Effects of old age on human skeletal muscle energetics during fatiguing contractions with and without blood flow

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We recently reported lower glycolytic flux (ATP_{GLY}) and increased reliance on oxidative ATP synthesis (ATP_{OX}) in contracting muscle of older compared to young humans. To further investigate this age-related difference in the pathways of ATP synthesis, we used magnetic resonance spectroscopy to determine the rates of ATP_{OX}, ATP_{GLY} and net phosphocreatine hydrolysis *in vivo* during maximal muscle contractions under free-flow (FF) and ischaemic (ISC) conditions in the ankle dorsiflexors of 20 young (27 ± 3 years; 10 male, 10 female) and 18 older (70 ± 5 years; 10 male, 8 female) adults. We hypothesized that ATP_{GLY} would be higher in young compared to old during FF contractions, but that old would be unable to increase ATP_{GLY} during ISC to match that of the young, which would suggest impaired glycolytic ATP synthesis with old age. Peak glycolytic flux during FF was lower in older (0.8 ± 0.1 mM ATP s^{-1}) compared to young (1.4 ± 0.1 mM ATP s^{-1}, $P < 0.001$) subjects. During ISC, peak ATP_{GLY} increased in old to a level similar to that of young (1.4 ± 0.2 mM ATP s^{-1}, 1.3 ± 0.2 mM ATP s^{-1}, respectively; $P = 0.86$), suggesting that glycolytic function remains intact in aged muscle *in vivo*. Notably, older adults fatigued less than young during both FF and ISC ($P \leq 0.004$). These results provide novel evidence of unimpaired *in vivo* glycolytic function in the skeletal muscle of older adults during maximal isometric dorsiflexion, and suggest a potential role for differences in metabolic economy and as a result, metabolite accumulation, in the fatigue resistance of the old.

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Although advanced age is commonly accompanied by losses of skeletal muscle mass and strength (Lexell, 1995), the effect of old age on muscle fatigability (i.e. the temporary decline in muscle force production during repeated muscle activity) is less clear. Numerous investigators have demonstrated greater muscle fatigue with advanced age (Davies & White, 1983; Cupido *et al.* 1992). In contrast, others have reported enhanced fatigue resistance with old age in many muscle groups (Narici *et al.* 1991; Bemben *et al.* 1996; Ditor & Hicks, 2000; Kent-Braun *et al.* 2002; Lanza *et al.* 2004). This apparent paradox may be explained in part by morphological changes to ageing muscle. For example, lower mass and strength may reduce intramuscular pressure and blood flow occlusion during contractions in older subjects (Wigmore *et al.* 2004), resulting in relatively greater perfusion during intense contractions in older compared to young muscle. The progressive shift to a greater proportion of oxidative type I muscle (Jakobsson *et al.* 1990) and lower motor unit discharge rates with old age (Kamen *et al.* 1995), may also contribute to an age-related fatigue resistance.

Several research groups have demonstrated age-related alterations in muscle bioenergetics that are consistent with increased resistance to fatigue with ageing. For example, during high intensity muscle contractions, young subjects show greater intramuscular acidification, phosphocreatine (PCr) depletion, and inorganic phosphate (P_i) accumulation than older adults (Coggan *et al.* 1993; Chilibeck *et al.* 1998; Kent-Braun & Ng, 2000). Furthermore, a recent study demonstrated a significant association between fatigue and diprotonated inorganic phosphate ($H_2PO_4^-$) during incremental isometric contractions in which older adults fatigued less than young (Kent-Braun *et al.* 2002). Collectively, these data suggest that age-related fatigue resistance may be related to the manner in which adenosine triphosphate (ATP) is synthesized to meet the energetic demands of muscular activity.

We recently used phosphorous magnetic resonance spectroscopy (^{31}P-MRS) to demonstrate significantly blunted glycolytic flux in older compared to young men during maximal ankle dorsiflexion (Lanza *et al.* 2005). While there are currently no clear explanations for the decreased glycolytic flux with old age, we propose two plausible mechanisms. First, the capacity for glycolytic ATP synthesis may be impaired with old age, as suggested

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by reduced glycolytic enzyme activities observed in older muscle (Larsson *et al.* 1978; Pastoris *et al.* 2000) and reduced lactate production in stimulated hindlimbs of older compared to young rats (Campbell *et al.* 1991; Hepple *et al.* 2004). Alternatively, older muscle may preferentially rely on oxidative phosphorylation due to its ability to adequately meet the energetic demands of contraction without increasing glycolytic flux to the same extent as young muscle, thus minimizing the accumulation of inhibitory metabolites.

The purpose of the present study was to determine whether glycolytic ATP production *in vivo* is impaired with old age. We used ^{31}P-MRS to determine the ATP flux through oxidative phosphorylation, anaerobic glycolysis, and net PCr breakdown during high intensity contractions performed with intact blood flow and during ischaemia where blood flow to the working muscle was restricted, thus limiting oxidative ATP synthesis. We hypothesized that (1) glycolytic flux would be higher in young compared to old during free-flow contractions (FF), (2) old would fatigue less than young during FF, consequent to their greater reliance on oxidative ATP synthesis and blunted metabolite accumulation compared to young, (3) old would be unable to increase glycolytic flux in response to ischaemia (ISC) to the same level of the young, consistent with the notion that glycolytic function is impaired with old age, and (4) old would fatigue more than young during ISC due to their inability to adequately meet the energetic demands of the contractions.

Methods

Subjects

Thirty-eight healthy, non-smoking young (10 male, 10 female, 27 ± 1 years, mean $\pm$ S.E.M.) and older (10 male, 8 female, 70 ± 1 years) subjects were studied. A subset of these data from the young age group ($n = 6$ male, 6 female) has been published previously (Lanza *et al.* 2006). Potential subjects were screened and excluded if they had a history of diabetes, cardiovascular disease, peripheral vascular disease (ankle : brachial systolic blood pressure < 1 (McDermott *et al.* 2001), stroke, or neurological disorders. Subjects taking anti-hypertensive, cardiac, or lipid-lowering medications were excluded due to the potential effects of these drugs on muscle function and blood flow. Each subject provided written informed consent in accordance with the procedures approved by the Human Subjects Review Boards at the University of Massachusetts and Yale University School of Medicine, and conforming to the standards set by the *Declaration of Helsinki*. Subjects were excluded from the study if they engaged in structured exercise more than 20 min per day, twice per week. To quantify physical activity level, each subject wore a uniaxial accelerometer (Manufacturing Technology, Fort Walton Beach, FL, USA) for seven consecutive days during waking hours, while maintaining their usual daily level of physical activity. Activity counts were averaged over a 5 day period and used to verify that activity levels were similar across age groups.

Experimental design

We used ^{31}P-MRS to measure intracellular concentrations of PCr, P_i, ATP, and phosphomonoesters (PME) in the dorsiflexors during three contraction protocols. First, oxidative capacity was determined from the kinetics of PCr recovery following a 16 s isometric maximal voluntary contraction (MVC) (Conley *et al.* 2000; Lanza *et al.* 2005). Next, the rates of ATP synthesis from oxidative phosphorylation, anaerobic glycolysis, and net PCr breakdown in the creatine kinase reaction were determined during an intermittent MVC protocol; once with intact blood flow and once with circulatory occlusion (Kemp *et al.* 1994; Lanza *et al.* 2006).

Subjects were tested on two occasions, separated by at least 48 h. The first visit consisted of a habituation session at the University of Massachusetts, where subjects were familiarized with the contraction protocols, and their ability to fully activate the dorsiflexor muscles was confirmed during an MVC. The second visit took place at the Magnetic Resonance Research Center at Yale University, where subjects performed the contraction protocols with metabolic measures using ^{31}P-MRS.

Habituation session

Subjects were positioned supine with either their left or their right foot (randomized) secured to a plate (ankle angle $\sim$120 deg) interfaced with a strain gauge for the measurement of dorsiflexion force (Kent-Braun *et al.* 2002). Electrodes for muscle stimulation and surface electromyography were attached to the lower leg to assess central activation, as previously described (Kent-Braun & Le Blanc, 1996). Subjects performed three baseline MVCs (3–4 s duration, 2 min recovery between each), the highest of which was taken as their isometric strength. Subjects were verbally encouraged and received visual force feedback from a series of light-emitting diodes.

Completeness of voluntary activation of the muscle was assessed during the final baseline MVC by superimposing a train (50 Hz, 500 μs pulse, 500 ms train duration) of supramaximal electrical stimuli. A central activation ratio (CAR) was calculated as MVC divided by the sum of MVC and any additional force produced by the superimposed train. A CAR less than 1.0 indicates incomplete voluntary activation. Two minutes following baseline MVC and CAR measures, subjects performed a contraction protocol consisting of six MVCs, each of 12 s duration, with 12 s rest

intervals between contractions. Verbal encouragement and visual feedback were provided.

The contralateral leg was tested in the same manner, but with circulatory occlusion to the lower leg induced 30 s prior to the initiation of the intermittent MVC protocol. Lower leg ischaemia was maintained by inflating a cuff around the proximal thigh to a pressure of 220 Torr using a rapid cuff inflator (Hokanson, Inc. Belleview, WA, USA). During the habituation and metabolic testing sessions, the free-flow protocol (FF) was always performed before the ischaemic protocol (ISC) to maximize subject compliance and minimize any effects of ischaemia on subsequent contractions. The protocols were randomized as to which protocol was performed by each leg.

The analog signal from dorsiflexion force was acquired and digitized at 500 Hz (DAQ pad 6020E, National Instruments, Austin, TX, USA). Custom data-processing programs (Matlab, Mathworks, Inc, Novi, MI, USA) were used to determine baseline strength and the force–time integrals (FTI) for each contraction during FF and ISC. Fatigue was calculated as the relative decline in FTI:

$$\mathrm{FTI}(\%) = \mathrm{FTI}/\mathrm{FTI}_{\mathrm{initial}} \times 100 \quad (1)$$

Metabolic testing

Subjects were instructed to avoid strenuous physical activity and alcohol during the 24 h preceding the lab visit, and to abstain from caffeine for 6 h prior to testing. Following a minimum of 4 h of fasting and ∼30 min prior to testing, each subject consumed a supplement bar (22 g carbohydrate, 6 g fat, 15 g protein) to standardize caloric intake. Subjects were positioned supine on the bed of a 4.0 tesla superconducting magnet (Bruker Biospin, Rheinstetten, Germany) with one leg affixed to an exercise apparatus, as described for the habituation session. A probe assembly consisting of a 6 cm diameter ^{1}H surface coil and a coplanar 3×5 cm elliptical ^{31}P surface coil was secured over the tibialis anterior muscle, which is the main dorsiflexor muscle. The subject was then positioned in the isocentre of the magnet, as confirmed using gradient-echo scout images. Magnetic field homogeneity was optimized by localized shimming on the proton signal of tissue water (width at half-maximal height of PCr $= 11.1 \pm 0.7$ Hz, $n = 38$). Prior to acquisition of MRS data, subjects performed two MVCs (3–4 s duration) separated by 2 min of rest to establish MVC force for that day.

Muscle oxidative capacity

Two minutes following the baseline MVCs, subjects performed a sustained MVC for 16 s. Phosphorous data were acquired (125 μs hard pulse, nominal 60 deg flip angle, 2 s repetition time, 2048 data points, 8000 Hz spectral width) during a 1 min rest interval prior to the contraction, during the contraction, and throughout a 10 min recovery period. Individual FIDs were averaged to yield 4 s resolution during the contraction, 8 s resolution during the first 5 min of recovery, and 30 s resolution during the last 5 min of recovery. Spectral analysis and peak integration were performed using NUTS software (Acorn NMR, Livermore, CA, USA) as described elsewhere (Lanza *et al.* 2005; Lanza *et al.* 2006). Phosphocreatine recovery following the contraction was fitted by a mono-exponential curve from which the rate constant, k_{PCr}, was used to calculate muscle oxidative capacity (Q_{max}, mM ATP s^{-1}) (Meyer, 1989; Conley *et al.* 2000):

$$Q_{\mathrm{max}} = [\mathrm{PCr}_{\mathrm{rest}}] \times k_{\mathrm{PCr}} \quad (2)$$

Free-flow ATP flux

Immediately following the oxidative capacity measure, subjects performed the FF contraction protocol (as described in habituation session) with simultaneous measures of muscle force and phosphorous metabolites. The ^{31}P-MRS measures were acquired 1 min before, during, and 10 min following the contraction protocol, with identical parameters to those described for muscle oxidative capacity. The rate of oxidative ATP synthesis ($\mathrm{ATP}_{\mathrm{OX}}$, mM s^{-1}) was determined from the rate of PCr recovery during the rest intervals between contractions (Boska, 1991; Kemp & Radda, 1994):

$$\mathrm{ATP}_{\mathrm{OX}} = \mathrm{dPCr}/\mathrm{d}t \quad (3)$$

Glycolytic flux ($\mathrm{ATP}_{\mathrm{GLY}}$, mM s^{-1}) was determined from the changes in pH and phosphorous metabolites during each 12 s contraction as previously described (Lanza *et al.* 2006). Intramuscular pH was calculated based on the chemical shift (σ) of $\mathrm{P_i}$ relative to PCr (Hoult *et al.* 1974). When distinct $\mathrm{P_i}$ splitting was evident, the pH corresponding to each $\mathrm{P_i}$ pool was calculated separately as previously described (Lanza *et al.* 2006). The pH change, after correcting for proton efflux (υ_{eff}), buffering capacity (β), the consumption of protons by the creatine kinase reaction (θ), and the small contribution from oxidative metabolism (m), reflects glycolytic flux (Walter *et al.* 1999; Kemp & Radda, 1994; Lanza *et al.* 2006):

$$\mathrm{ATP}_{\mathrm{GLY}} = 1.5(-\beta(\mathrm{dpH}/\mathrm{d}t) + \theta(\mathrm{dPCr}/\mathrm{d}t) - \mathrm{mATP}_{\mathrm{OX}} + \upsilon_{\mathrm{eff}}) \quad (4)$$

The rate of ATP synthesis from the net breakdown of PCr via the CK reaction ($\mathrm{ATP}_{\mathrm{CK}}$, mM s^{-1}) was determined from the change in PCr during each contraction. Since

the synthesis of ATP is stoichiometric with the hydrolysis of PCr in the creatine kinase reaction, the calculation of ATP_{CK} takes the simple form (Kemp & Radda, 1994):

$$ATP_{CK} = dPCr/dt \quad (5)$$

The overall rate of ATP turnover (ATP_{TOT} mM s^{-1}) was determined as the sum of the flux through the three pathways:

$$ATP_{TOT} = ATP_{OX} + ATP_{GLY} + ATP_{CK} \quad (6)$$

Ischaemic ATP flux

Subjects were removed from the magnet after the FF protocol and allowed to rest for ∼1 h before performing the baseline MVCs, 16 s MVC, and ISC protocol on the opposite leg, as described above. The cuff was released ∼5 s following the completion of the final contraction. During ISC, ATP_{OX} was assumed to be negligible based on studies showing that PCr does not recover following ischaemic contractions until the cuff is released, indicating that complete vascular occlusion is accomplished using cuff pressures similar to those used in the present study (Quistorff *et al.* 1993; Nakagawa *et al.* 2005). Furthermore, oxygen trapped within the muscle during cuff occlusion would be sufficient to generate only ∼4 mM ATP (Harris *et al.* 1975; Kemp *et al.* 1994), an amount that would be exhausted within ∼10 s during an MVC.

Glycolytic flux was calculated in a similar manner to that described for FF contractions, except that proton efflux and oxidative proton production were considered to be negligible:

$$ATP_{GLY} = 1.5\,(-\beta\,(dpH/dt) + \theta\,(dPCr/dt)) \quad (7)$$

The rate of ATP synthesis from PCr hydrolysis was calculated as described for the FF protocol, as was total ATP flux.

Spectral analyses

Free-induction decays were Fourier-transformed following 10 Hz line broadening. The resulting spectra were phased manually, and the underlying broad peak due to phosphorous in bone was removed by fitting a 5th order polynomial to the baseline region and subtracting this baseline from each spectrum. Peaks corresponding to PCr, PME, P_i, and γ-, α- and β-ATP were then fitted with Lorentzian-shaped curves (NUTS software) to quantify the area of each peak. When two distinct P_i peaks were observed, two Lorentzian-shaped curves were used, as previously described (Lanza *et al.* 2005, 2006). Corrections for partial saturation of each metabolite were applied (Lanza *et al.* 2005). Millimolar concentrations of phosphorous metabolites were calculated assuming that [PCr] + [Cr] = 42.5 mM and resting [ATP] = 8.2 mM (Harris *et al.* 1974), as previously described (Lanza *et al.* 2005, 2006).

Statistical analyses

The data were first examined for possible effects of sex and age-by-sex interactions on strength, fatigue, Q_{MAX}, ATP_{OX}, ATP_{GLY}, ATP_{CK} and ATP_{TOT} during FF and ISC using linear models, with sex treated as a categorical variable and age treated as a continuous variable. There were no significant effects of sex nor any age × sex interactions ($P > 0.05$), so all data were collapsed by sex and compared across age groups.

Our primary hypotheses were tested using a three factor (age, condition, time) repeated measures ANOVA to compare ATP_{GLY} across age groups during FF and ISC, and a two factor (age, condition) repeated measures ANOVA to examine fatigue across age groups during FF and ISC. In addition, Student's *t* test for unpaired data was used to compare baseline [PCr], [P_i], [ADP], [AMP], [PME], [ATP], [$H_2PO_4^-$], pH, MVC, Q_{max} and peak ATP_{GLY} across age groups. The non-normal distribution of CAR values necessitated non-parametric analyses using the Mann–Whitney *U* test.

To fully evaluate the metabolic response to the protocols, two-factor (age, condition) repeated measures ANOVA was used to compare end-exercise [PCr], [P_i], [ADP], [AMP], [PME], [ATP], [$H_2PO_4^-$], pH and fatigue in young and older subjects during FF and ISC. Two factor (age, time) repeated measures ANOVA was used to examine ATP_{OX} during the FF condition. Three factor (age, condition, time) repeated measures ANOVA was used to compare ATP_{CK} and ATP_{TOT} across age groups during FF and ISC. The appropriate covariance structures were defined for each analysis. *Post hoc* pairwise comparisons were conducted using Tukey's procedure where significant age-by-condition interactions were evident.

The relationship between FTI (% initial) and $H_2PO_4^-$ was determined for each subject using linear regression analysis. All analyses were performed using SAS software (SAS Institute, Inc., Cary, NC, USA). Data are presented as means ± S.E.M. with significance established at $P < 0.05$.

Results

Subject characteristics

The young and older groups were similar in height (172 ± 2, 171 ± 3 cm, respectively), mass (69 ± 3, 77 ± 4 kg, respectively), and physical activity level (265 ± 19, 295 ± 31 counts day^{-1} 1000^{-1}, respectively).

Table 1. Intracellular pH and metabolite concentrations measured prior to FF and ISC protocols

	Free-flow			Ischaemia		
Metabolites at rest	Young	Older	*P*	Young	Older	*P*
[PCr] (mM)	38.1 ± 0.2	38.2 ± 0.2	0.73	38.2 ± 0.3	37.8 ± 0.3	0.38
[P_i] (mM)	4.4 ± 0.2	4.3 ± 0.2	0.73	4.3 ± 0.3	4.7 ± 0.3	0.38
pH	7.00 ± 0.01	7.00 ± 0.01	0.95	7.02 ± 0.01	7.02 ± 0.00	0.60
$H_2PO_4^-$ (mM)	2.1 ± 0.6	1.5 ± 0.1	0.39	1.5 ± 0.1	1.6 ± 0.1	0.42
PME (mM)	2.9 ± 0.4	2.9 ± 0.3	0.97	2.7 ± 0.3	2.7 ± 0.2	0.99
ADP (mM)	0.008 ± 0.000	0.007 ± 0.007	0.46	0.008 ± 0.001	0.009 ± 0.001	0.36
AMP (μM)	0.007 ± 0.001	0.006 ± 0.001	0.60	0.007 ± 0.001	0.009 ± 0.002	0.38

Values are means ± S.E.M. *P*-values reflect comparisons across age groups using unpaired *t* tests.

Muscle oxidative capacity

Resting [PCr] was similar across age groups (Table 1). Young and older subjects depleted PCr to similar levels during the 16 s MVC prior to FF (young = 22.5 ± 0.6 mM, older = 23.5 ± 0.6) and ISC (young = 23.5 ± 0.5 mM, older = 23.6 ± 0.5). The rate constants of PCr recovery following the 16 s MVC were similar across the two legs (data not shown). Therefore, age-group comparisons of Q_{max} were performed using each subject's average value for the two trials. The average rate constant of PCr recovery was similar in young (0.027 ± 0.002) and older subjects (0.031 ± 0.001), as was oxidative capacity (Fig. 1).

Force and voluntary activation

Isometric strength (MVC) was similar in both age groups for the right (young, Y, 303 ± 22; older, O, 285 ± 14 N) and left legs (Y, 301 ± 20; O, 301 ± 17 N). All subjects, regardless of age, were able to achieve full voluntary activation of the dorsiflexors during baseline MVCs, as indicated by the CAR measure in the right (Y, 0.99 ± 0.01; O, 0.99 ± 0.00) and left legs (Y, 1.00 ± 0.00; O, 0.99 ± 0.01). Central activation was measured during the habituation sessions, but not during the metabolic testing sessions. For all habituation and testing protocols, subjects were well-motivated and received consistent instruction, verbal encouragement, and visual feedback during each contraction. Thus, we have no reason to expect that central activation was different across the two testing sessions. Young subjects fatigued more than older subjects during FF contractions (Fig. 2). All subjects fatigued more during ISC compared to FF contractions, with the older group once again fatiguing less than the young during this protocol (Fig. 2, $P_{age} < 0.001$, $P_{condition} < 0.001$, $P_{age\text{-}by\text{-}condition} = 0.20$).

Metabolite changes during FF and ISC

Resting pH and concentrations of all phosphorus metabolites were similar across age groups prior to FF and ISC (Table 1). Phosphocreatine decreased and P_i increased more in young compared to older subjects during FF but not during ISC (Fig. 3, Table 2). Intracellular pH declined more in young compare to old during FF and ISC, although there was a trend toward a significant age-by-condition interaction (Table 2), suggesting that the difference in pH between young and old was less pronounced during ISC compared to FF (Fig. 4, Table 2). Similarly, diprotonated inorganic phosphate increased more in young than older subjects during both protocols, with a trend toward a significant age-by-condition interaction (Table 2), suggesting a greater age-related difference in [$H_2PO_4^-$] in FF than ISC (Table 2). A trend toward greater phosphomonoester accumulation in young compared to older subjects was observed during FF and ISC (Table 2). Both ADP and AMP increased to a greater extent during ISC compared to FF, but these changes were similar in young and older subjects (Table 2). ATP did not change in either group during FF or ISC (data not shown).

To examine the role of intracellular metabolic changes in fatigue, the relationship between FTI (% initial) and $H_2PO_4^-$ (mM) was determined for each subject (Fig. 5). These regression analyses revealed strong linear

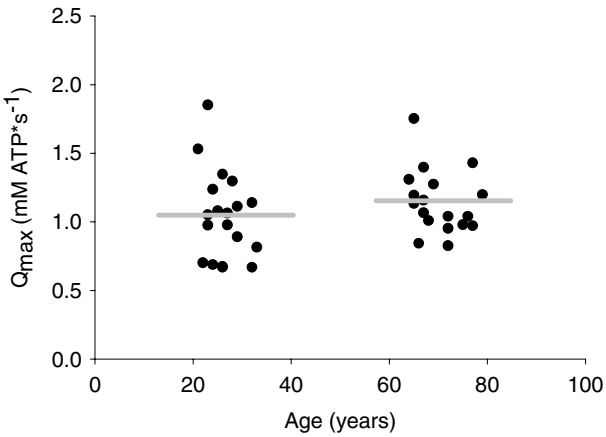

Figure 1. Muscle oxidative capacity (Q_{max}), determined from PCr recovery kinetics, was similar in older compared to young subjects
Data points represent individual subjects. Horizontal grey bars represent the mean values for each group.

associations between FTI and intracellular $H_2PO_4^-$ during FF in young ($r = 0.88 \pm 0.05$) and older ($r = 0.82 \pm 0.07$) subjects. Linear relationships were found also during ISC in young ($r = 0.90 \pm 0.05$) and older ($r = 0.82 \pm 0.06$) individuals.

ATP synthesis during FF and ISC

ATP_{OX} increased with time during FF contractions ($P_{time} = 0.004$) to a similar extent in young and older subjects (Fig. 6*A*). ATP_{CK} decreased similarly in young and older subjects during FF and ISC (Fig. 6*B*), and to a greater extent in ISC than FF ($P_{condition\text{-}by\text{-}time} < 0.001$). ATP_{GLY} increased with time during FF and ISC ($P_{time} < 0.001$, Fig. 6*C*). A significant age-by-condition interaction ($P_{age\text{-}by\text{-}condition} = 0.013$) indicated that ATP_{GLY} was lower in older compared to young subjects during FF ($P < 0.001$), but similar across age groups during ISC. Peak ATP_{GLY} was higher ($P < 0.001$) in young (1.4 ± 0.1 mM ATP s^{-1}) than older subjects (0.8 ± 0.1 mM ATP s^{-1}) during FF, but similar in both age groups during ISC (1.3 ± 0.2 mM ATP s^{-1}, 1.4 ± 0.2 mM ATP s^{-1}, respectively; $P = 0.86$). The overall ATP synthesis rate (ATP_{TOT}) was higher in young than older subjects during FF but not during ISC, as revealed by a significant age-by-condition interaction ($P_{age\text{-}by\text{-}condition} = 0.037$) and *post hoc* comparisons across age groups within both conditions (Fig. 6*D*). A significant condition-by-time interaction was observed for this variable ($P_{condition\text{-}by\text{-}time} < 0.001$), indicating that ATP_{TOT} decreased more in both age groups as the contractions progressed during ISC compared to FF.

Discussion

Despite blunted glycolytic flux in older compared to young adults during FF, older adults increased glycolytic flux to a level similar to that of young during ISC, when oxidative phosphorylation was no longer a viable source

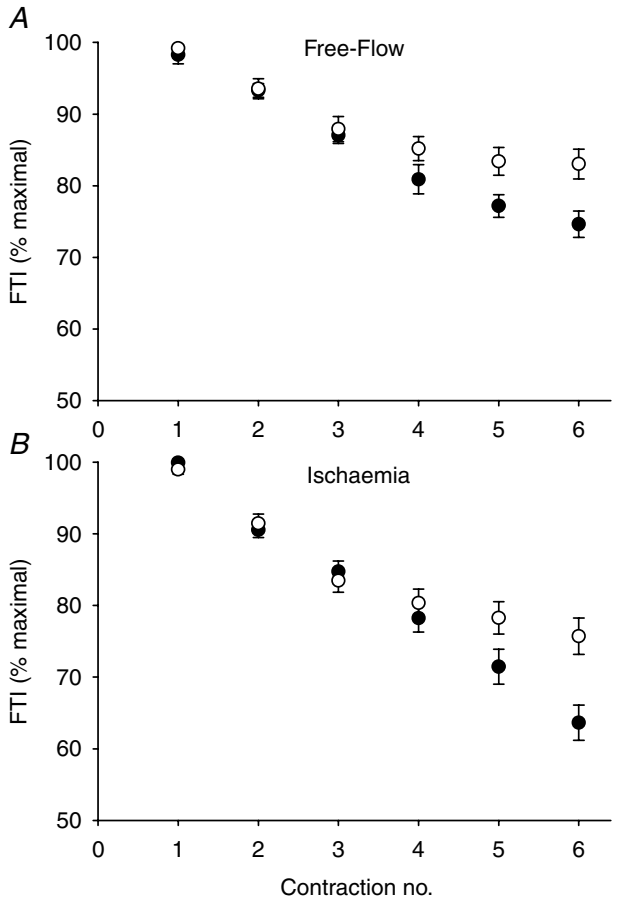

Figure 2. Changes in FTI during FF and ISC
The relative decline of the force–time integral during free-flow (*A*) and ischaemic (*B*) contractions in young (●) and older (○) subjects. Young fatigued more than older subjects during FF and ISC contractions. Data are presented as means ± S.E.M.

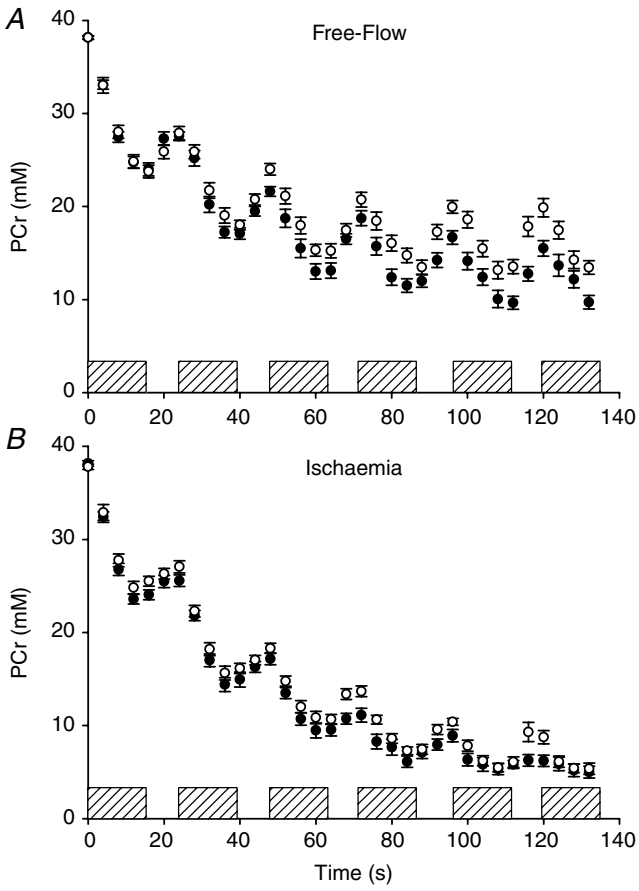

Figure 3. Phosphocreatine during FF and ISC protocols
A, phosphocreatine declined more in young (●) compared to older (○) subjects during free-flow contractions. *B*, similar PCr breakdown was observed across age groups during ischaemic contractions. Hatched bars represent each 12 s MVC. Data are presented as means ± S.E.M.

Table 2. Intracellular pH and metabolite concentrations measured at the end of FF and ISC

Metabolites at end-exercise	Free-flow		Ischaemia		*P* (age)	*P* (cond)	*P* (age-by-cond)
	Young	Older	Young	Older			
[PCr] (mM)	9.7 ± 0.7†	13.4 ± 0.7*†	5.0 ± 0.6	5.4 ± 0.7	—	—	<0.01
[P_i] (mM)	32.8 ± 0.7†	29.1 ± 0.8*†	37.5 ± 0.6	37.1 ± 0.6	—	—	<0.01
pH	6.71 ± 0.03	6.89 ± 0.03	6.59 ± 0.03	6.68 ± 0.03	<0.01	<0.01	0.09
$H_2PO_4^-$ (mM)	16.5 ± 0.9	11.9 ± 0.8	21.8 ± 0.8	19.6 ± 0.8	<0.01	<0.01	0.09
PME (mM)	9.1 ± 0.7	7.1 ± 0.7	10.5 ± 0.9	9.3 ± 0.8	0.06	<0.01	0.57
ADP (mM)	0.15 ± 0.02	0.12 ± 0.02	0.38 ± 0.08	0.33 ± 0.07	0.87	<0.01	0.41
AMP (μM)	2.6 ± 0.7	1.7 ± 0.6	17.0 ± 6.1	14.1 ± 560	0.64	<0.01	0.81

Values are means ± S.E.M. *P*-values reflect main effects of age, condition, and age-by-condition interactions from 2-way (age, condition) repeated measures ANOVA. *Significant (P <0.05) effects of age within a given protocol. †Significant effects of protocol within each age group.

of ATP. This metabolic adaptation suggests that glycolytic function remains intact in the ankle dorsiflexor muscles of heathly older adults. As expected, the greater fatigability of young compared to old during FF was related to inhibitory metabolite accumulation during contractions. The mechanisms of the age-related fatigue resistance during ISC are less clear. While our results provide further evidence for the role of muscular energetics in the age-related differences in muscle fatigue, they also suggest that increased metabolic economy may be a potential mechanism allowing older muscle to engage in maximal work with lower glycolytic flux compared to young muscle.

Muscle oxidative capacity

The rate constant of PCr recovery was similar in young and older subjects, as was the calculated oxidative capacity, Q_{max}. Although others have reported that oxidative capacity is impaired with old age *in vivo* (McCully *et al.* 1993; Taylor *et al.* 1997; Conley *et al.* 2000), the results of the present study are consistent with previous reports that oxidative capacity is preserved in the tibialis anterior (Kent-Braun & Ng, 2000; Lanza *et al.* 2005), forearm (Kutsuzawa *et al.* 2001), and plantarflexor muscles of older adults (Chilibeck *et al.* 1998). Studies *in vitro* reveal similar discrepancies with some reports of reduced oxidative enzyme activities (Coggan *et al.* 1992; Pastoris *et al.* 2000), increased mitochondrial DNA alterations (Short & Nair, 2001), and reduced mitochondrial protein synthesis (Rooyackers *et al.* 1996), while others found no effects of age on mitochondrial function by a variety of methods (Grimby *et al.* 1982; Aniansson *et al.* 1986; Barrientos *et al.* 1996). The diversity of muscle groups under investigation may be a primary explanation for these discrepant results. Indeed, numerous studies demonstrate that neural (Galea, 1996), structural (Grimby *et al.* 1982), mechanical (Lanza *et al.* 2003), and enzymatic (Houmard *et al.* 1998) changes with age exhibit muscle-group specificity. This consideration, as well as the impact of varying health characteristics and physical activity patterns on age-related changes in oxidative capacity, is reviewed elsewhere (Russ & Kent-Braun, 2004).

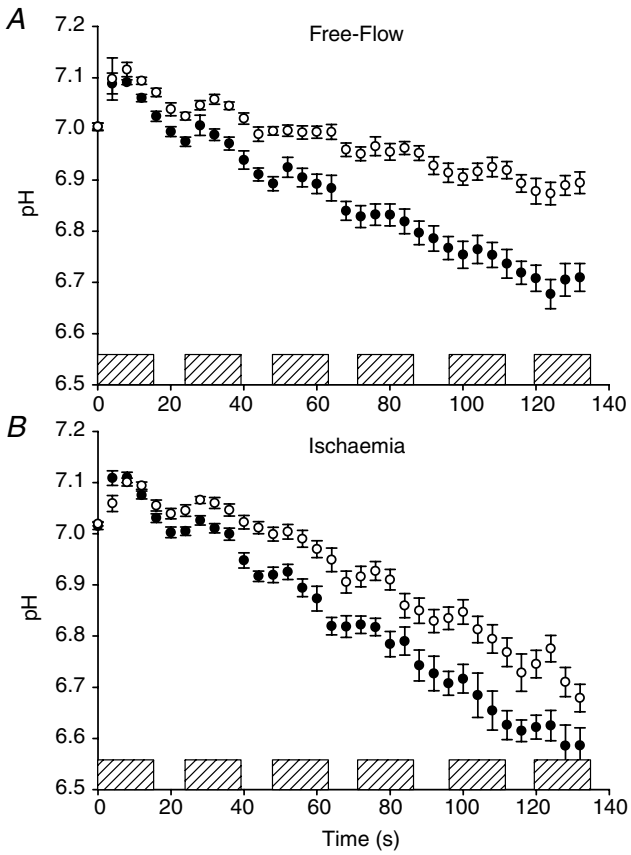

Figure 4. Intracellular pH during FF and ISC protocols
pH decreased to a greater extent in young (●) than older (○) subjects during free-flow contractions (*A*), and ischaemic contractions (*B*) Hatched bars represent each 12 s MVC. Data are presented as means ± S.E.M.

Pathways of ATP synthesis

The oxidative ATP synthesis rates were similar in young and older subjects during FF, as observed previously (Lanza *et al.* 2005), and were comparable to the rates reported in previous studies using similar methods (Boska, 1991; Jubrias *et al.* 2001; Lanza *et al.* 2005). The similarity in ATP_{OX} across age groups in the present study is consistent with our observations of preserved oxidative capacity with ageing. Phosphocreatine recovery is typically attributed exclusively to oxidative ATP synthesis, based on demonstrations that PCr does not recover in the absence of oxygen (Quistorff *et al.* 1993; Nakagawa *et al.* 2005). This dogmatic view has been challenged recently by reports of a transient portion of PCr recovery that can be attributed to anaerobic glycolysis (Crowther *et al.* 2002*b*; Lanza *et al.* 2006). As a result, attempts have been made to adjust the calculation of oxidative flux to account for this transient glycolysis. In two studies, glycolytic PCr resynthesis was quantified during ischaemic contractions and used to derive correction factors that were then applied during equivalent free-flow contractions (Jubrias *et al.* 2001; Lanza *et al.* 2006). However, these corrections assumed equal glycolytic flux during the postcontraction interval during FF and ISC, which may be inappropriate in the present study, where glycolytic flux was higher during ISC than FF in older subjects. Therefore, we did not correct for the glycolytic portion of PCr recovery in this study, and as a result have likely overestimated ATP_{OX}. This issue remains unresolved until more is known about the contribution of glycolysis to the postcontraction recovery of PCr.

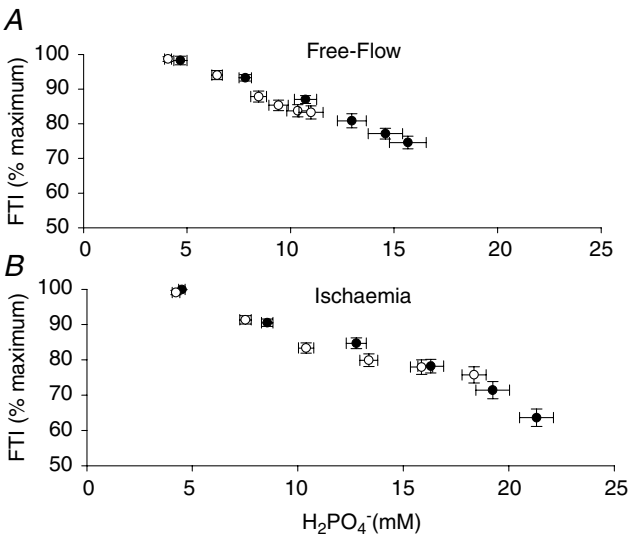

Figure 5. Relationships between FTI and diprotonated inorganic phosphate
A, the fall of FTI during free-flow contractions was strongly associated with the accumulation of $H_2PO_4^-$ in young (●) and older (○) subjects. *B*, although significant linear associations were also observed in both age groups during ischaemia, the older subjects appeared to reach a plateau in fatigue while $H_2PO_4^-$ continued to decline (see text for discussion).

The rates of ATP synthesis from net PCr hydrolysis in the creatine kinase reaction were similar in young and older subjects during FF and ISC. We have previously shown that ATP_{CK} is unaffected by old age during FF (Lanza *et al.* 2005). Using a steady-state saturation transfer MRS method, Horska *et al.* (2000) also found that flux through the creatine kinase reaction does not differ by age. In contrast to these studies *in vivo*, experiments using biopsy tissue have revealed significant age-related reductions in creatine kinase activity *in vitro* (Kaczor *et al.* 2006; Gelfi *et al.* 2006). Although the effects of ageing on the temporal ATP-buffering capacity of the creatine kinase reaction have not been studied extensively, the literature to date suggests that this pathway is unaffected by the ageing process *in vivo*.

Glycolytic flux was lower in older compared to young subjects during FF, as reported recently (Lanza *et al.* 2005) and inferred from blunted intracellular acidosis during muscle contractions (Coggan *et al.* 1993; Taylor *et al.* 1997; Chilibeck *et al.* 1998; Kent-Braun *et al.* 2002). Contrary to our hypothesis, ATP_{GLY} increased in older subjects when oxidative ATP synthesis was eliminated during ischaemia, suggesting that the functionality of glycolytic ATP synthesis *in vivo* remains robust with old age. Therefore, the blunted glycolytic flux observed during FF is likely to reflect the ability of older muscle to adequately meet energetic needs without increasing glycolytic flux to the same extent as young. It is important to note that we did not measure the capacity for anaerobic glycolysis, but rather the functionality of this pathway for ATP production under different conditions *in vivo*. It is certainly possible that the glycolytic flux we measured during maximal isometric contractions, even under ischaemic conditions, is below the upper limit that is possible *in vivo*. For example, dynamic contractions, which are more metabolically demanding than isometric contractions (Newham *et al.* 1995), may result in higher glycolytic flux than observed in the present study.

Studies of age-related alterations in glycolytic function in humans have been limited primarily to enzymatic analyses, which showed similar (Grimby *et al.* 1982; Coggan *et al.* 1992) or lower (Larsson *et al.* 1978; Pastoris *et al.* 2000) activity of glycolytic enzymes in old compared to young muscle. The ability to generate ATP glycolytically could decline with age as a result of reduced type II fibre area. By multiplying the proportional area of type I (Y, 64.5%; O, 81.1%) and type II fibres (Y, 35.5%; O, 18.9%; Jakobsson *et al.* 1990) by their respective PFK activities (type I, 25.8, type II, 49.4 mmol min^{-1} (kg dry weight)$^{-1}$; Essen *et al.* 1975), we estimate that the reduction in overall muscle PFK activity due to reduced type II fibre proportions is not likely to exceed ∼11% in old muscle. This prediction is higher than our earlier estimations

based on α-glycerol phosphate dehydrogenase (GPDH) activity; however, PFK is likely to be a better proxy for the potential for glycolytic flux than GPDH (Lanza *et al.* 2005). Regardless, both estimates suggest that the age-related fibre type shift cannot fully explain the observation that glycolytic flux is blunted in older compared to young under FF conditions.

Our results *in vivo* are in contrast to stimulated rat hind-limb experiments, which show reduced lactate production in older compared to young rats (Campbell *et al.* 1991; Hepple *et al.* 2004). However, this result was exclusive to white gastrocnemius muscle, whereas soleus, plantaris, and red gastrocnemius demonstrated no age-related impairment in glycolytic function. Thus,

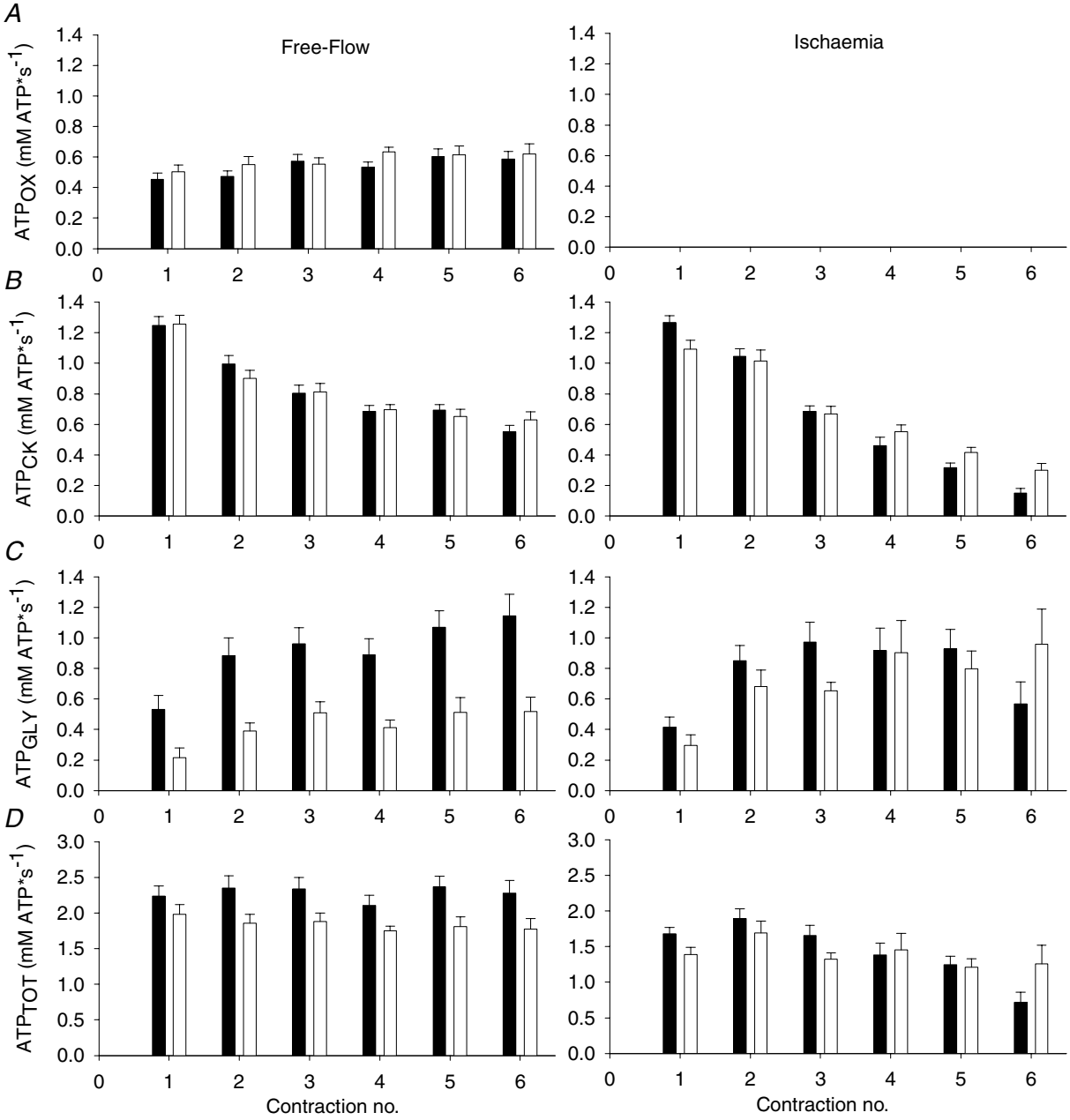

Figure 6. ATP synthesis rates
A, oxidative ATP synthesis (ATP_{OX}) was similar in young (■) and older (□) subjects during free-flow contractions (left panel) and assumed to be negligible during ischaemia (right panel). *B*, ATP derived from net PCr breakdown (ATP_{CK}) was similar across age groups during FF and ISC contractions. *C*, glycolytic flux (ATP_{GLY}) was higher in young than older subjects during FF, but similar in both age groups during ISC. *D*, total ATP synthesis rates (ATP_{TOT}) were lower in old during FF, but not during ISC. Data are presented as means ± S.E.M.

it seems that, at least in rat skeletal muscle, glycolytic function may be impaired with age in fast glycolytic fibres but preserved in more oxidative fibres. Nevertheless, despite the mixed composition of human tibialis anterior muscle (Jakobsson *et al.* 1990) we observed that glycolytic function is preserved with age in this muscle group. Further studies are warranted to determine if this result is consistent across other muscle groups with more glycolytic fibre composition.

Although our results oppose the notion of impaired glycolytic capacity as a mechanism to explain blunted glycolytic flux in old muscle, there remain numerous potential mechanisms that warrant discussion. We have previously proposed that glycolytic flux may be higher in younger subjects due to constricted blood flow during muscle contractions (Kent-Braun *et al.* 2002; Lanza *et al.* 2005). The larger, stronger muscles of younger individuals would be expected to generate greater intramuscular pressure and thus occlude blood flow to the working muscle to a greater extent than the smaller, weaker muscles of older subjects. However, this mechanism is unlikely to explain the greater glycolytic flux in young subjects in the present study since all subjects, regardless of age, generated $>60\%$ MVC during the free-flow protocol, which is a level force beyond which skeletal muscle perfusion is fully occluded during voluntary dorsiflexion (Wigmore *et al.* 2004).

Activation of glycolysis during muscle contraction is believed to be regulated by a 'dual control' model whereby intracellular calcium (Ca^{2+}) and metabolic by-products of muscle contraction (P_i, AMP, ADP) synergistically regulate glycolytic flux (Quistorff *et al.* 1993; Connett & Sahlin, 1996; Crowther *et al.* 2002*a*). Although [AMP] and [ADP] were similar in young and old during both protocols here, [P_i] was higher in the young during FF, but similar across age groups during ISC. During FF, [P_i] was at (older) or above (young) the reported Michaelis constant (K_m) of glycogen phosphorylase for P_i of $\sim$27 mM (Chasiotis *et al.* 1982), consistent with the notion of P_i as a regulator of age-related differences in glycolytic flux in FF. During ISC, [P_i] increased to a similar, high ($\sim$37 mM) level in young and old, and ATP_{GLY} was similar in both groups.

When examining the change in [P_i] in both groups from FF to ISC (Table 2), it is somewhat surprising to note the lack of increase in ATP_{GLY} in the young, given their $\sim$5 mM increase in [P_i] from FF to ISC. It is possible that [P_i] in young was sufficiently above the k_m during FF, such that a further increase in [P_i] during ISC had little effect on ATP_{GLY}. In contrast, the change in [P_i] from FF to ISC in the old ($\sim$8 mM) apparently occurred at a steeper portion of the saturation curve between P_i and ATP_{GLY}, and thus was able to further activate glycolysis in the old. Additionally, the inhibitory effects of intracellular acidosis on glycolytic flux (Hill, 1955; Chase & Kushmerick, 1988) may have been different in young and old across conditions. That is, the young may have reached an intracellular pH during both FF and ISC at which glycolysis became inhibited, while the older group may have attained this level of acidosis only during ISC.

While the differences in P_i accumulation and intracellular pH provide an attractive mechanism to explain the current results, the importance of intracellular Ca^{2+} in regulation of ATP_{GLY} should not be overlooked. It is possible that the age-related decline in motor unit discharge rates (MUDR) (Kamen *et al.* 1995; Connelly *et al.* 1999) and the leftward shift in the force–frequency relationship with old age (Ng & Kent-Braun, 1999; Allman & Rice, 2004) may combine to generate high force with relatively fewer activation pulses in the old. This effect would translate to less Ca^{2+} release from the sarcoplasmic reticulum, lower intracellular Ca^{2+}, and lower glycolytic flux during high-intensity contractions in older compared to young muscle. This possibility needs to be explored.

Total ATP flux

Because there was no change in [ATP] during these protocols, ATP_{TOT} can serve as a proxy for ATP demand. We observed that ATP_{TOT} was lower in old compared to young during FF. Although one might expect differences in absolute force production to contribute to the observed differences in the overall ATP demand, the similar strength of the two age groups, and the use of volumetric units (mM s^{-1}), render our flux measures independent of muscle size. Thus, our data suggest that the ATP requirements of force production are lower in older muscle, possibly as a result of more economical force production due to the combination of contractile slowing and lower MUDR mentioned above.

Previous reports of age-related changes in metabolic economy are scarce and thus far limited to animal studies, which have shown increased contractile economy with age (de Haan *et al.* 1993; Hepple *et al.* 2004). The age-related shift of muscle toward a slower, more oxidative and economical fibre-type composition (Jakobsson *et al.* 1990; Lexell, 1995; Hepple *et al.* 2004) is a potential source of increased metabolic economy with age. We are unaware of any studies that have investigated age-related changes in metabolic economy in humans to date.

Force and voluntary activation

As expected, young subjects fatigued more than older subjects during FF, in agreement with some previous studies (Bemben *et al.* 1996; Ditor & Hicks, 2000), but in contrast to others (Davies & White, 1983; Lennmarken *et al.* 1985; Cupido *et al.* 1992). In the present study, the strong association between fatigue and [$H_2PO_4^-$] during FF supports the contention of a metabolic basis for the

observed fatigue resistance with old age. We hypothesized that older subjects would fatigue more than young during ISC because of an inability to increase glycolytic flux in compensation for suppressed oxidative ATP synthesis. Although all subjects fatigued more during ISC compared to FF, the fatigue resistance of older subjects persisted despite occlusion of blood flow. As discussed above, older adults were capable of increasing their reliance on substrate-level phosphorylation during ISC as necessary to meet the energetic demands of the contraction protocol. Similar to FF, we again observed a strong association between fatigue and $[H_2PO_4^-]$ during ISC, suggesting that metabolite accumulation may still be a potent mechanism to explain greater fatigue in young than older muscle, even in the absence of blood flow. However, there was a trend for the age-related difference in $H_2PO_4^-$ accumulation to be less pronounced during ISC than FF, which suggests that something other than the direct effects of accumulating metabolites on muscle force development may have been contributing to the fatigue resistance of the older subjects. Recent observations from our laboratory (Chung *et al.* unpublished) reveal that greater central and peripheral activation failure in the young may explain a portion of the age-related fatigue resistance during ischaemic MVCs. However, we did not measure activation during fatigue in the present study, and thus cannot ascertain the neural contribution to fatigue.

In summary, we have shown that, during maximal voluntary ankle dorsiflexion, glycolytic flux was lower in older compared to young subjects during FF, but similar across age groups during ISC. These data suggest that glycolytic function remains intact with old age in this muscle group, and that the ankle dorsiflexors of older individuals retain the ability to meet the energetic demands of contractile activity under a variety of conditions. In addition, the results point to an age-related increase in metabolic economy as a potential mechanism that may allow older muscle to engage in maximal work with less fatigue compared to young. Further study is needed to determine whether the present findings are consistent across other morphologically and functionally distinct muscle groups.

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