Boys–men mean-power-frequency differences in progressive exercise to exhaustion, confounded by variability and adiposity

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Abbreviations

ANOVA – Analysis of Variance

ANCOVA – Analysis of Co-Variance

ATP – Adenosine Tri-Phosphate

EMG – Electromyography

EMG_{Th} – EMG Threshold

FR – Firing Rate

[**La**] **–** Lactate concentration

LBM – Lean Body Mass

Max – Maximum

Min – Minimum

MPF_{Min} – MPF's minimal value

MPF_{Peak} – MPF's maximal value

MPF_{Range} – MPF_{Peak} minus MPF_{Min}

MU – Motor Unit

MVC – Maximal Volitional Contraction

NS – Non-Significant

PCr – Phosphocreatine

PHV – Peak Height Velocity

RMS – Root Mean Square

RPE – Rating of Perceived Exertion

sEMG – Surface EMG

TTE – Time to Exhaustion

VL – Vastus Lateralis muscle

Abstract

Background: Only scant research has compared children's mean power frequency (MPF) to adults', with a clear overview still lacking. A significant obstacle has been MPF's high variability, which this study aimed to overcome in elucidating the MPF characteristics distinguishing boys from men in progressive exhaustive exercise.

Methods: Electromyographic (EMG) data of 20 men (23.5±2.5yrs) and 17 boys (10.2±1.0 yrs), who performed progressively exhausting, intermittent isometric knee extensions, underwent secondary MPF analysis. Participants' vastus-lateralis MPF data series were transformed to 3rd-order polynomial regressions and expressed as percentages of the peak polynomial MPF values (%MPF_{peak}). The resulting curves were compared at 5-% time-to-exhaustion (TTE) intervals, using repeated-measures ANOVA. Raw MPF_{peak} values were adiposity-corrected to 0% fat and used to convert the %MPF_{peak} data back to absolute MPF values (Hz) for estimating muscle-level MPF.

Results: No overall interaction or group effects could be shown between the %MPF_{peak} plots, but pair-wise comparisons revealed significantly higher men's values at 50–70%TTE and lower at 100%TTE, i.e., boys' shallower MPF rise and decline. The adiposity-corrected boys' and men's composite MPF values peaked at 125.7±2.5 and 166.0±2.4 Hz, respectively (110.7±1.7 and 122.5±2.1 Hz, uncorrected), with a significant group effect ($p<0.05$) and pair-wise differences at all %TTE points.

Conclusions: The boys were lower than the men in both the observed and, more so, in the adiposity-corrected MPF values that presumably estimate muscle-level MPF. The boys' shallower MPF rise and decline conform with children's claimed type-II motor-unit activation and/or compositional deficits and their related known advantage in muscular endurance.

Key Words: Fatigue, Endurance, EMG threshold, Child–adult differences, Neuromotor control, Motor-unit activation

Introduction

A muscle's contractile force and power depend on both motor-unit (MU) recruitment and firing rates. In fatiguing isometric contractions, increasing MU recruitment results in rising electromyographic (EMG) signal amplitude, while the mean power frequency (MPF) eventually decreases. Largely due to the type, duration, and intensity of contractions, MPF either declines from the onset (Halin et al. 2003, Moritani et al. 1985, Moritani et al. 1986), or is initially unchanged, or moderately increases (Bilodeau et al. 2003, Lenhardt et al. 2009). With exercise progression and increasing fatigue, MPF then typically decreases (Komi et Tesch 1979, Moritani et al. 1982, Bigland-Ritchie et al. 1986), likely due to MU fatigue and the increasing recruitment of the larger, slower-firing and more fatigable high-threshold MUs (Komi et Tesch 1979, Kupa et al. 1995, Taylor et al. 1997).

It has been shown that relative to adults, and beyond physical size, children are inferior in strength, speed, contractile velocity and power, and superior in muscle endurance, recovery, oxidative metabolism, and VO_2 kinetics (see Dotan et al. 2012 for a review). All of these have been hypothesized to be due, entirely or in part, to children's lower activation and/or muscle composition of higher-threshold, presumably type-II MUs (Dotan et al. 2012). This hypothesis was supported by several studies demonstrating a later onset of higher-threshold-MU activation during progressive exercise in children compared with adults (Pitt et al. 2015, Long et al. 2017,Woods et al. 2019, Woods et al. 2020). Congruent with that hypothesis, a shallower fatigue-induced MPF decline in sustained isometric contractions was demonstrated in boys vs. men (Halin et al. 2003). That is, lesser activation or lower composition of the more fatigable type-II MUs can be expected to induce lower rates of fatigue.

Although the pattern of declining MPF in sustained isometric contractions has been observed in both children (Halin et al. 2002, 2003; Moalla et al. 2006) and adults (Komi et Tesch 1979, Moritani et al. 1982, Bigland-Ritchie et al. 1986), this has not been as clear in other contraction types and protocols. In progressive, and particularly in cyclical or intermittent exercise, the simple MPF–fatigue relationship has not been as evident in adults (e.g., Chai et al.2019), while in children this has never been studied. The reasons for the inconsistent MPF–fatigue relationships have not been resolved. Contributing factors possibly include varying intensity–duration relationships, varying work–rest ratios in intermittent exercise, and fluctuations within the range of recruitment–FR interplay available for maintaining a given force output. Adding to these could be increasing fluctuations of the EMG-signal and force output observed with the onset of progressively increasing fatigue (Contessa et al. 2009), and the modulation of MU FR with

muscle length changes in dynamic contractions (Sato 1964), and with fascicle-length changes during isometric contractions (Martinez-Valdes et al. 2022).

Moreover, surface EMG (sEMG) amplitudes and frequencies have been shown to be much lower than the corresponding ones measured in a given muscle via indwelling wire electrodes. Backus et al. (2011), for example, found intra-muscular MPF to be twice higher than that derived from sEMG, while Green et al. (2017) showed a corresponding ~8-fold difference. The reason for these differences has mainly been attributed to the high-frequency filtering effect of the intervening muscle, skin, and particularly fat tissues between the monitored MUs and the sEMG electrode (De Luca 1997, Lowery et al. 2002). Thus, superimposed on the aforementioned temporal variability there is inter-muscle variability and the sEMG MPF variability, due to high inter-individual adiposity variance.

In the EMG data of our previous studies, that focused on EMG_{RMS} response to progressive exercise to exhaustion in cycling (Pitt et al. 2015, Long et al. 2017) or intermittent isometric contractions (Woods et al. 2019, Woods et al. 2020), we demonstrated that the EMG threshold (EMG_{Th}), defined as the onset of accelerated EMG_{RMS} increase, occurred at higher relative workload intensities in children than in adults. In those studies' data we also noticed much higher variability and pattern irregularities in MPF than in EMG_{RMS}, in both children and adults. Those MPF aberrations have never been reported or analyzed. The aim of the present study, therefore, was to re-analyze the EMG data in order to elucidate **a**. Characteristic MPF patterns; **b**. Whether such patterns are different in children than in adults; and **c**. The relationship between MPF and EMG_{Th}.

Methods

The current study's data are based on a secondary analysis of data collected by Woods et al. (2019). Twenty one men and 18 boys participated, but complete EMG_{RMS} and MPF data sets were available for 20 men and 17 boys. All participants were physically active, but none were athletes. The study was cleared by Brock University's Research Ethics Board. All participants and the boys' parents/guardians signed informed consent forms prior to participation.

Exercise Protocol

The protocol is described in detail in the original study (Woods et al. 2019). A custom-built, calibrated weights-based knee-extension dynamometer was used in conjunction with the Biodex System 3 (Shirly,

New-York) seat assembly. Participants were secured to the seat via tought restraining straps across the hips, over the thigh of the passive leg, and diagonally over the shoulders and across the chest. All testing was performed at a knee angle of 125–130°. One-repetition-maximum torque (MVC) was measured over at least 4 trials, with the highest value used for further analysis. EMG_{RMS} was recorded in a graded exercise test, from which EMG_{Th} and MPF data were derived. Starting at ~25 %MVC participants performed repeated sets of 5 isometric knee extensions (3s on, 2s off), successively incremented by ~3 %MVC and interspaced by 30-s recovery intervals to volitional exhaustion.

Anthropometry

All anthropometric measurements were performed by the same investigator. Standing and seated (boys only) height were measured to the nearest 1mm, using Ellard Instrumentation Ltd. Stadiometer. Body mass was determined, with only gym shorts on, to the nearest 0.1 kg, using the InBody 520 digital scale (Biospace CO., Ltd). Adiposity (%Fat) was determined by the same InBody 520 bio-electrical impedance apparatus.

The boys' maturity status (offset) was assessed using the Mirwald et al.'s equation (2002), producing estimates of the difference (years) between current age and age at peak height velocity (PHV).

EMG Data Acquisition

EMG was recorded using surface EMG double-differential tripolar electrodes (Delsys Inc., Boston, MA, USA), placed on the vastus lateralis (VL) at 2/3 the distance between the superior border of the patella and the anterior superior spina iliaca, in accordance with SENIAM guidelines. A reference electrode was placed over the 7th cervical vertebra. EMG data were sampled and recorded at 1000 Hz (EMGworks Acquisition, Delsys Inc., Boston, MA, USA) and band-pass filtered at 20–450 Hz, using the Bagnoli-4 bioamplifier (Delsys Inc., Boston, MA, USA).

Data Reduction

Data reduction of the original EMG/EMG_{Th} data was performed using MATLAB v.2017a (MathWorks, Natick, MA, USA). MATLAB v.2019a was used for the MPF data re-analysis.

Within each group, participants were divided into those who exhibited characteristic ('Expected') MPF patterns in the progressive exhaustive test series and those who manifested irregular (unexpected) patterns. 'Expected' was defined as a generally level or rising MPF in the initial stages of exercise, followed by a decline in the later stages (see Fig.1 for examples of 'Expected' vs. 'Irregular' MPF patterns

in men and boys). The 'Expected' sub-groups comprised 13 participants each. The physical characteristics of all ('All') and the 'Expected' boys' and men's cohorts are shown in Table 1.

[Figure 1] [Table 1]

As the total number of completed stages varied among participants (10 to 22), time to exhaustion (TTE) was normalized by defining the last stage of each series as 100 %TTE and assigning a %TTE value to each data point, accordingly.

To better evaluate the MPF pattern's form and reduce the large variability of the raw (measured) data, each participant's MPF data were expressed as percentages of peak value in the following manner. A 3rd-order polynomial regression was derived from each participant's raw MPF data. The highest polynomial value was defined as 100 %MPF (MPF_{Peak}) and a percentage value of that was calculated for every 5% of the 100 %TTE range (0, 5, 10, … 95, 100%); see example in Fig.2.

[Figure 2]

To control for large between- and within-group adiposity differences (Table 1) and to noninvasively estimate MPF at the muscle level, all raw EMG_{RMS} and MPF values were adiposity-corrected to a theoretical 0 %Fat, in two ways. a. Mean MPF (MPF_{Mean}) and EMG_{RMS} values were regressed against %Fat, separately for each group, to determine the respective correction factors (regression slopes). Raw MPF and EMG_{RMS} values were then recalculated as '%Fat-Corrected' and analyzed (Table 2). b. Non-corrected values were analyzed using ANCOVA, with %Fat as the covariate (see below).

[Table 2]

Statistical Analysis

Descriptive statistics were calculated for men and boys and group differences in physical characteristics and distinct MPF values (e.g., MPF_{Min}, MPF_{Peak}) were determined using an independent t-tests, as well as ANCOVA (%fat as covariate). Group differences in MPF pattern during exercise were examined using ANOVA or ANCOVA for repeated measures, as appropriate, with group as the between-subject effect and %TTE as the within-subject effect. Relationships between variables were examined using a linear regression. Data are reported as mean±SD. Statistical significance for all comparisons was defined as $p<0.05$.

Results

193 The men's body fat percentage (%Fat) was significantly greater than the boys' as was their MVC,
194 even when normalized to lean body mass (Table 1).

195 On average, the boys (All) completed 16.8±3.1 stages (range: 11–22), while the corresponding men's
196 values were 15.4±3.3 stages (range: 10–21), but the difference was not statistically significant.

197 MPF and EMG_{RMS} variabilities, defined a changes in trace direction, are exemplified in Fig.3. The
198 numbers of these changes were analyzed for all boys and men (n= 17 and 20, respectively), and the results
199 are depicted in Fig.4. ANOVA showed $p<0.001$ for the MPF vs. EMG_{RMS} effect, and $p=0.011$ as the group
200 effect. Change-of-direction pairwise comparisons showed statistically significant differences in both men
201 and boys, with the number of direction changes greater in MPF compared with EMG_{RMS} traces ($p<0.001$).
202 Moreover, the number of EMG_{RMS} direction changes was higher in boys ($p=0.005$), but the corresponding
203 difference in MPF did not reach statistical significance ($p=0.100$).

204 **[Figure 3, Figure 4]**

205 The MPF_{Mean}-vs.-%Fat relationship for all participants (n=37) is depicted in Fig.5. The men's
206 correction factor (slope) was –2.6445 $Hz \cdot \%Fat^{-1}$ and boys' was –1.4536 $Hz \cdot \%Fat^{-1}$. Each group's
207 regressions was stronger and statistically more significant than the combined men-boys regression,
208 implying that the two were distinct relationships and correction factors. The men's larger correction factor
209 was mainly due to size difference, since a given fat percentage constitutes a thicker fat layer in men than in
210 boys. Individual MPF and EMG_{RMS} values were corrected to 0 %Fat. The corresponding EMG_{RMS}
211 correction factors were –6.748 and –1.687 $\mu V \cdot \%Fat^{-1}$ for the men and boys, respectively.

212 The adiposity-corrected MPF_{Min} and MPF_{Peak} values were both highly correlated with body mass (as a
213 proxy for muscle size) ($r = 0.735$ and 0.688, respectively; $p<0.0001$). Notably, the corresponding
214 correlations with the observed, raw MPF values were essentially zero (–0.081, –0.033, respectively; NS)

215 **[Figure 5]**

216 Table 2 presents the experimental variables for the entire ('All') boys and men cohorts and for the
217 'Expected' sub-groups. MPF variables are presented for both the measured raw values and for the %Fat-
218 corrected ones. It is noteworthy that no statistically significant group differences could be shown in
219 measured MPF ($p=0.180–0.461$), or EMG_{RMS} ($p=0.052–0.073$), but those differences became highly
220 significant ($p<0.01$ to <0.0001) once corrected for adiposity. The EMG_{Th} values (taken from Woods et al.
221 2019) were significantly higher in the boys compared with the men, except in the 'All' group, when
222 expressed as %TTE ($p<0.1$). Note that the ANCOVA showed lower p values than those obtained via t-tests

of the %Fat-corrected values since the ANCOVA used the weaker combined (men-boys) regression (Fig.5), rather than the stronger group-specific regressions.

Avoiding the particularly high variability of the irregularly-patterned data, Fig.6 depicts only the 'Expected' cohorts' distribution of the measured MPF data and the respective 3rd-order polynomial regressions, demonstrating the data's fundamental high variability. Although the men's regression was statistically significant (p=0.005), it explained only 4% (0.200^2) of the observed variance. The boys' regression did not reach statistical significance, and as noted above, there was no statistically significant group difference between the means of the various MPF parameters (Table 2).

[Figure 6]

Expressing MPF values relative to each individual's peak MPF (exemplified in Fig.3), markedly diminished the variability stemming from the raw-data's dispersion, and highlighted group MPF-pattern differences, shown in Fig.7, which depicts each groups' $\%MPF_{Peak}$ as described earlier. There was a clear %TTE effect (p=0.001) and no significant group effect (p=0.98). The group–%TTE interaction did not reach statistical significance (p=0.188). However, statistically-significant pair-wise differences were detected throughout the 50–70 %TTE range, as well as at 100 %TTE.

[Figure 7]

Both MPF and EMG_{Th} have been considered fatigue-onset indicators (e.g., Hummel et al. 2005, Hanon et al. 1998, respectively), the respective mean locations of the $\%MPF_{Peak}$ maxima, and their corresponding EMG_{Th} values along the %TTE axis, are marked in Fig.7 by arrows. The boys' and men's $\%MPF_{Peak}$ curves both peaked at ~60 %TTE (59.7 and 59.9, respectively). The EMG_{Th}, however, was the same as the $\%MPF_{Peak}$ maximum only in the boys (60.0±12.8 %TTE), but preceded that value by nearly 12% (48.1±13.2 Hz) in the men (p<0.04) (see also Table 2**)**.

Fig.8_A is a composite of the groups' $\%MPF_{Peak}$ curves, as shown in Fig.7, and the mean measured MPF_{Peak} values for each group, given in Table 2 (i.e., $\%MPF_{Peak}$ x observed MPF_{Peak} for each group), which reverted MPF back to absolute (Hz) values. While the %TTE effect remained highly significant (p<0.001), there was no statistically-significant group effect (p=0.417), and the group–%TTE interaction barely reached significance at p=0.050. On the other hand, when adiposity-corrected MPF_{Peak} were plotted against %TTE (Fig.8_B), all differences turned significant (Group effect: p<0.01, %Fat effect: p<0.001, %TTE effect: p<0.001, and group–%TTE effect: p<0.001). The adiposity correction raised the men's peak

composite values by 35.5% (from 122.5±2.5 to 166.0±2.4 Hz) and by 13.6% in the boys (from 110.7±2.2 to 125.7±1.8 Hz).

[**Figure 8$_{A+B}$** (side by side, or one above the other)]

To gain additional perspective on the extent or limits of the observed MPF variability, we looked at the relationship between the adiposity-corrected MPF_{Min} and MPF_{Peak} in the entire (n=37) boys-men combined group, shown in Fig.9 ($r=0.961$, $p<0.0001$). The respective boys' and men's within-group correlations were 0.953 and 0.819 ($p<0.0001$).

[**Figure 9**]

Discussion

This study shows that during progressive, isometric contractions to exhaustion, and compared with men, boys had markedly lower MPF values (Figs.8_{A+B}) and their MPF response was shallower in both its rise and decline (Fig.7). Further, the study demonstrates and quantifies the adiposity effect on MPF. Distilling out the overarching boys–men MPF pattern and magnitude differences from the 'scattered cloud' of observed inter- and intra-individual MPF values (Fig.6) was facilitated by expressing MPF values in relative terms (% polynomial peak) rather than by the raw MPF values (Hz) and correcting for the suppressing effect of adiposity.

MPF Variability

A major source of inter-individual MPF variability is the wide range of muscle compositions. In a total of 305 men, Staron et al. (2000) and Simoneau et Bouchard (1989) reported VL compositional ranges of 18–85% type-I, 5–62% type-IIA, and 0–55% type-IIB fibres.

Within individuals, MPF has previously been shown to be undulating or fluctuating with exercise loads that are fixed (Sato 1965, Chai et al. 2019) or steadily increasing (e.g., Houtman et al. 2003). MPF's considerably greater variability than that of the EMG record from which it is derived, is clearly demonstrated in the present study – qualitatively in Fig.3 and quantitatively in Fig.4. Further attesting to MPF's greater variability is the fact that relatively stable MPF samples had to be limited to 1-s periods, while the corresponding EMG_{RMS} samples were 2-s long, and more importantly, that 11 participants had to be omitted from the main analysis due to inexplicably-irregular MPF patterns. As can be seen in Fig.4, MPF irregularities were 76% greater than those of EMG_{RMS} in the boys and 229% in the men. Also

relevant to this study is that the boys' MPF and EMG_{RMS} were 23 and 130% more irregular than the men's, respectively. The boys' greater variability is consistent with their previously-observed greater neuromotor and performance variabilities (e.g., Gerodimos et al. 2008 and Casamento-Moran et al. 2018, respectively).

A potential EMG-specific source of MPF variability is EMG amplitude cancellation, resulting in observed-frequency corruption (Dideriksen et Farina 2019, Tucker et Türker 2005). Unlike adiposity's effect on EMG amplitude and frequency, which can at least partly be accounted for (as done in this study), there is little that is *a priori* known about the prevalence, degree, or distribution of cancellation-related frequency aberrations. By and large, therefore, amplitude cancellation adds to MPF's variability in an unquantifiable manner.

Also, as noted earlier, the neuromotor system has some latitude in controlling the recruitment-vs.-FR interplay in producing a given force or power output. This interplay latitude is potentially an important source of MPF response variability, but we are aware of no proposed mechanism to explain how that interplay may be controlled.

Boys–Men Differences in MPF Magnitude

Contrary to several previous studies (e.g., Armatas et al. 2010, Yuen et al. 1989), the 'Expected' men's MPF values were higher than those of the boys' (7.9%), and when corrected for adiposity, that difference more than tripled, to 30.2%. These differences were nearly identical when the 'All' cohorts were compared (Table 2). In composite terms (Fig.8_{A+B}), the corresponding values were 10.7 and 30.1%, respectively.

Lower MPF values are congruent with boys' lower mean overall FR and FR at a given MU action-potential amplitude, as seen in discrete-MU analysis (Parra et al. 2020, McKiel et al. 2022, Woods et al. 2022). As important appears to be MPF's size dependency. The high body-mass correlations with the adiposity-corrected MPF_{Min} and MPF_{Peak} and the absence of comparable correlations with the observed, raw MPF values, lends support to the notion that MPF's muscle-level reality is obscured by adiposity.

The MPF picture presented in the literature, however, is mixed. In sustained 30-s elbow-flexion MVCs, Halin et al.'s (2003) boys, started lower than the men, had a shallower decline, and then, unlike our boys (Fig.8_{A+B}) finished with higher MPF values compared with the men. The difference in pattern between studies was likely due to protocol and employed-muscles differences. Adiposity was not accounted for but was similar in the two groups. Bax et al. (2021), on the other hand, using 25 wrist-flexions at 35%MVC, found similar men's and boys' MPF responses, but adiposity, which was 61%

greater in the men, was not corrected for, which would have markedly raised men's values relative to the boys', as we have found.

Armatas et al.'s (2010), used an intermittent maximal isometric knee-extension protocol to 50% of initial torque. MPF was not monitored during exercise, but the boys exhibited greater endurance performance than the men and their mean VL MPF immediately post exercise was, unlike ours, significantly higher than the men's (82.5 vs. 60.7 Hz, respectively). The groups' adiposities were similar and were not corrected for. Yuen et al. (1989) studied child–adult differences in masticatory-muscles' MPF of 5-s isometric MVC contractions, and found the boys' MPF to be 13% higher than the men's. Although the authors acknowledged the effect of subcutaneous-layer thickness, no adiposity data were reported and no corrections made. On the other hand, at the end of 12x90-s isometric knee-extension bouts at 30–40 %MVC in ~14-year-old trained and untrained boys, Callewaert et al. (2012) reported VL MPF values of 88.9 and 99.3 Hz, respectively. Adiposity was reported but not corrected for. Those MPF values were lower than ours (104 Hz; Table 2), but the untrained boys' 99.3-Hz MPF was relatively close. Inexplicable discrepancies occur, apparently, even within the same lab. Halin et al. (2002) tested elbow-flexor MPFs in gymnast vs. untrained prepubertal boys. Their respective MVC MPF_{Peak} values of ~136 and ~124 Hz, were markedly higher than the corresponding ~94-Hz MPF reported in the untrained boys of their subsequent study (Halin et al. 2003) that used a practically identical protocol. Both studies measured skinfold thicknesses that were used for muscle cross-sectional-area estimation, but not for MPF's adiposity correction.

To various degrees, all cited findings differ or contrast with our own. Whether or not adiposity corrected, our boys' MPF values (Hz) were markedly lower than the men's throughout the progressive exertion. Why some studies did not find the same is often inexplicable by differences in the employed muscles, exercise modes, or protocols. We presently can provide no further insight into that and it ought to be further investigated.

Boys–Men Differences in MPF Pattern

Distilled from the large inter- and intra-individual MPF variability (Fig.6), a most instructive finding of this study is the boys–men MPF pattern differences ($\%MPF_{Peak}$; Fig.7). In these relative terms, the boys' MPF reached lower peak values than the men's and then declined more moderately upon approaching exhaustion – a range of 6.4 vs. 12.7%, respectively.

The boys' shallower MPF decline in approaching exhaustion, is congruent with previous studies (Armatas et al. 2010, Halin et al. 2003, Tanina et al. 2017) and supports the hypothesis that adults can access relatively more of their type-II MUs, while children rely more on type-I MUs (Dotan et al. 2012, Pitt et al. 2015, Long et al. 2017, Woods et al. 2019, Woods et al. 2020). That is, type-I MUs have been demonstrated to be less fatigable (Hamada et al. 2003, Komi et Tesch 1979, Linssen et al. 1991), and children's rate of fatigue has been shown significantly lower than that of adults (Armatas et al. 2010, Birat et al. 2018, Paraschos et al. 2007, Ratel et al. 2015). Halin et al. (2003) previously arrived at a similar conclusion, suggesting that their observed men–boys MPF pattern difference was due to "a greater participation of Type II MU in men compared with boys". A direct relationship has been shown between MPF (or median frequency) and type-II fibre predominance among adults (Gerdle et al. 1991, 1992; Kupa et al. 1995; Tesch et al. 1983). Indeed, several studies (Lexell at al. 1992, Jansson 1996, Metaxas et al. 2019, Esbjörnsson et al. 2021) have shown children's type-I fibre compositions that are higher (type-II lower) than is typical of adults. The histological maturational effect, however, is not limited to fibre-type prevalence. There is also a concurrent trend of increasing type-II fibre size relative to type-I. Metaxas et al. (2019), for example, testing 11–15 year-old male soccer players, showed that as the prevalence of type-II_A fibres increased by 12.2% (from 41.7 to 46.8%) in the 11- and 15-year-olds, respectively, the corresponding type-IIA/type-I cross-sectional-area ratio increased by 8.4% (from 1.158 to 1.255). Children, presumably due to lesser reliance on higher-threshold MUs, attain lower intramuscular [La]s (Eriksson et al. 1971, 1973) which limit the decline of muscle-fibre conduction velocity (MFCV) and, consequently, MPF decline as well. Independently, the boys' lower maximal LBM-specific torque, only 72.8% of the men's (Table 1), and their 29.3% higher EMG_{Th} (Table 2; Woods et al. 2019), are both best explained by boys' lesser reliance on higher-threshold MUs.

Notably, when re-plotted with the 11 'irregular' participants (not shown), the men's and the boys' MPF-pattern characteristics (Fig.7) did not change, although statistical differences could no longer be demonstrated. Although child–adult MPF pattern and magnitude differences vary widely with different protocols and contraction types, the child–adult differential muscle activation hypothesis, if valid, can be expected to also affect child–adult MPF pattern differences in contraction modes and protocols other than those investigated in the present study.

Adiposity and the Intervening-Layer Effect

The suppressing effect of the MU-to-electrode intervening tissue on both the amplitude and frequency of the EMG-signal is well established and was elegantly modelled by Lowery et al. (2002). With the

intervening layer's muscle, fat, and skin constituents, Lowery et al. (2002) showed that when the thickness of the intervening layer increased from 7 to 20mm, the observed median frequency dropped >70 Hz. Nevertheless, corrections for this effect have rarely been applied in previous MPF studies in adults and never in children. In the present study (Fig.8_{A+B}), the boys–men MPF_{Peak} difference increased 29.1 Hz (from 10.1 to 39.2 Hz) when adiposity-corrected.

Although fat is but one of the intervening layer's 3 main constituents, it is by far the most variable and, by virtue of its typical quantities, also the most dominant (Lowery et al. 2002). Thus, although reliable layer thickness determinations, such as via ultrasound or magnetic resonance imaging, could provide more precise estimates, the sole use of adiposity (%fat) for assessing MPF-suppression is potent and should not be dismissed.

Regardless of its estimation method, the intervening-layer effect on observed EMG or MPF, could be larger than any technical or procedural effects. Thus, while our adiposity corrections were only approximations, they can still provide better estimates of the true, muscle-level nature of MPF than can surface-measured MPF on its own. Such corrections, then, ought to be applied, whenever possible, and particularly when comparing groups of differing adiposity. The present study is the first to apply these corrections in child–adult comparisons.

MPF as Fatigue Indicator

MPF decline at the later part of demanding exercise has long been claimed to reflect muscular fatigue (Camic et al. 2010, Komi et Tesch 1979, Moritani et al. 1982). The decline has partly been explained by MU synchronization, or 'grouping' at the higher contraction intensities (Furness et al. 1977, Lago et Jones 1977), and later also by diminishing MFCV (Arendt-Nielsen et Mills 1985, 1988; Bigland-Ritchie et al. 1981, Sadoyama et al. 1983), due to such factors as lactate-induced acidosis. The factors affecting the fluctuating MFCV–MPF relationship, however, have not been fully elucidated. A major confounding factor may be the different characteristics of recruitment strategy, fatigability, and MFCV differences between low-threshold, presumably type-I, and high-threshold, presumably type-II MUs. As these factors' effects are not necessarily linear, the resulting MPF response could be non-linear and possibly fluctuating as well, as seen in adults under the conditions of both sustained, submaximal isometric contractions (e.g., Houtman et al. 2003), and progressively increasing contraction intensities (e.g., Chai et al. 2019).

Indeed, some adult studies have failed to uphold an MPF–fatigue relationship. Nearly 60 years ago, Sato (1965) observed that, in sustained isometric contractions, EMG frequency components fluctuated

throughout the contractile duration and that these changes did not necessarily correspond to changes in measurable or subjective fatigue indicators. Similarly, in a series of intermittent submaximal isometric knee-extensions protocols, Bigland-Ritchie et al. (1986) observed that "the loss of force generating capacity seen during fatigue" could "not be explained by any of the usual factors thought to be responsible for fatigue". In constant-force isometric contractions, carried to ~35% muscle contractile failure, the authors observed no FR changes despite substantial EMG increases. Although MPF was not reported, the unchanged FR response suggests little or no change in the MPF response, as well.

More recently, Hummel et al. (2005) observed correlations of MPF with rating of perceived exertion (RPE; Borg 1982) which could not explain only 46–58% of the observed variance. Chai et al. (2019) investigated four upper- and lower-leg muscles in exhaustive, constant- and incremental-load running. They found the MPF–RPE correlations mean of only 0.192, ranging between –0.043 and 0.368, and explaining no more than 13.5% of the observed variance. It is thus unclear whether MPF can be used as an indicator of local or overall fatigue.

Antagonist cocontraction or coactivation were not assessed in this study, but have previously been linked to greater fatigue or compromised performance. Children have been shown to have a greater degree of cocontraction in locomotive / multi-joint exercise (Frost et al. 1997, Frost et al. 2002). However, in single-joint contractions, such as the knee extension used in this study, there is no significant difference between children and adults (see recent review by Woods et al. 2023). Thus, cocontraction/coactivation cannot be assumed to have played a roll in this study's findings.

MPF–EMG$_{Th}$ Congruence

In the realm of fatigue indication and in light of MPF's shortcomings, it is pertinent to ask how EMG_{Th}, also a fatigue-onset marker (Edwards et Lippold 1956, Lucia et al. 1999, Mäestu et al. 2006), is related to MPF. Fig.7 and Table 2 remarkably show that maximal $\%MPF_{Peak}$ occurred at 60 %TTE in both groups and coincided with the boys' EMG_{Th} (60.0 %TTE). The men's EMG_{Th}, however, occurred already at 48.1 %TTE. In terms of %MVC, the boys' EMG_{Th} was 13.0 %MVC higher than the men's (57.4 vs. 44.4 %MVC; Table 2; Woods et al. 2019). Thus, to the extent that these variables are taken to be fatigue-onset markers, the question is why did the men's MPF still rise after their EMG_{Th} had already been reached? As there is little to rely upon, we can only speculate that in the 48–60 %TTE zone the men already started to rely on their higher-threshold but still markedly oxidative type-IIA MUs, while the boys still relied on their lower-threshold, type-I. The type-IIA MUs, being more oxidative, less glycolytic and thus also less acidotic than their type-IIB counterparts, may have mitigated the men's MFCV drop, thus

postponing their MPF decline. Alternatively, the question could again be raised as to MPF's fidelity as a fatigue-onset marker and what factors, other than fatigue, may be affecting its decline.

While the typical MPF's decline at the end of exhaustive exercise is likely largely due to fatigue, the decline's onset (at MPF_{Peak}) may not necessarily be a good fatigue-onset indicator in the way the anaerobic-, ventilatory, or EMG thresholds are. Our boys' $\%MPF_{Peak}$–EMG_{Th} congruence and the men's apparent lack thereof, as discussed above, may suggest that although MPF may not be a reliable fatigue indicator, the boys' MPF behaviour may be more reflective of fatigue than is the men's.

MPF's Variability Limits

Given MPF's high variability (Figs. 3, 4), and the irregular, unexpected nature of many MPF responses (Fig.1), only low to moderate correlations could be expected among its various parameters. It was thus remarkable to find a very close MPF_{Min}–MPF_{Peak} relationship ($r=0.961$, $p<0.0001$; Fig.9) in the adiposity-corrected data, inclusive of the irregularly-patterned participants (n=37). The corresponding values for the individual boys' and men's groups were 0.953 and 0.819, respectively ($p<0.0001$).

Since the men's MPF_{Min}, MPF_{Peak} , or MPF_{Mean}, were all shown to be significantly higher than the boys', it appears likely that their high correlation with body mass are, at least partly, due to the wide range of muscle sizes (proxied by body mass). However, the significant boys–men MPF overlap seen in Fig.9, and the fact that the correlations with body mass explained only 47–54% of the observed variance, imply that one or more factors must be at play, as well.

Regardless, the high MPF_{Min}–MPF_{Peak} correlation suggests that the two are strongly inter-dependent and confined to a narrow range of possible fluctuations. MPF's observed high variability must, therefore, arise mostly from the highly variable inter-individual MPF_{Min}–MPF_{Peak} range differences (14.5±6.9 Hz, CV=48%) and from MPF's variability within those ranges, as shown in this study.

Limitations

The present study highlights MPF's pattern irregularities and greater variability than that of its EMG source, but it was not designed to elucidate the underlying reasons for this variability. Considering the high intra- and inter-individual MPF variability and the large number of observed MPF-pattern irregularities, larger sample sizes would have been beneficial. The correction accuracy of the intervening-layer's suppressing effect on measured MPF and EMG amplitude was likely limited by the use of whole-body adiposity estimates rather than direct thickness determination of the layer underlying the electrode. The

specifics of the intermittent isometric-contraction protocol, originally designed for EMG_{Th} detection, may not have been optimal for MPF determinations. Finally, due to the exclusive use of male data in this exploratory study, its findings cannot be generalized to include females.

Conclusions

Despite the high MPF variability, this study elucidated significant boys–men MPF pattern and magnitude differences in progressively-exhausting, intermittent, isometric exercise. The shallower rise and decline of the boys' MPF, compared with the men's, is congruent with children's hypothesized lesser reliance on the more fatigable, higher-threshold MUs, compared with adults. If true, these child–adult differences in muscle activation or composition can be expected to differentially affect MPF patterns of other contraction modes and protocols of fatiguing exercise.

The intervening-layer or adiposity effect is often overlooked, but can result in large MPF and EMG under-estimates. Correcting for this is essential for both proper group or inter-individual comparisons and for a more realistic muscle-level MPF and EMG amplitude assessment.

MPF, and particularly its decline onset, appears to be of low reliability as fatigue indicator, in general, but particularly when compared with EMG_{Th} in men. This, however, requires much further investigation.

Future research needs to systematically investigate the sources of MPF's variability and irregularity. Whether pattern irregularity is just another manifestation of variability, or a distinct phenomenon, is yet another open question.

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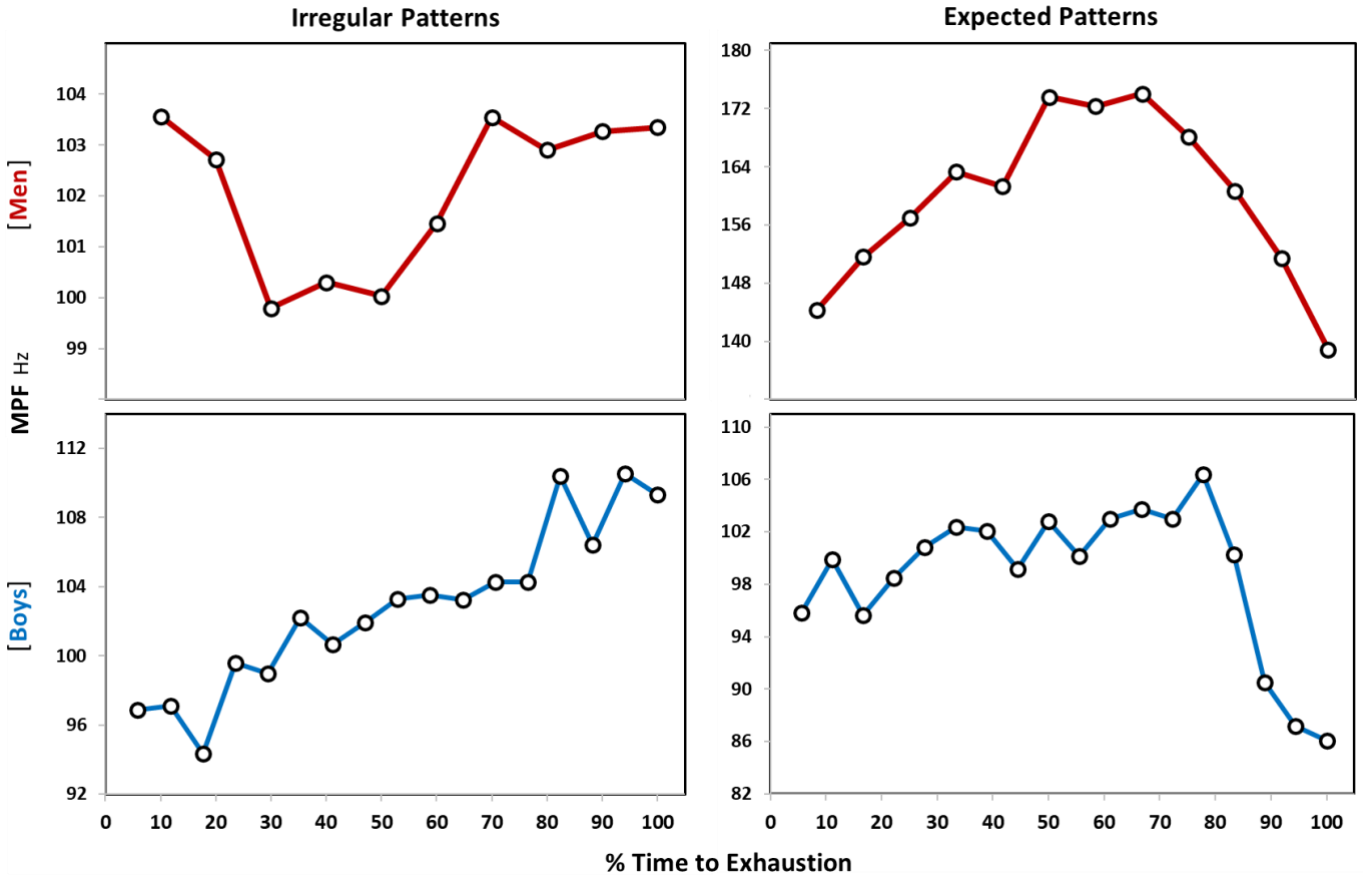
678 **Figure 1:**



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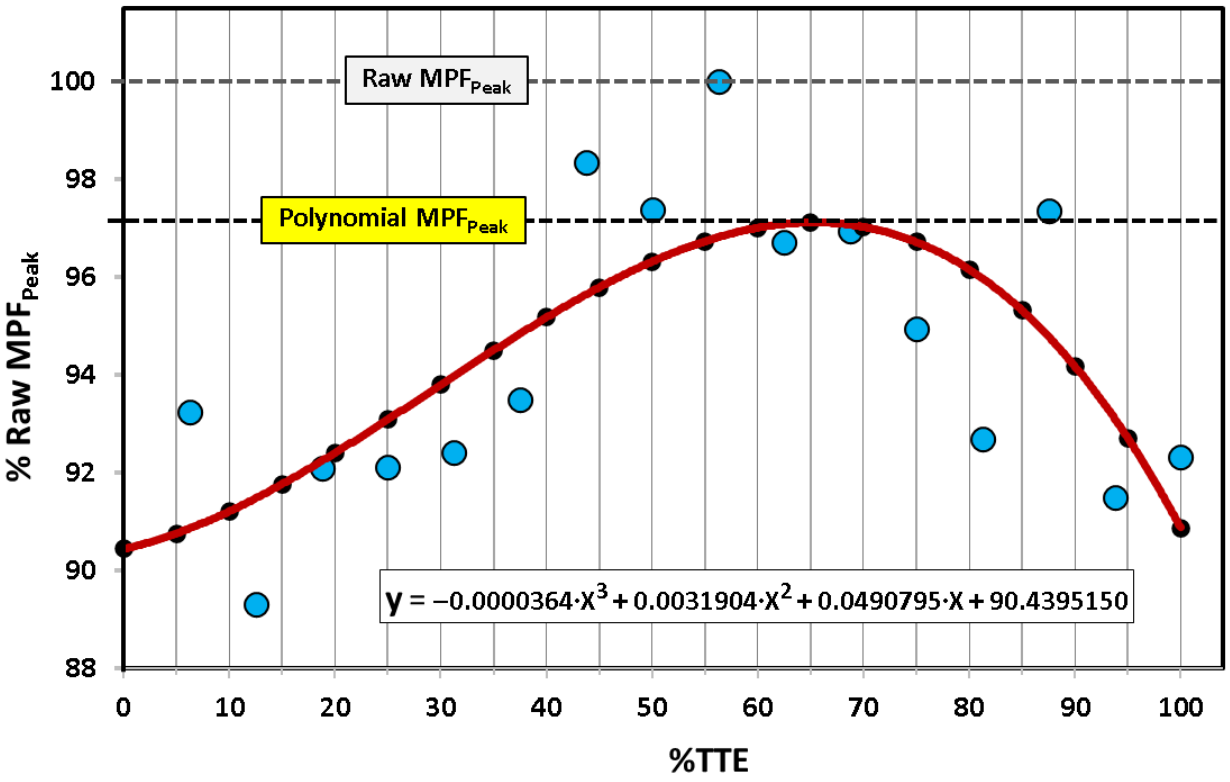
691 **Figure 2:**



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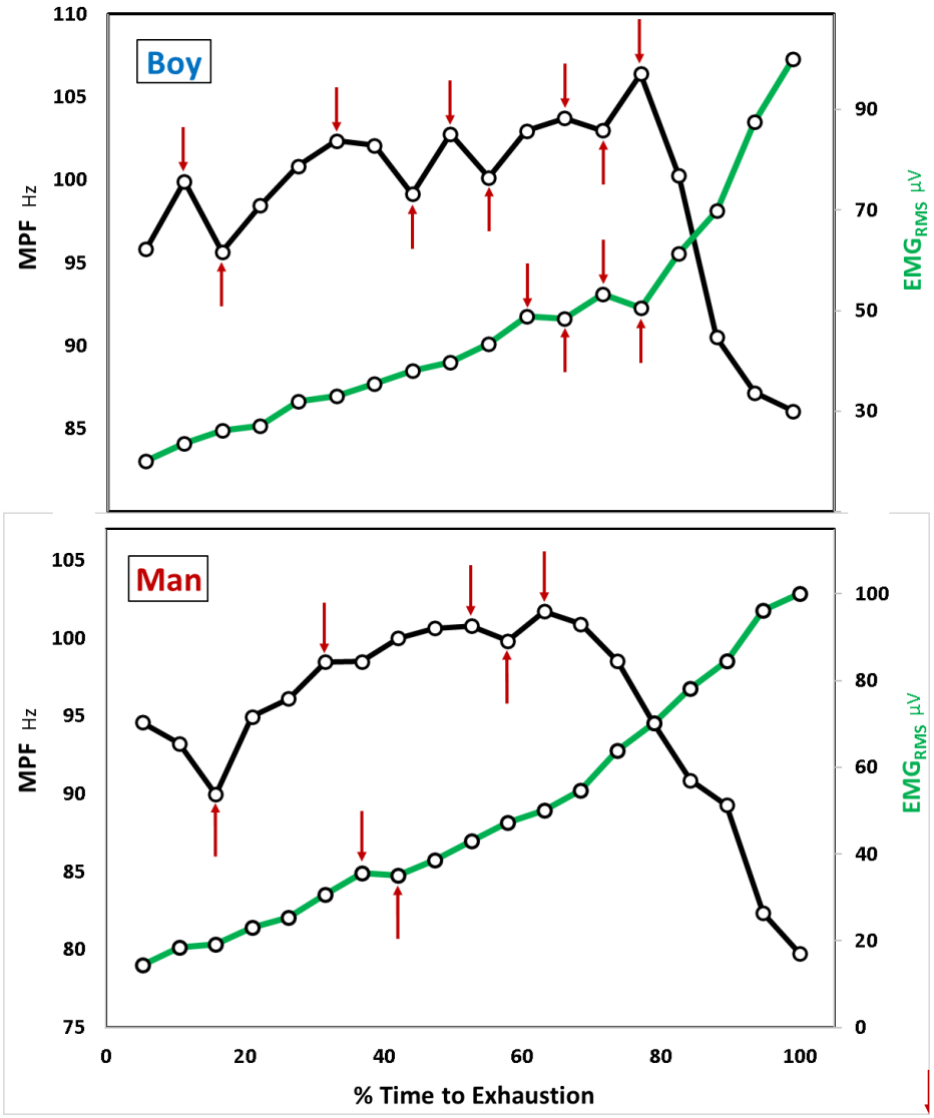

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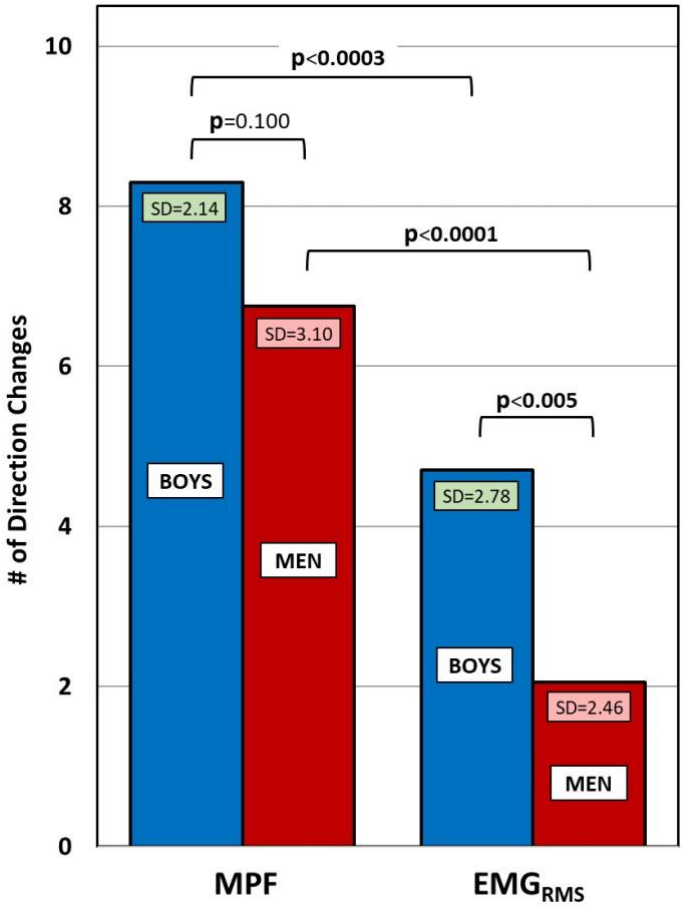
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# of Direction Changes
p<0.0003
p=0.100
p<0.0001
p<0.005
SD=2.14
SD=3.10
SD=2.78
SD=2.46
BOYS
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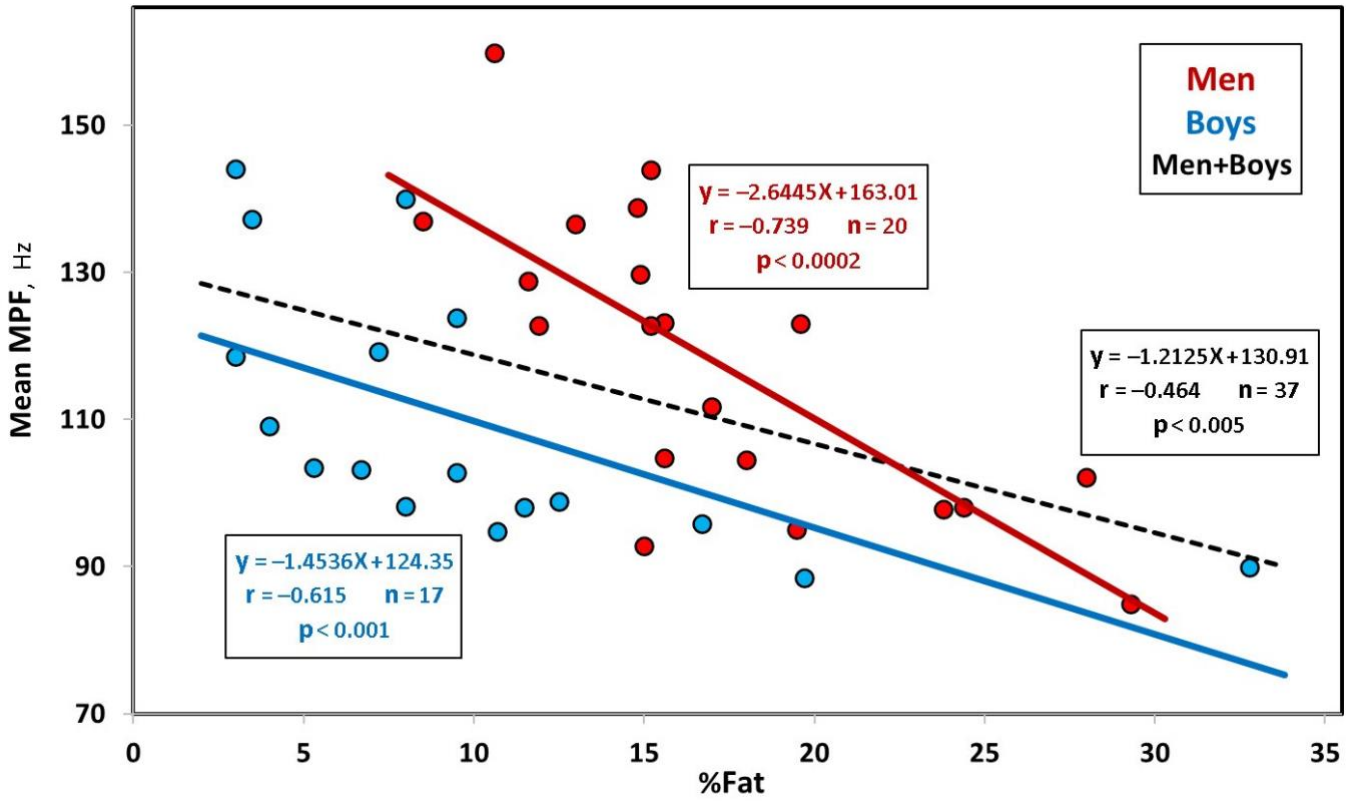
MPF
EMG RMS

705 Figure 5:



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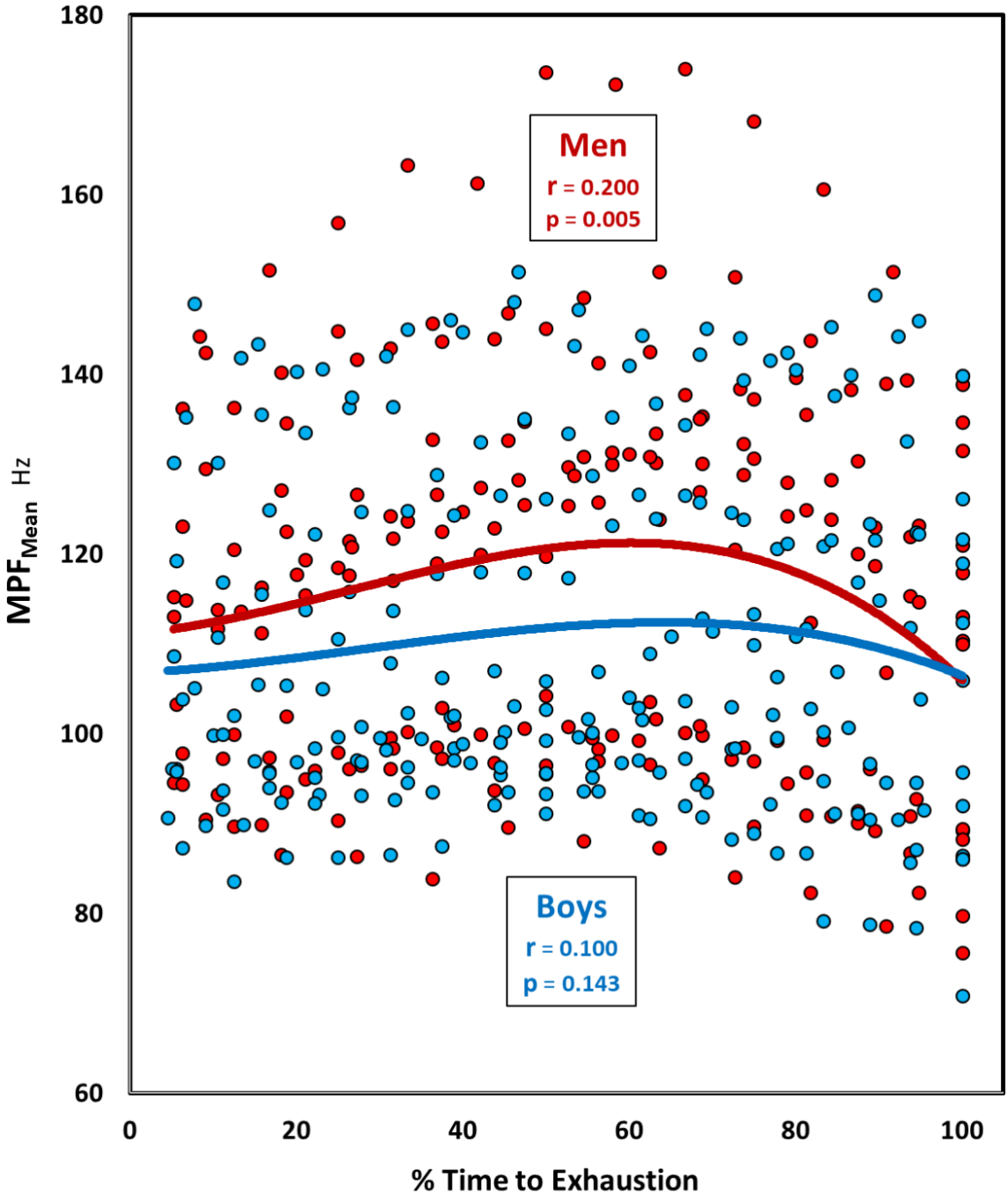

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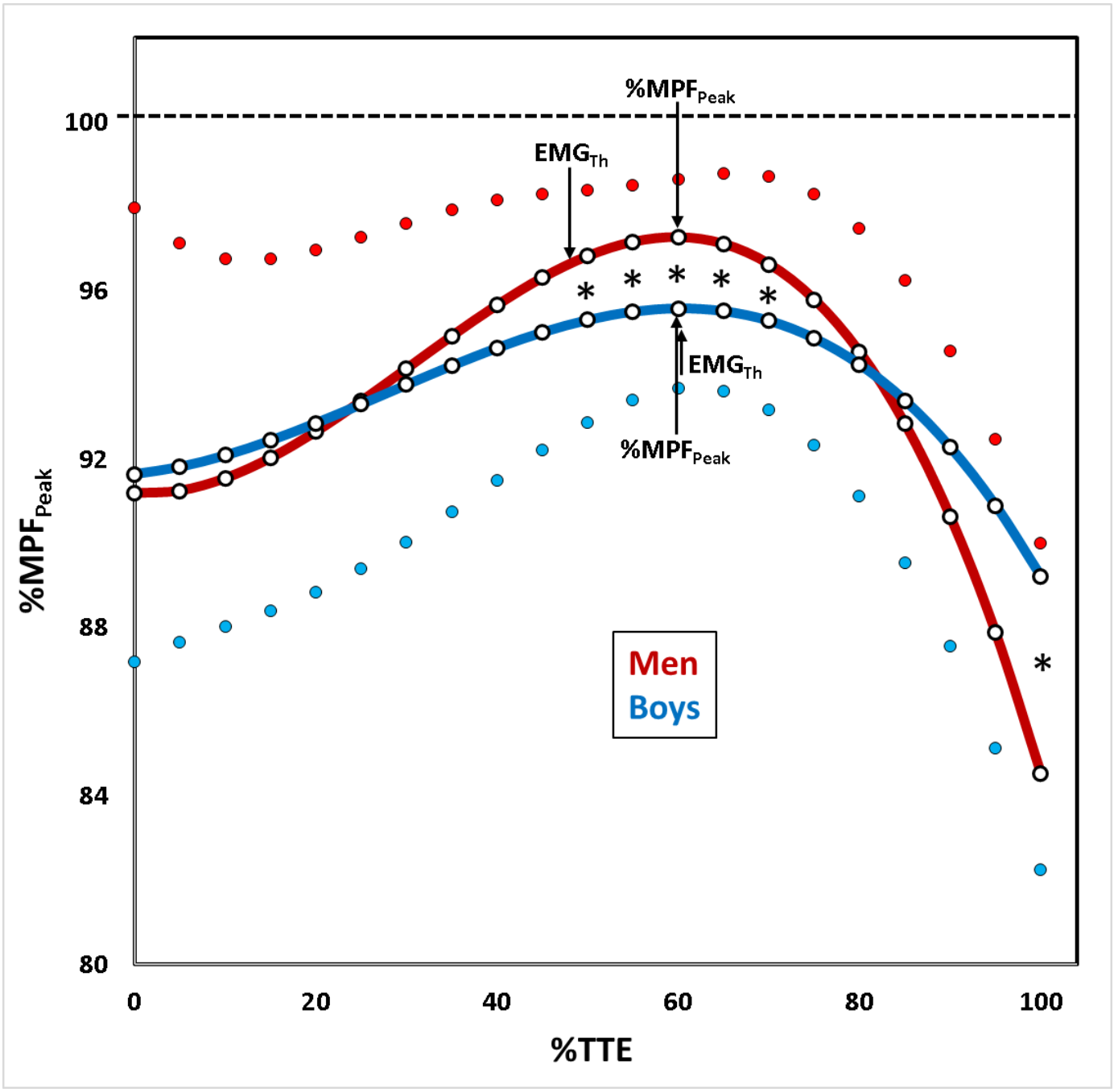

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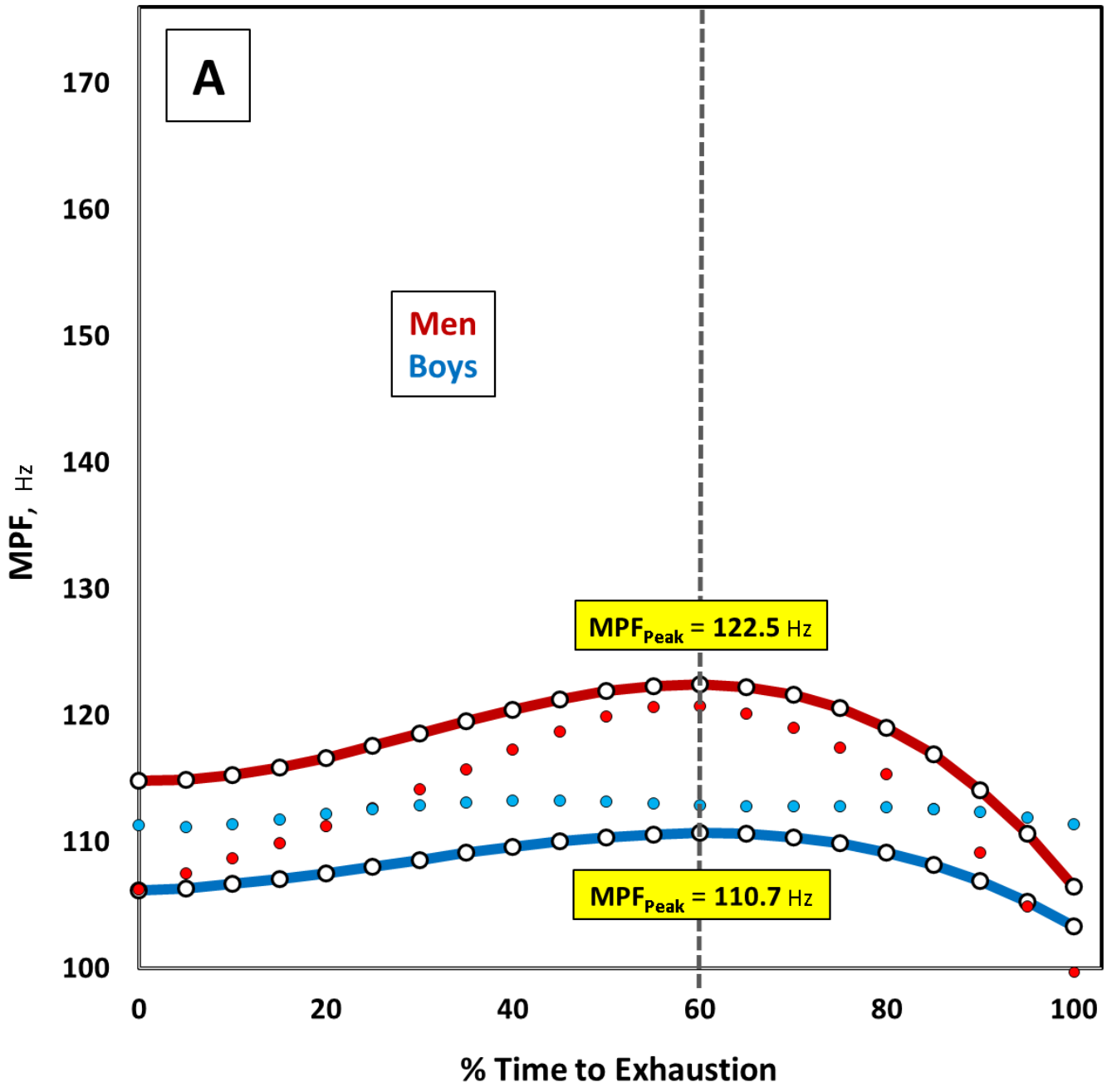

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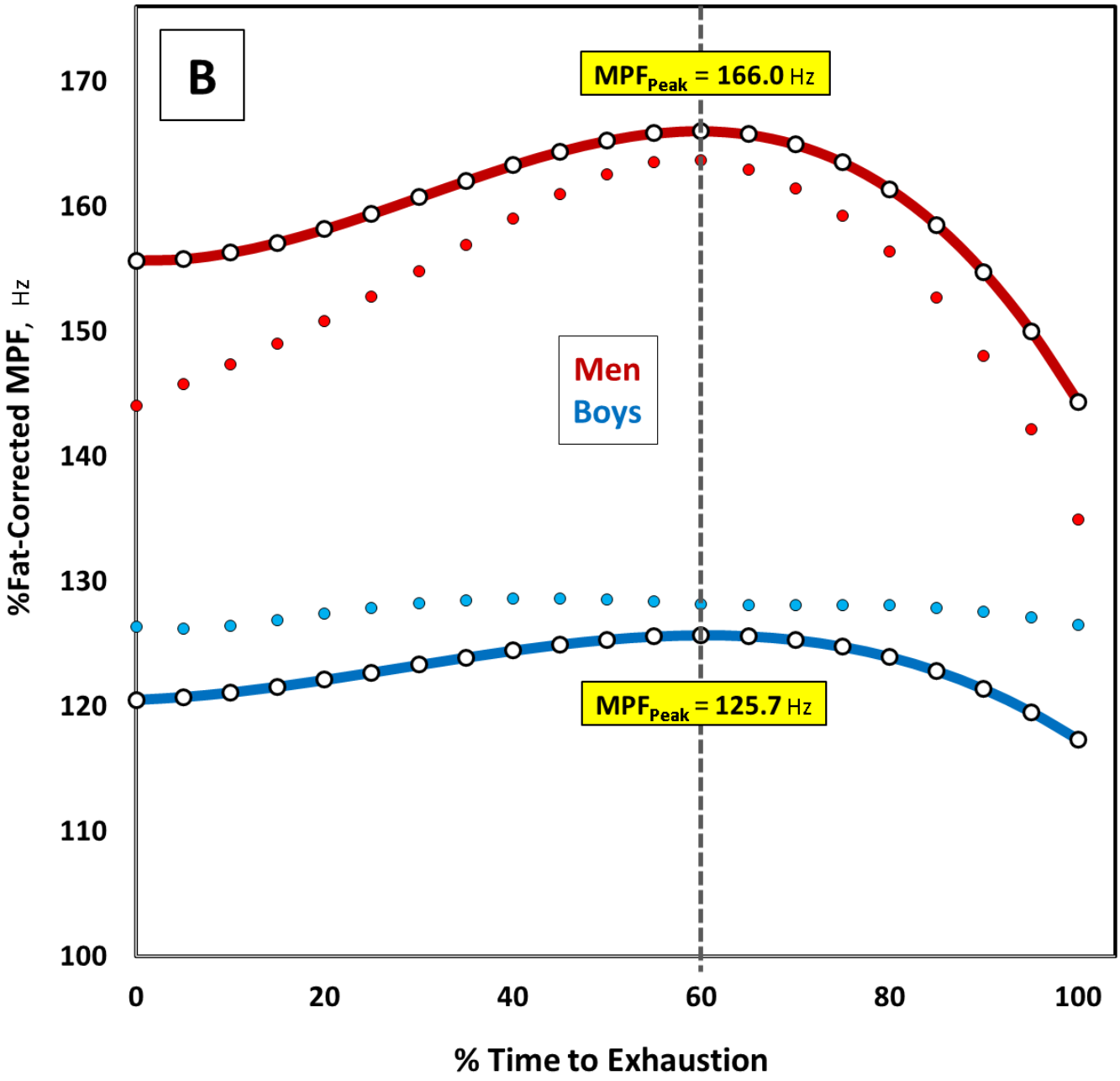

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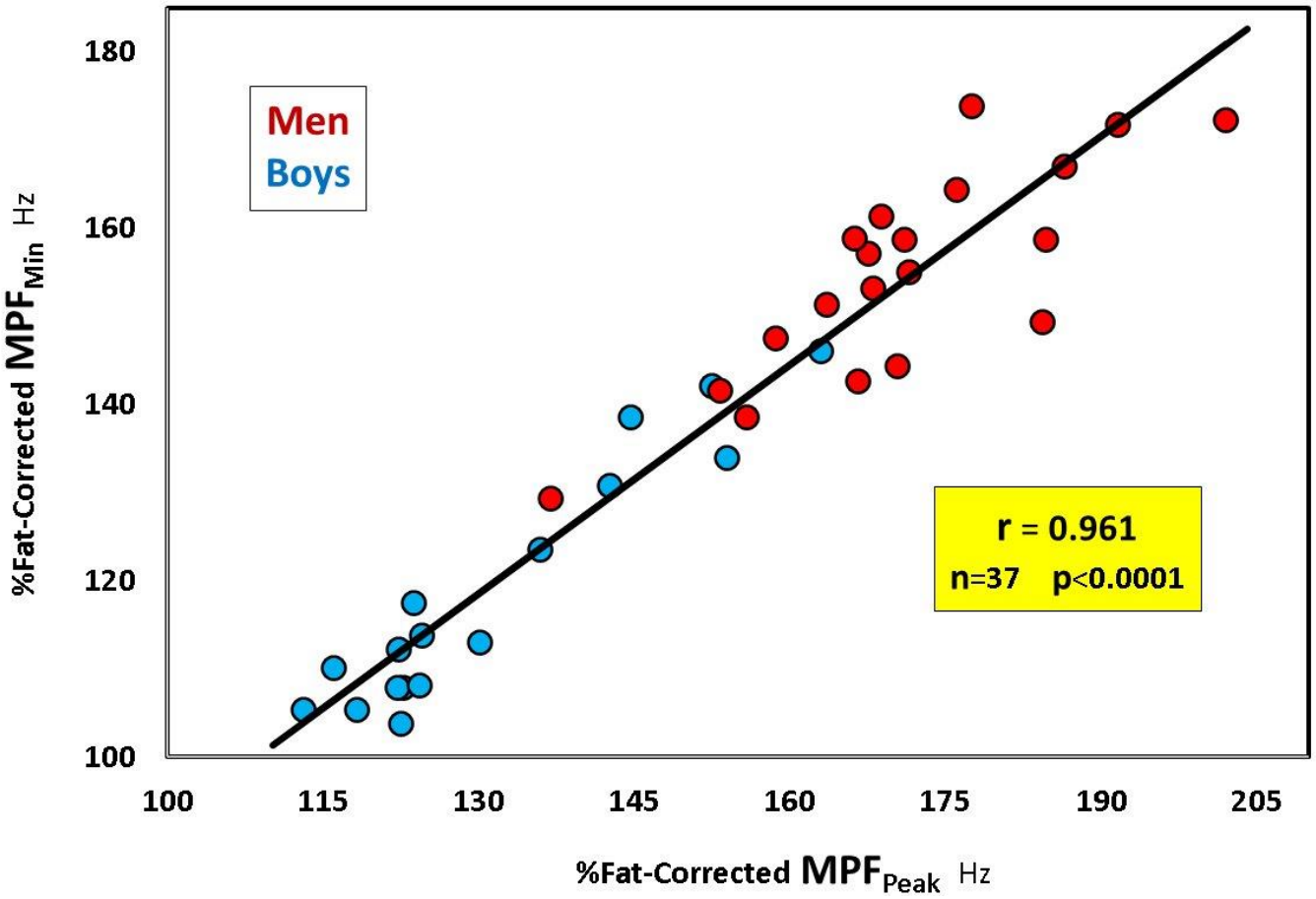
Men
Boys
r = 0.961
n=37 p<0.0001
%Fat-Corrected MPF Min Hz
%Fat-Corrected MPF Peak Hz
180
160
140
120
100
100
115
130
145
160
175
190
205

730

731

Table 1 – Participants' physical characteristics

		Boys	Men	p Value
n	All	17	20	–
	Expected	13	13	–
Age, years	All	10.2 ±1.0	23.5 ±2.5	**<0.0001**
	Expected	10.1 ±0.7	23.7 ±1.9	**<0.0001**
Maturity Offset, years *****	All	-3.03 ±0.65	–	–
	Expected	-3.10 ±0.59	–	–
Height, cm	All	143.4 ±7.6	177.8 ±6.8	**<0.0001**
	Expected	144.2 ±8.5	179.8 ±5.9	**<0.0001**
Body Mass, kg	All	35.3 ±8.0	77.8 ±10.8	**<0.0001**
	Expected	34.2 ±7.5	80.9 ±10.8	**<0.0001**
%Fat	All	10.1 ±7.2	17.1 ±5.5	**<0.005**
	Expected	10.8 ±8.1	16.9 ±5.9	**<0.05**
Lean Body Mass, kg	All	30.2 ±3.5	64.2 ±7.6	**<0.0001**
	Expected	30.7 ±3.5	66.8 ±7.5	**<0.0001**
MVC, Nm	All	103.9 ±22.7	300.5 ±42.7	**<0.0001**
	Expected	103.6 ±22.9	306.0 ±45.8	**<0.0001**
Specific MVC, $Nm \cdot kg_{LBM}^{-1}$	All	3.42 ±0.56	4.70 ±0.58	**<0.0001**
	Expected	3.35 ±0.47	4.60 ±0.62	**<0.0001**

'All' – Complete cohort

'Expected' – Participants with characteristic overall MPF patterns

***** – Years from age of peak height velocity (Mirwald et al. 2002)

Table 2 – MPF and EMG Variables

			Boys	Men	p value	
					t test	ANCOVA*
MPF_{Min} Hz	**Measured**	**All**	104.2 ±16.0	109.7 ±17.1	0.334	**0.002**
		Expected	104.0 ±16.5	109.4 ±19.0	0.461	**<0.03**
	%Fat-Corrected	**All**	118.8 ±13.7	154.8 ±11.7	**<0.0001**	–
		Expected	119.6 ±17.9	154.2 ±11.7	**<0.0001**	–
MPF_{Peak} Hz	**Measured**	**All**	116.7 ±18.1	125.9 ±21.6	0.180	**<0.001**
		Expected	115.8 ±18.1	125.9 ±23.8	0.253	**<0.01**
	%Fat-Corrected	**All**	131.4 ±14.3	171.1 ±14.2	**<0.0001**	–
		Expected	131.5 ±17.7	170.7 ±15.5	**<0.0001**	–
MPF_{Mean} Hz	**Measured**	**All**	109.7 ±17.1	117.9 ±19.5	0.200	**<0.001**
		Expected	109.0 ±17.7	117.6 ±21.6	0.294	**<0.01**
	%Fat-Corrected	**All**	124.4 ±13.5	163.0 ±13.1	**<0.0001**	–
		Expected	124.7 ±17.6	162.4 ±13.9	**<0.0001**	–
Mean EMG_{RMS} μV	**Measured**	**All**	70.4 ±23.7	97.5 ±53.9	0.071	**<0.001**
		Expected	67.2 ±19.6	100.4 ±52.7	0.052	**<0.005**
	%Fat-Corrected	**All**	87.5 ±20.3	212.5 ±40.4	**<0.0001**	–
		Expected	85.4 ±16.7	214.7 ±35.8	**<0.0001**	–
EMG Threshold %	**%MVC**	**All**	56.8 ±9.0	46.2 ±6.7	**<0.001**	–
		Expected	57.4 ±10.0	44.4 ±5.6	**<0.0005**	–
	%TTE	**All**	58.4 ±12.4	51.2 ±15.0	0.156	–
		Expected	60.0 ±12.8	48.1 ±13.2	**<0.04**	–

* – ANCOVA of the measured, values with %Fat as covariate

Figure Legends

Figure 1 – Examples of 'Expected' (characteristic) vs. 'Irregular' (unexpected) MPF patterns in our boys and men.

Figure 2 – A representative example of how individual regression points were determined. Note the difference between the raw MPF_{Peak} value and the polynomial MPF_{Peak}, calculated from 3rd-order polynomial regression. The values at 5% intervals of %TTE were used to calculate group means for each of the 21 points and construct the mean regressions for each group, shown in Fig.7.

Figure 3 – Boy's and man's representative examples of the relative pattern variability (fluctuations) of the EMG_{RMS} and MPF traces vs. % time to exhaustion (black=MPF, green=EMG_{RMS}). Red arrows mark each time a given trace's slope changes from positive to negative or vice versa. In these examples: the boy had 9 MPF trace changes vs. only 4 EMG_{RMS} ones, while the corresponding changes in the man were 5 vs. 2.

Figure 4 – MPF and EMG_{RMS} variability comparisons between the boys and the men. The boys show 30% more fluctuations in MPF, and 123% more in EMG_{RMS}, compared with the men. MPF was found ~1.8 times more variable than EMG_{RMS} in the boys and ~3.3 times in the men.

Figure 5 – The adiposity effect on surface-recorded MPF in the entire cohort ('All', n=37). The MPF_{Mean} vs. %Fat regression correlations for the men (red) and the boys (blue) were considerably higher than the corresponding correlation for the composite group (men + boys; black) correlation, indicating two distinct men and boys relationships (slope of – 2.645 vs. 1.454 $Hz \cdot \%Fat^{-1}$, respectively). These relationships were used to 'correct' the MPF values for the effect of %Fat, in each group (Table 2).

Figure 6 – MPF vs. %TTE data-point distribution in the 'Expected' subgroup (boys: n=13, blue; men n=13, red). Note the >100 Hz overall MPF variability range (70.8–174.1 Hz). No statistically significant group difference between the two regressions (p=0.303)

Figure 7 – Mean MPF percentage of the polynomial MPF_{Peak} (Fig.3) across exercise time (%TTE) for the men (red) and the boys (blue). Arrows mark the mean locations of maximal $\%MPF_{Peak}$ and

the EMG_{Th} in each group. Note that while peak $\%MPF_{Peak}$ occurred at ~60 %TTE in both groups, EMG_{Th} showed no such agreement, being nearly identical to $\%MPF_{Peak}$ in the boys, but occurring nearly 12 %TTE earlier in the men (See text). * = significant pairwise difference between groups ($p<0.05$). Dots denote the standard deviations of the means.

Figure 8 – Quantitative representation of the 'Expected' boys–men MPF differences in terms of both relative pattern and absolute magnitude. The $\%MPF_{Peak}$ values (Fig.7) were multiplied by the respective mean MPF_{Peak} values observed in boys and men (115.8 and 125.9 Hz, respectively) **(Fig.8$_A$),** or the respective adiposity-corrected values (131.5 and 170.7 Hz, respectively) (Fig.**8$_B$**). See also Table 2. The dots denote standard deviations of the means.

Figure 9 – The relationship between the %Fat-corrected MPF_{Min} and MPF_{Peak} for the entire cohort ('All', n=37), suggesting a strong size dependency between the two MPF parameters. The respective boys' and men's correlations were 0.953 and 0.819 ($p<0.0001$).