Skeletal muscle fiber-type distribution and habitual physical activity in daily life

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The capacity to perform physical activity largely depends on physical fitness. Muscle fiber-type distribution (MuscleFTD) is associated with physical fitness and may influence the capacity to perform physical activity. The purpose of this study was to determine whether habitual physical activity in daily life (PADL) and MuscleFTD are related. Thirty-eight healthy non-athletes (31 women, 7 men) were recruited. PADL was measured twice for 14 days using a tri-axial accelerometer for movement registration (Tracmor). From Tracmor output, the proportion of time subjects were physically active at low, moderate, and high intensities (%Low, %Moderate, and %High, respectively) was determined. A total activity index (PAindex) and sub-scores on work, leisure-time and sports were obtained using the Baecke questionnaire. MuscleFTD was determined using immuno-fluorescence against respective myosin heavy chain isoforms. No relationship was observed between PADL and MuscleFTD. %Low, %Moderate, and %High, as well as PAindex and its sub-scores, were not related to MuscleFTD either. The time spent on sports was associated with the proportion of type I and IIX fibers (P = 0.06 and P < 0.01, respectively). In conclusion, MuscleFTD probably cannot explain why some people are more prone to engaging in physical activities than others.

Activity-related energy expenditure is the most variable component of total energy expenditure (Ravussin & Swinburn, 1992) and appears to be an important determinant of energy balance (Schoeller et al., 1997). This implies that a reduced physical activity is a potentially important contributor to a predisposition to obesity (Heitmann et al., 1997; Weinsier et al., 1998; Esparza et al., 2000; Wardle et al., 2001; Ekelund et al., 2002). In a recent twin study, habitual physical activity in daily life (PA_{DL}) was determined using a tri-axial accelerometer for movement registration. Based on the difference in intra-pair correlation in PA_{DL} between monozygotic and dizygotic twins (R = 0.88 and 0.42, respectively), additive genetic factors were concluded to explain 78% of inter-subject variation in PA_{DL} (Joosen et al., 2005). This suggests that genes determine to a large extent whether a person is prone to engaging in physical activities. How PA_{DL} is affected by the genotype remains to be established.

One of the potential factors through which the genetic background could affect PA_{DL} is skeletal muscle fiber-type distribution (MuscleFTD) (Komi et al., 1977; Gollnick & Matoba, 1984; Simoneau et al., 1985; Lortie et al., 1986; Simoneau & Bou-
Materials and methods

Subjects

Based on an effect size of 0.25, a power calculation indicated that 33 subjects are required for a power of 0.8 in simple linear regression analyses. Taking a dropout rate of 15% into account, 38 healthy, non-smoking subjects (31 females, 7 males) aged 20 ± 2 years were recruited to participate in this study. Subjects were not using any medication except for oral contraceptives. Recruitment was carried out using flyers in the university building. Subjects spending over 2 h/week on endurance sports, or 5 h on sports in general, were excluded from participation to minimize the effect of exercise training on MuscleFTD. Subjects who were involved in sports but did not participate in sports or meet the aforementioned criterion. These subjects participated in sports on a recreational basis and in a wide range of sports. Information about the purpose and protocol of the study, as well as its risks and discomfort were provided orally and in writing. All subjects provided written informed consent before participating in the study. The study conformed to the standards set by the Declaration of Helsinki, and the local Ethics Committee approved the study. Subject characteristics (n = 38) are presented in Table 1.

PA_{DL} was measured using a tri-axial accelerometer for movement registration (Tracmor IV; Philips Research, Eindhoven, the Netherlands) sensitive to a wide range of body movements. The accelerometer has been validated against doubly labeled water, the gold standard for measuring energy expenditure in daily life (Plasqui et al., 2005). The Tracmor registers accelerations of the trunk along the antero-posterior, medio-lateral, and longitudinal axes using three uni-axial piezo-electric accelerometers (details are provided elsewhere; Plasqui et al., 2005). To ensure a valid reflection of long-term daily life activities, the accelerometer was worn for two 14-day periods under free-living conditions. Subsequently, PA_{DL} was acquired by summing the output of all three axes and is represented as Megacounts per day (Mcnts/day). PA_{DL} was defined as the average of both measurement periods.

Subjects were instructed to wear the Tracmor from the moment they woke up in the morning until they went back to bed at night. To verify whether subjects lived up to this instruction, waking hours and clock times of wearing the Tracmor were noted. To make sure only representative days were included, the difference between the total time the subject was awake and the time the accelerometer was worn was not allowed to exceed 75 min/day. The few days during which this difference was more than 75 min were excluded from the analysis. This resulted in an average of 26 representative days per subject. To make sure the subjects met the inclusion criterion concerning their participation in sports, the actual sporting hours were also recorded in the diary.

Using Tracmor data, the proportion of time subjects were physically active at a low, moderate, and high-intensity (%Low, %Moderate, and %High, respectively) was determined. The cut-off points for the intensity categories were determined in a pilot study (n = 5). The cut-off point for low-intensity physical activity was set by Tracmor outputs associated with walking on a treadmill at 3.5 km/h, which corresponds with approximately 3 metabolic equivalents (METs). For moderate-intensity physical activity, a Tracmor output associated with walking on a treadmill at 5 km/h was used, which corresponds with approximately 4.5 METs (Ainsworth et al., 2000). The relevant Tracmor outputs were 16.0 ± 3.0 Mcnts/min and 28.9 ± 3.0 Mcnts/min, respectively. The proportion of time per intensity category was calculated as the sum of all minutes per intensity category divided by the total duration of the measurement, i.e. 28 days minus the number of excluded days.

Using linear regression analysis in a population similar to the present study with respect to PA_{DL}, body composition and age, Plasqui et al. (2005) were able to predict physical activity level (PAL) with an explained variation of 70% using only PA_{DL}. This regression equation was used in the present study to estimate PAL.

Measures for physical activity during work, sports and leisure-time were obtained using the Baecke questionnaire (Baecke et al., 1982). Summing the scores of each section provided a total activity index (PA_{index}). Like the Tracmor, the Baecke questionnaire has been validated against doubly labeled water, with the PA_{index} explaining 45% of the variation in PAL (Philippaerts et al., 1999).

Muscle sample analysis

A muscle biopsy was obtained from the M. Vastus Lateralis under local anesthesia (xylocaine 2%) using a Bergström needle with suction (Bergstrom, 1975). The Vastus Lateralis was selected because of the absence of large vessels or nerves in the region, the presence of type I, II A, and II X muscle fibers in an ample amount (Staron et al., 2000) and the large inter-individual variation in MuscleFTD (Edstrom & Nystrom, 1969; Staron et al., 1994). Biopsies were frozen in melting isopentane and stored in a pre-cooled aluminum cryo-vial at −80 °C until analyzed.

Serial transverse cryosections were cut (5 μm) in a cryostat microtome (Leica; CM 3050, Rijswijk, the Netherlands) and thaw-mounted on uncoated pre-cleaned glass slides. After air-drying for ~120 min, sections were again stored at −80 °C until processing for routine immunofluorescent staining against distinct myosin heavy-chain (MHC) isoforms.

Muscle fibers were characterized as type I, II A, or II X using antibodies against the respective MHC isoforms. Briefly, air-dried cryosections were treated for 5 min with 0.5% triton X-100 in phosphate-buffered saline (PBS), and washed for 5 min with PBS. Thereafter, a 0.05% Tween20/PBS dilution containing the primary antibody for MHCI diluted 1:50 (A4.840; DSHB, Iowa city, Iowa, USA), MHCIIX diluted 1:50 (N2.261, DSHB) and anti-laminin diluted 1:200 (L-9393; Sigma, Zwijndrecht, the Netherlands) was applied for 45 min. After three 5-min washes with PBS the appropriate secondary antibodies [Alexa Fluor 555 Goat anti-Mouse IgM diluted 1:500 (A-21426), Alexa Fluor 488 Goat anti-Mouse IgG1 diluted 1:200 (A-21211), and Alexa Fluor 350 Goat anti-Rabbit IgG diluted 1:130 (A-11069) (Molecular Probes Invitrogen, Breda, the Netherlands)] were applied for 45 min at room temperature. Again, sections were washed with PBS.
three times for 5 min and embedded in Mowiol 4-88 (475904, Calbiochem, Amsterdam, the Netherlands).

After 24 h, the slides were examined using a Nikon E800 Fluorescence microscope (Uvikon, Bunnik, the Netherlands). Images were captured using a color CCD camera (Basler 113C, Basler vision technologies, Ahrensburg, Germany) with MHC in red, MHCIIA in green and laminin, a basement membrane marker to identify the myofiber boundary, in blue. All fibers without intracellular staining were considered to be type IIx muscle fibers.

Digitally captured images (× 20 magnification) were processed and analyzed using Lucia 4.8 software (Nikon; Düsseldorf, Germany). Muscle fiber typology was measured semi-automatically using a custom-written macro that identifies individual muscle fibers. Upon thresholding, red (MHC1), green (MHCIIA), and unstained fibers (MHCIIx) were identified and expressed as percentage of the total number of fibers.

Results

PA
d did not significantly differ between the two 14-day measurement periods (P = 0.14). Results on PA
d averaged over both periods, as well as on the proportion of time spent in each intensity category are presented in Table 2. PA\text{index} and Baecke subscores, as well as the weekly time spent on sports, MuscleFTD, VO2max and body composition are also shown in Table 2.

PA
d was comparable for men and women: 4128 vs 3704 Mcnts/day, respectively (Table 2). The proportion of time spent in each intensity category was comparable between genders as well, although.

Table 2. Data on physical activity, muscle fiber type distribution, and body composition

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>PA\text{DL} (Mcnts/day)</td>
<td>4128 ± 636</td>
<td>3704 ± 675</td>
</tr>
<tr>
<td>PAL</td>
<td>1.88 ± 0.10</td>
<td>1.80 ± 0.11</td>
</tr>
<tr>
<td>%Low (24 h)</td>
<td>96.5 ± 1.0</td>
<td>97.3 ± 1.0</td>
</tr>
<tr>
<td>%Moderate (24 h)</td>
<td>2.3 ± 0.5</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>%High (24 h)</td>
<td>1.2 ± 0.8</td>
<td>0.7 ± 0.4*</td>
</tr>
<tr>
<td>PA\text{index}</td>
<td>8.1 ± 1.0</td>
<td>8.6 ± 0.9</td>
</tr>
<tr>
<td>Baecke work</td>
<td>2.0 ± 0.3</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>Baecke sport</td>
<td>3.2 ± 0.7</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>Baecke leisure</td>
<td>3.0 ± 0.3</td>
<td>3.4 ± 0.4*</td>
</tr>
<tr>
<td>Sports (h/week)</td>
<td>2.2 ± 1.8</td>
<td>2.1 ± 1.7</td>
</tr>
<tr>
<td>%Type I</td>
<td>55.6 ± 12.9</td>
<td>59.6 ± 10.6</td>
</tr>
<tr>
<td>%Type IIa</td>
<td>39.4 ± 11.6</td>
<td>37.1 ± 10.3</td>
</tr>
<tr>
<td>%Type IIx</td>
<td>3.9 ± 3.9</td>
<td>3.3 ± 5.1</td>
</tr>
<tr>
<td>VO2max (L/min)</td>
<td>4.0 ± 0.8</td>
<td>2.7 ± 0.4*</td>
</tr>
<tr>
<td>VO2max (mL/min/kg BM)</td>
<td>51.1 ± 5.1</td>
<td>42.8 ± 4.7\textsuperscript{7}</td>
</tr>
<tr>
<td>VO2max (mL/min/kg FFM)</td>
<td>60.8 ± 4.8</td>
<td>58.4 ± 5.7</td>
</tr>
<tr>
<td>FFDM (kg)</td>
<td>66.3 ± 9.9</td>
<td>46.0 ± 4.6*</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>13.0 ± 6.3</td>
<td>17.1 ± 4.8</td>
</tr>
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</table>

\text{PA DL}, physical activity in daily life as measured using a tri-axial accelerometer during two periods of 2 weeks; Mcnts/day, Megacounts per day; PAL, physical activity level, i.e., the factor by which total energy expenditure differs from resting energy expenditure; %Low, %Moderate, and %High, proportion of time subjects were physically active at a low, moderate, and high-intensity respectively; PA\text{index}, total activity index measured with the Baecke questionnaire; Baecke Work, Sport, and Leisure, scores on each section of the Baecke questionnaire; Sports, weekly time spent on sports in hours; FFDM, fat-free mass; FM, fat mass; %Type I, %Type IIa, and %Type IIx, proportion of type I, IIa, and IIx muscle fibers; VO2max, maximal oxygen uptake, either absolute, or relative to body mass or FFDM; Data are means ± SD.

Statistics

Differences between men and women were tested using Student’s t-tests for unpaired samples. Differences in PA\text{DL} between the two measurement periods were evaluated using a Student’s t-test for paired samples. Bivariate correlation was used to test the association between parameters of physical activity and MuscleFTD. To evaluate the relationship between %Low, %Moderate, %High, and the time spent on sports with MuscleFTD, variables were log transformed. This transformation was applied to obtain a normal distribution of the residuals with homoskedasticity, i.e., an equal variance over the entire range of predicted values. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 11 for Macintosh OSX (SPSS Inc., Chicago, Illinois, USA). Data are expressed as means ± SD. For associations, unstandardized coefficients and 95% confidence intervals, as well as P-values are provided. P-values <0.05 were considered statistically significant.
High was significantly higher in men: 1.2% vs 0.7% in women \((P<0.01)\). Men and women combined were physically active at a low, moderate, and high-intensity for approximately 97%, 2%, and 1% of the time, respectively. This corresponds with 30 and 11 min of moderate and high-intensity physical activity per day. Application of the regression equation developed by Plasqui et al. (2005) to the present population showed that the PAL ranged from 1.62 to 2.04. Muscle\_FFTD was not significantly different between genders, which confirms the findings of previous studies (Bell et al., 1980; Kriketos et al., 1997; Everts et al., 1999; Staron et al., 2000; Jaworowski et al., 2002). When averaged for both genders, Muscle\_FFTD was approximately 59% type I, 38% type II\_A, and 3% type II\_X.

No difference was found between men and women for the PA\_ind and Baecke sub-scores on work and sports (Table 2). Only the Baecke sub-score for physical activity during leisure-time was significantly

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**Fig. 1.** Habitual physical activity in daily life and maximal oxygen uptake per kg fat-free mass as a function of muscle fiber-type distribution. (a–c) Show habitual physical activity in daily life (PA\_DL) as a function of the proportion of type I, II\_A, and II\_X muscle fibers, respectively. (d–f) Show maximal oxygen uptake (VO\_2\_max) expressed per kg fat-free mass (FFM) as a function of the proportion of type I, II\_A, and II\_X muscle fibers, respectively. (d) \(P<0.05\); (f) \(P = 0.05\).
different between genders: 3.0 ± 0.3 vs 3.4 ± 0.4 for men and women, respectively (P<0.05). The weekly time spent on sports did not differ between genders (Table 2). On average, subjects reported spending approximately 2 h/week on sports, which corresponds with approximately 17 min/day.

FFM is the strongest independent predictor of VO\textsubscript{2max}, alone explaining 86% of its variation (P<0.001). Although VO\textsubscript{2max} was significantly higher in men than in women, this difference did not remain after adjusting VO\textsubscript{2max} for FFM. Based on these results, both genders were combined for further analyses.

VO\textsubscript{2max} expressed per kilogram FFM was positively associated with %Type I (R\textsuperscript{2} = 0.13; P<0.05), and correlated borderline significantly with %Type II\textsubscript{X} (R\textsuperscript{2} = 0.10; P = 0.05) (Fig. 1). A trend towards a positive association was observed between PA\textsubscript{DL} and VO\textsubscript{2max} adjusted for FFM (P = 0.09). PA\textsubscript{DL} on the other hand was not significantly associated with Muscle\textsubscript{FTD} (P-values >0.7) (Fig. 1). No associations were found between %Low, %Moderate, and %High with Muscle\textsubscript{FTD} either (P-values ≥ 0.2). Moreover, neither the PA\textsubscript{index} nor the Baeeke subscores were significantly correlated with Muscle\textsubscript{FTD} (P-values ≥ 0.2). On the contrary, the time weekly spent on sports was negatively associated with %Type II\textsubscript{X} (R\textsuperscript{2} = 0.19; P<0.01) and tended to correlate positively with %Type I (R\textsuperscript{2} = 0.09; P = 0.06) (Fig. 2). All unstandardized regression coefficients, 95% confidence intervals and P-values are provided in Table 3.

**Discussion**

The capacity to perform physical activity was proposed previously to depend on physical fitness (Tikkanen et al., 1998; Hedman et al., 2002). Since Muscle\textsubscript{FTD} was shown to be strongly associated with physical fitness (Hedman et al., 2002), the former was considered to be a candidate to explain (part of) the inter-individual variation in PA\textsubscript{DL}. The aim of this study was thus to determine whether habitual physical activity in daily life was associated with Muscle\textsubscript{FTD}. To this end, PA\textsubscript{DL} was measured for two periods of 14 days using the Tracmor, a validated triaxial accelerometer for movement registration. The previously observed associations between physical activity and VO\textsubscript{2max} and between VO\textsubscript{2max} and Muscle\textsubscript{FTD} were confirmed in the present study. This affirms Muscle\textsubscript{FTD} as a candidate to influence the capacity to perform physical activity.

To prevent an effect of physical exercise training on Muscle\textsubscript{FTD}, subjects spending more than 2 h/week on endurance sports or more than 5 h/week on sports in general were excluded from participation. This resulted in an average engagement in sports of 2 h/week. Still, a wide range in PA\textsubscript{DL} was observed and the range in PAL observed indicates that sedentary as well as highly physically active subjects were recruited (Black et al., 1996; Westerterp, 2001).

No evidence was found for a relationship between PA\textsubscript{DL} and Muscle\textsubscript{FTD}. The accelerometer used in this study was also used by Joosen et al. (2005), who showed that the largest part of inter-subject variation in PA\textsubscript{DL} results from genetic variation. Hence, our results suggest that Muscle\textsubscript{FTD} probably

![Fig. 2. Time spent on sports in h/week and the proportion of type I (a), II\textsubscript{X} (b), and II\textsubscript{X} (c) muscle fibers after natural log (ln) transformation of both variables. (a) P = 0.06; (c) P<0.01.](image-url)
of type I, IIA and IIX muscle fibers. Moderate and High, proportion of time subjects were physically active at a low, moderate and high intensity, respectively; PAindex, total activity index on sports in hours; VO₂max, maximal oxygen uptake expressed in ml O₂ per minute per kg fat-free mass; %Type I, %Type IIA and %Type IIX, proportion of type I, IIA and IIX muscle fibers.

cannot explain the large inter-individual variation in PA大力, that results from genetic variation. In concurrence with this finding, no association was found between the PAindex and MuscleFTD. Moreover, no relationship was observed between %Low, %Moderate and %High, proportion of time subjects were physically active at a low, moderate and high intensity, respectively; PAindex, total activity index measured with the Baecke questionnaire; Baecke Work, Sport and Leisure, scores on each section of the Baecke questionnaire; Sports, weekly time spent on sports in hours; VO₂max, maximal oxygen uptake expressed in ml O₂ per minute per kg fat-free mass; %Type I, %Type IIA and %Type IIX, proportion of type I, IIA and IIX muscle fibers.

Rebux et al. actually resulted from sports rather than leisure-time per se. On average, subjects reported to spend 2 h/week on sports. A significant relationship was observed between the time weekly spent on sports and %Type II X. The power of this association was 0.84 (α = 0.05, one predictor, R² = 0.19, n = 38). Furthermore, the weekly time spent on sports tended to correlate positively with %Type I. Owing to the cross-sectional design of this study, no conclusions about causality can be drawn. However, previous studies showed that both endurance and strength training can induce a shift in MuscleFTD from type II X to type II A, i.e., toward a more oxidative phenotype (Andersen & Henriksson, 1977; Ingjer, 1979; Howald et al., 1985; Simoneau et al., 1985; Sale et al., 1990; Coggan et al., 1992; Staron et al., 1994). For example, Staron et al. (1994) showed that in untrained subjects, %Type II X decreased significantly already after four sessions of strength training within 2 weeks. This implies that even a small difference in training status may have resulted in the relationship found between the time spent on sports and %Type II X. The decreased %Type II X is therefore considered an effect rather than a cause of an increased time spent on sports.

Contrary to transitions from type II X to type II A, the majority of studies performed did not find an increased %Type I following a period of either endurance or strength training (Gollnick et al.,

<table>
<thead>
<tr>
<th>%Type I</th>
<th>%Type IIA</th>
<th>%Type IIX</th>
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<tbody>
<tr>
<td>B</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>PA大力</td>
<td>2.7</td>
<td>18.3 - 23.6</td>
</tr>
<tr>
<td>%Low (24/h)</td>
<td>-0.002</td>
<td>-0.02 - 0.02</td>
</tr>
<tr>
<td>%Moderate (24/h)</td>
<td>0.06</td>
<td>0.5 - 0.7</td>
</tr>
<tr>
<td>%High (24/h)</td>
<td>0.1</td>
<td>-1.1 - 1.3</td>
</tr>
<tr>
<td>PAindex</td>
<td>0.01</td>
<td>-0.02 - 0.04</td>
</tr>
<tr>
<td>Baecke work</td>
<td>-0.002</td>
<td>-0.01 - 0.008</td>
</tr>
<tr>
<td>Baecke sport</td>
<td>0.007</td>
<td>-0.01 - 0.03</td>
</tr>
<tr>
<td>Baecke leisure</td>
<td>0.005</td>
<td>-0.009 - 0.02</td>
</tr>
<tr>
<td>Sports (h/week)</td>
<td>0.9</td>
<td>0.05 - 1.9</td>
</tr>
<tr>
<td>VO₂max (mL/min/kg FFM)</td>
<td>0.2</td>
<td>0.02 - 0.3</td>
</tr>
</tbody>
</table>

$B$, Unstandardized regression coefficient; 95% CI, 95% confidence interval of $B$; PA大力, habitual physical activity in daily life in Megacounts per day; %Low, %Moderate and %High, proportion of time subjects were physically active at a low, moderate and high intensity, respectively; PAindex, total activity index measured with the Baecke questionnaire; Baecke Work, Sport and Leisure, scores on each section of the Baecke questionnaire; Sports, weekly time spent on sports in hours; VO₂max, maximal oxygen uptake expressed in ml O₂ per minute per kg fat-free mass; %Type I, %Type IIA and %Type IIX, proportion of type I, IIA and IIX muscle fibers.
1973; Saltin et al., 1976; Andersen & Henriksson, 1977; Ingjer, 1979; Coggan et al., 1992; Staron et al., 1994). For example, %Type I was not altered after 24 weeks of intensive endurance training in previously untrained women (Ingjer, 1979). Therefore, the increased time spent on sports may actually result from an increased %Type I. In other words: subjects with a higher %Type I may be more prone to engaging in sports. Since the time spent on sports was used as an exclusion criterion, its range was limited. This may explain why the association with %Type I did not reach significance.

In conclusion, MuscleFTD probably cannot explain why some people are more prone to engaging in physical activities than others. To generalize the findings, the measurements should be replicated.

Perspectives

The association between PA_DL and MuscleFTD was determined for the first time, using an objective and validated method to determine physical activity for a prolonged period of time. In spite of the association between physical fitness and MuscleFTD, no evidence was found for a relationship between PA_DL and MuscleFTD. This indicates that physical fitness and physical activity are two non-synonymous entities that should not be used interchangeably. It also suggests that variables other than MuscleFTD probably determine why some people are more physically active than others. Future research is required to identify these variables.

Key words: accelerometers, myosin heavy chain isoforms, sports, exercise, heredity.

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References


