Projects related to nutrition and DMD
Duchenne Parent Project Netherlands 2018

1. Dominic Wells, Royal College London, UK. Dietary manipulation for the amelioration of Duchenne muscular dystrophy. € 90.142

Duchenne Muscular dystrophy (DMD) is an inherited X-linked monogenic neuromuscular condition that exhibits muscle pathology including muscle fibre degeneration, regeneration, inflammation and fibrosis. A wide range of potential therapies are under investigation including restoring the missing or mutated dystrophin protein, through gene therapy, cell therapy, exon-skipping or compounds that read through premature stop mutations. Alternatively, the pathophysiology resulting from the loss of dystrophin could be targeted and inflammation is a key component that exacerbates disease progression. While nuclear factor kappa B (NF-κB) activation has been identified as one of the main players in the inflammatory cascade, recent studies show that the NOD-like receptor (NLR) family, pyrin domain-containing protein 3 (NLRP3) inflammasome is substantially upregulated in dystrophic muscle, and inhibiting this pathway reduces muscle pathology in the mdx mouse model of DMD. As the ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome–mediated inflammatory disease, this raises the possibility that a ketogenic diet may prove beneficial in patients with DMD. This proposal seeks to test this hypothesis by assessing the physiological and pathological changes seen with acute or chronic exposure of mdx mice to two versions of a ketogenic diet, one of which would offer simplified and fast translation to a treatment for children with DMD.

2./3. Maaike van Putten, LUMC Leiden, Netherlands and Olivier Dorchies, University of Geneva, Switzerland. Preclinical evaluation of branched chain amino acids to support protein metabolism in the early phase of muscular dystrophy: a consortium approach. € 123.102

Many Duchenne muscular dystrophy (DMD) patients use over-the-counter supplements, despite the fact that preclinical or clinical evidence of the benefits and risks is lacking. In addition, supplements may interfere with drugs used in standards of care, and impact their benefit/safety outcome. Nonetheless, food supplements may be a useful approach to provide key elements to support altered muscle metabolism according to the specific phase of the disease, serving as adjuvants to proper diet and drugs. To address this, we here propose a consortium approach to perform rigorous pre-clinical evaluation of two supplements. The goal of this specific application is to obtain scientific evidence for the usefulness of branched chain amino acids and the possible modulation of their efficacy when coadministered with corticosteroids.

Branched chain amino acids (BCAA; leucine, isoleucine and valine) was preselected, with the aim to address the hypothesis that the early necrotic phase increases the requirement for amino acids to sustain protein balance. The project will assess the outcome of this supplement with and without F-methylpredinisolone, in two sites (by our lab and the lab of Dr. Dorchies, University of Geneva) with complementary expertise to ensure independent validation. The study setup and core outcome measures will be standardized to assess efficacy, but each group will also use additional expertise and/or animal models to gain insight into mechanism of action. Data obtained will provide fundamental insights into the specific working hypothesis; the integration with the results obtained by other two sites (University of Bari (UniBa) and Baylor College of Medicine (BCM)) that will independently test L-citrulline, using the same approach, will serve to validate a platform for future testing of other supplements.

4./5. Annemaria De Luca, Universitity of Bari, Italy and Martha Fiorotto, Baylor College of Medicine, USA. A consortium approach for a de-risking preclinical assessment of efficacy of dietary supplements in muscular dystrophy: L-citrulline to support protein metabolism in the early necrotic phase of the disease. € 124.128

Many Duchenne muscular dystrophy (DMD) patients use over-the-counter supplements, despite the lack of preclinical or clinical evidence of their benefits and risk. In addition, supplements may interfere with drugs used in standards of care, and impact their benefit/safety outcome. Nonetheless, food supplements may be a useful approach to provide key elements to support altered muscle metabolism according to the specific phase of the disease, serving 2 as adjuvants to proper diet and drugs. To address this, we here propose a consortium approach to perform rigorous and de-risking pre-clinical evaluation of two supplements.
The goal of this specific application is to obtain scientific evidence for the usefulness of L-citrulline in mitigating development of dystrophic symptoms and the possible modulation of its efficacy when co-administered with corticosteroids. L-citrulline was preselected, with the aim of addressing the hypothesis that the early necrotic phase of the disease increases the requirement for this amino acid precursor to sustain the increased need for arginine, NO production, and for protein turnover. The project will assess the outcome of feeding the supplement to mdx mice, with and without F-methylpredinisolone (PDN), at two sites (our lab and the lab of Prof. Fiorotto, Baylor College of Medicine) with complementary expertise to ensure independent validation. The study setup and core outcome measures will be standardized, but each group will also incorporate additional expertise to gain insight into possible mechanisms of action. Data obtained will provide fundamental insights into the specific working hypothesis; the integration with the results obtained by other two sites (Leiden University Medical Center and University of Geneva) that will independently test branched chain amino acids, using the same approach, will serve to validate a platform for future testing of other supplements.

6. Gordon Lynch, University of Melbourne, Australia. Evaluating a sulforaphane-based nutraceutical to alleviate gastrointestinal dysfunction in DMD. € 99.744

Gastrointestinal (GI) upsets including constipation and bloating are common among DMD patients and cause discomfort, pain and altered quality of life. Our previous DPP NL supported research applied sophisticated video imaging and spatiotemporal mapping to identify similar GI issues in mdx dystrophic mice, including altered motor patterns in the small intestine and colon. Patients and their families try all manner of nutritional formulations to alleviate the symptoms of DMD. This rationale is wellmeaning and based on claims that some nutraceuticals can potentially exert anti-inflammatory and/or antioxidant effects. This may have a scientific basis but unfortunately the merit of many nutritional supplements is based solely on anecdotal evidence. Nutraceuticals with potential anti-inflammatory benefits must be evaluated rigorously before being advocated to DMD patients and their families. Sulforaphane (SFN), a compound within the isothiocyanate group of organosulfur compounds, is found in cruciferous vegetables like broccoli, Brussels sprouts, and cabbages. Three preliminary studies in mdx mice showed SFN had anti-inflammatory and anti-fibrotic effects in muscle. Whether a SFN-based nutraceutical could alleviate GI dysfunction in muscular dystrophy has not been evaluated. To capitalise on the success of our recently completed study that identified GI dysfunction in mdx mice, this proposal will rigorously evaluate the therapeutic potential of SFN to alleviate colonic motility issues in muscular dystrophy. Our ability to evaluate GI dysfunction using spatiotemporal mapping as well as skeletal muscle and heart defects, will allow us to rigorously evaluate the therapeutic potential of SFN as a nutraceutical for DMD. The findings will determine whether SFN should advance to clinical trials to address the pain and discomfort caused by these symptoms of DMD.

7. Zoe Davidson, Monash University, Australia. A weighty problem: tackling obesity in Duchenne muscular dystrophy. € 93.325

Recent advances in Duchenne muscular dystrophy (DMD) care have extended life expectancy into the third decade of life. Despite these advances in clinical management, obesity continues to be a significant issue with approxiamately one in two boys with DMD classified as obese.

Obesity seriously affects the physical and psychosocial wellbeing of boys with DMD and their families. Whilst families receive the best available evidence-based nutrition advice, the complexities of managing a child's weight in the context of a chronic and life-limiting disease hinder weight management success. To prevent this

relatively new population of young adults and men living with DMD being burdened by additional metabolic co-morbidities related to obesity, it is crucial that we understand and address childhood obesity in this population **now.**
The aim of this research fellowship is to understand the determinants and impact of obesity in boys with DMD to inform the development of a weight management strategy designed to improve clinical outcomes and quality of life for these children. The weight management strategy will be evaluated, and a dissemination plan developed so that the findings can assist boys with DMD and their families around the world.
My dietetic background, established neuromuscular collaborations combined with my proven expertise and substantial track record in nutrition and DMD attest to my ability to complete this program of research. I have access to the 1) necessary support systems; 2) an expanding research team; 3) funding; 4) established collaborations for the realisation of this research program.

8. Martha Fiorotto, Baylor College of Medicine, USA. Protein requirements to support growing dystrophic muscle. € 103.486

There are currently no recommendations concerning the optimal protein intake for boys with Duchenne muscular dystrophy (DMD). This is especially of concern for the youngest patients because due to their stage of development their already high requirement could be increased further by the dystropathology. If these increased needs are not met, growth and the capacity for muscle repair likely would be compromised. The *hypotheses* to be tested are:
1. A blunted response in muscle protein synthesis to feeding in the dystrophin-deficient mdx mouse promotes greater amino acid catabolism and an increase in protein requirements.

2. The need for continuous muscle repair in young, growing mdx mice increases their protein requirements which, if not met, will promote earlier and more severe muscle degeneration and greater fat deposition.

The *specific aims* of the experiments are:

1. To quantify in vivo rates of muscle protein synthesis and whole body amino acid oxidation in juvenile (24-days-old) mdx mice in the fasted and fed states using isotope-labelled amino acid tracers.

2. To identify if low, “standard”, or high protein intakes beginning preweaning impact the onset, severity, and/or progression of muscle

degeneration and body composition in male mdx mice. This will be assessed through measurement of biomarkers of muscle damage, histology, and in vivo strength and locomotor function tests.

The outcomes will reveal whether the adequacy of dietary protein intake affects the onset and progression of muscle damage in a preclinical model. The results will provide information necessary for designing effective diets to test in young DMD patients.

9. **Fast Track: Andrea Farini, University of Milan, Italy. Characterization of intestinal inflammation and microbiota richness in mdx mice as keys to modulate the pathogenesis of DMD. € 24.820**

   In our gut and intestines are living a lot of microorganisms, like bacteria (the gut flora or microbiota) that are important for, amongst others, the digestion of food and our immune system. The loss of dystrophin in DMD also has effects on our gut, thereby dysregulating the gut flora and causing immune responses to these microorganisms, which could be harmful. Many Duchenne patients have intestinal problems, like disturbances in movement of the intestines and constipation. In this project the microbiota and inflammation in the intestines of a DMD mouse model will be characterised, which could help to ameliorated the disease pathology.

10. **Fast Track: Fabio Iannotti, Institute Biomolecolar Chemistry, Pozzuoli, Italy. Unravelling new pathways and innovative perspectives: a pilot study on gut microbiota-endocannabinoids interaction in Duchenne's muscular dystrophy. € 25.000**

    The body itself also produces cannabinoids and has a whole system regulating their effects (the so called 'endocannabinoid system'). The group of Fabio Iannotti has shown in earlier research that inhibition of this system can have beneficial effects on muscle and improved the pathology in a mouse model for DMD. In addition, it is known that the gut flora (microorganisms, like bacteria, living in the gut) has an effect on muscle. Corticosteroids (*e.g.* prednisone and deflazacort) have an effect on this gut flora, which after prolonged use can have negative effects on the gut. In this research project will be looked in a DMD mouse model at the relationship between the gut flora and the endocannaboid system This knowledge may be useful to ameliorate the DMD pathology and reduce the side effects of steroids in the gut.