Skeletal Muscle Morphology and Exercise Response in Congenital Generalized Lipodystrophy

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OBJECTIVE — Congenital generalized lipodystrophy (CGL) is an autosomal recessive genetic disorder characterized by almost complete absence of adipose tissue, muscular appearance, and severe insulin resistance since birth. We investigated whether insulin resistance in CGL patients is associated with abnormal muscle morphology and whether increased muscularity imparts increased muscle strength and exercise capacity.

RESEARCH DESIGN AND METHODS — We obtained quadriceps muscle biopsies to study muscle fiber types and capillary density in three African-American women (aged 17–20 years) with CGL. We also assessed quadriceps muscle strength, muscle metabolism, and maximal O2 consumption in the patients.

RESULTS — Quadriceps muscle biopsies revealed a markedly higher percentage of type II (fast-twitch glycolytic) muscle fibers in patients with CGL versus sedentary young women (75–78 vs. 47–57%, respectively). The capillary-to-fiber ratio (2.7–3.0), however, was normal. Cross-sectional areas of type I (slow-twitch oxidative) (1,262–2,685 µm²) and type II (2,304–3,594 µm²) fibers were far below the normal values (3,811–4,310 and 3,115–4,193 µm², respectively), suggesting muscle hyperplasia but not hypertrophy. The quadriceps muscle strength, as measured by Cybex, was below average; the maximal O2 consumption (23–32 ml·kg⁻¹·min⁻¹) was also below average. 31P nuclear magnetic resonance spectroscopy of the forearm muscles revealed normal pH and metabolic responses to static and dynamic exercises.

CONCLUSIONS — We conclude that insulin resistance in patients with CGL is associated with an increased proportion of type II muscle fibers but not reduced capillary density. Increased muscularity in CGL is due to muscle hyperplasia and is not associated with increased muscle strength.

RESEARCH DESIGN AND METHODS

Subjects
Three young women of African-American origin (aged 17–20 years) with CGL were studied. Two of the patients belonged to the CG 800 pedigree, and one was of the CG 900 pedigree. The clinical features included extreme paucity of body fat, muscular appearance, insulin-resistant diabetes, acanthosis nigricans, acromegoid features, and hirsutism. The detailed clinical characteristics of the patients have been published previously (9–11). Briefly, all of them had severe hypertriglyceridemia, eruptive xanthomas, and hepatosplenomegaly. Diabetes was diagnosed during early adolescence in each of them. Insulin requirements ranged from 180 to 500 U/day. One of the subjects had irregular menstrual periods. Focal lytic lesions in the appendicular skeleton were noted in all of the patients (10). One patient died at the age of 24 years (11). All three patients were sedentary and did not engage in any strenuous physical activity. The protocol for this study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center in Dallas, and each patient gave written informed consent. The following studies were conducted in each of the patients.

Skeletal muscle morphometric analyses
Skeletal muscle biopsy was obtained from the quadriceps femoris muscle through a small (3-mm) skin incision after anesthetizing the overlying skin and fascia with 1% lidocaine. The tissue was quickly frozen in Freon-22 cooled to liquid nitrogen tem-
Muscle morphology in lipodystrophy

Table 1—Skeletal muscle fiber types and capillary morphometrics in patients with CGL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type I</th>
<th>Type II</th>
<th>Capillaries per square millimeter</th>
<th>Capillary-to-fiber ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Diameter (µm²)</td>
<td>% Diameter (µm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG 800.7</td>
<td>23</td>
<td>2685</td>
<td>77</td>
<td>3594</td>
</tr>
<tr>
<td>CG 800.8</td>
<td>22</td>
<td>2074</td>
<td>78</td>
<td>2522</td>
</tr>
<tr>
<td>CG 900.8</td>
<td>12</td>
<td>1262</td>
<td>75</td>
<td>2304</td>
</tr>
<tr>
<td>Normal values</td>
<td>43–52*</td>
<td>3,811–4,310*</td>
<td>47–57*</td>
<td>3,115–4,193*</td>
</tr>
</tbody>
</table>

Data for normal values are ranges or means ± SD. *Values from references 16–20; †values of the cross-country skiers are from reference 14.

RESULTS — Skeletal muscle biopsies obtained from rectus femoris (quadriceps) muscles revealed normal architecture. All three patients with CGL had a markedly higher percentage of type II skeletal muscle fibers than that reported in sedentary young women (75–78% of the total muscle fibers vs. 49–57%, respectively) (Table 1 and Fig. 1). The proportion of type I skeletal muscle fibers, therefore, was much reduced (22–25 vs. 43–52%, respectively) (Table 1 and Fig. 1). The mean cross-sectional areas of both type I (1,262–2,685 µm²) and type II (2,304–3,594 µm²) skeletal muscle fibers were also much below the mean values reported for the sedentary young women (3,811–4,310 and 3,115–4,193 µm², respectively) (Table 1). The skeletal muscle capillary-to-fiber ratio in patients with CGL ranged from 2.7 to 2.95 compared with the value of 3.0 in the elite cross-country skiers from our laboratory (14) (Table 1). Capillary density in patients with CGL was 280–461 capillaries/mm² compared with the mean value of 411 capillaries/mm² in the male and female skiers from our laboratory (14) (Table 1). Using hematoxylin-eosin staining in 24 men and 14 women, Toft et al. (21) reported a capillary-to-fiber ratio of 1.5 ± 0.6 and a capillary density of 211 ± 79 per mm². In a study by Nyholm et al. (22), a capillary-to-fiber ratio of 1.9 ± 0.1 and a capillary density of 395 ± 18 per mm² were found in 13 men and 8 women by use of amylase-periodic acid-Schiff staining.

Patients with CGL had reduced quadriceps muscle strength. Using Cybex 340, peak torque assessment of the right and left quadriceps was 99 ± 41 and 96 ± 23 N·m (normal value 200 N·m); total work was 1,120 ± 281 and 977 ± 377 J, respectively (normal value 2,000 J), and the endurance ratio was 133 ± 36 and 110 ± 3, respectively (normal value 75). VO₂max of patients with CGL was 23–32 ml·kg⁻¹·min⁻¹, which
is also below the mean values of 34–41 ml · kg\(^{-1}\) · min\(^{-1}\) reported in the healthy young sedentary women (17,19,20). \(^{31}\)P NMR spectroscopy of the forearm muscles, however, revealed a normal pH and metabolic response to both static and dynamic exercise (Fig. 3). The chemical shift positions and the ratio of the areas of the phosphocreatine (PCr) to inorganic phosphorus (Pi) to \(-\)ATP peaks in spectra from resting muscle were not different from those in healthy subjects. The change in the chemical shift distance between PCr and Pi and the ratio of these peaks were also not different from those in healthy subjects.

**CONCLUSIONS** — Muscular appearance is noticeable in patients with CGL since birth. Although this appearance may be accentuated by lack of subcutaneous adipose tissue, there seems to be an increase in muscle mass. The mechanisms for the increased muscle mass and severe insulin resistance, however, are not clear. Because levels of plasma growth hormone, IGF-I, and testosterone are not elevated (23; A.G., unpublished data), it is possible that extreme hyperinsulinemia due to severe insulin resistance may be a contributory factor to muscular growth through a “specificity spill-over” phenomenon via IGF-I receptors. In other states of insulin resistance, such as obesity and type 2 diabetes, an increased proportion of type II (fast-twitch glycolytic) muscle fibers and reduced proportion of type I (slow-twitch oxidative) muscle fibers has been reported (2–7). Therefore, we investigated whether insulin resistance in CGL patients is associated with such abnormal skeletal muscle morphology.

The most striking finding in our study was the homogeneity of skeletal muscle morphology in all three patients with CGL. Specific findings on quadriceps femoris biopsy included an increase in type II muscle fibers (75–77%) and a relative reduction in type I muscle fibers (22–25%). For comparison, mean values of 43–52% type I muscle fibers have been reported in vastus lateralis muscle of sedentary young women (16–20). Even after considering a wide range in muscle fiber composition in healthy women, our patients with CGL had a strikingly low proportion of type I muscle fibers. Some investigators believe that subjects of African origin tend to have a higher proportion of type II skeletal muscle fibers than subjects of Caucasian origin. Because all three of our subjects were African-American, it can be argued that they were racially predisposed to have a high proportion of type II skeletal muscle fibers. However, this notion is based on one study, which showed an increased proportion of type II fibers in black West Africans compared with white Canadians (24), whereas two other studies have shown no such racial differences in skeletal muscle morphology (25,26). Therefore, it is unlikely that these striking changes in skeletal muscle morphology in our patients with CGL are merely due to racial predisposition.

Another argument can still be made that our patients with CGL were studied...
after the development of diabetes and that having hyperglycemia for several years could have affected skeletal muscle morphology. However, whether skeletal muscle fiber composition changes in subjects who progress from impaired glucose tolerance to type 2 diabetes or whether such changes are related to diabetes duration remains unclear. Nonetheless, documentation of similar findings in younger patients with CGL who have not yet developed overt diabetes would be of interest.

The mechanisms by which an increased proportion of type II skeletal muscle fibers occurs in both patients with CGL and other patients with extreme insulin resistance and patients who have insulin resistance resulting from obesity or type 2 diabetes (2–7) remain unclear. In vitro studies in rat revealed greater insulin binding, higher insulin-mediated glucose uptake, and increased GLUT4 levels in muscles with predominantly type I fibers versus type II fibers (27–29). Therefore, it is likely that a higher proportion of type II fibers may also be associated with reduced insulin responsiveness in humans.

In the study by Lillioja et al. (5), insulin sensitivity more strongly correlated to capillary density than to muscle fiber type. Thus, CGL patients with extreme insulin resistance should be expected to have markedly low capillary density; however, this case was not observed. Both the skeletal muscle capillary-to-fiber ratio and capillary density per square millimeter were lower in the normal to high range in all three patients. Therefore, reduced capillary density does not seem to be a consistent feature in patients with CGL.

The precise mechanisms of insulin resistance in patients with CGL are not known. In a recent study, we measured intramyocellular lipid concentrations by 1H-magnetic resonance spectroscopy in four patients with CGL and found those concentrations to be twice as high as those in control subjects (19.8 ± 4.6 vs. 10.7 ± 1.4 µmol/g, respectively) (30). Increased intramyocellular lipid concentrations have been associated with insulin resistance in nondiabetic subjects and in offspring of patients with type 2 diabetes (31–34). Therefore, high intramyocellular lipid concentrations and increased proportion of type II skeletal muscle fibers may contribute to insulin resistance in CGL patients. Interestingly, some investigators have observed a post-insulin receptor tyrosine kinase defect in fibroblasts from CGL patients (35,36); however, insulin receptor, IGF-I receptor, and insulin receptor substrate 1 genes have been excluded as candidate genes for CGL (37,38). Recently, we performed linkage analysis studies in 17 pedigrees and localized one of the genes responsible for the disorder (CGL1) to human chromosome 9q34 (8). The CGL gene(s) remains to be identified.

Another important observation from our study related to the reduced and not increased cross-sectional skeletal muscle fiber size of both types I and II skeletal muscle fibers in all three patients. It is generally believed that the muscular phenotype in patients with CGL is primarily due to skeletal muscle hypotrophy (1); however, our data do not support this contention. In fact, the data support the alternative hypothesis that the increased skeletal muscle mass in patients with CGL is mainly due to an increase in fiber number or hyperplasia. This pattern is in contrast to that observed in athletes who undergo strength training in which an increase in skeletal muscle mass is mainly due to skeletal muscle fiber hypertrophy (39).

Previous studies have consistently revealed normal histology and ultrastructure of skeletal muscle fibers in patients with CGL (40–45). However, studies of fiber types, capillary density, and fiber size were not conducted. Afifi et al. (45) reported subsarcolemmal and interfibrillary aggregates of normal mitochondria on electron microscopy of gastrocnemius muscle from three patients with CGL. The significance of our findings and those of Afifi et al. (45) to hypermetabolism in patients with CGL is not clear.

However, the quadriceps muscle strength in our patients, as measured by Cybex and VO2max, were below average. The reduction in muscle strength apparently was not due to abnormal skeletal muscle metabolism during exercise, as documented by normal responses to both static and dynamic exercise during 31P NMR spectroscopy. In both types of exercise, the pattern and degree of reduction in \([PCr]/[PCr] + [P_i]\) was not different from that which occurred in normal healthy subjects (46). For these patients, for any given exercise rate, the shift in the balance of high-energy phosphates (e.g., from ATP to ADP) was not different from that of normal healthy subjects. This means that CGL had no detectable effect on the bioenergetic cost of skeletal muscle exercise. Therefore, although there was no detectable bioenergetic effect, patients with CGL appear to have a discrepancy between skeletal muscle hyperplasia and strength.

In conclusion, insulin resistance in patients with CGL is associated with an increased proportion of type II muscle fibers but not with reduced capillary density. Muscular phenotype in CGL appears to be due to skeletal muscle hyperplasia and not hypertrophy. Despite skeletal muscle hyperplasia, muscle strength in patients with CGL may not be increased.
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References


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