Clinical Nutrition 32 (2013) 386–390

Contents lists available at SciVerse ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Effect of glutamine on glucose metabolism in children with Duchenne muscular dystrophy

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ARTICLE INFO

Article history:
Received 26 April 2012
Accepted 22 August 2012

Keywords:
Glucose metabolism
HOMA
Glutamine
Insulin sensitivity
Duchenne muscular dystrophy
Body composition

SUMMARY

Background & aims: Glutamine is a potent gluconeogenic precursor and stimulates insulin secretion. Glutamine's effect on glucose metabolism in Duchenne muscular dystrophy (DMD) has never been studied. To determine plasma glucose and insulin concentrations measured during and after glutamine administration in DMD boys. We hypothesized that glutamine can modulate whole body glutamine–glucose metabolism in DMD, a genetically determined disease.

Methods: As secondary endpoints of a randomized crossover trial in 30 prepubertal DMD boys, we measured fasting blood glucose, insulin and the Homeostasis Model Assessment (HOMA) index after daily oral glutamine (0.5 g kg^{-1} d^{-1}) for 4 months versus placebo. In a separate time series trial in 6 prepubertal DMD boys, we measured the same endpoints as well as plasma glutamine and whole body glucose turnover (Ra,glc) (primed continuous i.v. infusion of D-[6,6-^{2}D]glucose), while participants received acute oral glutamine (0.5 g kg^{-1} d^{-1}) continuously for 5 h.

Results: In the randomized trial, baseline measurements of HOMA correlated with age ($r = 0,51$, $p = 0.007$) and percent fat estimated by bioelectrical impedance analysis (BIA) ($r = 0.39$, $p = 0.047$). After 4 months glutamine supplementation, we observed no treatment or order effect on HOMA or insulin. During acute glutamine for 5 h (time series trial), plasma glutamine doubled and was associated with increased plasma insulin concentration (10.42 ± 2.54 vs 7.32 ± 1.86, $p = 0.05$) with no effect on plasma glucose, HOMA or Ra,glc.

Conclusions: Acute glutamine transiently stimulates insulin secretion in DMD boys, which could be mediated by plasma glutamine concentrations. Fasting insulin concentration and HOMA might provide quantifiable indices of disease progression.

1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked disease characterized by a progressive and severe muscle mass loss. At the age of 10 years, DMD children have lost up to 75% of their muscle mass resulting in a decrease in muscle function and loss of ambulation at the age of 15 years without steroid therapy. In a group of 9 DMD children aged 10 ± 1 years, Haymond et al. showed that postabsorptive whole body glucose turnover was not affected by

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http://dx.doi.org/10.1016/j.clnu.2012.08.019

DMD in spite of a dramatic muscle mass loss i.e. source of gluconeogenic amino acids,[1] lowered plasma alanine concentrations and whole body alanine turnover. They hypothesized that gluconeogenesis was maintained because of increased proteolysis and glutamine availability. It is noteworthy that in the same study, 7 out of 9 DMD children showed abnormal glucose tolerance on an oral glucose tolerance test suggesting some degree of insulin resistance. Another group reported higher free fatty acid and ketone body concentrations in DMD patients with low creatine phosphokinase suggesting energy metabolism change with disease progression.[2] A similar phenomenon occurs during starvation in order to spare muscle from amino acid release and subsequent hepatic gluconeogenesis.[3]

Glutamine (GLN) is a potent gluconeogenic precursor.[4,5] Glutamine contribution to gluconeogenesis increases from 8% to 16% after an 18 h and a 48 h fast respectively.[4] During fasting, amino acids routed to gluconeogenesis come from muscle. Although DMD is a genetically determined disease, we have shown that doubling plasma glutamine concentration was able to inhibit estimates of whole body protein degradation[6] and that this effect remained more than 12 h after the last dose of oral glutamine i.e. when plasma glutamine concentration had recovered normal values.[7] The effect of glutamine on glutamine–glucose metabolism has never been studied in children with DMD. Therefore the objective was to test whether an increase in plasma GLN concentration was able to affect glucose turnover or insulin secretion and if this putative effect remained 1 day after the last glutamine ingestion when plasma glutamine concentration had returned to normal.

As secondary endpoints of a randomized crossover trial, we measured plasma insulin and glucose to test insulin resistance after daily oral glutamine supplementation for 4 months in DMD boys.[8] Because fasting blood samples were taken the day after glutamine administration, we also present glucose metabolism data (never published) from a time series experiment in which DMD boys received oral glutamine continuously for 5 h[6] to investigate the effect of an acute increase in plasma glutamine concentrations on insulin resistance and whole body glucose turnover.

Our hypothesis was that glutamine was able to modulate whole body glutamine–glucose metabolism even though DMD was a genetically determined disease.

2. Materials and methods

2.1. Randomized crossover trial: long-term daily glutamine administration (4 months)

The study was part of a randomized crossover trial of 4 months oral GLN versus placebo on muscle mass and function in ambulant DMD boys (ClinicalTrials.gov NCT00296621).[8] Subjects were selected on the basis of the following eligibility criteria: Pediatric neurologist diagnosis of DMD by clinical history, muscle biopsy (absence of dystrophin confirmed by immunohistochemistry or Western blot) and molecular biology, as well as the ability to walk 170 m. Exclusion criteria included: wheelchair dependency, body weight >60 kg, hepatic or renal insufficiency, or surgery scheduled in the year following the first visit. Steroid treatment was not an exclusion criterion but patients were asked to remain at the same dose throughout the study. The protocol was approved by the Paris-Bichat Ethics Committee (Comité Consultatif pour la Protection de la Personne dans la Recherche Biomédicale). All participating families gave their written informed consent after a thorough explanation of the study protocol to both the parents and the children. Subjects were recruited among those followed in multidisciplinary outpatient facilities from University hospitals in Paris, Poitiers and Lille, France. Subjects were randomly assigned in a double blind crossover manner to 4 months of taking oral GLN (0.5 g kg^{-1} d^{-1}) followed by 4 months of taking placebo (maltodextrin of equal weight) or vice versa. Supplements were identically packaged in 5 g sachets (maximum 6 sachets qd). The two 4-month periods were separated by a 1-month wash-out. The order of treatment allocation was randomized. Participants, investigators, physicians, assessors and data analysts were blinded throughout the trial to the order of treatment assignment. The GLN dose (0.5 g kg^{-1} d^{-1}) was selected based on previous studies in DMD boys[6,9,10] and in healthy humans[9,11] that showed a 2-fold increase in plasma glutamine concentrations during glutamine administration.

Fasting blood glucose and insulin were collected at 0, 2, 4, 5, 7, 9 months. HOMA index (HOmestasis Model Assessement) was calculated as a rough estimate of insulin resistance: fasting insulinemia (UI L^{-1}) × fasting glycemia (mmol L^{-1})/22.5.[12,13] Weight, height and body mass index *Z*-score for age and sex were calculated using French references for growth.[14,15] Body composition was estimated using monofrequency (50 kHz) bioelectrical impedance analysis (BIA; 101 Q; RJL systems, Clinton Township, MI or Quantum/S; Akern SRL, Pontassieve, Italy) under standardized conditions.[16]

Plasma insulin, glucose and HOMA were presented as medians and quartiles. Baseline characteristics were presented as means ± SD. For a crossover design we evaluated treatment and order effects as well as the interaction. The effect of treatment with glutamine was assessed using repeated-measures analysis of variance. Normality of residuals and absence of heteroscedasticity were verified, otherwise logarithmic transformations were applied to the dependent variable. The correlations between fasting insulin, HOMA, body fatness and age were tested (Pearson's correlation). Significance was set at $p < 0.05$. The study was designed and powered to detect a 10% increase in walking speed (primary outcome) after a 4-month daily glutamine administration. Data were double entered and analysis was carried out by a blinded statistician using SAS® version 9.1 (Cary System).

2.2. Time series trial: acute glutamine administration (5 h)

In the randomized crossover trial (presented above), because fasting blood samples were taken the day after glutamine administration, plasma glutamine concentrations were normal as demonstrated in a previous trial in DMD boys under similar conditions.[7] Thus in order to investigate the effect of a 2-fold increase in plasma glutamine concentrations on fasting plasma glucose and insulin, we report unpublished glucose metabolism data from a time series study of acute oral glutamine (5 h) on whole body protein and glutamine metabolism in DMD boys.[6] The Nemours Children's Clinic Research Committee and the Baptist Medical Center Institutional Review Committee approved the protocol. The protocol was explained to the children and their parents and written informed consent was obtained. Subjects were recruited from the Nemours Children's Clinic, Jacksonville, Florida. Six prepubertal boys with DMD were studied on 2 consecutive days in the postabsorptive state after 14 h of fasting while drinking every 30 min for 5 h 80 mL of flavored water (Kool-Aid®) only, the first study day, and glutamine dissolved in the same flavored water (800 μmol kg^{-1} h^{-1} of L-glutamine, dose equivalent to 0.6 g kg^{-1} d^{-1}) the second study day. The study procedure is described elsewhere.[6] In order to measure glucose metabolism, children received a primed, continuous i.v. infusion of D-[6,6-^{2}D]glucose (7 mg kg^{-1} as a bolus followed by 7 mg kg^{-1} h^{-1}) for 5 h using a calibrated syringe-pump starting at 08:00 h. D-[6,6-^{2}D]glucose solution (Cambridge Isotopes, Woburn, MA; 98% ^{2}D) was determined to be pyrogen-free (Limulus lysate assay), sterile (plate culture), passed