a fatigue run as a percentage of the pre-incubation value. Fatigue resistance represents the peak tetanus force of the 25th tetanus in a fatigue run as a percentage of that of the first tetanus in a fatigue run. Rate of tetanus stress production 1 was calculated post-incubation from the first tetanus in the fatigue run. Rate of tetanus stress relaxation 1 was calculated post-incubation from the first tetanus in the fatigue run.

Table 3. Effect of 10 min incubation in 10 μmol l^{-1} dantrolene on mechanical properties of rat lateral gastrocnemius muscle

	Dantrolene	Control	*P*
Acute peak tetanus force production (%)	68.6±4.2	88.4±2.2	*<0.001*
Fatigue resistance (%)	64.8±5.0	53.3±2.2	0.12
Rate of tetanus stress production 1 (kN m^{-2} ms^{-1})	2.77±0.33	6.22±0.32	*<0.001*
Rate of tetanus stress production 25 (kN m^{-2} ms^{-1})	1.80±0.16	4.65±0.38	*<0.001*
Rate of tetanus stress relaxation 1 (kN m^{-2} ms^{-1})	1.06±0.14	2.02±0.15	*0.001*

Values are means ± s.e.; *P*, Student's paired *t*-test. *N*=9. *P*-values in italics are significant ($P<0.05$).

Acute force production represents peak tetanus force of the first tetanus in a fatigue run as a percentage of the pre-incubation value. Fatigue resistance represents the peak tetanus force of the 25th tetanus in a fatigue run as a percentage of that of the first tetanus in a fatigue run. Rate of tetanus stress production 1 was calculated post-incubation from the first tetanus in the fatigue run. Rate of tetanus stress production 25 was calculated post-incubation from the 25th tetanus in the fatigue run. Rate of tetanus stress relaxation 1 was calculated post-incubation from the first tetanus in the fatigue run.

muscle physiology. In any case, our findings demonstrate that the widely used systems for classification of muscle fibre type may be too simplistic to predict muscle performance. The basic systems for classification of muscle fibre types are based upon differences in glycolytic and oxidative metabolic capacities, where slow, endurance fibres are thought to be more oxidatively poised and fast fibres have greater glycolytic capacity (Bottinelli and Reggiani, 2000). Nonetheless, the molecular traits used to categorize muscle fibre types often do not correspond to the expected patterns of muscle performance and sprint–endurance trade-offs (Gibb and Dickson, 2002; James et al., 2005; Plomgaard et al., 2006; Seebacher and James, 2008). Our data indicate that the reason for this may be that actual muscle contractile performance is determined not only by metabolic capacity but also by interactions between ecological factors and multiple molecular traits.

Calcium cycling and muscle mechanics

We hypothesized that inter-individual variation in the dynamics of calcium handling would be associated with variation in muscle mechanics. We found that muscles with greater SERCA2 mRNA concentrations had longer relaxation times. However, total SERCA activity did not influence muscle mechanics, such that SERCA2 mRNA concentrations were not indicative of total SERCA activity. A possible reason for this is that SERCA activity is regulated by phospholamban, and unphosphorylated phospholamban reduces SERCA activity (Verboomen et al., 1992). Phosphorylation of phospholamban by protein kinase A or calmodulin kinase is therefore a principal pathway for regulation of SERCA activity (East, 2000; Gustavsson et al., 2011). Phosphorylation of phospholamban is at least partly under sympathetic control *via* increases in cAMP concentration following stimulation of β_2-adrenoreceptors (McCormick et al., 2010). Hence, different levels of phospholamban phosphorylation will influence SERCA activity beyond protein and mRNA concentrations. More definite support for a role of SERCA in muscle performance and sprint–endurance trade-offs comes from our pharmacological studies: SERCA inhibition with thapsigargin reduced fatigue resistance, but did not influence tetanic force production. These findings were paralleled by the near-significant correlation between SERCA activity and fatigue resistance. Interestingly, inhibition of ryanodine receptors with dantrolene had the opposite effect; that is, it reduced force production but did not affect fatigue resistance. These different roles of Ca^{2+} release and re-sequestration mechanisms may partly explain the trade-off between muscle endurance and sprint performance, particularly because there is evidence from cardiac muscle that increases in SERCA1 expression cause concomitant decreases in ryanodine receptor and L-type Ca^{2+} channel density and activity (Lalli et al., 2001; Periasamy and Kalyanasundaram, 2007). Additionally, high ryanodine receptor activity is advantageous for fast muscle contraction, but it also leads to Ca^{2+} store depletion, which can accelerate muscle fatigue (Allen et al., 2008). High ryanodine receptor activity would require increased rates of Ca^{2+} re-sequestration into the sarcoplasmic reticulum to decrease relaxation times and thereby maintain muscle performance over time. High ryanodine receptor activity may therefore be incompatible with high SERCA2 concentrations, because our results and those of others (Sumbilla et al., 1999) indicate that elevated SERCA2 concentrations slow muscle relaxation. Hence, there is a functional trade-off between ryanodine receptor activity and both SERCA 1 and 2 isoforms.

The results from our dantrolene treatment are consistent with the negative correlation between ryanodine receptor mRNA concentration and contraction times, indicating that in this case mRNA concentration is representative of receptor activity. An increased density of ryanodine receptors in muscle has previously been correlated with increased shortening velocity and force in isolated muscle (Kandarian et al., 1992; Mänttäri and Järvilehto, 2005), and has been associated with training-induced improvements in trout endurance swimming performance (Anttila et al., 2008). Use of dantrolene to block ryanodine receptors in rat lateral gastrocnemius muscle demonstrated

the importance of the calcium release channel in affecting rates of tetanus stress production. Dantrolene is used clinically in humans to inhibit calcium release *via* ryanodine receptors from the sarcoplasmic reticulum, for example to reduce the likelihood of death of malignant hyperthermia patients during anaesthesia (Mackrill, 2010). Dantrolene is also used clinically to reduce the incidence of the skeletal muscle syndrome exertional rhabdomyolysis in racehorses (Edwards et al., 2003). Therefore, these drug treatments highlight the importance of SERCA activity and ryanodine receptors in influencing inter-individual differences in sprint and endurance performance. The next step is to determine the significance of these mechanisms for the sprint–endurance trade off at the level of the whole animal, which could provide the mechanistic explanation for sporting success and inter-individual variation in ecologically important measures of performance.

ACKNOWLEDGEMENTS

Thanks to two anonymous referees whose comments helped to improve this manuscript.

FUNDING

This research was supported by an Australian Research Council grant to F.S. R.S.J. was supported by a Royal Society International Short Visit grant.

REFERENCES

Allen, D. G., Lamb, G. D. and Westerblad, H. (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiol. Rev.* **88**, 287-332.
Anttila, K., Järvilehto, M. and Mänttäri, S. (2008). The swimming performance of brown trout and whitefish: the effects of exercise on Ca^{2+} handling and oxidative capacity of swimming muscles. *J. Comp. Physiol. B* **178**, 465-475.
Berchtold, M. W., Brinkmeier, H. and Müntener, M. (2000). Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. *Physiol. Rev.* **80**, 1215-1265.
Bonting, S. L., Simon, K. A. and Hawkins, N. M. (1961). Studies on sodium-potassium-activated adenosine triphosphatase. I. Quantitative distribution in several tissues of the cat. *Arch. Biochem. Biophys.* **95**, 416-423.
Bottinelli, R. and Reggiani, C. (2000). Human skeletal muscle fibres: molecular and functional diversity. *Prog. Biophys. Mol. Biol.* **73**, 195-262.
Dickinson, M. H., Farley, C. T., Full, R. J., Koehl, M. A. R., Kram, R. and Lehman, S. (2000). How animals move: an integrative view. *Science* **288**, 100-106.
East, J. M. (2000). Sarco(endo)plasmic reticulum calcium pumps: recent advances in our understanding of strucutre/function and biology. *Mol. Membr. Biol.* **17**, 189-200.
Edwards, J. G. T., Newton, J. R., Ramzan, P. H. L., Pilsworth, R. C. and Shepherd, M. C. (2003). The efficacy of dantrolene sodium in controlling exertional rhabdomyolysis in the thoroughbred racehorse. *Equine Vet. J.* **35**, 707-711.
Farah, C. S. and Reinach, F. C. (1995). The troponin complex and regulation of muscle contraction. *FASEB J.* **9**, 755-767.
Fleming, J. R., Crockford, T., Altringham, J. D. and Johnston, I. A. (1990). Effects of temperature acclimation on muscle relaxation in the carp: a mechanical, biochemical, and ultrastructural study. *J. Exp. Zool.* **255**, 286-295.
Fruen, B. R., Mickelson, J. R. and Louis, C. F. (1997). Dantrolene inhibition of sarcoplasmic reticulum Ca^{2+} release by direct and specific action at skeletal muscle ryanodine receptors. *J. Biol. Chem.* **272**, 26965-26971.
Galli, G. L. J., Taylor, E. W. and Shiels, H. A. (2006). Calcium flux in turtle ventricular myocytes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **291**, R1781-R1789.
Gibb, A. C. and Dickson, K. A. (2002). Functional morphology and biochemical indices of performance: is there a correlation between metabolic enzyme activity and swimming performance? *Integr. Comp. Biol.* **42**, 199-207.
Golden, K. L., Marsh, J. D., Jiang, Y., Brown, T. and Moulden, J. (2003). Gonadectomy of adult male rats reduces contractility of isolated cardiac myocytes. *Am. J. Physiol. Endocrinol. Metab.* **285**, E449-E453.
Gustavsson, M., Traaseth, N. J., Karim, C. B., Lockamy, E. L., Thomas, D. D. and Veglia, G. (2011). Lipid-mediated folding/unfolding of phospholamban as a regulatory mechanism for sarcoplasmic reticulum Ca^{2+}-ATPase. *J. Mol. Biol.* **408**, 755-765.
Irschick, D. J. and Losos, J. B. (1998). A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean anolis lizards. *Evolution* **52**, 219-226.
James, R. S., Wilson, R. S., De Carvalho, J. E., Kohlsdorf, T., Gomes, F. R. and Navas, C. A. (2005). Interindividual differences in leg muscle mass and pyruvate kinase activity correlate with interindividual differences in jumping performance of *Hyla multilineata*. *Physiol. Biochem. Zool.* **78**, 857-867.
Jayne, B. C. and Bennett, A. F. (1990). Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**, 1204-1229.
Kandarian, S., O'Brien, S., Thomas, K., Schulte, L. and Navarro, J. (1992). Regulation of skeletal muscle dihydropyridine receptor gene expression by biomechanical unloading. *J. Appl. Physiol.* **72**, 2510-2514.
Kurebayashi, N. and Ogawa, Y. (2001). Depletion of Ca^{2+} in the sarcoplasmic reticulum stimulates Ca^{2+} entry into mouse skeletal muscle fibres. *J. Physiol.* **533**, 185-199.
Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.
Lalli, M. J., Yong, J., Prasad, V., Hashimoto, K., Plank, D., Babu, G. J., Kirkpatrick, D., Walsh, R. A., Sussman, M., Yatani, A. et al. (2001). Sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) 1a structurally substitutes for SERCA2a in the cardiac sarcoplasmic reticulum and increases cardiac Ca^{2+} handling capacity. *Circ. Res.* **89**, 160-167.
Le Galliard, J. F., Clobert. J. and Ferrière, R. (2004). Physical performance and Darwinian fitness in lizards. *Nature* **432**, 502-505.
Mackrill, J. J. (2010). Ryanodine receptor calcium channels and their partners as drug targets. *Biochem. Pharmacol.* **79**, 1535-1543.
Mänttäri, S. and Järvilehto, M. (2005). Comparative analysis of mouse skeletal muscle fibre type composition and contractile responses to calcium channel blocker. *BMC Physiol.* **5**, 4.
McCormick, C., Alaxanre, L., Thompson, J. and Mutungi, G. (2010). Clenbuterol and formoterol decrease force production in isolated intact mouse skeletal muscle fibre bundles through a β_2-adrenoreceptor-independent mechanisms. *J. Appl. Physiol.* **109**, 1716-1727.
Méndez, J. and Keys, A. (1960). Density and composition of mammalian muscle. *Metabolism* **9**, 184-188.
Periasamy, M. and Kalyanasundaram, A. (2007). SERCA pump isoforms: their role in calcium transport and disease. *Muscle Nerve* **35**, 430-442.
Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **29**, e45.
Piersma, T. (1998). Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight? *J. Avian Biol.* **29**, 511-520.
Plomgaard, P., Penkowa, M., Leick, L., Pedersen, B. K., Saltin, B. and Pilegaard, H. (2006). The mRNA expression profile of metabolic genes relative to MHC isoform pattern in human skeletal muscles. *J. Appl. Physiol.* **101**, 817-825.
Ramnanan, C. J. and Storey, K. B. (2008). The regulation of thapsigargin-sensitive sarcoendoplasmic reticulum Ca^{2+}-ATPase activity in estivation. *J. Comp. Physiol. B* **178**, 33-45.
Rupert, J. L. (2003). The search for genotypes that underlie human performance phenotypes. *Comp. Biochem. Physiol.* **136A**, 191-203.
Seebacher, F. and James, R. S. (2008). Plasticity of muscle function in a thermoregulating ectotherm (*Crocodylus porosus*): biomechanics and metabolism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, 1024-1032.
Seebacher, F., Guderley, H., Elsey, R. M. and Trosclair, P. L., 3rd (2003). Seasonal acclimatisation of muscle metabolic enzymes in a reptile (*Alligator mississippiensis*). *J. Exp. Biol.* **206**, 1193-1200.
Seebacher, F., Brand, M. D., Else, P. L., Guderley, H., Hulbert, A. J. and Moyes, C. D. (2010). Plasticity of oxidative metabolism in variable climates: molecular mechanisms. *Physiol. Biochem. Zool.* **83**, 721-732.
St-Pierre, J., Charest, P. and Guderley, H. (1998). Relative contribution of quantitative and qualitative changes in mitochondria to metabolic compensation during seasonal acclimatisation of rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* **201**, 2961-2970.
Sumbilla, C., Cavagna, M., Zhong, L., Ma, H., Lewis, D., Farrance, I. and Inesi, G. (1999). Comparison of SERCA1 and SERCA2a expressed in COS-1 cells and cardiac myocytes. *Am. J. Physiol.* **277**, H2381-H2391.
Van Damme, R., Wilson, R. S., Vanhooydonck, B. and Aerts, P. (2002). Evolutionary biology: performance constraints in decathletes. *Nature* **415**, 755-756.
Van Winkle, W. B. (1976). Calcium release from skeletal muscle sarcoplasmic reticulum: site of action of dantrolene sodium? *Science* **193**, 1130-1131.
Vanhooydonck, B., Van Damme, R. and Aerts, P. (2001). Speed and stamina trade-off in lacertid lizards. *Evolution* **55**, 1040-1048.
Verboomen, H., Wuytack, F., De Smedt, H., Himpens, B. and Casteels, R. (1992). Functional differences between SERCA2a and SERCA2b Ca^{2+} pumps and their modulation by phospholamban. *Biochem. J.* **286**, 591-596.
Walter, I. and Seebacher, F. (2009). Endothermy in birds: underlying molecular mechanisms. *J. Exp. Biol.* **212**, 2328-2336.
Wilson, J. A., Kronfeld, D. S., Gay, L. S., Williams, J. H., Wilson, T. M. and Lindinger, M. I. (1998). Sarcoplasmic reticulum responses to repeated sprints are affected by conditioning of horses. *J. Anim. Sci.* **76**, 3065-3071.
Wilson, R. S. and James, R. S. (2004). Constraints on muscular performance: trade-offs between power output and fatigue resistance. *Proc. R. Soc. B* **271**, S222-S225.
Wilson, R. S., James, R. S. and Van Damme, R. (2002). Trade-offs between speed and endurance in the frog *Xenopus laevis*: a multi-level approach. *J. Exp. Biol.* **205**, 1145-1152.
Wilson, R. S., James, R. S., Kohlsdorf, T. and Cox, V. M. (2004). Interindividual variation of isolated muscle performance and fibre-type composition in the toad *Bufo viridus*. *J. Comp. Physiol. B* **174**, 453-459.
Zaykin, D. V., Zhivotovsky, L. A., Westfall, P. H. and Weir, B. S. (2002). Truncated product method for combining P-values. *Genet. Epidemiol.* **22**, 170-185.