

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 O_2 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 μm^2) and type II (2,304–3,594 μm^2) fibers were far below the normal values (3,811–4,310 and 3,115–4,193 μm^2 , respectively), suggesting muscle hyperplasia but not hypertrophy. The quadriceps muscle strength, as measured by Cybex, was below average; the maximal O_2 consumption (23–32 $ml \cdot kg^{-1} \cdot min^{-1}$) was also below average. ^{31}P 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.

Diabetes Care 23:1545–1550, 2000

Congenital generalized lipodystrophy (CGL) (Berardinelli-Seip syndrome, Mendelian Inheritance in Man no. 269700) is a rare autosomal recessive genetic disorder characterized by almost complete absence of adipose tissue, muscular appearance, and severe insulin resistance since birth (1). The pathologic mechanisms for the muscular phenotype

and whether it imparts increased muscle strength and exercise capacity to patients with CGL are not known. Recent studies have reported abnormal skeletal muscle morphology, i.e., reduced skeletal muscle capillary density and an increased proportion of type II (fast-twitch glycolytic) muscle fibers in other states of insulin resistance, such as obesity, impaired glucose tolerance,

or type 2 diabetes (2–7). Therefore, the primary aim of the study was to investigate whether severe insulin resistance in patients with CGL is associated with similar abnormalities in skeletal muscle morphology, as reported in other states of insulin resistance. Another aim was to study any relationships of such morphological abnormalities with muscle strength, muscle metabolism, and exercise capacity.

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 (8). The clinical features included extreme paucity of body fat, muscular appearance, insulin-resistant diabetes, acanthosis nigricans, acromegalooid 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 at 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-

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Abbreviations: CGL, congenital generalized lipodystrophy; NMR, nuclear magnetic resonance; PCr, phosphocreatine; P_i , inorganic phosphate.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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

Patient	Muscle fiber types				Capillaries per square millimeter	Capillary-to-fiber ratio
	Type I		Type II			
	%	Diameter (μm^2)	%	Diameter (μm^2)		
CG 800.7	23	2685	77	3594	406	2.8
CG 800.8	22	2074	78	2522	461	2.7
CG 900.8	23	1262	75	2304	280	2.95
Normal values	43–52*	3,811–4,310*	47–57*	3,115–4,193*	411 \pm 27†	2.9 \pm 0.2†

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

perature (-180°C). No cryoprotectant was used. All samples were stored at -80°C in airtight containers for later analyses.

Adjacent cross sections ($5\ \mu\text{m}$ thick) were cut on a cryostat (Reichert 2800; Reichert-Jung, Neu Bloch, Germany) at -20°C . Sections were mounted on coverslips and dried overnight at 4°C before staining. Types I and II muscle fibers were classified from the staining pattern produced by a room temperature alkaline preincubation at pH 10.35 for myofibrillar actomyosin ATPase (12,13). A ulex lectin stain with a fast green counterstain was used to identify capillaries (14).

Quantitative analyses of muscle fiber area, percent muscle fiber type, capillary density, and capillary-to-fiber ratio were performed directly from stained tissue sections using a Magiscan IIA image analyzer (Joyce-Loebl Nikon; Nikon, Garden City, NY). At least 50 fibers of each fiber type were counted in each biopsy. Both cross-sectional and longitudinal profiles of capillaries were counted.

Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy was performed on a 1.89-T horizontal bore magnet (30 cm diameter) (Oxford Instruments, Oxford, U.K.) interfaced to an NT-80 console (Broker Instruments, Billerica, MA) as previously described (15). A single-turn radio frequency surface coil with a 2-cm diameter that was singly tuned to the ^{31}P resonance frequency of 32.5 MHz was used to collect NMR data.

At the start of the study, the maximal voluntary contraction handgrip force was determined for the dominant upper extremity (right hand in all three patients) for each subject. A rhythmic isometric handgrip exercise was performed at 100% of the maximal voluntary contraction. The

exercise periods consisted of 10 s of static handgrip alternating with 10 s of relaxation (i.e., 3 contractions/min) for 10 min. NMR spectra were obtained every 2 min during the exercise period and for 6 min after exercise during the recovery period.

The static handgrip exercise was conducted at 30% of the maximal voluntary contraction for a period of 3 min. NMR spectra were obtained every minute during exercise and for 6 min during the recovery period after the exercise.

Cybox

Isokinetic torque was measured in both lower extremities with the Cybex 340 extremity-testing system (Medway, MA). The muscles tested included the quadriceps group for knee extension. The patients were tested with a standard protocol of four warm-up (trial) repetitions followed by three maximal repetitions at 60°/s with 60 s rest before the next set of four trial repetitions and three maximal repetitions at 180°/s. This procedure was followed by 4 trial repetitions and 25 maximal repetitions at 300°/s. Peak torque was normalized to body weight for each speed, and total work at 300°/s was calculated for extension of both knees.

Maximal O_2 consumption

Maximal oxygen consumption ($\text{VO}_{2\text{max}}$) was determined by an incremental ($12.5\ \text{W}/2\text{-min}$ stage) cycle ergometry to volitional exhaustion, with an open circuit system for measuring expired gas concentrations and ventilation. Oxygen concentration of expired gas was measured by an Ametek 2A Oxygen Analyzer (Ametek, Pittsburgh, PA). The carbon dioxide concentration was measured with an Ametek CO_2 Analyzer. Ventilation was measured with the Rayfield version of a Parkinson-Cowen Gasometer (Rayfield Equipments, Waitsfield, VT).

Oxygen uptake, carbon dioxide production, respiratory quotient, and ventilation were determined for the highest power output sustained. The peak power output and oxygen uptake sustained for 60 s were designated as peak power and peak O_2 consumption. Heart rate and ventilation associated with peak O_2 consumption are reported as peak values. Heart rate was measured by telemetry (Polar electrode). The data were analyzed on line with an Apple IIa computer using Rayfield software.

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 [16–20]) (Table 1 and Fig. 1). The proportion of type I skeletal muscle fibers, therefore, was much reduced (22–25 vs. 43–52%, respectively [16–20]) (Table 1 and Fig. 1). The mean cross-sectional areas of both type I ($1,262\text{--}2,685\ \mu\text{m}^2$) and type II ($2,304\text{--}3,594\ \mu\text{m}^2$) skeletal muscle fibers were also much below the mean values reported for the sedentary young women ($3,811\text{--}4,310$ and $3,115\text{--}4,193\ \mu\text{m}^2$, respectively [15–19]) (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 the laboratory (14) (Table 1 and Fig. 2). Capillary density in patients with CGL was 280–461 capillaries/ mm^2 compared with the mean value of 411 capillaries/ mm^2 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 mm^2 . 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 mm^2 were found in 13 men and 8 women by use of amylose-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 $96 \pm 23\ \text{N}\cdot\text{m}$ (normal value $200\ \text{N}\cdot\text{m}$); total work was $1,120 \pm 281$ and $977 \pm 377\ \text{J}$, respectively (normal value $2,000\ \text{J}$), and the endurance ratio was 133 ± 36 and 110 ± 3 , respectively (normal value 75). $\text{VO}_{2\text{max}}$ of patients with CGL was $23\text{--}32\ \text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, which

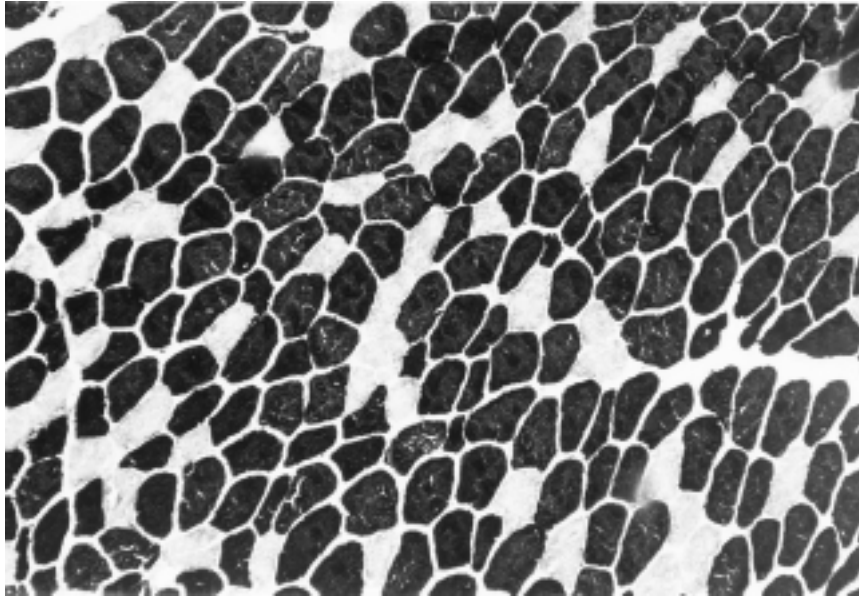


Figure 1—Section of quadriceps femoris muscle from a patient with congenital generalized lipodystrophy after alkaline preincubation for a myofibrillar actomyosin ATPase stain. The section shows both an increase in type II fibers and small fiber size. Lighter fibers are type I and darker fibers are type II skeletal muscle fibers.

is also below the mean values of $34\text{--}41\text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ 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 β -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 the areas 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

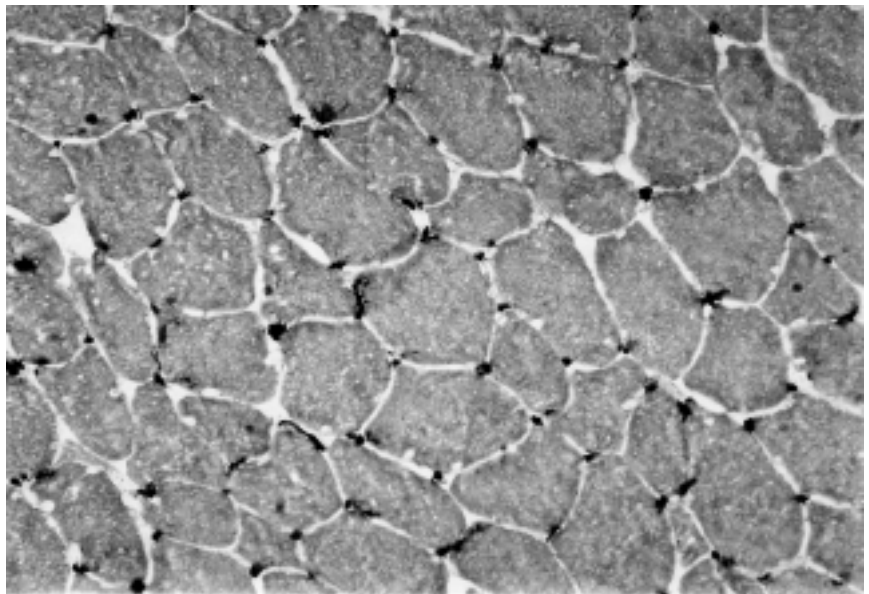


Figure 2—Section of quadriceps femoris muscle from a patient with congenital generalized lipodystrophy incubated with ulex lectin and counterstained with fast green for capillary measurements. The section shows normal capillary density.

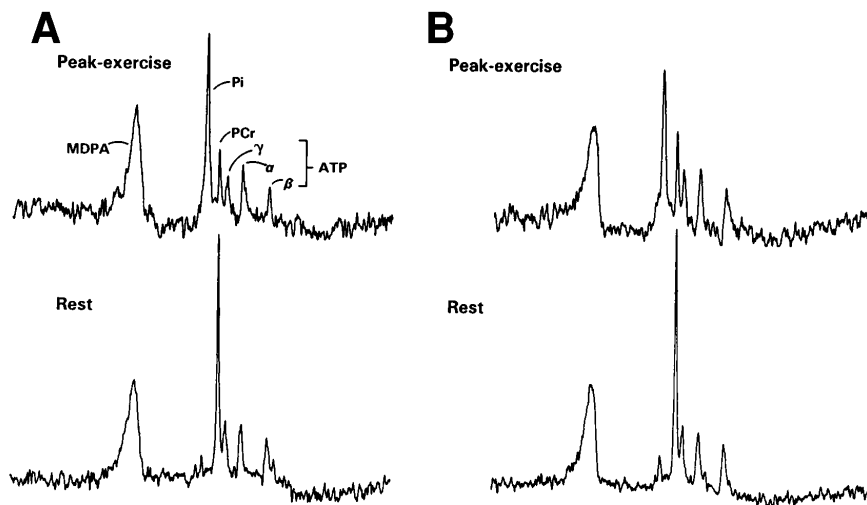


Figure 3—Typical ^{31}P NMR spectra collected during rest and at peak of rhythmic handgrip exercise at 100% maximal voluntary contraction from a healthy subject (A) and a patient with CGL (B). Spectral peaks (from left to right) correspond to methylenediphosphonate (MDPA) (an external standard), Pi, PCr, and the γ , α , and β peaks of ATP.

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 who have 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

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 $\mu\text{mol/g}$, 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 hypertrophy (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 $\text{VO}_{2\text{max}}$, 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 ^{31}P NMR spectroscopy. In both types of exercise, the pattern and degree of reduction in $[\text{PCr}]/[\text{PCr}] + [\text{P}_i]$ 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.

Acknowledgments— The work was supported in part by grants from the National Institutes of Health (R01-DK54387, M01-RR-00633, RR-02584, and HL-07360) and a nutrition fellowship from the St. Paul Medical Center, Dallas, Texas.

We are indebted to Scott M. Grundy, MD, PhD, for helpful suggestions; to Margaret Arnecke, Wyman Schultz, and Andrea Katz for technical support; and to the nursing and dietetic staff of the General Clinical Research Center of the University of Texas Southwestern Medical Center at Dallas for providing care to the patients.

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