# Muscle Fiber Phenotype: A Culprit of Abnormal Metabolism and Function in Skeletal Muscle of Humans with Obesity

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## ABSTRACT

The proportion of the different types of fibers in a given skeletal muscle contributes to its overall metabolic and functional characteristics. Greater proportion of Type I muscle fibers is associated with favorable oxidative metabolism and function of the muscle. Humans with obesity have lower proportion of Type I muscle fibers. We discuss how lower proportion of Type I fibers in skeletal muscle of humans with obesity may explain metabolic and functional abnormalities reported in these individuals. These include lower muscle glucose disposal rate, mitochondrial content, protein synthesis, and quality/contractile function, as well as increased risk for heart disease, lower levels of physical activity, and propensity for weight gain/resistance to weight loss. We delineate future research directions and the need to examine hybrid muscle fiber populations, which are indicative of a transitory state of fiber phenotype within skeletal muscle. We also describe methodologies for precisely characterizing muscle fibers and gene expression at the single muscle fiber level to enhance our understanding of the regulation of muscle fiber phenotype in obesity. By contextualizing research in the field of muscle fiber type in obesity, we lay a foundation for future advancements and pave the way for translation of this knowledge to address impaired metabolism and function in obesity.

**Key Words**: obesity, skeletal muscle, muscle fibers, myosin heavy chain, metabolism

## INTRODUCTION

Obesity is recognized as a disease associated with abnormal skeletal muscle and whole body-metabolism, including insulin resistance, Type 2 diabetes, heart disease, stroke and cancer (1-3). Addressing the obesity epidemic has been proven a great challenge (4). Skeletal muscle comprises a large fraction of total body mass and is a plausible regulator of obesity (5). Moreover, skeletal muscle is a main site for protein metabolism in the body and serves as the primary depot for protein “storage”. The myofibrillar protein pool, comprising the various isoforms of the protein myosin heavy chain (MHC), accounts for approximately half of the total protein in muscle.

The three most abundant isoforms of MHC proteins expressed in human skeletal muscle are MHC-I (gene: MYH7), MHC-IIa (gene: MYH2), and MHC-IIx (gene: MYH1). MCH isoforms provide a marker to characterize muscle fibers (6), and most experimental approaches employ the relative expression of MHC isoforms to identify muscle fiber types, namely slow-Type I (i.e., MHC-I), fast-Type IIa (i.e., MHC-IIa), and fast-Type IIx (i.e., MHC-IIx). The proportion of these types of fibers in skeletal muscle determines the metabolic responses and function of a given muscle. Physiologically, the muscle fibers are part of a continuum and they rank in the order Type I ↔ Type IIa ↔ Type IIx for oxidative metabolism and fatigue resistance, with Type IIx fibers having the lowest capacity for oxidative metabolism and highest fatigability. Specific structural, metabolic, and functional features of the different types of fibers in skeletal muscle have been previously comprehensively reviewed (7-9). It is recognized that some muscle fibers express more than one MHC isoform, in which case they are classified as hybrid fibers.

## EXPERIMENTAL APPROACHES EMPLOYED TO DETERMINE FIBER TYPES IN SKELETAL MUSCLE

Current knowledge on skeletal muscle fiber diversity stems from the contribution of several experimental methods that have been applied over the years to study muscle phenotype. These include measurements of enzyme activities, enzymatic ATPase staining, immunostaining, and protein sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (6-8, 10, 11). In the most basic form, muscle fibers are classified as Type I and Type II or “slow” and “fast”, respectively, with the latter terminology deriving from the contraction speed of their respective myosins. Some rather crude approaches to characterize muscle fiber types are based on the activity of metabolic enzymes measured in muscle homogenates and classified as oxidative (i.e., succinate dehydrogenase) and glycolytic (i.e., α-glycerophosphate dehydrogenase) (10). In general, their differential expression in muscle fibers was a readout for a high proportion of Type I/low proportion of Type II and low proportion of Type I/high proportion of Type II fibers, respectively. Experimental methods employing biochemical approaches or histochemistry expanded the spectrum of muscle fibers to include, besides slow/Type I, two different fast fibers, namely Type IIa, and Type IIx. Also, Type I, Type IIa, and Type IIx muscle fibers can be distinguished based on myosin ATPase-based histochemistry, which results in differential staining at different pH levels (8). ATPase-based histochemistry assays are limited in their ability to accurately describe muscle fiber types (12). Myosin within myofibrils is partially replaced with myosin from the cytosol with a half-life of hours, whereas the MHC protein itself has a turnover rate

of days (13), which may explain why fiber typing using ATPase-based assays may produce different results than methods that measure MHC content. A breakthrough in the field of muscle fiber type determination was the generation of large-scale use of monoclonal antibodies specific for different MHC isoforms, which allowed for the precise determination of the proportion of isoforms of MHC in different muscle samples (7, 14).

However, none of these methodologies can identify hybrid fibers in muscle samples. Strengths and limitations of the various experimental approaches used over the years to determine fiber types in skeletal muscle have been the subject of recent comprehensive reviews (15, 16). Despite their limitations, however, these experimental approaches have helped establish a link between altered proportion of types of muscle fibers and defective muscle and whole-body metabolism that characterizes obesity.

## SKELETAL MUSCLE FROM HUMANS WITH OBESITY HAS DECREASED PROPORTION OF TYPE I FIBERS

Generally, obesity is linked to considerable changes in skeletal muscle structure (17), metabolism (5), and function (18). Substantial amount of evidence shows that obesity is associated with reduced percentage of Type I fibers in muscle, which was first reported more than 30 years ago (19). Numerous investigations have since confirmed the inverse relationship between the proportion of Type I fibers and increased body mass (20-28). This association is observed in conjunction with a positive correlation between the proportion of Type IIX fibers and increased body mass (29), suggesting a shift of muscle fibers in skeletal muscle of humans with obesity from slow-Type I to fast-Type II fibers.

Because Type II fibers seem to be larger (30), a shift toward Type II muscle fiber phenotype can result in an increase in total muscle mass, as Type II fibers now collectively comprise a greater proportion of total muscle volume. In addition, it has been shown that obesity increases the size of muscle fibers independent of fiber type (24, 31), which is also likely to contribute to an increase in muscle volume regardless of the shift in muscle fiber types in obesity. These lines of evidence collectively may explain the apparent increase in muscle mass reported in humans with obesity (32, 33).

It has not been feasible to determine whether altered distribution of fibers in skeletal muscle of humans with obesity is a cause or a result of obesity. Using dietary interventions as experimental approaches to understand the development of obesity, it has been shown that long-term diet high in fat and sugar results in a “slow-to-fast” fiber type distribution in non-human primates, as documented by corresponding decrease MHC-I gene expression in skeletal muscle (34). In humans, a high fat diet for nine days decreases the synthesis rate of the MHC-I isoform to a larger extent than that of the MHC-II isoform (35). Moreover, hyperinsulinemia, a common manifestation in obesity, increases MHC IIx gene expression (36). These lines of evidence can be interpreted to suggest that lower percentage of Type I fibers seen in humans with obesity are a consequence of the metabolic environment of obesity. However, there is also evidence that muscle fiber type distribution has a genetic component, with muscle fiber type being influenced by genetic factors by 40-50% (37), and with Type I fibers being more heritable than Type II muscle fibers (38). Also, down-regulation of genes controlling the expression of Type I fibers in muscle in transgenic mice induces functional and metabolic effects seen in obesity (i.e., impaired glucose metabolism, low physical

activity) (39), suggesting a causal role of muscle fiber phenotype in determining impaired metabolism and function in obesity. Regardless of the cause of lower proportion of Type I fibers in the muscle of humans with obesity (i.e., genetics versus environment such as diet or lack of physical activity), a substantial amount of evidence discussed below associates impaired metabolism and function in skeletal muscle and whole body in obesity to lower proportion of Type I muscle fibers.

Not all studies report significantly lower proportion of Type I fibers in obesity (40-43). As previously discussed (44), these discrepant findings may be partially attributable to the experimental methods employed to classify muscle fibers, differences in the age and ethnicity of the participants studied, and the inclusion of participants at the lower end of body mass index range for obesity. For instance, it has been argued that the myosin ATPase histochemical approach is limited in its ability to determine muscle fiber phenotype accurately (12, 45). The fact that obesity spans a broad spectrum of morphological and physiological traits, including "healthy" obesity (46), may potentially contribute to these differences. A recent comprehensive review provides an excellent summary of current evidence showing that humans with obesity have lower proportion of Type I fibers concurrent with higher proportion of Type IIX fibers in skeletal muscle compared to lean humans (29).

## IMPAIRED METABOLISM AND FUNCTION IN HUMANS WITH OBESITY IS LINKED TO DECREASED PROPORTION OF TYPE I MUSCLE FIBERS

A comprehensive synthesis of extensive evidence reveals a compelling link between altered responses at the whole-muscle and whole-body metabolism and function, as

well as in body weight regulation, in individuals with obesity and low proportion of Type I muscle fibers in these individuals. This associative evidence is supported by altered molecular mechanisms at the whole-muscle level in humans with obesity resulting from lower proportion of Type I fibers in muscle of these individuals, and as illustrated in **Figure 1**, and discussed in greater detail below. Consequently, restoring a higher proportion of Type I fibers in muscle may be essential for addressing whole-muscle and whole-body pathophysiology that sustains impaired metabolism and function in humans with obesity.

**Reduced Glucose Disposal**

At the molecular level, whole-muscle glucose uptake is largely regulated by the overall availability of proteins involved in insulin signal transduction, and glucose transport and metabolism in muscle. Compared to Type II fibers, Type I muscle fibers have a greater capacity to metabolize glucose as evidenced by higher levels of insulin receptor and glucose transport protein 4 (GLUT4), as well as enzymes involved in glucose handling (23, 26, 40, 47). These lines of evidence imply that a lower percentage of Type I muscle fibers can play a crucial role in lowering the overall muscle's glucose-handling capacity in humans with obesity, and as illustrated in **Figure 1**. In support of this argument, insulin sensitivity in muscle is related to the proportion of Type I muscle fibers (20, 23, 48).

Females have a higher plasma glucose disposal to muscle than males (49, 50), and this may be due to a larger fraction of Type I muscle fibers generally reported in skeletal muscle of females (51-54). However, it is interesting that this effect cannot not

be simply attributed to biological sex, as females with a smaller fraction of Type I muscle fibers also have lower muscle glucose disposal (55), supporting rather a direct link between Type I fibers and muscle glucose metabolism. More direct evidence shows that pharmacological intervention (i.e., β3-adrenergic receptor agonist mirabegron) in individuals with obesity that enhances plasma glucose disposal to muscle occurs concurrently with an increase in the proportion of Type I fibers in muscle (56). Also, treatment of diet-induced obesity in mice with the dietary component sesamol increases the fraction of Type I muscle fibers while improving glucose absorption in muscle (57). Such lines of evidence strengthen the association between lower proportion of Type I muscle fibers and impaired muscle glucose disposal in obesity.

**Increased Risk for Heart Disease**

Research dating back more than 30 years has revealed that individuals with heart failure had lower percentage of Type I fibers in skeletal muscle (58). Subsequent reports have confirmed this link between skeletal muscle fiber phenotype and heart disease (59-62). The mechanism(s) linking low Type I muscle fibers to heart disease remain largely unknown. Biological effects resulting from an increased percentage of Type I muscle fibers on blood pressure (63) and plasma cholesterol concentration (64) may contribute to the decreased risk for heart disease. Also, low sympathetic tone may mediate the inverse relationship between proportion of Type I fibers in skeletal muscle and resting heart rate (60). Greater capacity of Type I muscle fibers to utilize fatty acids can promote uptake of circulating triglycerides and favorably modify the plasma cholesterol in high density lipoprotein particles (65). In summary, heart disease may be

linked to obesity-associated alterations in skeletal muscle fiber composition, notably a lower proportion of Type I fiber in the muscle of humans with obesity.

**Lipid Accumulation in Skeletal Muscle**

Accumulation of lipids in skeletal muscle is a hallmark of obesity, and it is documented as accumulation of both intermuscular adipose tissue (IMAT) and intramyocellular (IMCL) lipids (66-69). Interestingly, IMCL is elevated not only in obesity, but also in healthy, endurance exercise-trained individuals (70), and lipid accumulation is greater in Type I muscle fibers in such circumstance (71). However, proteins regulating IMCL metabolism, including oxidation of lipids are also more abundant in Type I fibers of these individuals (71). Therefore, lipid accumulation, per se, in a given muscle fiber is not the only determinant of metabolic responses of the muscle fiber. In fact, oxidative capacity is also important facilitator with respect to determining the relationship between abnormal metabolism (i.e., insulin resistance) and muscle lipid accumulation (70). Therefore, Type I fibers because of their greater oxidative capacity are better positioned to manage greater amounts of intracellular lipid when compared to Type II fibers.

IMAT accumulates in the skeletal muscle of overweight/obese individuals, as seen in the case of the polycystic ovary syndrome patients, in the presence of decreased proportion of Type I fibers (72). In response to a diet-induced obesity, lipids begin to accumulate in skeletal muscle first outside of the muscle fibers (73), possibly making IMAT accumulation a more prevalent characteristic of the skeletal muscle in obesity than IMCL accumulation. Evidence describing the accumulation of IMAT and

IMCL, as discussed above, indicates an inverse relationship between Type I muscle fibers and accumulation of lipids at the whole-muscle level, and where greater proportion of Type I muscle fibers prevents lipid accumulation in muscle (73). From mechanistic point of view, and because Type I muscle fibers have greater oxidative and lipid-handling capacity than Type II (particularly Type IIx) fibers, lower proportion of Type I fibers in muscle reduces the flexibility for overall lipid metabolism, resulting in lipid accumulation within and between muscle fibers in muscle (30, 74, 75), and as depicted in **Figure 1**.

**Low Content of Mitochondria in Skeletal Muscle**

It is known that at the whole-muscle level, muscles with a higher proportion of slow-Type I fibers have higher mitochondrial content than muscles with higher proportion of fast-Type IIx fibers (76). This is due to the greater abundance of mitochondria within the individual Type I muscle fibers (7). Thus, lower mitochondrial content reported at the whole-muscle level in humans with obesity (77-80) may result, in part, from lower proportion of Type I fibers in muscle of these individuals, and as depicted in **Figure 1**.

**Low Protein Synthesis in Skeletal Muscle**

Several studies have shown that the rate of protein synthesis at the whole-muscle level is lower in humans with obesity compared to lean controls (81-84). Moreover, protein degradation is lower in human primary myotubes propagated from muscle biopsy samples obtained from humans with obesity, and this is observed

231 together with lower proportion of MHC-I protein (28). Because Type I muscle fibers have
232 the highest rate of protein synthesis among the other types of muscle fibers (85), low
233 proportion of Type I fibers in the muscle of humans with obesity may explain the low
234 protein turnover in muscle of these individuals. Indeed, muscles characterized by low
235 proportion of Type I muscle fibers have lower rate of protein synthesis (86-88). At the
236 molecular level, Type I muscle fibers have two- to six-fold higher amounts of total RNA
237 (89), as well as greater amounts of ribosomal and proteasome-associated proteins (90,
238 91), all of which would support greater protein turnover rate in these muscle fibers, and
239 as depicted in **Figure 1**. Since Type I motor units are the first to be activated in skeletal
240 muscle (92), a higher rate of protein synthesis in type I muscle fibers may be due to
241 their greater rate of activation.
242
243 **Propensity for Weight Gain/Resistance to Weight Loss**
244 Individuals with higher percent of Type I fibers in muscle accumulate less body
245 fat in response to overfeeding (93), and are more prone to diet-induced obesity even in
246 the presence of reduced caloric and fat intakes (27). On the other hand, study
247 participants with obesity having higher proportion of Type I fibers in muscle lose more
248 weight in response to dietary intervention (24) or weight-reduction surgery (25).
249 It is important to note that the proportion of Type I fibers (94) and MHC-I content
250 (95) in muscle does not change after gastric bypass intervention in humans with
251 obesity, and similar findings have been reported following caloric restriction
252 interventions, as reviewed previously (44). Failure to modify the muscle fiber phenotype
253 in response to such interventions may explain the inability of humans with obesity to

sustain weight loss after weight loss interventions (96, 97) and the tendency for weight re-gain after gastric bypass (98). It has been argued that understanding and treating a metabolic programming related to epigenetic and/or genetic variables that defines the muscle fiber phenotype in individuals with obesity (99), is essential for resolving resistance to weight loss.

Muscle fiber type-specific energy expenditure may be the molecular link between muscle fiber phenotype and resistance to weight loss and/or propensity for weight gain in humans with obesity. Characteristically, increased content of Type I muscle fibers in transgenic mice (i.e., via activation of the peroxisome proliferator-activated receptor delta) prevents high-fat diet-induced obesity in parallel with higher oxygen consumption (100). This response may in turn be linked to the higher protein turnover rate of Type I muscle fibers and given that protein synthesis is the most energy-intensive process in resting muscle (101, 102).

**Low Muscle Quality and Contractile Function**

Evaluation of whole-muscle quality in humans is generally based on measuring the maximal force produced per unit of muscle mass (33, 103), and relevant evidence shows poor muscle quality in young adults with obesity (33). No study to date has examined contractility/function in obesity at the single muscle fiber level. However, studies using isolated whole-muscle preparations in rodents provide pertinent insights into the potential role of obesity in affecting muscle function. Muscle quality, evaluated as force per unit muscle area, is reduced in the extensor digitorum longus (i.e., low percentage of Type I fibers) but not in the soleus (i.e., high percentage of Type-I fibers)

muscle of mice with obesity (104). In zebrafish, diet-induced obesity reduces the proportion of Type I fibers in muscle concurrently with reduction in the contractile function of the whole-muscle (105). Although, these findings provide probable mechanistic support for the lower muscle quality reported in humans with obesity (33), there is clear need for human studies to determine how obesity affects the contractile function at the level of individual muscle fibers.

**Low Physical Activity**

Humans with obesity engage in less spontaneous physical activity than normal-weight controls (106-108). Also, the long-term compliance with structured physical activity is poor in individuals with obesity (109) and the rate of decline in their physical activity levels is significantly greater in these individuals (110). Because participation in everyday physical activity is sustained by the recruitment of Type I muscle fibers (111), and given that these muscle fibers are well-suited to sustain muscle contractions at a far lower energy cost than Type II muscle fibers (112, 113), a lower proportion of Type I muscle fibers may account for the lower physical activity in humans with obesity. In support of this argument, studies in humans show a direct correlation between the amount of time a muscle remains physically active and the percentage of Type I fibers present (114).

Experiments with transgenic mice show that increasing the expression of Type I fibers increases resistance to fatigue and enhances physical endurance (100), which may be linked to the biochemical composition of Type I muscle fibers, which utilize oxidative metabolism to maintain a continuous energy turnover (86), and thus sustain

300 locomotor performance/physical activity. Also, it has been suggested that Type I muscle
301 fibers can more efficiently sustain prolonged contractions compared to Type II muscle
302 fibers because of their longer optimal sarcomere resting lengths as a result of having
303 longer thin filaments (115). Collectively, this evidence indicates higher capacity to
304 sustain physical activity in the presence of higher proportion of Type I fibers in muscle.
305 Studies employing exercise training in humans with obesity have reported
306 inconsistent effects of exercise on muscle fiber phenotype, and this evidence has been
307 summarized previously (44). In a recent study, improved muscle mitochondrial
308 metabolism in humans with obesity in response to exercise training occurred
309 concomitantly with a trend (i.e., $P = 0.066$) for increased proportion of Type I fibers in
310 muscle (116). It is likely that it takes longer time to observe changes in the overall Type
311 I fiber proteome in muscle, and given that contractile proteins have an overall turnover
312 rate that is lower than that of mitochondrial proteins (117). Therefore, although exercise
313 improves substrate metabolism in muscle of humans with obesity (99, 116), studies of
314 longer duration are needed to determine the conditions under which exercise training
315 increases MHC-I content and the proportion of Type I fibers in muscle.
316
317 **Disassociation between Muscle Metabolism and Muscle Fiber Phenotype**
318 In contrast to the evidence discussed above, muscle metabolism and muscle
319 fiber phenotype may not always be linked, as seen after gastric bypass, which
320 enhances insulin sensitivity and decreases muscle lipid accumulation without altering
321 the muscle fiber type distribution (94). Following hyperthyroidism treatment, the mRNA
322 of MHC-I increases in human muscle but that of mitochondrial genes regulating muscle

metabolism does not (118). Animal gene mutation studies show that the expression of genes regulating energy metabolism (e.g., citrate synthase) become uncoupled from the expression of genes regulating MHC isoforms (119), while mice deprived of food for twenty-four hours exhibit elevated mRNA levels of mitochondrial genes in muscle along with elevated mRNA levels of MHC-II, but not MHC-I (120). Moreover, carnitine palmitoyltransferase knock out mice with a loss in mitochondrial long-chain fatty acid oxidation capacity exhibit a shift in their muscle bioenergetics profile toward Type II muscle fibers, but without corresponding changes in muscle MHC isoforms expression (121). Finally, acute exercise increases muscle insulin sensitivity in humans with obesity (122), whereas short-term experimental increase in plasma lipids decreases muscle insulin sensitivity (123), and both responses evidently occur without concurrent changes in muscle MHC isoforms. Collectively, these lines of evidence suggest that the fiber type distribution in skeletal muscle does not necessarily predict the muscle's metabolic characteristics.

In order to retain muscle energy homeostasis, compensatory processes under acute circumstances (e.g., exercise, fat infusion) or as a result of gene mutations or experimental manipulations that do not reflect normal physiology are likely to disassociate muscle metabolism from muscle MHC isoform composition. We note that our review is focused on the relationship between muscle fiber phenotype and muscle metabolism under "static" conditions, in which skeletal muscle metabolism is not disrupted by circumstances that modify the body's metabolic milieu. Also, under "dynamic" conditions resulting from repeated stimuli (i.e., exercise training), muscle fiber phenotype changes progressively (16), and where metabolic changes precede changes

346 in the contractile apparatus (7, 124). The latter is expected given that proteins that
347 regulate metabolism (i.e., mitochondria) have faster turnover rate than those that
348 regulate contractile function in skeletal muscle (117). Investigating how the muscle fiber
349 proteome may (or may not) change over time under “dynamic” conditions associated
350 with exercise, dietary, and bariatric surgery interventions in humans with obesity will
351 undoubtedly enhance our understanding of the strength of the link between metabolic
352 and contractile functions in the muscle of humans with obesity.
353

354 **FUTURE DIRECTIONS TO IMPROVE OUR UNDERSTANDING OF THE MUSCLE**
355 **FIBER PHENOTYPE IN HUMANS WITH OBESITY**

356 **Characterize Hybrid Fibers in Skeletal Muscle of Humans with Obesity**

357 Investigations on skeletal muscle of humans with obesity discussed in the
358 sections above have largely focused on “pure” muscle fibers characterized by a
359 predominance of one isoform of MHC. However, it is known that skeletal muscle
360 contains a variable portion of “hybrid” fibers (7), which express comparable amounts of
361 more than one MHC isoform, such as MHC-I/IIa or MHC IIa/IIx. The existence of hybrid
362 muscle fibers has been recognized for more than three decades, and their prevalence in
363 muscle has been linked to muscle plasticity in the context of both exercise and disuse.
364 Endurance exercise-trained individuals have a higher percentage of MHC-I/IIa-
365 containing fibers, whereas sedentary individuals have a higher percentage of MHC-
366 IIa/IIx-containing fibers (125). It has been reported that the proportion of hybrid fibers in
367 the muscle of a sedentary person can be 10 times greater than that in the muscle of an
368 exercise-trained person (126). The challenge in establishing a definitive link between

specific metabolic characteristics of skeletal muscle and muscle fiber phenotype when sedentary individuals are studied (54) may be attributable, in part, to the limitation of identifying the muscle fibers as only Type I, Type IIa and Type IIx. In this respect, identifying fibers across the spectrum of muscle fiber types, which includes hybrid fibers, may enable the discovery of a stronger link between a given muscle fiber phenotype and the metabolic characteristics of the whole-muscle.

Hybrid fibers appear to possess features that are intermediate to those of single MHC isoform-dominant fibers (127), and likely contribute to “in-between” control of overall muscle physiology. Hybrid muscle fibers are thought to facilitate the transition of muscle fibers within the muscle in response to certain stimuli, such as exercise training (16). However, a higher fraction of hybrid fibers in skeletal muscle may also reflect a phenotypically permanent state in relation to the fiber types in skeletal muscle (128). Our current understanding of the fiber phenotype in muscle of humans with obesity is limited, due to the lack of quantitative evidence describing the expression of hybrid fibers in muscle of these individuals. This is likely due in part to current observations in populations where much research on characterizing muscle fiber phenotype has been conducted (i.e., exercise-trained individuals) and where hybrid fibers do not contribute significantly to the total fiber phenotype in muscle (129).

Experimental paradigms that lessen full weight-bearing loads on muscle, such as spaceflight (130) or bed rest (131) show that decrease in the percentage of Type I fibers occurs concomitantly with increase in the percentage of hybrid fibers in muscle, indicating that hybrid fibers in muscle are prevalent under certain circumstances. In older adults, up to ~50% of muscle fibers contain more than one MHC isoform (132,

133), a proportion that is considerably greater than the 25% reported in younger individuals (134). These lines of evidence suggest that "unhealthy" muscle physiology is associated with an increase in the proportion of hybrid fibers in skeletal muscle. However, the effects of aging on the fraction of hybrid fibers in skeletal muscle may not be applicable to obesity. This is because the skeletal muscle in obesity exhibits a slow-to-fast muscle fiber shift (as discussed above), whereas skeletal muscle in aging exhibits a fast-to-slow muscle fiber shift (135, 136). Nonetheless, evidence showing an increased proportion of hybrid fibers in the skeletal muscles of genetically obese (i.e., ob/ob) mice (137) or mice with high-fat diet-induced obesity (138), raises the question of whether and how hybrid fibers differ in skeletal muscle of humans with obesity.

If the increased proportion of hybrid fibers observed in the skeletal muscle of rodents with obesity (137, 138) is indicative of transitions in muscle fiber types, then these fiber transitions in muscle in obesity appear to involve loss of Type I fibers and gain of Type IIx fibers, given that the distribution of Type IIx muscle fiber is increased in obesity (29). This is physiologically important because Type IIx fibers are associated with least favorable metabolic responses, such as lower glucose handling capacity (40, 139). Possible variations in the distribution of hybrid fibers in muscle of humans with obesity may affect metabolism and function in skeletal muscle in these individuals, with higher distribution of Type I/IIa fibers being associated with more favorable muscle metabolism and function than higher distribution of Type IIa/IIx fibers. However, in order to gain a mechanistic understanding of these effects, it is necessary to precisely quantify the extent of hybrid fibers and the biological mechanisms responsible for their potential prevalence in the muscle of humans with obesity. Such research is crucial for

gaining a thorough understanding of how skeletal muscle phenotype in humans with obesity impacts the metabolism and function in these individuals.

## New Methodologies to Determine Fiber Phenotype and Metabolism of Individual Fibers in Skeletal Muscle

Experimental methodologies employed to date to understand fiber type distribution in skeletal muscle have improved our understanding for a “slow-to-fast” shift in the proportion of fibers in skeletal muscle of humans with obesity, and as discussed in the sections above. For a thorough understanding of the role of skeletal muscle in health and disease, however, it is crucial to study single muscle fibers (15, 16). By analyzing the MHC isoform composition of single muscle fibers, isolated muscle fibers can be categorized as either pure or hybrid. Regarding the resolution of hybrid muscle fibers based on their MHC content, SDS-PAGE is regarded as the "gold standard" technique (15). Using this approach, the relative contribution of an MHC isoform within a given muscle fiber can also be assessed, revealing important information regarding the transitional state of the hybrid fibers in the muscle of humans with obesity. Mass spectrometry-based proteomics research shows that the majority of muscle fibers express at least two MHC isoforms, albeit with the minority MHC isoforms in very small amounts (140). Experimental grouping of fibers that have a dominating percentage of a single MHC isoform (i.e., Type I if MHC-I > 80%) has allowed contrasting the proteomes of different types of muscle fibers (140). Analyses of different types of muscle fibers characterized in this manner have shown that aging results in differential modifications of metabolic pathways in specific types of muscle fibers (90), and it is likely that

corresponding alterations exist in certain types of muscle fibers in humans with obesity. Moreover, the analysis of individual muscle fibers allows the characterization of the metabolic machinery of the different, including hybrid, types of muscle fibers in response to exercise or bariatric surgery in individuals with obesity and the determination of whether observed metabolic changes in given muscle fibers occur in the absence of changes in their MHC composition.

Although isolating individual fibers to understand fiber types in muscle has undeniable advantages over other methods described above, it is crucial to be aware of potential limitations. It is important to know the extent to which individual muscle fibers preserve their biochemical and functional integrity during processing and until pertinent measurements are collected. It is also essential to ensure that data obtained from a given number of muscle fibers analyzed produce information that is reproducible. Because isolating individual muscle fiber is laborious and time-consuming, it has been suggested that characterization of muscle fibers may be accomplished with as few as 25 muscle fibers (141). This may be sufficient when examining muscle from individuals with relatively homogenous pools of few types of muscle fibers. However, isolating and describing a small number of muscle fibers may not be sufficient to accurately characterize a muscle composed of various pools of hybrid muscle fiber types, as may be the case in obesity.

Another concern when analyzing single muscle fibers is that the fiber's molecular characteristics may vary along the length of an individual muscle fiber, so that information obtained in one section of the fiber (i.e., the section from which the biopsy is taken) may not reflect characteristics of other sections of the fiber or the entire fiber.

Independent lines of evidence demonstrating changes over the length of single muscle fibers at the levels of the proteome, as well as the MHC mRNA and protein isoforms, support this notion (128, 140, 142).

Despite any limitations, studying individual muscle fibers with state-of-the art approaches like -omics technologies can undoubtedly offer significant amounts of information to dissect and understand the metabolism and function of the skeletal muscle in humans with obesity. Given the possibility of a high proportion of hybrid muscle fibers in obesity, examining a large number of individual muscle fibers should ensure the generation of data that are reproducible in terms of muscle fiber type, metabolism, and function.

## Conclusions

Higher proportion of Type I fibers in skeletal muscle is associated with favorable health outcomes because of inherent favorable metabolic and functional features characterizing the Type I muscle fibers. Although a clear cause-and-effect relationship remains to be established, lower proportion of Type I fibers in muscle of humans with obesity may contribute to the metabolic and functional abnormalities seen at the whole-muscle and whole-body levels of humans with obesity. This review proposes a comprehensive and mechanistic characterization of the type and function of single fibers in muscle of humans with obesity, with an emphasis on the population of muscle fibers that represent hybrid fibers. We suggest future directions to enhance our understanding of the differential expression of the different types of muscle fibers in

obesity. This information will be crucial for the design of effective strategies to combat obesity and its detrimental metabolic and functional outcomes.

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## AUTHOR CONTRIBUTIONS

NS and CSK conceived and designed research; NS drafted manuscript and prepared figures; JPKH, JAH, MM and CSK edited and revised manuscript; NS, JPKH, JAH, MM and CSK approved final version of manuscript.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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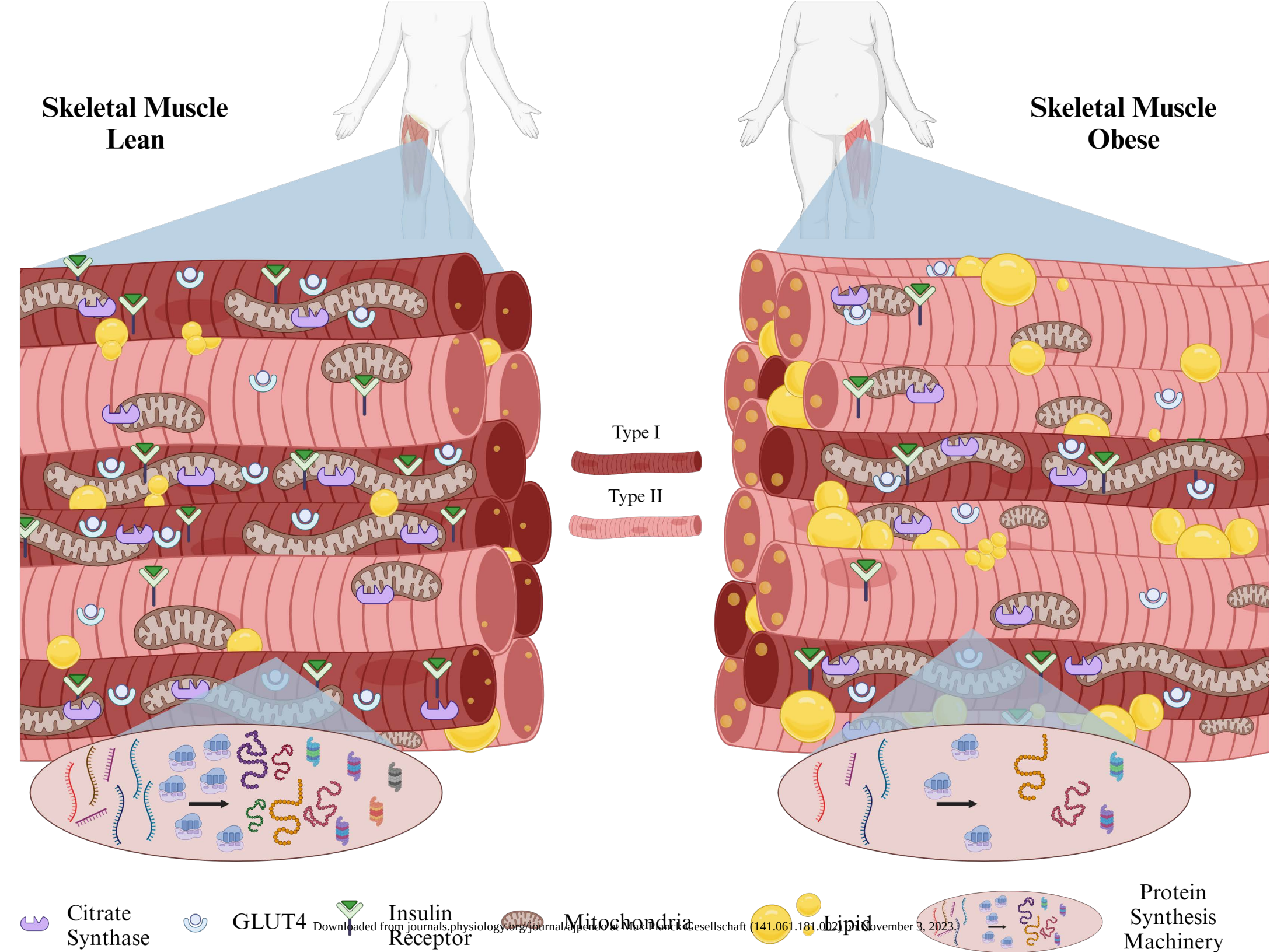
## FIGURE LEGENDS

**Figure 1** Illustration of molecular differences at the whole-muscle level in lean humans and human with obesity, which can be explained by lower proportion of Type I vs Type II muscle fibers, and as a result of the inherent molecular differences between Type I and Type II muscle fibers. For simplification purposes, only selected key molecular differences are depicted, while further molecular differences are discussed in text. (Created with BioRender.com).



# Low Proportion of Type I Fibers in Skeletal Muscle May Explain Metabolic and Functional Abnormalities in Humans with Obesity