

Blocked Muscle Fat Oxidation During Exercise in Neutral Lipid Storage Disease

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Objective: To determine whether impaired exercise capacity in neutral lipid storage disease with myopathy is solely caused by muscle weakness or whether a defect in energy metabolism (blocked fat oxidation) may also play a role.

Design: We studied a 37-year-old woman with neutral lipid storage disease with myopathy, who cycled while lipid oxidation was assessed using U-¹³C palmitate tracer dilution technique. The effect of a glucose infusion during exercise was also studied.

Setting: Neuromuscular research unit.

Results: The exercise-induced increase in fat oxidation was virtually abolished in the patient. Treatment with intravenous glucose infusion improved maximal oxygen uptake from 23 to 27 mL × kg⁻¹ × min⁻¹, and maximal workload from 75 to 100 W.

Conclusions: These results demonstrate that in addition to fixed weakness, neutral lipid storage disease with myopathy is also characterized by a profound block in fat oxidation, which limits exercise tolerance.

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NEUTRAL LIPID STORAGE diseases (NLSDs) are disorders of endogenous triglyceride catabolism due to deficiencies of hormone-sensitive lipases, which normally hydrolyze triglycerides, diglycerides, and monoglycerides. One of these enzymes, adipose triglyceride lipase (ATGL), specifically catalyzes the first step in the hydrolysis of triacylglycerol, generating free fatty acids (FFAs) and diacylglycerol.¹ This enzyme requires the activator protein CGI-58, located on the surface of cytoplasmic lipid droplets.

Mutations in the gene coding for CGI-58 (*ABHD5*) are responsible for the pediatric disease NLS with ichthyosis, which is also called Chanarin-Dorfman syndrome.² Neutral lipid storage disease with myopathy (NLSM) is caused by mutations of ATGL (*PNPLA2* gene), with only 12 reported cases in the literature.³⁻⁶ Most patients have progressive muscle weakness involving both proximal and distal limb muscles. Cause of death is often related to heart failure or arrhythmia. Exercise intolerance was never reported as a predominant clinical feature, but several patients presented an early fatigability.^{4,5}

Large accumulations of lipids are always observed in the leukocytes (Jordan anomaly), heart, and skeletal muscle of patients with NLSM. Although exercise-

induced muscle pain and rhabdomyolysis have not been reported in patients with NLSM, the impaired mobilization of fatty acids (FAs) caused by ATGL deficiency likely impairs energy supply to skeletal muscle during exercise. Using stable isotope techniques, we investigated fatty acid oxidation (FAO) and the effects of glucose on exercise capacity in a patient with NLSM.

METHODS

A 37-year-old woman, in whom early motor development was normal, had no exercise intolerance and developed subacute right-sided foot drop and hand weakness at age 27 years. Neurologic examination revealed a severe, asymmetric distal lower and upper limb weakness, with fatty replacement of affected muscles on computed tomographic scanning. Muscle weakness remained stable, and the patient is still ambulatory. Echocardiography and vital capacity were normal. Serum creatine kinase levels were elevated (8-fold). Deltoid muscle biopsy analysis revealed a vacuolar myopathy with a massive lipid accumulation. Lipid vacuoles were also detected in leukocytes. Genetic analysis revealed a homozygous nonsense mutation (865C→T) in exon 7 of the gene encoding ATGL.³

Four healthy subjects (3 women and 1 man matched for age to the patient) performed the same exercise and stable isotope interventions as the patient.

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MAXIMAL WORK CAPACITY

Maximal oxidative capacity ($\dot{V}O_{2max}$) was determined by an incremental exercise test to exhaustion on the first day. Tests were performed on a stationary cycle ergometer (Tunturi Oy Ltd) and cardiopulmonary gas exchanges were measured via a mask connected to a gas and airflow analyzer (Quark b²; Cosmed).

SUBSTRATE TURNOVER

The patient had a carbohydrate-rich breakfast before the experiment. Two venous catheters were used for stable isotope infusion and blood sampling. A heating pad was used to arterialize the venous blood samples. A primed, constant-rate infusion of [¹³C] palmitate (0.0026 mg/kg/min, primed by a 0.085 mg/kg sodium bicarbonate [¹³C] bolus) was delivered and 2 hours later, the subjects cycled at a workload of 50% to 60% of $\dot{V}O_{2max}$ for 30 minutes. Heart rate and ratings of perceived exertion were monitored every other minute during exercise. Preparation of tracers, sampling of gases for ¹³CO₂ enrichment, and tracer calculations were performed as previously described.⁷ Analyses of glycerol, FFAs, and epinephrine were determined as previously described.^{7,8}

INTRAVENOUS GLUCOSE INFUSION

On separate days, a priming dose of saline (first day) or 125 mL of 10% glucose (second day) was infused 5 minutes before exercise and followed by a constant-rate infusion of 300 mL per hour. The patient was blinded to the infusion used. The patient cycled at a constant workload of 60% of $\dot{V}O_{2max}$ for 30 minutes, after which the workload was gradually increased in a straightforward ramp test from 35 W until exhaustion.

STATISTICAL ANALYSIS

Findings are described quantitatively in comparison with findings in healthy subjects.

RESULTS

MAXIMAL WORK CAPACITY

Maximal oxidative capacity in the patient with NLSDM was low compared with healthy subjects (mean [SD], 23 vs 39 [2] mL × kg⁻¹ × min⁻¹). Accordingly, the constant workload was lower in the patient with NLSDM than control subjects (mean [SD], 35 vs 86 [14] W).

SUBSTRATE TURNOVER

Free fatty acid concentration, rates of appearance, and disappearance of palmitate were lower in the patient with NLSDM during exercise than in healthy control subjects (**Figure 1**). Palmitate oxidation was normal at rest in the patient but did not increase during exercise as seen in healthy subjects (Figure 1). In line with this, the respiratory exchange rate increased during exercise in the patient with NLSDM and was higher in the patient compared with healthy control subjects, indicating a higher reliance on carbohydrate oxidation in the patient (**Figure 2**). Plasma palmitate and glycerol (lipolysis) increased with exercise in the healthy subjects, but they tended to decrease with exercise in the patient with NLSDM (Figures 1 and 2).

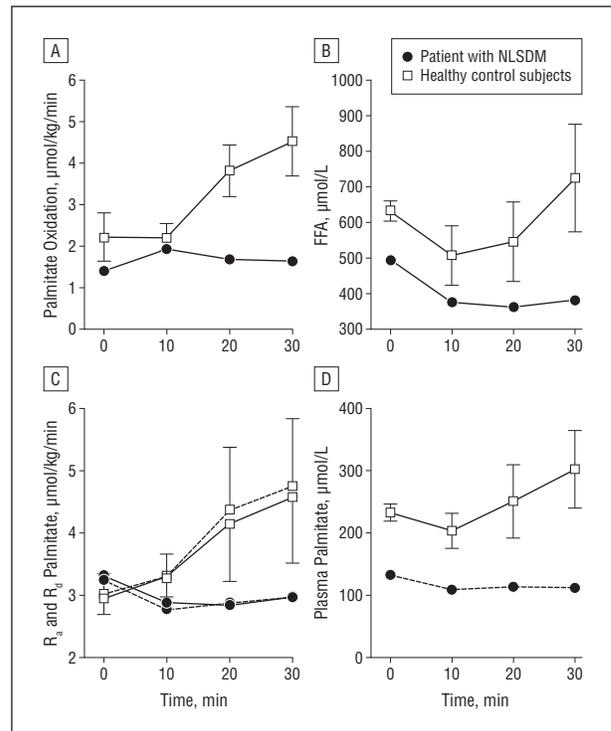


Figure 1. Comparisons between a patient with neutral lipid storage disease with myopathy (NLSDM) vs 4 healthy control subjects concerning palmitate oxidation (A), plasma free fatty acid (FFA) (B), palmitate rate of disappearance (R_d) and rate of appearance (R_a) (C, dotted lines), and plasma palmitate concentration (D) at rest and during exercise at 50% to 60% of maximal oxidative capacity.

Plasma epinephrine levels increased from 48 pmol/L at rest to 67 pmol/L (to convert to picograms per milliliter, divide by 5.459) after 30 minutes of exercise.

Plasma insulin was lower at rest in the patient with NLSDM (8 IU/L) vs healthy subjects (mean [SD], 33 [4] IU/L) and did not change significantly during exercise. Plasma glucose concentrations at rest and during exercise were generally lower in the patient with NLSDM vs healthy subjects (Figure 2).

INTRAVENOUS GLUCOSE INFUSION

Intravenous glucose improved exercise tolerance in the patient as indicated by decreased heart rate (mean [SD], 9 [4] beats/min) and decreased Borg ratings (mean [SD], 2.2 [0.3]) (**Figure 3**). It is known that glucose infusion at this work intensity has no influence on heart rate and perceived exertion in healthy subjects.⁸ The patient with NLSDM exercised 4 minutes longer on glucose, and the glucose infusion increased $\dot{V}O_{2max}$ (from 23 to 27 mL × kg⁻¹ × min⁻¹) and maximal workload (from 75 to 100 W). Peak exercise heart rate and Borg rating were lower on glucose at the workload at which the patient had to stop exercise while receiving saline.

COMMENT

The main objective of this study was to clarify the implication of the ATGL defect for fat mobilization during exercise, thus for fat availability and oxidation. A low-

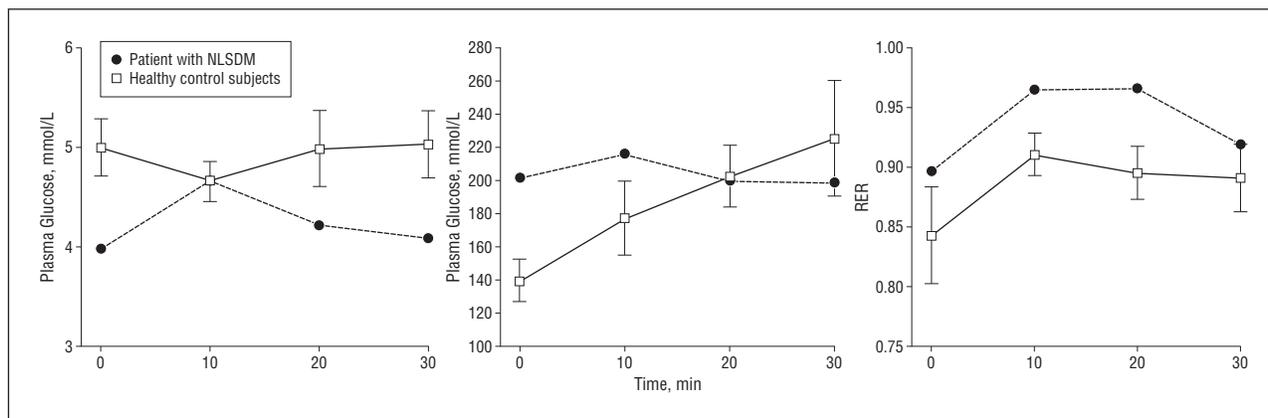


Figure 2. Plasma glucose concentration, plasma glycerol, and respiratory exchange rate (RER) in a patient with neutral lipid storage disease with myopathy (NLSDM) and 4 healthy control subjects at rest and during exercise at 50% to 60% of maximal oxidative capacity.

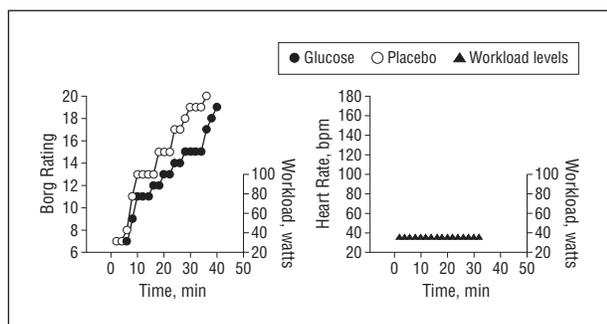


Figure 3. Heart rate and score of perceived exertion (Borg rating) with intravenous glucose and placebo during exercise at 60% of maximal oxidative capacity followed by an incremental test to exhaustion in a patient with neutral lipid storage disease with myopathy. BPM indicates beats per minute.

intensity, prolonged exercise protocol was used to facilitate fat use, and FAO was assessed using a stable isotope technique. The principal finding is that the exercise-induced increase in FAO was completely blocked in the patient with NLSDM, suggesting that exercise capacity in NLSDM is not only caused by fixed weakness but also by a profound shortage of energy. Mobilization of fat was absent despite a normal sympathetic activation as shown by the elevation of plasma epinephrine levels during exercise. The energy shortage could be partially reversed by supplying intravenous glucose.

An impaired increase in FAO during exercise has also been observed in patients with very-long-chain acyl-CoA dehydrogenase and carnitine palmitoyltransferase II deficiencies,^{7,9} but unlike these 2 conditions, the block in FAO during exercise in NLSDM appears to be caused by the poor availability of circulating FAs as a result of the defect in mobilization of FA from triglycerides. It is well known from healthy subjects that palmitate oxidation is directly correlated with the concentration of palmitate in plasma.¹⁰ Therefore, palmitate oxidation is also directly inhibited by the much lower palmitate availability, levels of which were 3-fold lower in the patient with NLSDM vs control subjects. In contrast, FA availability in very-long-chain acyl-CoA dehydrogenase and carnitine palmitoyltransferase II deficiencies is normal; in these conditions, FAO is impaired because of the defective transport of FAs into the mitochondrion (carnitine palmitoyltransferase II defi-

ciency) or the defect of FA β -oxidation (very-long-chain acyl-CoA dehydrogenase deficiency). The absence of rhabdomyolysis episodes triggered by exercise, fasting, or fever in NLSDM could be due to the absence of a potentially toxic effect of accumulated long-chain FAs in this disease in contrast to other FAO disorders.

Studies in mice lacking ATGL have shown that maximal running velocity and endurance capacity were reduced although muscle weakness was not reported.¹¹ This points to an important contribution of energy shortage to the lowering of exercise capacity in NLSDM, as found in this study. Similarly to what we observed in our patient with NLSDM during steady state exercise, plasma FA levels were low in exercising mice. Rapid depletion of muscle and liver glycogen stores and an increased respiratory exchange ratio, as seen in our patient, suggested a compensatory increase in carbohydrate metabolism in the mice.¹¹ A severely impaired activation of peroxisome proliferator-activated receptor α target genes in cardiac and skeletal muscles of mice models has also been recently found.¹² These genes are involved in FAO, and their downregulation could also contribute to the severely impaired palmitate oxidation that we observed in the patient.

Intravenous glucose improved maximal oxygen uptake and workload, and it alleviated some of the exercise-induced symptoms, as indicated by the ratings of perceived exertion. This stresses the importance of carbohydrate metabolism in these patients for energy production in muscle. Carbohydrate metabolism is spontaneously increased in NLSDM, as indicated by the mice data just described and the low glucose levels in plasma of our patient. The findings suggest that it is important to maintain adequate stores of glycogen in muscle and liver, which is best achieved by adopting a diet high in carbohydrates. This diet would probably be well tolerated because insulin and glucose concentrations of our patient with NLSDM suggested normal insulin sensitivity despite massive lipid accumulation in muscle. This notion is consistent with the finding that ATGL knockout mice showed increased glucose tolerance and increased insulin sensitivity, regardless of lipid deposition in muscle, supporting the view that lipid accumulation per se does not induce insulin resistance.¹³

Oxidative capacity, but not FAO, was assessed in another patient with NLSDM using cycle exercise.⁶ In this

young and clinically unaffected patient, there was a normal oxidative capacity; however, short-duration, high-intensity exercise is associated with an almost exclusive combustion of carbohydrate so that a mild defect of FAO may be easily overlooked. Further exercise studies are needed to confirm the severe block in FAO observed in our patient with NLSDM. Our study shows that assessment of FAO by a stable isotope tracer dilution technique is a powerful method, not only to describe the metabolic basis of NLSDM, but also as a potential outcome measure in future therapeutic trials with drugs such as peroxisome proliferator-activated receptor α agonists¹² for this currently untreatable disorder.

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