**Diet for Dads Sperm (D4Ds study)**

**Background**

The proportion of Australian men who are classified as obese has tripled since the late 1970s, now affecting 28.7% of men (1). Men who are obese are more likely to have children with higher fat mass during the first 12 months of life, and who grow into children and adults with obesity (2). This effect goes beyond the lifestyle behavioural influence of a shared raising environment.

We have demonstrated that reactive oxygen species (ROS) concentrations in sperm, are elevated in men who are obese and are implicated in the transmission of obesity from fathers to offspring (3). Sperm are highly susceptible to ROS due to a lack of intracellular antioxidants and high levels of polyunsaturated fats which reduces their capacity to repair ROS related damage, especially to their DNA. This ROS induced DNA damage in sperm is transmitted at conception, compromising offspring health (4, 5).

It is assumed that weight loss in these men will assist in restoring sperm quality. The current clinical guidelines set by The Royal Australian College of General Practitioners recommends that men who are obese lose 5-10% of their body weight prior to conception (6). Some interventions that are effective in reducing weight, including bariatric surgery and restriction of energy intake have been shown to have unintended consequences for sperm quality (reductions in sperm count, motility and morphology) (7-11). Furthermore, data from our undernutrition mouse model also shows negative effects on subsequent generation of F1 offspring metabolic and reproductive health (12). *Thus the current clinical guidelines for obese men prior to conception may therefore not have the intended effects, and may increase obesity risk to their children.*

**Lessons from our murine models of male obesity:** We have assessed diet and exercise lifestyle interventions in a male mouse model of obesity (13). Exercise, without change in adipose tissue mass improved sperm quality and function and reduced sperm DNA damage (13). Further, in comparison to those mice who were subjected to calorie restriction, the phenotype of the F1 offspring in the “exercise group” were considerably less likely to have excess adiposity (14-17). In undernourished male mice (70% caloric restriction), micronutrient supplementation to fathers pre-conception improved the metabolic and reproductive phenotypes of their F1 offspring (12). More recently, we have showed in our male obesity model that micronutrient supplementation of the high fat diet (HFD) restored sperm quality and ROS concentrations while partially restoring fetal weights without the need for weight loss (18). Accordingly, *we proposed that high nutrient quality diets* *will prevent obesity in children of fathers with obesity while weight loss alone may not be beneficial and may be deleterious particularly if the diet is nutritionally suboptimal.*

**Evidence to date in men:** There is currently limited direct evidence that poor sperm quality associated with obesity is reversible through lifestyle interventions in humans. A study of 43 obese men during a 14 week residential weight loss program (dietary and exercise advice) showed increases to sperm count and sperm morphology (19). A case study of six men undergoing fertility treatment reported a reduction in sperm DNA damage and subsequent positive pregnancy with reduction of abdominal adiposity after dietary advice (20). Further, a study of 105 men who were obese found that sperm DNA damage was reduced 25% following interventions that focused on healthy diet and exercise (21). However, whether the improvements in these studies were due to the improvements to lifestyle factors such as nutrition or the weight loss could not be determined. *The current understanding of how lifestyle interventions in males who are obese, therefore, are limited by the inability to distinguish the effects of weight loss vs improvements to dietary nutrition and by measures of sperm quality that are unrelated to offspring health as ROS concentrations of sperm as far as we are aware have never been measured***.**

**Exploring a new hypothesis for humans:** This evidence has led to our hypothesis that: **Feeding men who are obese with a suboptimal diet, a nutrient dense diet will reduce sperm ROS concentrations without requiring weight loss.**

**PILOT STUDY**

A parallel arm factorial RCT design is proposed to compare the effects on sperm quality of a nutrient dense diet without weight loss (NQ) in men who are obese. Participants will be randomly allocated in a 1:1 ratio (N=16 per group) for a period of 12 weeks.

**Group 1 (Control) (N=16): Given current Australian guidelines on healthy eating and exercise.**

**Group 2 (Intervention- NQ) (N=16): Nutrient dense diet/weight maintenance (± 2kg).**

The nutrient dense diet has been developed specifically for men and used previously to improve erectile dysfunction and diabetes in men who are obese (22, 23). The diet is based on the Commonwealth Scientific Industrial and Research Organisation (CSIRO) Total Wellbeing Diet with a nutrient composition high in protein and low in saturated fat with high micronutrient content (24). We will utilise a 12 week intervention period as it approximates one full round of spermatogenesis in the human (~64 days) with this length of time able to improve sperm count, motility and morphology in men undergoing lifestyle interventions previously (19, 21).

Men randomised to ***Group 1: Control*** will receive Standard Care booklets that are freely available from the clinical trials coordinator at their dietary consultation. The SA Health brochures for men SNAPS Resources (i) Live life, sit less, move more, (ii) Alcohol- take control, (iii) Good sleep = good health and (iv) Fuel right, function better, which provides limited healthy lifestyle information. Provision of these resources results in minimal change in weight or diet quality in men (A/Prof Heilbronn, NCT03689608)

Men randomised to **Group 2*: Intervention- NQ*** will be prescribed the CSIRO Total Well Being Diet for Men (24). This nutrient dense diet has been adapted specifically for men and previously used by Professor Wittert and Dr Taylor to improve erectile dysfunction and diabetes in men with obesity (22, 23). The macronutrient proportions comprising of protein (35% of calories), low saturated fat (<10%), and total fat (<30%). It includes 300g of lean meat/poultry/fish/non-meat equivalent (100g lunch and 150g dinner), and three servings/day of cereals/bread and low-fat dairy foods and two fruit and five vegetable serves per day. The diets will be isocalorically prescribed by a study dietician based on their calculated daily energy requirements using the Schofield Energy Prediction Equation to ensure calorie sufficiency (25). Men will receive a list of foods that they can choose, including recipes for the study duration and adapted for their dietary preferences (e.g. vegetarian) as well as easy food swaps and alternatives. A daily food intake checklist will be provided listing the required foods by type and volume. Food selections will be based on readily available foods and/or products within the marketplace. Consultations with study dieticians will follow the Australian dietetics management plan (baseline, 2 weeks, 6 weeks and 12 weeks) for a total of 4 consultations.

**Inclusion Criteria**

* Men aged 18-50 years of age. This is the age range for the target population and sperm quality remains stable within this age range (26).
* Obese defined by a BMI of ≥30 kg/m2 and a waist circumference of ≥102cm (27).
* Dietary confirmation of a suboptimal low nutrient diet as determined by the CSIRO Healthy Diet Score survey ≤60 out of 100 (28) (~80% of all Australian men). The diet scoring system assess the quantity, quality and variety of foods consumed, where an individual’s diet score reflecting their overall compliance with the Australian Dietary Guidelines, with a higher score reflecting better compliance. <https://www.totalwellbeingdiet.com/au/health-tools/quizzes/healthy-diet-score>
* Able to read and write English.

**Exclusion Criteria**

* Prior vasectomy or vasectomy reversal.
* Un-descendent testes and suspected pathologies related to sub-fertility (i.e. varicoceles) or known genetic disorders that effect weight and fertility (i.e. Klinefelter's Syndrome, Prader-Willi or Larence-Moon-Bardet-Biedel).
* Cigarettes or illicit drug use.

**Determining Nutritional intake**

**Dietary intake** - Self-reported dietary intake will be recorded by a 3-day weighed food records (29) every 2 weeks during the intervention via the mobile App Research Food Diary. An estimate of kilojoules consumed, and core and non-core food groups will be derived. Study dietitians will monitor results.

**Randomisation**

Eligible participants will be randomly allocated in a 1:1 ratio using covariate adaptive randomisation. Pre-programed software will assign participants to particular treatment groups by considering BMI, sperm ROS concentrations and previous assignments of participants. This type of randomization uses the method of minimization by assessing the imbalance of sample size among several covariates.

**Recruitment/Monitoring**

Men will be recruited by community radio announcements, social media advertisement, direct promotion through Healthy Male and other Stakeholder organisations, and the FCMHW, which all have subscriber-based mailing lists.

Men will be prompted to complete an online pre-screening anonymous questionnaire (enter in current body weight and height, respond to inclusion and exclusion criteria and complete the CSIRO Healthy Diet Score survey via RedCap (online portal). If pre-screening is successful, they will be directed to the study consent form via the online portal. The clinical trials coordinator will then contact the participants to organise a first assessment. The first assessment will include online medical and sexual history questionnaires, height, weight and waist circumference, body image analyser (BIA), whole blood collection (for measurements of glucose, cholesterol and Trigs), blood pressure and a semen analysis (either brought in from home or produced on site at level 4 clinical research facility in AHMS).

Participants will be scheduled for a dietary consultation within the following week, by telehealth and will receive information on their study group allocation and any dietary prescription and education for the interventions through the clinical trials coordinator (*Group 1* - *control*) and study dietitian (*Group 2* – *NQ*) with additional information deposited online via the study portal. During the week between first assessment and first consultation, men will be required to complete their first round of 3-day weighted food diaries and be fitted with the actigraphy device to record physical movement. Study dietitians will review the 3-day food diaries, body weights and waist circumferences for all participant in *Group 2 – NQ* and will provide follow up advice if required. If it is found that a participant in *Group 2 – NQ* has lost more than 2kg of their original body weight at weekly weigh in, modifications to dietary prescriptions and recalculations of dietary needs will occur to ensure participants hit prescribed goals.

Post intervention participants will be invited back for study end-point collections (Table 1). Post intervention, those men allocated to Group 1: *Control* will meet with a clinical dietitian for education and administering of nutrient diet plans.

**Outcomes (Table 1)**

Men will be asked to abstain from ejaculation 2-4 days prior to their screening assessment, and post – intervention collections. Semen collections can occur at home or in clinical research facility.

**Primary Outcome: Clinical Sperm Quality Markers – ROS induced DNA damage**

1) DNA fragmentation – using the Halosperm®, clinical diagnostic test (Halotech)

2) Oxidation-reduction potential of semen –MiOXSYS System, clinical diagnostic test (30).

**Secondary Outcomes: Clinical Sperm Quality Markers – basic semen analysis**

Sperm count, motility and morphology will be analysed on CASA semi-automatic system for semen analysis (Microptic SCA System) located within CI McPherson research laboratory and conducted in accordance with the 2010 WHO manual for the processing of human semen (31).

**Secondary Outcomes: Clinical Sperm Quality Markers – ROS research tools**

A number of research measures of ROS in sperm will be assessed including super oxide generation – using MitoSox Red indicator, lipid peroxidation – using BODIPY indicator (Thermofisher Scientific), total intracellular ROS generation – using Cell ROX indicator and 8OHdG DNA lesions (ROS generated DNA damage, antibody mediated).

**Other Outcomes/characterisation of study population:**

1. **Metabolic profile** –fasted whole blood collection for serum measurements of glucose, cholesterol and triglycerides. Up to 15 ml of whole blood will be collected per participant per time point. Blood pressure will also be measured.
2. **Body composition –** both BMI (height (cm) and weight (kg)) and waist circumference (cm) calculated at standard collection times. Additional body weights will be measured weekly by all participants in the comfort of their own home. Body composition will be measured by a body image analyser (BIA) located in A/Prof Heilbronn laboratory at standard collection times.
3. **Exercise monitoring**–participants will be fitted with an actigraphy device (wGT3X-BT provided by A/Prof Heilbronn’s laboratory) to monitor daily movement and sleeping/waking patterns for a week.
4. **Health Surveys -** Short form of the International Index of Erectile Dysfunction (IIED), Berlin Sleep Apnoea Questionnaire, SF36 Health Status Survey, Alcohol Frequency Questionnaire, International Physical Activity Questionnaire, Perceived Stress Scale, The Coping Self-efficacy Scale, Regulate Exercise Scale, Regulate Eating Habits Scale, The Sleep Self-efficacy Scale, The reproductive history scale.

***Table 1: Study schedule and measures***

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|  | **Pre-screen**  **Online Portal** | **Screen/Base-line**  **CTU** | **12 week Diet intervention** | | | | | | | | | | | | **Post-intervention**  **CTU** |
| **Study Week** | **1** | **2** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
| **Weight, height, waist circumference** | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **Adiposity (BIA)** |  | X |  |  |  |  |  |  |  |  |  |  |  |  | X |
| **Semen collection** |  | X |  |  |  |  |  |  |  |  |  |  |  |  | X |
| **Diet Intervention** |  |  | X | X | X | X | X | X | X | X | X | X | X | X |  |
| **Dietetics consultation**  **(Group 2 only)** |  |  | X |  | X |  |  |  | X |  |  |  |  | X |  |
| **Healthy Diet Score** | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Food Diaries** |  | X | X |  | X |  | X |  | X |  | X |  | X |  |  |
| **Whole blood sampling** |  | X |  |  |  |  |  |  |  |  |  |  |  |  | X |
| **Health survey** |  | X |  |  |  |  |  |  |  |  |  |  |  |  | X |
| **Exercise monitoring** |  |  | X |  |  |  |  |  |  |  |  |  |  | X |  |

CTU – Adelaide Health and Medical Science Building, Level 4 Clinical Trials Unit

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