Klinefelter syndrome (XXY) occurs in 1 in 600 males, resulting in testosterone deficiency and a high prevalence of insulin resistance. Testosterone deficiency in men is a known cause of insulin resistance, and mitochondrial dysfunction is hypothesized to mediate this relationship. The aim of this cross-sectional study was to evaluate muscle mitochondrial function in XXY compared with male controls. Twenty-seven boys with XXY (age 14.7 $\pm$ 1.8 years) were compared with 87 controls (age 16.9 $\pm$ 0.9). In-vivo calf muscle mitochondrial function was assessed via phosphorus magnetic resonance spectroscopy (^{31}P-MRS) following 90 s of isometric 70% maximal exercise. Multiple linear regression was used to compare ^{31}P-MRS outcomes (ADP and phosphocreatine (PCr) time constants, rate of oxidative phosphorylation (Oxphos), and Q_{max} or the maximal mitochondrial function relative to mitochondrial density) between groups after adjusting for age differences. There were no statistically significant differences in the mitochondrial outcomes of ADP, Oxphos, PCr, and Q_{max} between the groups. There were also no differences in a sensitivity analysis within the XXY group by testosterone treatment status. In this study, in-vivo postexercise skeletal muscle mitochondrial function does not appear to be impaired in adolescents with XXY compared with controls and is not significantly different by testosterone treatment status in XXY.