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Prolonged exercise training increases intramuscular lipid content and perilipin 2 expression in type I muscle fibres of patients with type 2 diabetes

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Running Head: exercise training and intramuscular lipid

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Abstract

The aim of the present study was to investigate changes in intramuscular triglyceride (IMTG) content and perilipin 2 expression in skeletal muscle tissue following 6 months of endurance type exercise training in type 2 diabetes patients. 10 obese, male type 2 diabetes patients (age 62±1 y, BMI 31±1 kg/m^2) completed 3 exercise sessions per week, consisting of 40 min of continuous endurance type exercise at 75% $VO_{2\ peak}$, for a period of 6 months. Muscle biopsies collected at baseline and after 2 and 6 months of intervention were analysed for IMTG content and perilipin 2 expression using fibre type specific immunofluorescence microscopy. Endurance type exercise training reduced trunk body fat by 6±2% and increased whole-body oxygen uptake capacity by 13±7% (P<0.05). IMTG content increased two-fold in response to the 6 months exercise training in both type I and type II muscle fibres (P<0.05). A three-fold increase in perilipin 2 expression was observed from baseline to 2 and 6 months of intervention in the type I muscle fibres only (1.1±0.3, 3.4±0.6, and 3.6±0.6% fibre stained, respectively; P<0.05). Exercise training induced a 1.6-fold increase in mitochondrial content after 6 months of training in both type I and type II muscle fibres (P<0.05). In conclusion, this is the first study to report that prolonged endurance type exercise training increases the expression of perilipin 2 alongside increases in IMTG content in a type I muscle fiber type specific manner in type 2 diabetes patients.

Key words: Exercise, lipid metabolism, intramuscular triglyceride, ADRP, adipophilin, insulin sensitivity

Introduction

Skeletal muscle insulin resistance is a defining characteristic of type 2 diabetes and is associated with intramuscular triglyceride (IMTG) accumulation. The concept of lipid-induced insulin resistance was initially derived from cross-sectional studies which demonstrated a correlation between high IMTG concentrations and insulin resistance (30). However, trained endurance athletes are generally highly insulin sensitive despite having substantially elevated IMTG levels (13, 46). Consequently, IMTG accumulation appears to relate to insulin resistance when accompanied by a sedentary lifestyle and low oxidative capacity (5). It is postulated that a low turnover of the intramuscular lipid pool and a resultant elevation in the concentration of lipid metabolites, such as diacylglycerol and ceramides, mediates impairments in the insulin signaling pathway which are responsible for reduced insulin sensitivity (28).

The benefits of prolonged endurance type exercise training on cardiovascular and metabolic health have been well established (17), and provide a basis for prescribing exercise in the prevention and treatment of type 2 diabetes (33). Exercise training interventions that enhance oxidative capacity and improve the storage and packaging of IMTG are likely to facilitate the improvement in skeletal muscle insulin sensitivity. In accordance, recent studies demonstrate an increase in mitochondrial density and intrinsic mitochondrial function in response to prolonged endurance type exercise training in type 2 diabetes patients (18, 31). The impact of endurance type exercise training on IMTG content is less clear, with studies showing an increase (10, 35), no change (6), or a decrease (4, 42) in muscle lipid storage in older obese individuals and obese type 2 diabetes patients. Changes in IMTG deposition can be assessed by the use of biochemical TG extraction of muscle tissue as well as immunohistochemical analyses of oil red O-stained muscle cross sections (43). The latter approach has shown a 3-4 fold greater lipid content in type I versus type II muscle fibres (45). This method has been applied frequently to evaluate fibre type specific differences in IMTG content across different populations and in response to exercise (46, 47). Therefore it is important to utilize techniques that allow IMTG content and associated proteins to be analysed in a muscle fibre type specific manner.

Lipid droplets (LDs) containing IMTG are viewed as a dynamic organelle which play a role in a variety of cellular functions including lipid homeostasis and cell signaling (for recent reviews see (11, 12)). This notion is supported by the discovery of a family of proteins associated with the phospholipid monolayer of LDs, referred to as the perilipin proteins (numbered 1 to 5; (22)). Perilipin 1 is relatively well-characterized and appears to regulate lipolysis through its interaction with lipases and co-activators at the surface of the LD (15, 48), however its expression is reported to be limited to adipocytes and steroidogenic cells (24). Perilipin 2 (formerly known as adipocyte differentiation-related protein; ADRP or adipophilin) on the other hand is ubiquitously expressed and present in skeletal muscle tissue. Perilipin 2 content is closely related to IMTG concentrations and is more abundantly expressed in the type I muscle fibres (3, 27, 39). Although the exact function of perilipin 2 remains to be established, *in vitro* data suggest that its presence on the lipid droplet surface can limit the LD-association with adipose triglyceride lipase (ATGL) (1, 23). Therefore, TG accumulation in cells expressing perilipin 2 has been attributed to the subsequent lowering of basal lipolytic rates which also promotes tissue insulin sensitivity (1). In agreement, human studies demonstrate that perilipin 2 gene expression is higher in insulin sensitive versus insulin resistant individuals (8) and improvements in insulin mediated glucose disposal in response to weight loss and the pharmacological treatment of type 2 diabetes alters the expression of perilipin 2 in skeletal muscle (27, 32). However, the impact of prolonged endurance type exercise training on fibre type specific perilipin 2 protein expression remains to be assessed.

We hypothesized that prolonged endurance type exercise training increases muscle lipid storage and upregulates the expression of perilipin 2. Given the importance of considering muscle fibre type when investigating IMTG and related proteins, we applied immunofluorescence microscopy techniques to investigate muscle fibre type specific changes in IMTG, perilipin 2 and cytochrome *c* oxidase (COX) content following 2 and 6 months of endurance type exercise training in type 2 diabetes patients.

Materials and Methods

Participants

Ten type 2 diabetes patients participated in the current study (62±1 y, BMI 31.2±0.9 $kg.m^{-2}$). Participants had been diagnosed for at least 12 months, were all being treated with oral blood-glucose-lowering medication and were sedentary. The study was approved by the medical ethics committee of the Virga Jesse Hospital, Belgium and written informed consent was obtained from all participants. The patients in the current study were part of a larger project (clinical trial registration: ISRCTN32206301) investigating the impact of prolonged endurance type exercise training in a cohort of fifty type 2 diabetes patients, described in detail elsewhere (16).

Study design

Participants completed a 6 month endurance type exercise training program. Prior to commencement of the study, and after 2 and 6 months of the intervention, oxidative capacity, body composition and oral glucose tolerance were assessed as described previously (16). Muscle biopsies were taken from the *vastus lateralis* in the morning and following an overnight fast and were analysed for mitochondrial content, IMTG, and perilipin 2 expression. The measurements at 2 and 6 months were performed at least 4 d after the last exercise session. Oral blood glucose and/or lipid-lowering medication were stopped 3 d prior to these measurements.

Training intervention

Participants undertook 3 supervised training sessions per week in the rehabilitation centre of the hospital. Each exercise session consisted of walking, cycling, and cross-country ski-type exercise and was performed for 40 min at a heart rate corresponding to exercise performed at 75% of $VO_{2\ peak}$. The relationship between $VO_{2\ peak}$ and heart rate was reassessed after 2 months, and training intensity was adjusted accordingly.

Immunohistochemistry

Muscle samples were dissected free of fat and connective tissue, before being embedded in Tissue-Tek OCT Compound (Sakura Finetek Europe, The Netherlands) and frozen in liquid nitrogen-cooled isopentane. Cryosections of 5 μm thickness were fixed in 3.7% formaldehyde and permeabilised for 5 min in 0.5% Triton-X 100. Sections were then incubated for 1 h with a mouse monoclonal anti-ADRP/perilipin2 antibody (Progen, Germany) as described previously (38, 39). As a key protein in the electron transport chain, identification of cytochrome C oxidase using a mouse monoclonal anti-OxPhos Complex IV (COX) antibody (Invitrogen, UK) was also used as a marker of the mitochondrial network of skeletal muscle. Fibre type determination was achieved through incubation of muscle sections with mouse anti-myosin heavy chain type I (A4.840-c, DSHB, developed by Dr. Blau). Sections were then incubated with either an Alexa Fluor goat anti-mouse IgG_{2a} 594 (for OxPhos Complex IV) or an Alex Fluor goat anti-mouse IgG_1 594 (for perilipin 2) in combination with an Alexa Fluor goat anti-mouse IgM 488 (for MHC I) (Invitrogen, UK) for 30 min. Coverslips were mounted with a glycerol and mowiol 4-88 solution in 0.2 M Tris buffer (pH 8.5) (including 0.1% DABCO anti-fade medium). When IMTG visualisation was undertaken, the neutral lipid dye oil red O staining protocol in combination with immunofluorescence was used (47). In this respect, oil red O was applied to sections for 30 min following incubation with antibodies for fibre type determination.

Image capture, processing and data analysis

Image capture was performed in a blinded fashion on a widefield Nikon E600 microscope with a 40x 0.75 NA objective, coupled to a SPOT RT KE colour 3 shot CCD camera (Diagnostic Instruments Inc., USA) for the fibre type-specific determination of IMTG and perilipin 2. FITC (465-495 nm) and Texas Red (540-580 nm) excitation filters were used to view the Alexa Fluor 488 and 594 fluorophores, respectively. The Texas Red excitation filter was also used to view sections stained with oil red O. An inverted confocal laser scanning microscope (Leica DMIRE2, Leica Microsystems) with a 63x 1.4 NA oil immersion objective was used to obtain digital images of mitochondria, IMTG and perilipin 2. A Helium-Neon laser was used to excite the Alexa Fluor 594 fluorophore and oil red O, and an argon laser was used to excite the Alexa Fluor 488 fluorophore.

Image processing was undertaken using Image-Pro Plus 5.1 software (Media Cybernetics, MD, USA). Widefield images were used to assess fibre-type specific content of IMTG and perilipin 2. Confocal images were used to assess COX content, LD size and to visualize the subcellular distribution of perilipin 2. Fibres positively stained for MHC I were considered type I muscle fibres and non-stained fibres were considered type II muscle fibres. Identification of COX, IMTG and perilipin 2 was achieved through the selection of an intensity threshold that was used uniformly for all images in that series. COX, IMTG and perilipin 2 content was expressed as the percentage fibre area positively stained. IMTG and perilipin 2 density were expressed as number of positively stained 'spots' corrected for fibre area (μm^2). Mean LD size was calculated by dividing the total number of objects by the total area stained. A total of 100±12, 72±6 and 86±7 fibres were analysed per muscle cross-section for COX, IMTG and perilipin 2 analysis respectively.

Statistics

All data are expressed as means±SEM. Significance was set at the 0.05 level of confidence. Changes in whole-body characteristics, exercise capacity, body composition and insulin sensitivity were analysed were using a one-way repeated measures ANOVA, with the within-subject factor as '*time*' (0 vs 2 vs 6 months). Changes in COX, IMTG and perilipin 2 were assessed using a two-way repeated measures ANOVA, with two within-subject factors '*fibre*' (type I vs type II fibres) and '*time*' (0 vs 2 vs 6 months). Significant main effects or interactions were assessed using Bonferroni adjustment post hoc analysis.

Results

Participants

Participant characteristics are displayed in Table 1. Significant reductions in body mass and BMI were observed with training ($P<0.05$; Table 1) which were accompanied by a reduction in relative trunk fat and leg fat percentage of 6±2% and 5±2% post-training, respectively ($P<0.05$). Maximal oxygen uptake demonstrated a significant increase over time ($P<0.05$) and was 16±3% higher after 2 months of training (from 23.4±1.5 to 27.1±1.7 $mL \cdot kg^{-1} \cdot min^{-1}$, $P<0.01$), and remained 13±7% higher

compared to baseline values after 6 months of intervention (26.5±1.9 $mL.kg^{-1}.min^{-1}$, P=0.08). Although the total cohort showed significant improvements in glycemic control, as shown by reduced levels of HbA_{1c} (16), in this small subcohort of 10 subjects the decrease in HbA_{1c} following training failed to reach significance (7.0±0.4, 6.7±0.3 and 6.5±0.2%, for 0, 2 and 6 months respectively; P=0.20). No significant changes in fasting plasma glucose and insulin concentrations, 2 h post-OGTT glucose concentrations or HOMA index of insulin sensitivity were observed in response to training (P>0.05).

Immunohistochemical analysis

Cytochrome c oxidase: Muscle fibre type specific COX expression was significantly greater in type I compared with type II muscle fibres at all time points (P<0.01; Figure 1). Six months of endurance type exercise training induced a time-dependent increase in COX expression in both type I and type II fibres (P<0.01). Following 6 months of exercise training, COX expression was higher than baseline in both type I (8.9±2.1 vs 14.0±2.9% fibre stained) and type II muscle fibres (5.4±1.8 vs 8.8±2.4% fibre stained). The confocal micrographs of COX stained muscle fibres at baseline, 2 months and 6 months are presented in Figure 1b. These images demonstrate greater COX density in both intermyofibrillar and subsarcolemmal regions of the muscle fibres after 6 months of training, and are most prominent in the subsarcolemmal regions.

Intramuscular triglyceride: IMTG content, expressed as the area fraction stained, differed between type I and type II muscle fibres at each time point (Figure 2A; P<0.01). Six months of endurance type exercise training induced a time dependent increase in IMTG content in both type I and II muscle fibres. IMTG content had increased ~1.9-fold in type I fibres (2.3±0.4 vs 4.3±0.5%; P<0.01) and type II fibres (0.9±0.1 vs 1.7±0.3%; P<0.01) following 6 months of training. The increase in IMTG content was mirrored by significant increases in IMTG density after 6 months of training in type I fibres (0.051±0.007 and 0.079±0.008 LDs/μm^2 for 0 and 6 months, respectively; P<0.01) but not in type II muscle fibres (0.022±0.002 and 0.031±0.003 LDs/μm^2 for 0 and 6 months, respectively; P=0.056). No significant changes in IMTG content or density were apparent after 2 months of training in either fibre

type (P>0.05). There were no differences in lipid droplet size as determined by confocal microscopy between fibre types or in response to endurance type exercise training (P>0.05).

Perilipin 2. At baseline, perilipin 2 expression calculated as the percentage area stained did not differ between type I and type II muscle fibres (Figure 2B; P>0.05). Six months of exercise training induced a time dependent increase in perilipin 2 expression in type I muscle fibres only (P<0.05). In comparison to baseline, perilipin 2 expression in type I muscle fibres had increased ~3-fold after 2 months of training (1.1±0.3 vs 3.6±0.6%, respectively; P<0.05) with no further increase observed after 6 months of training. This fibre type specific training response resulted in greater perilipin 2 expression in type I compared with type II muscle fibres after 2 and 6 months of training (P<0.05). Representative immunofluorescence images of perilipin 2 expression in type I and type II muscle fibres at baseline, and after 2 and 6 months of training are shown in figure 3. In comparison to baseline, perilipin 2 density in type I fibres also increased ~2-fold after 2 and 6 months (0.018±0.003, 0.033±0.004 and 0.035±0.005 perilipin 2 objects/μm^2 for 0, 2 and 6 months respectively; P<0.05) whereas perilipin 2 density in type II muscle fibres did not change (P>0.05). Therefore, perilipin 2 density was higher in type I than type II muscle fibres after 2 and 6 months of training only. Higher magnification images of perilipin 2 were obtained using confocal laser scanning microscopy and are shown in Figure 3. These images show a clear increase in perilipin 2 expression after 6 months of training. These images demonstrate that distinct rings of perilipin 2 can frequently be observed and are more abundant after prolonged exercise training.

Discussion

Prolonged endurance type exercise training is known to improve insulin-stimulated glucose uptake and glycaemic control in type 2 diabetes patients (17). In this study we demonstrate that endurance type exercise training also increases both IMTG deposition and COX expression, which are higher in type I muscle fibres. In accordance, we show for the first time that training induces a greater expression of perilipin 2 in type I muscle fibres.

Insulin sensitivity is enhanced by regular physical activity which explains why significant improvements in glycaemic control were observed in the previous study after 6 months of endurance type exercise training in a large cohort of type 2 diabetes patients (16). In the subset of participants used in this study there was no significant change in glycaemic control as evident from the HbA_{1c} levels after 6 months of training (Table 1). Nevertheless a decline from 7.0±0.4% down to 6.5±0.2% in HbA1c is of great clinical significance, as it would translate into a >10% reduction in the risk of premature death, a 5-10% reduction in the risk of myocardial infarction and a ~20% reduction in the risk of microvascular disease (25).

Skeletal muscle oxidative capacity and whole body fatty acid oxidation are good predictors of muscle insulin sensitivity (5, 13, 14, 19). Obese individuals with insulin resistance and type 2 diabetes commonly display a reduced capacity for oxidative metabolism (2, 20, 21, 36). Thus, it is likely that increased oxidative capacity following exercise interventions are mechanistically linked to improvements in metabolic health in this population. Accordingly, we observed a ~1.6-fold increase in COX expression in skeletal muscle following 6 months of endurance type exercise training (Figure 1). The increase in COX expression in this subset of patients is in agreement with the 50% increase in COX and citrate synthase activities observed in the full cohort of patients reported previously (16). We extend on these previous data by the application of immunofluorescence microscopy, allowing us to assess oxidative capacity in a muscle fiber type specific manner. Furthermore, we also assessed subcellular localisation of the observed increases in oxidative capacity (Figure 1). The present work shows that increases in the content of the mitochondrial enzyme COX can be observed in both the subsarcolemmal and intermyofibrillar region of the type I muscle fibres. In agreement with previous data investigating mitochondrial content following a 10 week training intervention in type 2 diabetes patients using transmission electron microscopy (29), we show that increased COX expression is prominent in subsarcolemmal regions of type I fibres after prolonged endurance type exercise training.

The exercise training-induced increase in skeletal muscle oxidative capacity was accompanied by a ~2-fold elevation in skeletal muscle lipid deposition in both type I and type II muscle fibres (Figure 2). This is the first study to show a type I muscle fibre specific increase in IMTG content following prolonged exercise training in type 2 diabetes patients. These findings tend to be in line with several recent studies demonstrating IMTG accretion coupled to increased oxidative capacity in older, obese insulin resistant individuals following 12-16 weeks of exercise training (10, 35). Although IMTG content is already elevated in obese type 2 diabetes patients, these levels still remain below those observed in endurance-trained athletes who are highly insulin sensitive (13, 46). The high IMTG content in combination with a reduced oxidative capacity in type 2 diabetes patients likely mediates the reduction in muscle insulin sensitivity rather than merely elevated IMTG stores. Accordingly, exercise training-induced increases in mitochondrial content, coupled to IMTG accretion appear to enhance insulin sensitivity. For example, a recent study has demonstrated that training-induced increases in IMTG concentrations and improvements in insulin sensitivity are coupled to a reduction in the concentration of diacylglycerol and ceramide (9). Therefore it has been hypothesized that the process of IMTG synthesis consumes the lipid metabolites that are precursors to IMTG and impair skeletal muscle insulin signaling. In further support, the high IMTG synthesis rates observed in the period after endurance type exercise protects against the development of insulin resistance during (intra)lipid infusion (37). The present study adds to this growing body of evidence by demonstrating greater IMTG storage and improved glycaemic control in response to 6 months training in type 2 diabetes patients. Some studies employing a shorter training duration have failed to observe a significant increase in type 1 muscle fibre IMTG content following training in type 2 diabetic patients (26). Therefore, it is possible that a more prolonged intervention, such as the 6 month endurance training programme applied in the current study, is required before increases in IMTG deposition are observed in type 2 diabetes patients. The duration of the training intervention, in addition to the method of IMTG analysis, may also explain the discrepancy across the many studies investigating changes in IMTG content.

The increase in total IMTG content following training in the present study was accompanied by an increase in the number of LDs in type I fibres, whereas there was no change detected in LD size. This is in agreement with a previous electron microscopy study in young males and females, where the increase in total IMTG content with training was due to an increase in LD density while LD size remained unchanged (44). IMTG expansion through an increase in the number of smaller LDs would befit a metabolic advantage as the surface area available for the interaction of lipolytic enzymes with the regulatory proteins contained on the LD surface would be enhanced. This would maximize the capacity for rapid LD turnover, allowing more efficient lipid mobilization and therefore oxidation during exercise.

One of the regulatory proteins that reside on the surface of the LD monolayer is perilipin 2. In the current study, despite observing a ~2-fold higher IMTG concentration in the type I muscle fibres, (Figure 2A) there was no difference in perilipin 2 expression between type I and type II muscle fibres prior to endurance type exercise training (Figure 2B). However, training induced a significant increase in perilipin 2 expression in type I muscle fibres. The perilipins are important in the packaging of lipid droplets and *in vitro* studies demonstrate that perilipin 2 expression increases cellular TG and improves insulin sensitivity. The presence of perilipin 2 at the LD surface appears to limit the association of ATGL with the LD surface, reduce basal lipolytic rates and therefore promote TG storage (1, 23).

We show that when type 2 diabetes patients are physically active, type I muscle fibres exhibit a greater expression of perilipin 2 than type II muscle fibres. This is in agreement with our previous observations of a greater perilipin 2 expression in the type I muscle fibres of sedentary individuals and trained cyclists (39, 40). The increase in perilipin 2 expression in type I muscle fibres is likely to result in enhanced coverage of the LD surface with perilipin 2. This adaptation would limit rates of basal lipolysis and promote IMTG storage in the basal state. Furthermore, hormone sensitive lipase translocates to perilipin 2-coated LDs during muscle contraction and adrenaline stimulation (34) and perilipin 2-associated LDs are depleted during exercise (40). We hypothesize that an increase in

perilipin 2 surface coverage of the LD, along with the greater total LD surface area available and the enhanced mitochondrial density, would aid the mobilization and oxidation of the IMTG pool during exercise. This proposed improvement in the regulation of IMTG turnover both at rest and during exercise may go some way to explaining why insulin sensitivity can be enhanced despite further accumulation of IMTG with training. However, it should be noted that neither intramuscular lipolysis nor lipid oxidation rates were assessed in the present study, therefore further research is required to fully explore the relationship between changes in perilipin 2 expression and intramuscular lipid oxidation.

A non-exercise control group was not included in the present study, however reductions in fat mass, and improvements in VO_{2max} and muscle oxidative capacity are not seen in a similar time frame in non-exercising controls (7, 41). Therefore we can be confident that the related changes in perilipin 2 expression and IMTG storage in the present study are specific adaptations to the exercise intervention. As perilipin 2 is one of four perilipin proteins present in skeletal muscle, additional investigations examining other perilipins are required to fully understand the role of IMTG metabolism in the development insulin resistance and the insulin sensitizing effect of endurance type training.

In conclusion, prolonged endurance type exercise training increases intramuscular lipid storage in a muscle fibre type dependent manner in type 2 diabetes patients. Importantly, the increase in IMTG content is accompanied by a type I muscle fiber specific increase in perilipin 2 expression. The greater perilipin 2 expression following prolonged endurance type exercise training in combination with increased oxidative capacity may explain the improved turnover of the skeletal muscle lipid pool with regular physical activity, and likely contributes to the improvements in skeletal muscle insulin action and subsequent glycaemic control.

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Disclosures

There are no conflicts of interests declared by the authors

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Figure Legends

Figure 1. Fibre type specific COX expression. A. Mean fibre type-specific COX expression (expressed as percentage fibre area stained) at baseline, and following 2 and 6 months of exercise training. Data represent means±SEM. Time effect, P=0.006; fibre effect, P<0.001; interaction of time and fibre, P=0.112. * Significant effect of time; † significant difference between fibre types (P < 0.05). B. Upper panel, representative images of mitochondrial network, stained with anti-COX and viewed and quantified with confocal immunofluorescence microscopy; Scale bar, 40 μm. Lower panel, representative images of subsarcollemal and intermyofibrillar mitochondria areas. Scale bar, 1 μm.

Figure 2. Fibre type specific intramuscular triglyceride content and perilipin 2 expression. Mean fibre type-specific intramuscular triglyceride (IMTG) content (A) and perilipin 2 expression (B) at baseline, and following 2 and 6 months of exercise training (expressed as percentage fibre area stained). Data represent means±SEM. **A.** Time effect, P=0.001; fibre effect, P<0.001; interaction of time and fibre, P=0.003. **B.** Time effect, P=0.017; fibre effect, P<0.001; interaction of time and fibre, P= 0.01. * Significant effect of time; † significant difference between fibre types; # significant difference from baseline (P<0.05).

Figure 3. Immunofluorescence images of fibre type specific perilipin 2 protein expression. A. Representative images of perilipin 2 at baseline and 6 months, stained using anti-perilipin 2 in combination with ant-myosin heavy chain type I and wheat germ agglutinin 350 (WGA350) and viewed and quantified with widefield immunofluorescence microscopy. Scale bar, 50 μm. B. Representative confocal images of perilipin 2 at baseline and 6 months, rings of perilipin staining are clearly visible in both images. Scale bar, 10 μm.

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568 **Tables**

Table 1. *Subject Characteristics*

Characteristic	Time			
	Baseline	**2 months**	**6 months**	**Effect of time (P value)**
Age (years)	62 ± 1	-	-	
Height (m)	1.72 ± 0.02	-	-	
Body weight (kg)	92.9 ± 3.4	91.7 ± 3.4	91.3 ± 3.4	<0.05
Body mass index ($kg.m^{-2}$)	31.2 ± 0.9	30.8 ± 0.9	30.7 ± 0.9	<0.05
Insulin sensitivity				
Fasting glucose ($mmol.L^{-1}$)	9.2 ± 0.7	9.0 ± 0.7	8.7 ± 0.7	0.632
2 h glucose ($mmol.L^{-1}$)	17.9 ± 1.6	16.5 ± 1.6	15.5 ± 1.7	0.367
Fasting insulin ($\mu IU.mL^{-1}$)	17.8 ± 2.3	16.8 ± 2.3	17.7 ± 2.1	0.855
HOMA index	7.5 ± 1.4	7.0 ± 1.5	7.1 ± 1.2	0.865
HbA_{1c} (%)	7.0 ± 0.4	6.7 ± 0.3	6.5 ± 0.2	0.200
Exercise capacity				
$VO_{2\ peak}$ ($L.min^{-1}$)	2.15 ± 0.14	2.46 ± 0.15$^{\#}$	2.41 ± 0.19	<0.05
$VO_{2\ peak}$ ($mL.kg^{-1}.min^{-1}$)	23.4 ± 1.5	27.1 ± 1.7$^{\#}$	26.5 ± 1.9	<0.05
W_{max} (W)	180 ± 12	189 ± 11	189 ± 11	0.134
Body composition				
% Trunk fat	38.4 ± 1.4	37.3 ± 1.7	35.8 ± 1.3$^{\#}$	<0.01
% Leg fat	23.2 ± 1.6	22.4 ± 1.5	21.8 ± 1.3	<0.05

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570 Data provided represent means±SEM (n=10). $^{\#}P$<0.05 vs. baseline.

Figure 1

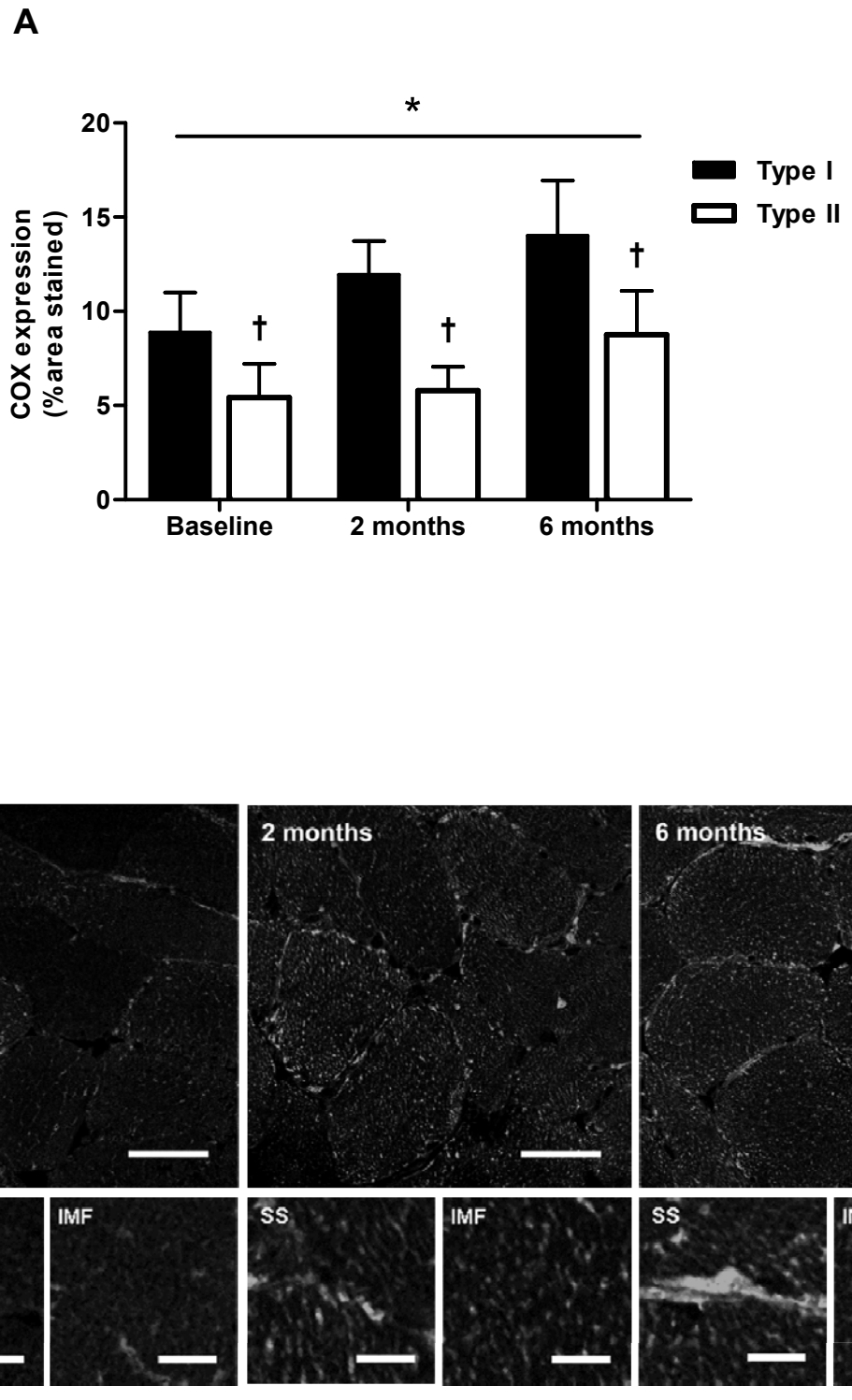

A
*
20
15
10
5
0
COX expression
(%area stained)
Type I
Type II
†
†
†
Baseline
2 months
6 months
B
Baseline
2 months
6 months
SS
IMF
SS
IMF
SS
IMF

Figure 2

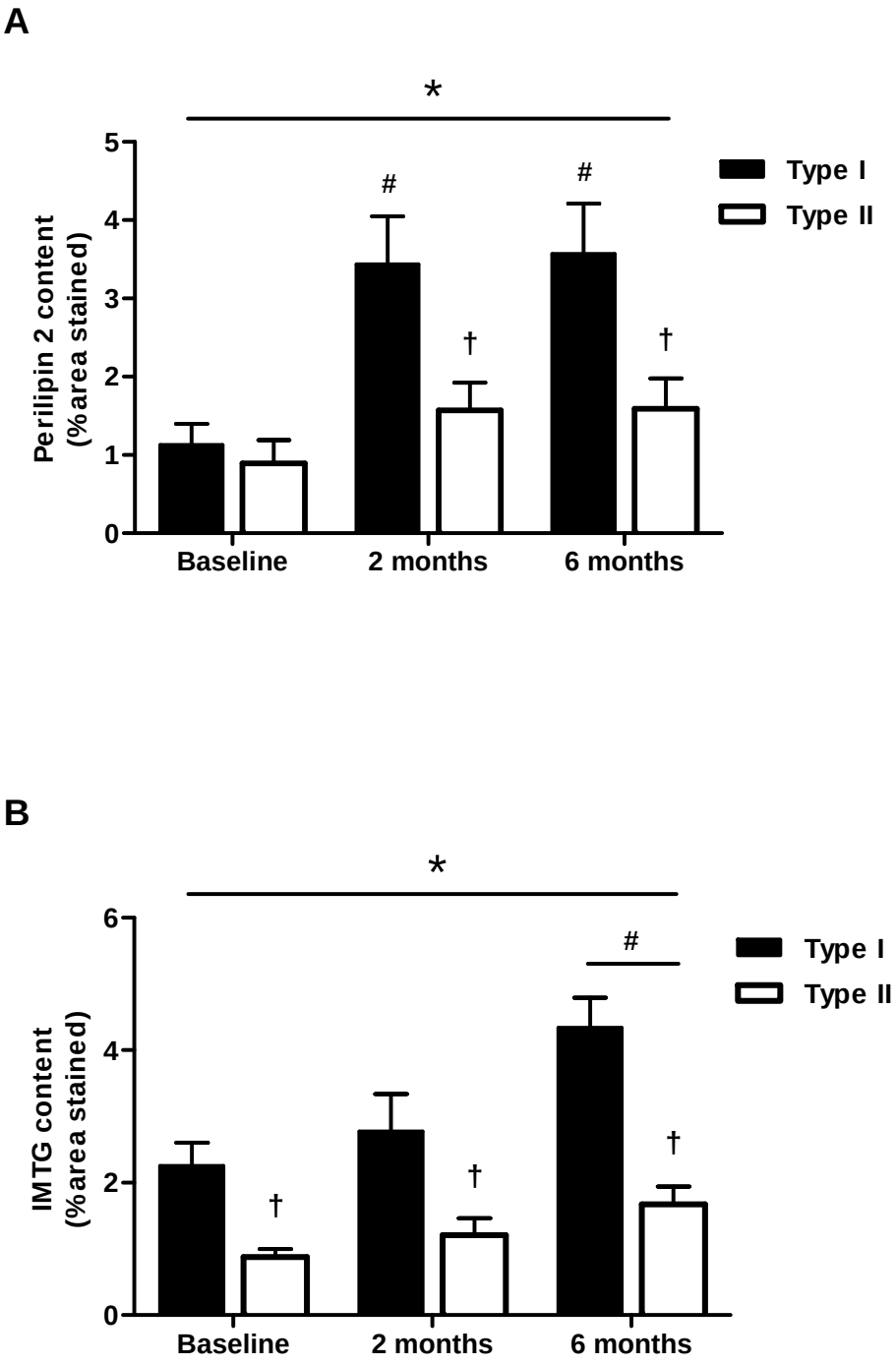
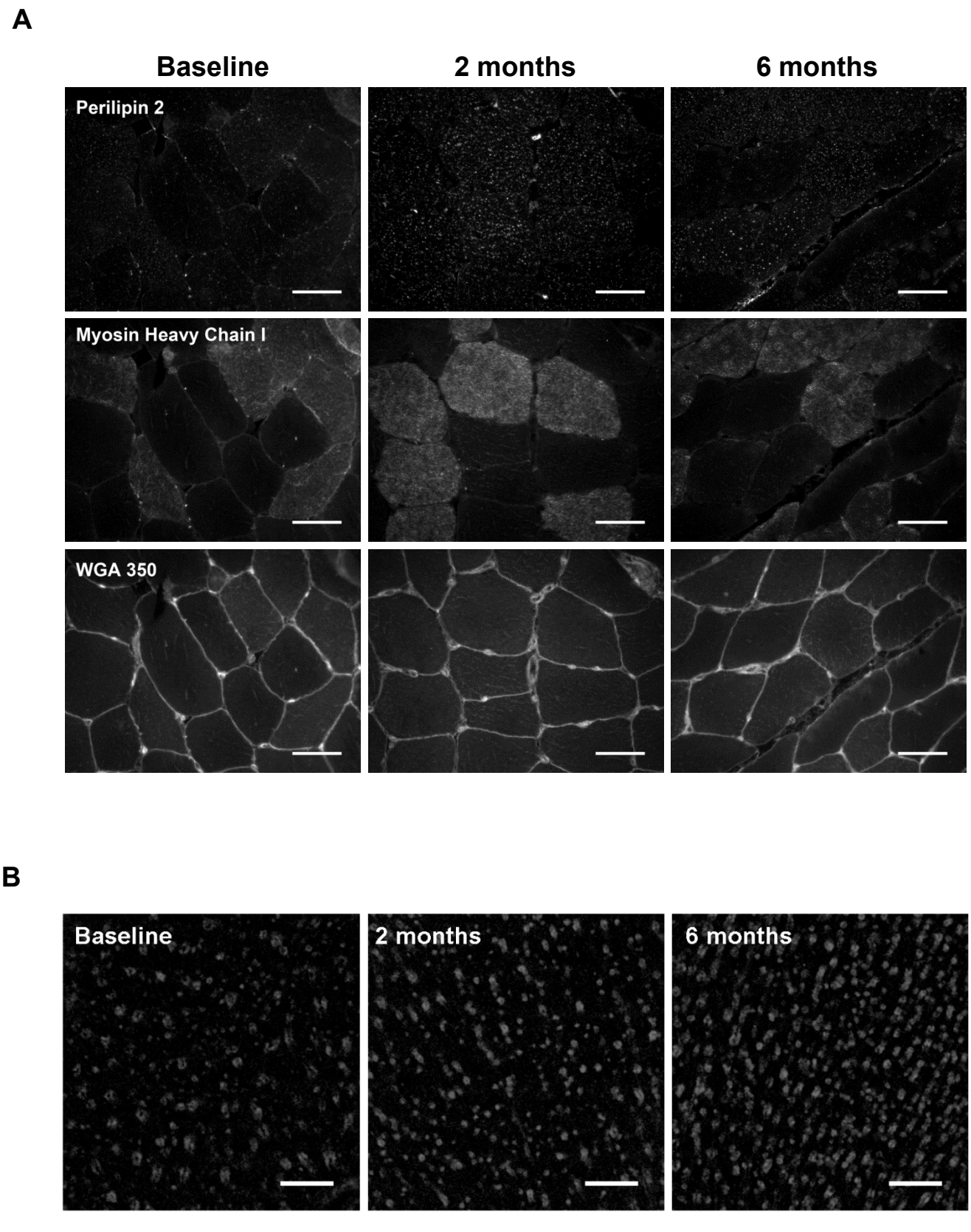

A
*
Perilipin 2 content
(%area stained)
5
4
3
2
1
0
#
#
†
†
Type I
Type II
Baseline
2 months
6 months
B
*
IMTG content
(%area stained)
6
4
2
0
#
†
†
†
Type I
Type II
Baseline
2 months
6 months

Figure 3


A
Baseline
2 months
6 months
Perilipin 2
Myosin Heavy Chain I
WGA 350
B
Baseline
2 months
6 months