# Can muscle typology explain the inter-individual variability in resistance training adaptations?

Running title: Influence of muscle typology on resistance training adaptations

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## First author profile

First author profile: Kim Van Vossel is PhD candidate under the supervision of Prof. Dr. Wim Derave in the Exercise Physiology and Sports Nutrition lab at the Department of Movement and Sports Sciences, Ghent University. Her current research focuses on the personalization of training based on muscle typology and on elucidating the underlying determinants of human variability in training adaptations.

## Key points

- This study investigated the influence of muscle typology (= muscle fiber type composition) on the variability in resistance training adaptations and on its role in the individualization of resistance training frequency.
- We demonstrate that an individual's muscle typology cannot explain the inter-individual variability in resistance training induced increases in muscle volume, maximal dynamic strength and fiber cross-sectional area when repetitions are performed to failure.
- Importantly, slow typology individuals performed a significantly higher training volume to obtain similar adaptations compared to fast typology individuals.
- Muscle typology does not determine the most appropriate resistance training frequency. However, regardless of muscle typology, an additional weekly training (3x/week vs 2x/week) increases muscle hypertrophy but not maximal dynamic strength.
- These findings expand on our understanding of the underlying mechanisms for the large inter-individual variability in resistance training adaptations.

## Abstract

Considerable inter-individual heterogeneity exists in the muscular adaptations to resistance training. It has been proposed that fast-twitch fibers are more sensitive to hypertrophic stimuli and thus that variation in muscle fiber type composition is a contributing factor to the magnitude of training response. This study investigated if the inter-individual variability in resistance training adaptations is determined by muscle typology and if the most appropriate weekly training frequency depends on muscle typology. In strength-training novices, 11 slow (ST) and 10 fast typology (FT) individuals were selected by measuring muscle carnosine with proton magnetic resonance spectroscopy. Participants trained both upper arm and leg muscles to failure at 60% 1RM for 10 weeks, whereby one arm and leg trained 3x/week, the contralateral arm and leg 2x/week. Muscle volume (MRI-based 3D segmentation), maximal dynamic strength (one-repetition maximum, 1RM) and fiber-type specific cross-sectional area (vastus lateralis biopsies) were evaluated. The training response for total muscle volume (+3 to +14%), fiber size (-19 to +22%) and strength (+17 to +47%) showed considerable inter-individual variability, but these could not be attributed to differences in muscle typology. However, ST individuals performed a significantly higher training volume to gain these similar adaptations as FT individuals. The limb that trained 3x/week had generally more pronounced hypertrophy than the limb that trained 2x/week, and there was no interaction with muscle typology. In conclusion, muscle typology cannot explain the high variability in resistance training adaptations when training is performed to failure at 60% of 1RM.

## Visual abstract

Abstract figure legend:  This study investigated if muscle typology can explain the high variability in resistance training adaptations. Slow and fast typology resistance training novices were selected to participate in this study by the non-invasive measurement of muscle carnosine with proton magnetic resonance spectroscopy. After the chronic training period a high inter-individual variability was observed in changes in muscle volume, maximal dynamic strength and fiber cross-sectional area. However, this high inter-individual variability could not be explained by muscle typology for any of the outcomes.  Visual abstract created with BioRender.

## Introduction

Resistance training is a preventive and therapeutic strategy against several chronic diseases and is widely used in athlete populations to improve performance (Kraemer *et al*., 2002; Sawan *et al*., 2022). It is the primary utilized exercise form to increase muscle volume and strength. However, considerable heterogeneity exists in the physiological, performance and health related adaptations to resistance training. Following the same training program, some individuals show no responsiveness while others demonstrate large progression in whole-muscle cross-sectional area (-2% to +59%) and maximal dynamic strength (0% to +250%) (Hubal *et al.*, 2005; Ahtiainen *et al.*, 2016). The magnitude of response to resistance training can be influenced by a broad range of factors like dietary protein intake and genetics (Thalacker-Mercer *et al.*, 2013; Morton *et al.*, 2015).

Recent evidence suggests that muscle typology might be a key determinant in predicting and understanding training adaptations (Haun *et al.*, 2019*a*; Deshmukh *et al.*, 2021). Human skeletal muscle is composed of different cell types which can be distinguished based on their contractile protein myosin heavy chain: slow-twitch fibers (type I) and fast-twitch fibers (type IIa and type IIx). Based on numerous studies, both type I and type II fibers can show hypertrophy. However, the majority of the results point towards a greater hypertrophic capacity in type II fibers which might even be 50% higher compared to type I fibers (Fry, 2004; Adams & Bamman, 2012). The latter should be interpreted with caution as recent studies investigating the influence of training load on fiber type specific hypertrophy showed equal hypertrophy in type I and type II fibers when resistance training was performed to failure (Mitchell *et al.*, 2012; Morton *et al*., 2016; Grgic *et al.*, 2020).

Interestingly, large inter-individual differences exist in fiber type composition ranging from individuals with only 15% type II fibers (slow typology individuals; ST) to individuals with 85% type II fibers (fast typology individuals; FT) in certain muscles (Saltin *et al.*, 1977). Fiber-type specific hypertrophic capacity has been extensively studied (Staron *et al.*, 1989; Wang *et al.*, 1993; McCall *et al.*, 1996; Kosek *et al.*, 2006; Bickel *et al.*, 2011). However, studies that made the translation from muscle fiber level to whole muscle level are scarce and have equivocal results with regards to the hypertrophic and strength potential of ST and FT individuals. Schoenfeld *et al.*, (2020) showed for example no differences in hypertrophy between the soleus (typical slow-twitch muscle) and the gastrocnemius medialis (mixed fiber type muscle) while Haun *et al.*, (2019) found a positive relationship between the percentage of type II fibers and vastus lateralis hypertrophy. Similar, contradictory results were found in relation to strength as Dons *et al.*, (1979) found a higher increase in dynamic strength in FT individuals while Thorstensson *et al.*, (1976) reported a higher increase in isometric strength in ST individuals after chronic resistance training. Therefore, it is needed to further elucidate the role of muscle typology on resistance training induced whole muscle adaptations.

In addition to the potentially different hypertrophy capacity of type I and type II fibers, it is known that type II fibers are less fatigue-resistant compared to type I fibers (Burke & Edgerton, 1975; Szentesi *et al.*, 2001; Li *et al.*, 2002). Also, FT individuals are found to fatigue more, to need longer recovery time and to be more prone to overreaching compared to ST individuals (Lievens *et al.*, 2020; Bellinger *et al.*, 2020). The American College of Sports Medicine guidelines recommend a training frequency of 2 or 3 times per week for resistance training novices (Ratamess *et al.*, 2009). Based on the fiber and muscle typology characteristics, an additional weekly training (3x/week), which is accompanied by a higher weekly training volume and less recovery time, might be beneficial for ST individuals but less for FT individuals. More specifically, it could be hypothesized that if training volume is too high and training sessions succeed too quickly, FT individuals might built up fatigue over the training sessions which may impair their performance in the next training sessions and subsequently decrease their muscular adaptations (Bellinger et al., 2020). Therefore, further research is needed to better define the optimal training frequency and to optimize muscle hypertrophy and strength adaptations in individuals with divergent muscle typologies.

Regardless of the optimal resistance training modalities, muscle hypertrophy can only occur in the presence of a positive net muscle protein balance and sufficient amino acid availability (Biolo *et al.*, 1995; Phillips *et al.*, 1997). Post-exercise muscle blood flow is known to be an important contributor to muscle protein synthesis (Biolo *et al.*, 1995; Fujita *et al.*, 2006; Timmerman *et al*., 2010) and a suppressed post-exercise blood flow may lead to reduced resistance training adaptations as proposed by post-exercise muscle cooling studies (Roberts *et al.*, 2015; Fyfe *et al.*, 2019; Fuchs *et al.*, 2020). However, it remains to be investigated if the blood flow following training sessions of different training loads is different between ST and FT individuals. This could help explain potential differences in chronic adaptations to resistance training between muscle typologies.

The main purpose of this research was to investigate if the inter-individual variability in resistance training adaptations can be explained by muscle typology. First, an acute study was performed to investigate muscle typology-based differences in post-exercise blood flow in the 2h following a resistance training session as post-exercise blood flow contributes to muscle protein synthesis. The acute training was performed at 40, 60 and 80% of one-repetition maximum (1RM) and the training load eliciting the greatest differences in post-exercise blood flow between typology groups would be used in the chronic training study. It was hypothesized that post-exercise blood flow would be higher in FT individuals. In the chronic study, a 10-week whole-body resistance training program was performed to investigate if chronic adaptations to resistance training (muscle volume, maximal dynamic strength, fiber cross-sectional area (fiber CSA)) are dependent on muscle typology. Greater training adaptations were expected in FT individuals. Lastly, by using a within-subject study design we wanted to investigate if training 2x or 3x/week is more beneficial for a specific muscle typology. It was hypothesized that ST individuals would respond better to a higher weekly training frequency compared to FT individuals.

# Methodology

## Ethical approval

A total of 86 subjects participated in this study. All subjects gave their written informed consent. The study was conducted according to the Declaration of Helsinki and was approved by the local ethics committee of Ghent University Hospital, Belgium (Ethics Applications BC-08006 and BC-10253). The chronic study was registered on ClinicalTrials.gov (ID: NCT05108181).

## Subjects

This study consists of an acute and a chronic resistance training study. In the acute and chronic study respectively 36 and 50 healthy, young Caucasian subjects without resistance training experience were invited to estimate their muscle typology by measuring muscle carnosine with proton magnetic resonance spectroscopy ($^1$H-MRS) (Baguet *et al*., 2011). Based on their carnosine concentration we excluded the intermediate typology individuals (IT) and only ST and FT individuals were included in both studies. In the acute study 21 subjects were selected, 11 ST (6 males, 5 females) and 10 FT individuals (5 males, 5 females). Similarly but in another study population, 11 ST (6 males, 5 females) and 10 FT individuals (5 males, 5 females) were included in the chronic resistance training study (carnosine cut-off values: see further). None of the participants were involved in both studies. Participant characteristics are displayed in Table 1. Exclusion criteria included previous resistance training experience, smoking, chronic drug use, having a cardiovascular or neuromuscular disease, being vegetarian or vegan and the intake of creatine, carnosine, beta-alanine or any other supplement 3 months prior or during the study. Only women using oral contraceptives were included in the study to control for hormonal fluctuations which may possibly affect resistance training adaptations (Kissow *et al.*, 2022). Female participants were asked to inform the researchers about their continued use of contraceptives throughout the intervention period.

**Table 1.** Baseline characteristics of slow typology (ST) and fast typology individuals (FT) in the acute and chronic study.

| | Acute study | | | Chronic study | | |
|---|---|---|---|---|---|---|
| | **ST** | **FT** | **P – value** | **ST** | **FT** | **P – value** |
| **Age (y)** | 24.5 ± 2.0 | 22.2 ± 1.2 | 0.005* | 21.9 ± 2.9 | 22.3 ± 1.0 | 0.674 |
| **Height (m)** | 1.74 ± 0.08 | 1.78 ± 0.09 | 0.118 | 1.79 ± 0.07 | 1.72 ± 0.10 | 0.027* |
| **Body mass (kg)** | 68.46 ± 9.48 | 74.42 ± 8.62 | 0.079 | 68.33 ± 6.96 | 69.02 ± 9.85 | 0.867 |
| **BMI (kg.m$^{-2}$)** | 22.58 ± 2.07 | 23.50 ± 2.14 | 0.337 | 21.46 ± 2.37 | 23.53 ± 4.06 | 0.140 |
| **Fat percentage (%)** | 19.2 ± 6.5 | 18.9 ± 5.5 | 0.718 | 20.3 ± 9.0 | 21.8 ± 9.6 | 0.647 |
| **$VO_{2peak}$ (mL.min$^{-1}$.kg$^{-1}$)** | 44.9 ± 9.4 | 43.5 ± 6.0 | 0.598 | 42.2 ± 8.7 | 39.8 ± 7.1 | 0.441 |
| **Mean carnosine Z-score** | -0.95 ± 0.37 | +1.16 ± 0.55 | | -0.97 ± 0.32 | +0.93 ± 0.42 | |

Data are presented as mean ± SD. *Significant difference between ST and FT individuals

## Baseline characteristics

### Muscle typology screening

Muscle carnosine content was measured in the right m. vastus lateralis, m. soleus and m. gastrocnemius medialis by proton magnetic resonance spectroscopy ($^1$H-MRS) to estimate muscle typology (Baguet *et al.*, 2011). A 3T whole-body magnetic resonance imaging scanner (Siemens, Healthineers AG, Erlangen) was used to perform the measurements as previously described (Lievens *et al*., 2021). The carnosine concentration of each muscle was converted to a z-score relative to an age- and sex-matched control population of active, healthy non-athletes. The control population for the m. soleus and m. gastrocnemius consisted of 163 males and 112 females and for the m. vastus lateralis of 70 males and 56 females. The mean of the carnosine z-scores of all scanned muscles was calculated  and is referred to as 'mean carnosine z-score' in the manuscript. Based on their mean carnosine z-score subjects were divided into 3 groups: ST individuals with a mean carnosine z-score ≤ -0.5, IT individuals with a mean carnosine z-score between −0.5 and +0.5 and FT individuals with a mean carnosine z-score ≥ +0.5. IT individuals were not included in the acute and chronic study.

### Anthropometry

Body mass was measured using a digital scale (0.1 kg, Tanita Body Composition Analyzer, DC-360, Tokyo, Japan) and height with a portable stadiometer (0.1 cm, Seca 213 Portable). In the acute resistance training study, the upper leg fat free mass (cm$^3$, ULFFM) of the participants' right leg was estimated based on anthropometric measurements (skin folds, limb lengths and circumferences) as described by Layec *et al.*, (2014). All blood flow data were normalized for ULFFM as post-exercise blood flow was related to or tended to be related to ULFFM in the 80% (r = 0.481, p = 0.028) and 60% (r = 0.416, p = 0.061) load condition. Participants' body fat percentage was estimated by a bioelectrical analysis system (BIA) (Tanita Body Composition Analyzer, DC-360) in both the acute and chronic training study. As an extra verification, body fat percentage was also estimated in the chronic study by the measurement of the biceps, triceps, subscapular and suprailiac skinfolds following the formula of Durnin and Rahaman (1967) (Harpenden Skinfold Calipers; Baty). Pearson correlation revealed a strong relationship between BIA and anthropometrical estimations (r = 0.97).

### Fitness level

All participants performed an incremental ramp test to exhaustion on a cycling ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) to assess their baseline fitness level. Subjects performed a 6-minute warming-up (males: 100W, females: 80W) followed by 2 minutes of rest and a 4-minute warming-up at 50W, after which the incremental test started. During the test the work rate increased continuously every minute by 25W until volitional exhaustion. Volitional exhaustion was defined as the moment the cycling cadence dropped below 60 revolutions per minute despite strong verbal encouragement. Whole-body peak oxygen uptake ($VO_{2peak}$) was measured breath-by-breath using a metabolic system (Metalyzer 3B, Cortex Biophysik GmbH, Leipzig, Germany). $VO_{2peak}$ was defined as the highest 30 second-average achieved value during the test.

## Acute study

### Acute resistance training study design

The acute study consisted of 6 test days interspersed by at least 48 hours to examine if blood flow during the recovery of an acute resistance training is dependent on muscle typology. On the first day anthropometrical data were collected (body mass, height, fat percentage) and an incremental cycling test until exhaustion was performed to ensure a similar aerobic fitness level among participants. During test day 2 and 3, the 1RM of the subjects' right leg was determined in duplicate for the leg extension and leg curl exercise. During the following 3 test days, participants came to the lab via passive transport. The participants lay down in supine position for 30 minutes after which femoral arterial blood flow at rest was measured 10 minutes, 5 minutes and immediately before the start of the resistance training. These three values were averaged to determine the participants' blood flow at rest. The training consisted of 3 sets of leg extensions and 3 sets of leg curls to failure. Training load was different and randomized on the 3 test days: 80% 1RM, 60% 1RM or 40% 1RM. Immediately after the last repetition, the participants lay down again for two hours and post-exercise blood flow was measured at 1 minute post-exercise followed by measurements every 10 minutes (Figure 1).

**Figure 1**. Overview of study design (Created with BioRender)

## Maximal dynamic strength assessment

Maximal dynamic strength was determined as the maximal weight the subjects could lift unilaterally in one repetition (1RM) over a full range of motion with proper technique for the knee extension (leg extension exercise) and knee flexion (leg curl exercise). The 1RM protocol was similar for every exercise. Subjects started with a 5-minute warming-up on a rowing ergometer followed by 2 x 10 exercise specific submaximal repetitions with 1 minute of rest in between. Thereafter, subjects received 5 attempts to lift the highest possible load with proper technique. Subjects received 2 minutes of rest between attempts (Baechle & Earle, 2008). All subjects reached their 1RM within 5 attempts for every exercise.

## Acute resistance training protocol

Participants performed 3 sets of knee extensions (accurate to 2.5 kg, Technogym, Selection 900 Leg extension) and 3 sets of knee flexions (2.5 kg, Technogym, Selection 900 Leg curl) to failure with their right leg at a training load of 40%, 60% or 80% 1RM. Concentric and eccentric contractions had a duration of respectively 1s and 2s which was controlled with the real-time biofeedback of the Unity Mini interface (Technogym). Participants received respectively 2 and 3 minutes recovery between sets and exercises. Failure was defined as the inability to perform another repetition over the full range of motion or with a proper technique.

## Post-exercise blood flow

Pre- and post-exercise blood flow was measured in the right femoral artery with Doppler Ultrasound (Xario 100, Canon Medical Systems Europe, Zoetermeer, The Netherlands) and a 11L4 linear probe. We used an imaging frequency of 8.4 MHz and a Doppler frequency of 4.0 MHz. The artery was always insonated below 60° (at the lowest possible angle), distal to the inguinal ligament and, to avoid turbulence, approximately 3 cm above the bifurcation where the artery splits into its superficial and deep part. The sample volume was maximized to the artery diameter and a standard low-velocity rejection filter was applied. Femoral artery diameter was measured thrice during systole with the built-in calipers. Doppler traces were averaged over 39 seconds at each measurement time point and the iAUC (incremental Area Under the Curve) was calculated with the trapezoidal method as a measurement of total 2 hours post-exercise blood flow (Van der Stede, 2021).

## Chronic study

### Chronic resistance training study design

At baseline, 4 experimental test days were performed interspersed by at least 48 hours. On test day 1 the anthropometry of the subjects was assessed followed by the performance of an incremental cycling test. On test day 2, the muscle volume of the upper leg muscles (m. quadriceps femoris and m. hamstrings) and the upper arm muscles (m. biceps brachii and m. triceps brachii) was measured bilaterally by magnetic resonance imaging (MRI). During the third test day, the participants' 1RM was determined unilaterally (separate determination for right and left limb) for the knee extension (leg extension exercise), knee curl (leg curl exercise), elbow flexion (biceps curl exercise) and elbow extension (lying triceps extension exercise). Based on the 1RM their maximal dynamic strength and the exercise weight corresponding to a training load of 60% 1RM were determined. Chronic training was performed at 60% 1RM since the biggest differences between typologies in post-exercise blood flow were found at the moderate load of 60% in the acute study. 1RM was re-assessed at the end of week 3 and week 6 and training weights were adapted accordingly to ensure a training load of approximately 60 % 1RM over the whole training period. The 1RM assessment protocol was the same as described in the acute study. The last test day, a muscle biopsy was collected from the right and left m. vastus lateralis to determine muscle fiber type specific adaptations. The post-training testing was identical to the baseline tests. Only the incremental exercise test was not repeated post training (Figure 1).

At least 5 days after the biopsy collection, participants started with the 10 weeks resistance training period. Participants came to the lab 3 times per week to perform a supervised training session primarily targeting the knee extensor (quadriceps femoris), knee flexor (hamstrings), elbow flexor (biceps brachii) and elbow extensor (triceps brachii) muscles. A 5-minute warming-up was performed on a Concept 2 rowing ergometer followed by unilateral knee extensions (2.5 kg, Technogym, Selection 900 Leg extension), knee flexions (2.5 kg, Technogym, Selection 900 Leg curl), elbow flexions (0.5 kg, dumbbell weights) and elbow extensions (0.5 kg, dumbbell weights) in randomized order. Each training consisted of 3 or 4 sets per exercise until muscular failure at 60% 1RM. Repetitions were performed with a 1s concentric and 2s eccentric phase. Two minutes of recovery were afforded between sets and 3 minutes between exercises. During the training period a within-subject design was used to investigate if training 2x/week or 3x/week is more suited for a certain muscle typology. One leg and arm trained 3 times per week with at least 48 hours of rest (e.g. Monday – Wednesday – Friday) while the contralateral leg and arm trained 2 times per week with at least 72 hours of rest (e.g. Monday – Friday). This resulted in a higher training volume and less recovery time for the limb that performed an additional training per week. Allocation of training frequency per limb was randomly performed ensuring that an equal amount of dominant and non-dominant limbs trained 3 times per week. All trainings were supervised by research staff to ensure a proper execution of the exercises. Total number of repetitions was recorded per performed set to quantify total training volume (total number of repetitions x weight lifted). In both the acute and chronic study participants were not allowed to exercise the day before or during test days nor to perform any resistance related exercises during the chronic study.

## Muscle volume

Muscle volumes of the upper leg and upper arm muscles were obtained from a 3T whole-body magnetic resonance imaging scanner (Siemens, Healthineers AG, Erlangen). A multi-slide 2D gradient echo sequence with proton density weighted contrast and minor T1 weighting was used to acquire the leg images. Scans were taken from the T12 vertebra to the tibial tuberosity covering both legs while the participants lay in supine position. The images were acquired with the built-in body coil in 3 or 4 stacks with 45 slices per stack, slice thickness of 5.0 mm, interslice gap of 0.0 mm, repetition time of 498.0 ms and echo time of 2.98 ms. Total acquisition time per stack was 1.12 minutes. The analyzed muscles were the rectus femoris, vastus lateralis, vastus medialis and vastus intermedius of the quadriceps femoris and the biceps femoris long head, biceps femoris short head, semimembranosus and semitendinosus of the hamstrings. All muscles were segmented using a combination of an AI-based algorithm and manual vetting (Handsfield *et al.*, 2014; Ni *et al.*, 2019) (Springbok Analytics, Charlottesville, VA). The arm images were acquired with a T1-weighted VIBE DIXON water only sequence. Scans were made separately for the right and left arm from the top of the humerus to mid forearm with the subjects laying in supine position. The images were acquired with the built-in body coil in 1 or 2 stacks with 80 slices per stack, slice thickness of 5.0 mm, interslice gap of 0.0 mm, repetition time of 8.76 ms, echo time 1 of 2.46 ms and echo time 2 of 3.69 ms. Total acquisition time per stack was 4.09 minutes. The arm muscles were manually segmented by tracing the margins of the biceps and triceps muscles in every axial, coronal and sagittal slice using the image analysis software ITK-SNAP (University of Pennsylvania, Philadelphia, PA; www.itksnap.org) (Yushkevich et al., 2006). As it was not always possible to identify the borders between the biceps, brachialis and brachioradialis, these muscles were analyzed together and referred to as ‘biceps brachii’ in the manuscript. On average, the test-retest CV for the quadriceps femoris was 0.63% and for the hamstrings 0.86%. The interrater variability for biceps brachii and triceps brachii was 1.01% and 0.74%.

## Supplementation and dietary pattern

On test and training days subjects abstained from caffeine and alcohol. Immediately after every resistance training session of the chronic training study, participants ingested 0.3 g of whey protein isolate per kg body mass (Etixx High Protein Shake, Etixx Sports NV, Merelbeke, Belgium) to help meet recommended daily protein intake. Participants were asked to maintain their normal dietary pattern and were not allowed to take any supplements during the entire chronic training study. Dietary compliance was assessed thrice on non-training days, once at baseline and once in week 3 and week 6 by self-reported food questionnaires. The questionnaires were processed using the Nubel software (Nutriënten België, www.nubel.com) to calculate daily energy and macro nutrient intake (proteins, fats and carbohydrates). The average daily energy intake, protein intake, fat intake and carbohydrate intake did not differ between ST and FT individuals (Supplementary table S1).

## Indices of fatigability

*Capillary blood samples:* During one training of the chronic study, capillary blood samples were collected before the training, one minute post warming-up, one minute post training and 5 minutes post training. Blood samples were analyzed for lactate, pH, bicarbonate and blood glucose (Radiometer ABL90 FLEX, Zoetermeer, The Netherlands).

*Wellness questionnaires:* The physical and mental well-being of the participants was examined before the start of every training of the chronic study via a visual analogue scale (1-10), with the value 1 representing the most negative outcome. The questionnaire consisted of scales for muscle soreness in general or per limb, fatigue in general or per limb, readiness to train, sleep quality, physical wellbeing and mood (Bellinger *et al*., 2020)

## Muscle biopsy sampling analysis

Pre and post the chronic training period a small incision was made in the middle portion of the right and left m. vastus lateralis under local anesthesia (0.5 mL xylocaine, Aspen Netherlands B.V., Gorinchem, The Netherlands). Muscle biopsy samples were taken in each leg using a 5 mm Bergström needle with suction (Bergström, 1975). The fiber bundles of the muscle sample were oriented perpendicularly, then mounted in embedding medium (OCT Compound Tissue-Tek, Sakura Finetek, Zoeterwoude, The Netherlands), frozen in liquid nitrogen cooled isopentane and then frozen in liquid nitrogen. All muscle samples were stored at -80°C until processing.

The embedded muscles samples were cut into muscle cross-sections (8 µm) using a cryostat (Epredia HM560), mounted on microscope glass slides, air dried for 15 minutes at room temperature and then stored back at – 20 °C upon analysis. Pre and post samples were mounted on the same glass slide to exclude staining variability. After 30 minutes of air-drying, samples were fixated for 10 minutes with 4% paraformaldehyde solution and washed 3 x 5 minutes in phosphate buffer saline (PBS). Subsequently, samples were incubated with Wheat Germ Agglutinin to identify the myofiber membranes (1:25, CF®405M, Biotium, Cat# 29028-1) and again washed 3 x 5 minutes in PBS. Next, samples were blocked with 5% goat serum (Jackson ImmunoResearch, 005-000-001) diluted in PBS, 0.5% Bovin Serum Albumin (Sigma-Aldrich, A7030) and 0.02% Triton X-100 (Sigma-aldrich, T9284) for 30 minutes. This was followed by overnight incubation with primary antibodies against MyHC-I (1:50, DSHB Cat# BA-F8, RRID:AB_10572253), MyHC-IIa (1:20, DSHB Cat# A4.74, RRID:AB_528383) and MyHC-IIx (1:100, DSHB Cat# 6H1, RRID:AB_1157897) diluted in PBS, 0.5% Bovin Serum Albumin and 0.02% Triton X-100. The next morning, cryosections were washed in PBS for 5 minutes and then incubated with secondary antibodies (1:500) for MyHC-I (Thermo Fisher Scientific Cat# A-21146, Lot# 2273676, RRID:AB_2535782), MyHC-IIa (Thermo Fisher Scientific Cat# A-21124, Lot# 2506092, RRID:AB_2535766) and MyHC-IIx (Thermo Fisher Scientific Cat# A-21042, Lot# 2160416, RRID:AB_2535711) and finally mounted with polyvinyl alcohol mounting medium with DABCO (Sigma-Aldrich, 10981).

Stained muscle cross-sections were visualized using a fluorescence microscope with a 10x objective (Zeiss Axioscan 7, Zeiss). Fiber type composition and fiber CSA were measured using ImageJ software (National Institutes of Health) with FIJI extension. The total number of fibers per fiber type (I, IIa, IIx, hybrid fibers) was counted per muscle cross-section to determine muscle fiber composition and on average 939 ± 277 fibers per muscle cross-section were counted. Fiber CSA ($\mu m^2$) was measured by randomly selecting 100 well-oriented fibers per fiber type per biopsy and manually encircling their fiber membranes. Due to the difficulty to reliably separate type IIa and type IIx stained fibers, both fiber types are included in the total type II percentage in this manuscript. Type I/IIa hybrid fibers accounted for less than 1 percent of all fibers and were excluded from the analysis. Longitudinally oriented fibers, fibers with an unclear cell membrane and fibers on the boundary of the biopsy were also not included in the analysis. Moreover, only fibers with an appropriate circularity (4 x fiber CSA/(fiber perimeter)$^2$ of > 0.70 were accepted to be analyzed (Charifi *et al.*, 2004). Mean circularity did not differ between the pre and post biopsies (0.828 ± 0.015 vs 0.830 ± 0.016, $p = 0.191$). The percentage type II area (% type II area) was calculated as the product of the mean CSA of the type II fibers and the total number of the type II fibers divided by the total CSA of the type I and type II fibers.

## Statistical analysis

Shapiro-Wilk's tests and Levene's tests were used to control for respectively normality and homogeneity of variance and the data were log10 transformed in case of violations of these assumptions. Participant baseline characteristics, differences in blood flow at rest, baseline 1RM, baseline muscle volume and baseline fiber CSA were analyzed by a one-way ANCOVA with covariate for sex. Similarly differences between ST and FT individuals in the number of repetitions performed during the acute study, hypertrophy, increase in 1RM, change in fiber CSA, total training volume, blood parameters and nutritional intake were analyzed by a one-way ANCOVA with covariate for sex. Two-way repeated measures ANCOVAs with sex as a covariate were used for analysis of the blood flow data (training load x muscle typology), the optimal training frequency (frequency x muscle typology) and the wellness questionnaires (time x muscle typology). Post hoc pairwise comparisons tests with Bonferroni correction were performed when appropriate. Pearson correlations were conducted to reveal relationships between estimations of fat percentage with BIA or skinfold measurements, between the mean carnosine z-score and the post-exercise iAUC, between hypertrophy in the 3x/week limb and the 2x/week limb, between the increase in 1RM in the 3x/week limb and the 2x/week limb, between total hypertrophy in the quadriceps femoris muscle and increases in fiber CSA, between the baseline fiber type composition of the right and left vastus lateralis, between the invasive and non-invasive measurement of muscle typology, between baseline and post carnosine values in the vastus lateralis and between the total number of repetitions and hypertrophy/increase in 1RM. When muscle typology was not taken into account, paired sample t-tests or Wilcoxon paired rank tests were used to evaluate overall increases from baseline to post in muscle volume and maximal dynamic strength, differences in hypertrophy between training 2x and 3x/week and baseline-post differences in circularity of the fibers. Data are presented as mean ± SD or as individual values. All statistical analyses were performed using Graphpad Prism (Version 9.3.1, GraphPad Software) or SPPS (SPSS 28.0). Effect sizes for AN(C)OVA were estimated using partial eta squared, 95% confidence intervals (CI) are shown and statistical significance was accepted as $p \leq 0.05$. Data are available upon request.

# Results

## Acute study

### Post-exercise blood flow is affected by muscle typology

At rest, the femoral artery blood flow during the three test days did not differ between ST (0.035 ± 0.013 $mL.min^{-1}.cm^{-3}$) and FT individuals (0.031 ± 0.011 $mL.min^{-1}.cm^{-3}$; p = 0.333, ES = 0.052, CI = -0.005 to 0.015). Muscle blood flow increased approximately 5-fold from baseline to 1 minute post-exercise and remained elevated for about 30-70 minutes post-training (Figure 2A). A main effect of muscle typology (p = 0.022, ES = 0.260) revealed a higher post-exercise iAUC in FT individuals (Figure 2B). This was most pronounced in the 60% condition (ST: 1.913 ± 1.053 $mL*cm^{-3}$, FT: 3.068 ± 1.064 $mL*cm^{-3}$, p = 0.051, ES = 0.280 CI = -2.128 to -0.243, Figure 2B). The post-exercise iAUC was on average 39.1%, 60.4%, 37.4% higher in FT individuals compared to ST individuals in respectively the 40%, 60% and 80% condition. This finding was confirmed by significant correlations between the mean carnosine z-score and the post-exercise iAUC at 60% 1RM (r = 0.507, p = 0.019, CI = 0.096 to 0.770) and also at 40% 1RM (r = 0.443, p = 0.044, CI = 0.014 to 0.734) but not at 80% 1RM (r = 0.344, p = 0.127, CI = -0.103 to 0.675). The post-exercise blood flow was elevated for an extended period of time in FT individuals at 80% (ST: 30min, FT: 70min) 60% (ST: 20min, FT: 40min, Figure 2A) and 40% (ST: 30min, FT: 60min). No interaction effect was found between muscle typology and training load (p = 0.343) (Figure 2B) and total number of repetitions was not related to iAUC at any training load (p > 0.05). Collectively these data indicate that muscle typology influences blood flow during the recovery of an acute resistance training as FT individuals show a higher blood flow compared to ST individuals. The size of the effect depends on the training load, and since the difference was most pronounced in the 60% 1RM condition, this load was chosen for the subsequent chronic training study.

**Figure 2. Post-resistance training blood flow**
A) Time course of the two hours post-resistance training blood flow after resistance training at 60% 1RM for ST (n = 11) and FT individuals (n = 10). Data are expressed as mean ± SD. *significant increase in blood flow relative to baseline (p ≤ 0.05). B) Difference between ST (n = 11) and FT individuals (n = 10) in total blood flow (iAUC) after resistance training at 40%, 60% and 80% 1RM. Data are expressed as mean ± SD + individuals values.

## Chronic study

### High inter-individual variability in chronic resistance training adaptations

Total training adherence during the 10-week resistance training study was 99.65% (range: 93 to 100%). After the training program, significant increases in muscle volume and maximal dynamic strength were observed in all trained muscles in both the limbs that trained 3 times per week and 2 times per week (p <0.005). However similar to previous research, we found considerable inter-individual variability in both hypertrophy and increase in maximal dynamic strength. Total muscle volume (all trained muscles and both frequency conditions combined) increased between 3.0% and 13.7%. Total dynamic strength increased between 17.4% and 47.1% (See visual abstract). Moreover, the inter-individual variability differed between all trained muscles and exercises. Mean increases and ranges in muscle volume and maximal dynamic strength for the limbs that trained 3 times per week are shown in Figure 3. Similar results were found for the 2 times per week condition (Figure S1). Strong relationships were found between the total hypertrophy of the limbs that trained 3x/week and 2x/week ($r = 0.775$, $p < 0.0001$, CI = 0.515 – 0.904). Similar results were demonstrated for the increase in maximal dynamic strength (r = 0.615, p = 0.003, CI = 0.249 – 0.827) indicating that high-responders in the one limb/condition are also higher responders in the other limb/condition.

**Figure 3. Inter-individual variability in chronic training adaptations in the 3x/week condition**
Individual increase in muscle volume of the A) quadriceps femoris, B) hamstrings, C) biceps brachii and D) triceps brachii muscle and individual increase in maximal dynamic strength for the E) knee extension, F) knee flexion, G) elbow flexion and H) elbow extension exercise in the limbs that trained 3x/week. Data are presented as mean increase (dotted horizontal line) + individual values (bars). Green bars represent the FT individuals and blue bars with stripes the ST individuals. P-values: comparison between ST and FT individuals with respect to increases in muscle volume and muscle strength. n = 21

## Influence of muscle typology on the variability in chronic resistance training adaptations

Baseline muscle volumes (Table 2) and 1RM (Table 3) did not differ between ST and FT individuals. Similarly, after normalization of muscle volume for body mass and height (Handsfield *et al.*, 2014), still no differences were found ($p > 0.05$). Following the training period ST and FT individuals showed on average similar increases in muscle volume and maximal dynamic strength and this in both the 3x/week condition (Table 2, Table 3, Figure 3) and the 2x/week condition (Table S2, Table S3, Figure S1). High and low responders were present in both ST and FT individuals (Figure 3). This indicates that muscle typology is not the main determinant for the magnitude of increases in muscle volume and maximal dynamic strength when training is performed to failure in previously untrained individuals.

**Table 2**. **Baseline and post-training muscle volumes and muscle volume increases (%) for ST (n = 11) and FT individuals (n = 10) in the 3x/week condition.** All pre-post differences were significant. No significant differences were found between ST and FT individuals neither for baseline values, post values or delta values.

| | **ST** | | | **FT** | | |
|---|---|---|---|---|---|---|
| | **Baseline (mL)** | **Post (mL)** | **Delta (%)** | **Baseline (mL)** | **Post (mL)** | **Delta (%)** |
| **Muscle Volume** | **3x/week** | | | | | |
| **Quadriceps femoris** | 1807 ± 304 | 1927 ± 314 | 6.82 ± 3.71 | 1779 ± 346 | 1889 ± 360 | 6.32 ± 2.89 |
| Rectus femoris | 261 ± 55.9 | 278 ± 62.2 | 6.38 ± 7.55 | 227 ± 53.0 | 247 ± 51.4 | 9.29 ± 7.43 |
| Vastus intermedius | 241 ± 37.2 | 256 ± 38.0 | 7.34 ± 13.2 | 223 ± 48.4 | 237 ± 45.6 | 6.74 ± 7.46 |
| Vastus lateralis | 866 ± 151 | 927 ± 151 | 7.48 ± 5.59 | 882 ± 172 | 942 ± 185 | 6.92 ± 4.27 |
| Vastus medialis | 439 ± 79.4 | 466 ± 89.5 | 5.95 ± 5.22 | 446 ± 92.7 | 463 ± 102 | 3.58 ± 2.88 |
| **Hamstrings** | 646 ± 147 | 720 ± 150 | 12.1 ± 6.27 | 626 ± 119 | 690 ± 133 | 10.3 ± 4.21 |
| Biceps femoris: long head | 181 ± 46.0 | 199 ± 45.9 | 10.9 ± 6.51 | 169 ± 26.1 | 184 ± 29.9 | 9.15 ± 5.59 |
| Biceps femoris: short head | 89.0 ± 27.4 | 103 ± 25.8 | 17.9 ± 11.8 | 81.0 ± 26.4 | 92.7 ± 24.1 | 16.5 ± 863 |
| Semimembranosus | 205 ± 43.1 | 213 ± 48.6 | 3.37 ± 2.89 | 213 ± 53.6 | 222 ± 53.8 | 4.41 ± 4.88 |
| Semitendinosus | 171 ± 47.2 | 206 ± 50.5 | 21.5 ± 12.4 | 163 ± 29.3 | 191 ± 41.2 | 16.8 ± 10.1 |
| **Biceps brachii** | 295 ± 72.5 | 318 ± 73.4 | 8.20 ± 3.50 | 303 ± 101 | 333 ± 115 | 9.51 ± 4.14 |
| **Triceps brachii** | 333 ± 78.0 | 398 ± 91.5 | 19.6 ± 4.34 | 361 ± 125 | 433 ± 139 | 20.8 ± 5.29 |

Data are presented as mean ± SD.

**Table 3. Baseline and post-training maximal dynamic strength and increases in maximal dynamic strength (%) for ST (n = 11) and FT individuals (n = 10) in the 3x/week condition**. All pre-post differences were significant. No differences were found between ST and FT individuals neither for baseline values, post values or delta values.

| | ST | | | FT | | |
|---|---|---|---|---|---|---|
| | **Baseline** | **Post** | **Delta (%)** | **Baseline** | **Post** | **Delta (%)** |
| **Maximal dynamic strength (kg)** | **3x/week** | | | | | |
| **Knee extension** | 48.4 ± 9.24 | 59.3 ± 12.8 | 22.3 ± 10.37 | 46.0 ± 8.51 | 55.8 ± 9.21 | 21.8 ± 8.61 |
| **Knee flexion** | 34.3 ± 6.62 | 42.7 ± 7.02 | 25.5 ± 12.3 | 30.5 ± 7.80 | 41.0 ± 10.0 | 35.7 ± 22.6 |
| **Elbow flexion** | 13.6 ± 4.39 | 19.2 ± 5.08 | 48.7 ± 38.6 | 15.2 ± 3.77 | 20.1 ± 4.86 | 34.0 ± 19.7 |
| **Elbow extension** | 7.82 ± 2.82 | 11.6 ± 3.05 | 53.7 ± 20.8 | 8.80 ± 4.02 | 13.4 ± 4.43 | 63.8 ± 41.1 |

Data are presented as mean ± SD.

## Muscle fiber type specific adaptations

The baseline CSA area of the type I fibers and type II fibers was not different between ST and FT groups (Table 4, Table S3) (Type I: 3x/week: $p = 0.910$, ES = 0.001, CI = -842.456 to 755.248; 2x/week: $p = 0.177$, ES = 0.099, CI = -1179.989 to 234.610; Type II: 3x/week: $p = 0.297$, ES = 0.060, CI = -464.733 to 1434.886; 2x/week: $p = 0.633$, ES = 0.013, CI = -1007.272 to 628.553). Training did not increase the average fiber CSA measured from muscle biopsies in either type I or type II fibers in either typology group or frequency condition ($p > 0.05$, Table 4, Table S4). Thus, muscle hypertrophy was observed at the whole muscle volume level (based on MRI imaging), but not at the fiber level. Nevertheless, the training-induced change in the fiber size of all fibers was positively correlated to the change in quadriceps muscle volume ($r = 0.512$, $p = 0.018$, CI = 0.104 to 0.773) (Figure 4B).

Similar to the whole muscle adaptations, a high variability in the training induced change in CSA of the type I and type II fibers was observed both in the 3x/week condition (Figure 4C, 4D) and the 2x/week condition (Figure S2A, S2B). Also when both frequency conditions and both fiber types were analyzed together, total CSA hypertrophy ranged from -19.1% to 21.5% (See visual abstract). No significant differences were found in fiber CSA adaptations between ST and FT groups (Type I 3x/week: $p = 0.318$, ES = 0.055, CI = -23.812 to 8.171; type II 3x/week: $p = 0.096$, ES = 0.146, CI = -26.088 to 2.342; type I 2x/week: $p = 0.667$, ES = 0.011, CI = -15.513 to 23.685; type II 2x/week: $p = 0.435$, ES = 0.034, CI = -11.107 to 24.740) (Table 4, Table S4).

**Figure 4.** A) Representative image of a muscle cross-section with type I fibers colored in blue and type II fibers colored in green. B) Relationship between the increase in CSA of all fibers (mean of 3x/week and 2x/week) and increase in whole quadriceps femoris muscle volume (mean of 3x/week and 2x/week) (n = 21). C) Individual change in type I fiber CSA in ST (n = 11) and FT individuals (n = 10) in the 3x/week condition. D) Individual change in type II fiber CSA in ST (n = 11) and FT individuals (n = 10) in the 3x/week condition. E) Relationship between the non-invasively measured concentration of carnosine in the vastus lateralis and biopsy-based % area of type II fibers (n = 21). Data are presented as individual values. The dotted line represents the average change in fiber CSA. P-values represent the comparison in fiber CSA change between ST and FT individuals.

## Direct versus indirect assessment of muscle typology

The non-invasive estimation of muscle carnosine in the vastus lateralis muscle showed a positive relationship with the percentage area occupied by type II fibers of the two baseline vastus lateralis biopsies ($r = 0.731$, $p = 0.0002$, CI = 0.437 to 0.884) (Figure 4E). Moreover, the percentage area occupied by type II fibers was significantly higher in FT compared to ST (FT: 61.63 ± 13.29%; ST: 53.06 ± 14.93%, $p = 0.050$, ES = 0.197, CI = -0.012 to 18.89). Lastly, a good correlation was found between pre and post training period measurements of carnosine in the vastus lateralis ($r = 0.869$, $p < 0.0001$, CI = 0.697 to 0.946).

**Table 4. Baseline and post-training CSA values and increase in fiber CSA (delta (%)) of the type I, type II and all fibers (type I + type II) in ST (n = 11) and FT individuals (n = 10) in the 3x/week condition**. No differences were found between ST and FT individuals neither for baseline values, post values or delta values.

| | ST | | | FT | | |
|---|---|---|---|---|---|---|
| | **Baseline** | **Post** | **Delta (%)** | **Baseline** | **Post** | **Delta (%)** |
| **Fiber CSA (µm²)** | **3x/week** | | | | | |
| Type I | 4396 ± 927 | 4548 ± 962 | 5.17 ± 19.9 | 4376 ± 849 | 4231 ± 941 | -2.89 ± 13.5 |
| Type II | 4140 ± 933 | 4408 ± 986 | 7.38 ± 16.4 | 4614 ± 1095 | 4401 ± 1111 | -4.01 ± 13.5 |
| All fibers | 4267 ± 799 | 4478 ± 833 | 6.24 ± 16.8 | 4495 ± 941 | 4316 ± 895 | -3.42 ± 10.8 |

Data are presented as mean ± SD.

## Total training volume

Total training volume was expressed as the total number of performed repetitions during the entire training period multiplied by the weight lifted. In general, total training volume in the 3x/week condition was significantly higher compared to the 2x/week condition (3x: 109331 ± 26311 kg; 2x: 70312 ± 18022 kg, $p < 0.0001$). Total training volume was significantly higher in the ST individuals when the 3x/week and 2x/week condition were combined (ST: 198548 ± 47470 kg – FT: 158847 ± 29252 kg, p = 0.033, ES = 0.229, CI = 3487 to 73326) and in the 3x/week condition (p = 0.054, ES = 0.191, CI = 424 to 42800) and 2x/week condition separately (p = 0.017, ES = 0.277, CI = 3433 to 31003) (Figure 5A, 5B and Supplementary Figure S3A). The difference in total training volume occurred mainly in the knee flexion exercise (Figure 5C and Figure S3B), while the total number of repetitions was also higher in the elbow flexion exercise (3x: p = 0.012; 2x: p = 0.033). However, total training volume was not related to total hypertrophy (3x, r = -0.125, p = 0.590, CI = -0.592 to 0.264; 2x, r = -0.340, p = 0.132, CI = -0.673 to 0.108) or total increase in maximal dynamic strength (3x, r = -0.074, p = 0.750, CI = -0.500 to 0.381; 2x, r = -0.233, p = 0.311, CI = -0.613 to 0.234) (Figure 5D and Figure S3C). Similar findings were found when total training volume was expressed as total number of repetitions.

**Figure 5. Total training volume differs between ST and FT individuals**

A) Difference in total training volume between ST (•, n = 11) and FT (▲, n = 10) individuals in the 3 times per week (3x) and 2 times per week (2x) condition. B) Overview of the total weekly training volume of ST and FT in the 3 times per week condition. C) Difference in total training volume per exercise between ST and FT in the 3 times per week condition. D) Relationship between total training volume and total hypertrophy in the 3 times per week condition (n = 21). Data are presented as mean + individual values (A,C), as mean ± SD (B) and as individual values (D).

## An additional weekly training benefits hypertrophy independent of muscle typology

To investigate if a certain muscle typology benefits more from an additional training per week, a within-subject design was used with one leg and arm training 2 times per week and the contralateral leg and arm training 3 times per week. Regardless of muscle typology, a higher hypertrophic response was found in the quadriceps femoris (p = 0.018, ES = 0.251, CI = 0.3174 to 2.962), biceps brachii (p = 0.0002, ES = 0.499, CI = 0.989 to 2.726) and triceps brachii muscles ($p < 0.0001$, ES = 0.585, CI = 2.875 to 6.597) that trained 3x/week (Figure 6A). Results for the hamstrings pointed in the same directions but are not significant (p = 0.056, ES = 0.170, CI = -0.061 to 4.234). Unlike hypertrophy, an additional training seems not beneficial to further increase maximal dynamic strength in the knee extension (p = 0.250, ES = 0.066, CI = -9.134 to 2.516), knee flexion (p = 0.211, ES = 0.077, CI = -9.948 to 1.908), elbow flexion (p = 0.413, ES = 0.034, CI = -9.769 to 9.992) and elbow extension exercise (p = 0.103, ES = 0.128, CI = -17.38 to 1.254) (Figure 6B).

No significant interaction effect (muscle typology x training frequency) was found for hypertrophy in the quadriceps femoris ($p = 0.899$), hamstrings ($p = 0.644$), biceps brachii ($p = 0.707$) and triceps brachii muscles ($p = 0.577$) (Figure 6C-F). Similar results were found for the increase in 1RM of the knee extension ($p = 0.576$), elbow flexion ($p = 0.342$) and elbow extension exercise ($p = 0.176$) while training 2x/week led to higher 1RM increases in the hamstring muscles of the FT individuals but not in the ST individuals ($p = 0.050$) (Figure S4). In general, this indicates that an individual's optimal resistance training frequency is not affected by muscle typology.

The results were supported by the VAS-scores completed on the wellness questionnaires. No interaction effects were found (muscle typology x VAS score) for muscle soreness in general or per limb, fatigue in general or per limb, readiness to train, sleep quality, physical wellbeing or mood ($p > 0.05$). These findings indicate that the perceptual feeling of soreness or fatigue during the training period did not differ between ST and FT individuals. Moreover during a specific training session, capillary blood was collected to measure pH, bicarbonate, lactate and glucose. No significant differences were found between ST and FT individuals at baseline, 1 minute post warming-up and 1 and 5 minutes post training for all four parameters ($p > 0.05$) indicating similar metabolic perturbation in both groups despite a higher number of repetitions in the ST group.

**Figure 6. Optimal resistance training frequency**

A,B) Increase in (A) muscle volume and (B) 1RM per training frequency (n = 21). Data are presented as mean + individual values. P-values represent the comparison between frequencies. C, D, E, F) Differences in hypertrophy of the (C) quadriceps femoris, (D) hamstrings, (E) biceps brachii and (F) triceps brachii between the 2x/week and 3x/week frequency in ST (n = 11) and FT individuals (n = 10). Data are presented as mean + individual values. P-values represent the comparison between frequencies per muscle typology.

## Discussion

This study provides novel insights on the influence of muscle typology on the variability in whole-body resistance training adaptations and on the role of muscle typology in the individualization of resistance training frequency. The main finding of the present study is that muscle typology is seemingly not an important factor to explain the inter-individual variability in hypertrophy and strength adaptations when training is performed at 60% 1RM to failure.

To examine the influence of muscle typology on the heterogeneous responsiveness to resistance training, we first performed an acute study. In this study, post-exercise blood flow was measured at different training loads. A 37 to 60% higher post-exercise blood flow was found in FT compared to ST individuals (Figure 2B). The elevation of muscle blood flow in the hour(s) following a resistance training bout may be an important factor and/or marker for the magnitude of resistance training adaptations. A selection of studies found a positive association between post-exercise hyperemia and muscle protein synthesis rates (Fujita *et al*., 2006; Timmerman *et al*., 2010). Cold water immersion studies, in which blood flow is reduced (Mawhinney *et al.*, 2017), provide further evidence in this regard by showing attenuated resistance training induced adaptations in muscle volume, strength and fiber CSA in the cold water immersed limb (Roberts *et al.*, 2015; Fyfe *et al.*, 2019). The latter may be the result of a diminished amino acid delivery to the muscle and consequent suppressed myofibrillar protein synthesis rates (Biolo *et al.*, 1995; Roberts *et al.*, 2015; Fyfe *et al.*, 2019; Fuchs *et al.*, 2020). The higher blood flow in FT individuals might therefore be indicative of a higher muscle protein synthesis potential and consequently superior resistance training induced whole-muscle adaptations in these individuals. Moreover, this might be a possible underlying factor in explaining the high inter-individual responses to resistance training. To investigate these hypotheses, a 10-week whole-body resistance training follow-up study was conducted to assess the influence of muscle typology on whole-muscle resistance training adaptations. Since the biggest differences between typologies in post-exercise blood flow were found at the moderate load of 60% 1RM, this load was employed in the chronic training study.

The muscle typology mediated difference in post-exercise blood flow could  however not be translated in the 10-week whole muscle adaptations. Similar to previous research, a considerable variability was observed in hypertrophy (all muscles combined: +3% to +14%) and maximal dynamic strength increases (all exercises combined: +17% to +47%) in all trained muscles and both frequency conditions (Figure 3, Figure S1) (Hubal *et al.*, 2005; Ahtiainen *et al.*, 2016). Despite these large heterogeneous responses, no differences between ST and FT individuals were found for any of the outcome variables. To the best of our knowledge, this is the first study investigating whole muscle adaptations in two distinct muscle typology groups in both the individual upper leg and arm muscles.

Our findings are (partially) in line with the results of Häkkinen *et al*. (1998) who found no relationship between fiber typology and strength increases and with the study of Mobley *et al.*, (2018) who demonstrated no differences in fiber composition between subjects with high or low increases in vastus lateralis muscle volume. Similarly, Schoenfeld *et al*., (2020) showed comparable hypertrophy in the slow-twitch soleus muscle and the mixed fiber gastrocnemius medialis muscle but the inter-individual fiber type composition of the participants' muscles was not measured in this study. Contrary to our results, Häkkinen *et al*., (1998) also reported a negative relationship between the type I fiber percentage and rectus femoris hypertrophy. However, both young and old subjects were included in this correlation analysis with the old subjects mainly showing the lowest hypertrophy and the highest type I percentage which might have distorted the findings. Similar to Häkkinen, Haun *et al.*, (2019) showed that low responders to resistance training had a higher baseline type II fiber percentage combined with a smaller type II fiber cross-sectional area. However, training was not performed to failure in this study and involved trained individuals which may explain the disparity with our findings. Lastly, Dons et al., (1979) reported in a study with a very small sample size (n = 6) a positive relationship between the type II fiber percentage and dynamic strength increases when training was performed at 80% 1RM but not at 50% 1RM. This would indicate that training at higher loads is more beneficial for FT to optimize strength gains. However, similar to Haun but in contrast to our study, these authors did not let participants perform training repetitions to failure during the training period (Dons *et al*., 1979).

Fiber-type specific differences in hypertrophy are often found when training is not performed to failure (Netreba *et al*., 2013; Vinogradova *et al*., 2013) but not when training is performed to failure (Mitchell *et al*., 2012; Morton *et al*., 2016; Lim *et al*., 2019). Therefore, when repetitions are not performed to muscular failure, this may lead to suboptimal training stimuli and adaptations in certain individuals. The latter is confirmed in our study as ST individuals performed a higher number of repetitions and a higher training volume to gain similar adaptations as FT individuals (Figure 5 and Figure S3). The lower number of performed repetitions in FT individuals might be related to the fact that they fatigue more rapidly (Burke & Edgerton, 1975; Thorstensson & Karlsson, 1976; Lievens *et al.*, 2020). More specifically, type II fibers are characterized by a higher sarcoplasmatic reticulum $Ca^{2+}$ pump and cross-bridge ATP consumption (Szentesi *et al.*, 2001; Periasamy et al., 2007; Westerblad *et al*., 2010) and the sarcoplasmatic reticulum $Ca^{2+}$ function is more suppressed in fatigued state with increasing type II percentage (Li *et al.*, 2002). Moreover, type II fibers mostly rely on the glycolytic energy pathway for the production of ATP. However, no differences were found in acute pH or lactate accumulation between the two muscle typology groups in this study. Taken together, muscle typology is not a determining factor in resistance training adaptations when training is performed to failure at 60% 1RM. Importantly, our findings indicate that FT individuals have a higher muscle response per repetition. One could expect that in a study design where we would have matched for absolute training volume (number of repetitions), the responses would have been larger in the FT than ST group. However, this would have been a less ecologically relevant study, as in practice most resistance training is performed until failure.

Similar to whole muscle volume adaptations, a large heterogeneity in type I and type II fiber size responses was found. At group level, this resulted in no increases in type I and type II fiber CSA. A recently performed comparable training protocol found similar inter-subject ranges and also no significant hypertrophy in type I (range: -20.6% to +27.5%) or type II fiber CSA (range: -21.7% to 60.2%) (Thomas *et al.*, 2022). Although on average no fiber-type specific hypertrophy occurred, the increase in fiber type CSA significantly correlated with quadriceps hypertrophy. The discrepancy between the observed changes in fiber CSA and whole muscle hypertrophy is in alignment with other findings showing that microscopic and macroscopic measurements of hypertrophy poorly correspond (Esmarck *et al.*, 2001; Morton *et al.*, 2016; Ruple *et al.*, 2022). However, in contrast to our results, increases in fiber CSA often exceed increases in muscle mass. The disagreement between the two measurement methods can have different causes (Haun *et al.*, 2019*b*; Ruple *et al.*, 2022). First, it might be possible that whole-muscle hypertrophy occurred without fiber diameter enlargement but via increases in pennation angle or via longitudinal growth of the muscle fibers by increasing sarcomere length or by adding new sarcomeres in series  (Ruple *et al.*, 2022; Ema *et al.*, 2016; Jorgenson *et al.*, 2020). Second, although on average 939 fibers were counted per fiber cross-section, this still represents only a very small part of total number of vastus lateralis muscle fibers (~ 600.000) and might not be representative for whole muscle adaptations (Lexell *et al.*, 1988). Third, it is not possible to measure the same muscle fibers pre an post training period. This may have led to increased variability in the fiber cross-sectional area data (Horwath *et al.*, 2021). Lastly, our biopsies were taken in the quadriceps muscle, the muscle group with the smallest increase in muscle volume (4.9% – 6.6%) of all trained muscle groups in the present study. This increase is possibly too small to outweigh the variability in biopsy-based fiber hypertrophy.

In the second aim of the present study we investigated if muscle typology can be used to individualize and optimize the resistance training frequency prescriptions. Despite earlier findings of higher recovery needs (Lievens *et al.*, 2020) and higher susceptibility for overtraining in FT individuals (Bellinger *et al.*, 2020), we demonstrated that FT individuals can also cope with a higher resistance training frequency of 3x/week. This is an important finding as further analysis of the data, regardless of muscle typology, revealed an advantage of training 3x/week to optimize muscle hypertrophy in all trained muscles. This finding is in line with the meta-analysis of Schoenfeld *et al.*, (2019) demonstrating higher training frequencies enhance hypertrophy in non-volume equated studies (studies in which the training volume is not equated between the different training frequencies) like the present study. The present results create an added value to the current body of literature as almost all studies included in the meta-analysis of non-volume equated studies are performed in middle-aged or older individuals and used mostly indirect measurements for hypertrophy. A similar dose-response relationship has been demonstrated between strength gains and training frequency or volume (Ralston *et al.*, 2017; Grgic *et al.*, 2018). However, this could not be confirmed in our study as no differences were found in strength increases between the two frequency conditions for none of the exercises. In summary, both ST and FT individuals resistance training novices should preferentially train 3x/week to optimize muscle hypertrophy while training 2x/week seems sufficient for optimizing strength gains in single-joint exercises.

Our study has a few limitations. First, due to methodological constraints muscle typology was not measured in the arm muscles. However, as significant correlations between the carnosine z-scores of arm and leg muscles and an across-muscle phenotype with regards to fiber composition exist (Vikne *et al.*, 2012; Bex *et al.*, 2017), it can be assumed that the leg carnosine measurements are representative for the upper arm muscles. Secondly, our findings are specific to the training modalities in the present study. Therefore we cannot exclude that muscle typology is of importance in individualizing other resistance training variables like contraction speed or exercise types like endurance training. Moreover, it cannot be ruled out that differences would occur between ST and FT individuals when training is performed at an even higher weekly frequency. However, this would be less applicable in resistance training novices. Lastly, our within-subject design consisted of two bilateral training sessions and one unilateral training session per week. We acknowledge that in the unilateral training session, a cross-education effect may have occurred, in which neural-mediated strength gains can transfer to the non-training contralateral limb (Munn *et al.*, 2004). However, to the best of our knowledge this effect has not yet been demonstrated when both limbs are trained concurrently with different training stimuli. In addition, given the high inter-individual variability, a within-subject design is superior in controlling inter-subject differences like nutrition and training responsiveness.

In conclusion, we demonstrate that an individual's muscle typology cannot explain the variability in chronic resistance training adaptations when training is performed to failure. Our findings negate that a fast typology is beneficial to yield superior resistance training adaptations. However, the ST group performed significantly more repetitions and a higher training volume than the FT group to obtain the same muscle gains. Secondly, individualization of resistance training frequency per week should not be based on muscle typology. However, regardless of muscle typology, training 3x/week is more beneficial than 2x/week to enhance muscle hypertrophy but not maximal dynamic strength in the upper arm and upper leg muscles in strength-training novices.

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## Additional information

### Data availability statement

The data that support the findings of the present study will be made available upon reasonable request to the corresponding author (WD). Figures and tables concerning the 2x/week frequency condition can be found in supplementary data.

### Competing interests

The authors declare that they have no competing interest.

### Author contributions

KVV, EL and WD designed the study. KVV, JH and AW performed the experiments. KVV and JH analyzed the data and interpreted the data together with TVDS, FVDC, EL, JB, SB and WD. KVV, EL and WD drafted the manuscript. All authors revised the manuscript critically for important intellectual content. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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### Keywords

Resistance training, muscle typology, blood flow, hypertrophy, dynamic strength, fiber cross-sectional area, resistance training frequency, resistance training volume

## Figure Legends

**Figure 1.** Overview of study design (Created with BioRender)

**Figure 2. Post-resistance training blood flow**
A) Time course of the two hours post-resistance training blood flow after resistance training at 60% 1RM for ST (n = 11) and FT individuals (n = 10). Data are expressed as mean ± SD. *significant increase in blood flow relative to baseline ($p \leq 0.05$). B) Difference between ST (n = 11) and FT individuals (n = 10) in total blood flow (iAUC) after resistance training at 40%, 60% and 80% 1RM. Data are expressed as mean ± SD + individuals values.

**Figure 3. Inter-individual variability in chronic training adaptations in the 3x/week condition**
Individual increase in muscle volume of the A) quadriceps femoris, B) hamstrings, C) biceps brachii and D) triceps brachii muscle and individual increase in maximal dynamic strength for the E) knee extension, F) knee flexion, G) elbow flexion and H) elbow extension exercise in the limbs that trained 3x/week. Data are presented as mean increase (dotted horizontal line) + individual values (bars). Green bars represent the FT individuals and blue bars with stripes the ST individuals. P-values: comparison between ST and FT individuals with respect to increases in muscle volume and muscle strength. n = 21

**Figure 4.** A) Representative image of a muscle cross-section with type I fibers colored in blue and type II fibers colored in green. B) Relationship between the increase in CSA of all fibers (mean of 3x/week and 2x/week) and increase in whole quadriceps femoris muscle volume (mean of 3x/week and 2x/week) (n = 21). C) Individual change in type I fiber CSA in ST (n = 11) and FT individuals (n = 10) in the 3x/week condition. D) Individual change in type II fiber CSA in ST (n = 11) and FT individuals (n = 10) in the 3x/week condition. E) Relationship between the non-invasively measured concentration of carnosine in the vastus lateralis and biopsy-based % area of type II fibers (n = 21). Data are presented as individual values. The dotted line represents the average change in fiber CSA. P-values represent the comparison in fiber CSA change between ST and FT individuals.

**Figure 5. Total training volume differs between ST and FT individuals**
A) Difference in total training volume between ST (•, n = 11) and FT (▲, n = 10) individuals in the 3 times per week (3x) and 2 times per week (2x) condition. B) Overview of the total weekly training volume of ST and FT in the 3 times per week condition. C) Difference in total training volume per exercise between ST and FT in the 3 times per week condition. D) Relationship between total training volume and total hypertrophy in the 3 times per week condition (n = 21). Data are presented as mean + individual values (A,C), as mean ± SD (B) and as individual values (D).

**Figure 6. Optimal resistance training frequency**
A,B) Increase in (A) muscle volume and (B) 1RM per training frequency (n = 21). Data are presented as mean + individual values. P-values represent the comparison between frequencies. C, D, E, F) Differences in hypertrophy of the (C) quadriceps femoris, (D) hamstrings, (E) biceps brachii and (F) triceps brachii between the 2x/week and 3x/week frequency in ST (n = 11) and FT individuals (n = 10). Data are presented as mean + individual values. P-values represent the comparison between frequencies per muscle typology.

**Abstract figure legend:** This study investigated if muscle typology can explain the high variability in resistance training adaptations. Slow and fast typology resistance training novices were selected to participate in this study by the non-invasive measurement of muscle carnosine with proton magnetic resonance spectroscopy. After the chronic training period a high inter-individual variability was observed in changes in muscle volume, maximal dynamic strength and fiber cross-sectional area.

However, this high inter-individual variability could not be explained by muscle typology for any of the outcomes. Visual abstract created with BioRender.

## Table legends

**Table 1.** Baseline characteristics of slow typology (ST) and fast typology individuals (FT) in the acute and chronic study.

**Table 2**. **Baseline and post-training muscle volumes and muscle volume increases (%) for ST (n = 11) and FT individuals (n = 10) in the 3x/week condition.** All pre-post differences were significant. No significant differences were found between ST and FT individuals neither for baseline values, post values or delta values.

**Table 3. Baseline and post-training maximal dynamic strength and increases in maximal dynamic strength (%) for ST (n = 11) and FT individuals (n = 10) in the 3x/week condition**. All pre-post differences were significant. No differences were found between ST and FT individuals neither for baseline values, post values or delta values.

**Table 4. Baseline and post-training CSA values and increase in fiber CSA (delta (%)) of the type I, type II and all fibers (type I + type II) in ST (n = 11) and FT individuals (n = 10) in the 3x/week condition**. No differences were found between ST and FT individuals neither for baseline values, post values or delta values.

## Supplementary Figure Legends

**Supplementary Figure S1. Inter-individual variability in chronic training adaptations in the 2x/week condition**
Individual increase in muscle volume of the A) quadriceps, B) hamstrings, C) biceps and D) triceps muscle and individual increase in dynamic strength for the E) knee extension, F) knee flexion, G) elbow flexion and H) elbow extension exercise in the limbs that trained 2x/week. Data are presented as mean increase (dotted horizontal line) + individual values (bars). Green bars represent the FT individuals and blue bars with stripes the ST individuals. P-values: Comparison between ST and FT individuals with respect to increases in muscle volume and muscle strength.

**Supplementary Figure S2. Fiber CSA**
A) Individual changes in type I fiber CSA in ST and FT individuals in the 2x/week condition. B) Individual changes in type II fiber CSA in ST and FT individuals in the 2x/week condition. Data are presented as individuals values (A, B). The dotted line represents the average change in fiber CSA. P-values represent the comparison in fiber CSA change between ST and FT individuals.

**Supplementary Figure S3. Total training volume differs between ST and FT individuals in the 2x/week condition**
A) Overview of the total weekly training volume of ST (•) and FT individuals (▲) in the 2 times per week condition. B) Difference in total training volume per exercise between ST and FT individuals in the 2 times per week condition. C) Relationship between total training volume and total hypertrophy in the 2 times per week condition. Data are presented as mean + SD (A), as mean ± SD + individual values (B) or as individual values (C).

**Supplementary Figure S4. Optimal resistance training frequency**
Differences in increase in maximal dynamic strength (Δ1RM) of the (A) knee extension, (B) knee flexion, (C) elbow flexion and (D) elbow extension between the 2x/week and 3x/week frequency in ST (n = 11) and FT individuals (n = 10). Data are presented as mean + individual values.

## Supplementary tables

**Supplementary Table S1.** Average daily energy, protein, carbohydrate and fat intake for ST and FT individuals.

| | ST | FT | P – value |
|---|---|---|---|
| **Energy intake (kcal/kg FFM/day)** | 34.5 ± 7.41 | 40.5 ± 10.0 | 0.145 |
| **Protein intake (g/kg BM)** | 1.31 ± 0.26 | 1.29 ± 0.23 | 0.887 |
| **Carbohydrate intake (g/kg BM/day)** | 3.24 ± 0.82 | 3.60 ± 1.24 | 0.389 |
| **Fat intake (g/kg BM/ day)** | 1.05 ± 0.23 | 1.22 ± 0.47 | 0.253 |

Data are presented as mean ± SD. FFM = Fat free mass. BM = Body mass. P-value = difference between ST (n = 11) and FT individuals (n = 10).

**Supplementary Table S2. Baseline and post muscle volumes and increases in muscle volume (%) for ST (n = 11) and FT individuals (n = 10) in the 2x/week condition**. No differences were found between ST and FT individuals neither for baseline values, post values or delta values.

| | ST | | | FT | | |
|---|---|---|---|---|---|---|
| | **Baseline (mL)** | **Post (mL)** | **Delta (%)** | **Baseline (mL)** | **Post (mL)** | **Delta (%)** |
| **Muscle Volume** | **2x/week** | | | | | |
| **Quadriceps femoris** | 1820 ± 301 | 1910 ± 306 | 5.09 ± 2.72 | 1773 ± 351 | 1850 ± 332 | 4.77 ± 3.61 |
| Rectus femoris | 261 ± 56.3 | 276 ± 60.5 | 5.60 ± 4.99 | 230 ± 50.9 | 245 ± 51.8 | 6.92 ± 6.88 |
| Vastus intermedius | 247 ± 41.9 | 258 ± 43.1 | 4.53 ± 6.49 | 232 ± 51.7 | 243 ± 51.0 | 5.15 ± 5.33 |
| Vastus lateralis | 872 ± 136 | 923 ± 134 | 6.05 ± 2.97 | 878 ± 182 | 920 ± 163 | 5.41 ± 4.86 |
| Vastus medialis | 439 ± 86.0 | 453 ± 85.8 | 3.41 ± 3.20 | 433 ± 93.4 | 442 ± 85.6 | 2.72 ± 4.35 |
| **Hamstrings** | 649 ± 123 | 708 ± 120 | 9.58 ± 4.00 | 623 ± 143 | 675 ± 146 | 8.73 ± 3.58 |
| Biceps femoris: long head | 181 ± 43.0 | 194 ± 42.2 | 7.76 ± 5.51 | 170 ± 32.1 | 179 ± 34.0 | 5.51 ± 3.50 |
| Biceps femoris: short head | 88.1 ± 21.5 | 98.5 ± 21.2 | 12.7 ± 7.13 | 78.6 ± 26.6 | 89.2 ± 26.8 | 14.8 ± 8.22 |
| Semimembranosus | 211 ± 43.8 | 219 ± 46.9 | 3.89 ± 3.64 | 214 ± 55.5 | 224 ± 54.6 | 4.99 ± 4.44 |
| Semitendinosus | 169 ± 32.2 | 197 ± 35.1 | 16.7 ± 7.45 | 161 ± 39.2 | 183 ± 43.2 | 14.5 ± 7.46 |
| **Biceps brachii** | 305 ± 86.3 | 321 ± 81.8 | 6.19 ± 4.58 | 302 ± 102 | 326 ± 112 | 7.82 ± 3.91 |
| **Triceps brachii** | 342 ± 88.0 | 392 ± 95.3 | 15.3 ± 6.03 | 363 ± 115 | 416 ± 124 | 15.5 ± 3.77 |

Data are presented as mean ± SD.

**Supplementary Table S3. Baseline and post muscle volumes and increases in maximal dynamic strength for ST (n = 11) and FT individuals (n = 10) in the 2x/week condition**. No differences were found between ST and FT individuals neither for baseline values, post values or delta values.

| | ST | | | FT | | |
|---|---|---|---|---|---|---|
| | **Baseline** | **Post** | **Delta (%)** | **Baseline** | **Post** | **Delta (%)** |
| **Maximal dynamic strength (kg)** | **2x/week** | | | | | |
| **Knee extension** | 49.1 ± 9.76 | 59.1 ± 12.6 | 20.5 ± 12.0 | 46.0 ± 8.51 | 53.8 ± 10.2 | 16.8 ± 7.51 |
| **Knee flexion** | 34.1 ± 6.55 | 42.7 ± 6.17 | 26.8 ± 11.8 | 32.3 ± 9.24 | 40.5 ± 11.2 | 25.9 ± 13.1 |
| **Elbow flexion** | 13.9 ± 4.16 | 19.4 ± 4.52 | 44.4 ± 26.2 | 14.8 ± 4.16 | 20.0 ± 4.62 | 38.9 ± 26.9 |
| **Elbow extension** | 7.82 ± 2.75 | 11.4 ± 2.74 | 51.5 ± 22.6 | 9.10 ± 3.70 | 13.2 ± 4.58 | 49.3 ± 21.0 |

Data are presented as mean ± SD.

**Supplementary Table S4. Baseline and post CSA values and increase in fiber CSA (delta (%)) of the type I, type II and all fibers (type I + type II) in ST (n = 11) and FT (n = 10) in the 2x/week condition**. No differences were found between ST and FT neither for baseline values, post values or delta values.

| | ST | | | FT | | |
|---|---|---|---|---|---|---|
| | **Baseline** | **Post** | **Delta (%)** | **Baseline** | **Post** | **Delta (%)** |
| **Fiber CSA ($\mu m^2$)** | **2x/week** | | | | | |
| Type I | 4598 ± 752 | 4635 ± 717 | 1.89 ± 14.5 | 4120 ± 750 | 4248 ± 839 | 5.96 ± 26.0 |
| Type II | 4530 ± 886 | 4378 ± 928 | -2.51 ± 14.3 | 4314 ± 958 | 4395 ± 882 | 4.64 ± 23.6 |
| All fibers | 4564 ± 740 | 4506 ± 745 | -0.44 ± 13.6 | 4217 ± 795 | 4321 ± 785 | 4.99 ± 23.6 |

Data are presented as mean ± SD

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