Title: Diet and exercise in an obese mouse fed a high fat diet improves metabolic health and reverses perturbed sperm function

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Running head: Diet and exercise improve sperm function in obese mice

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**ABSTRACT**

Male obesity is associated with reduced sperm motility, morphology and increased sperm DNA damage and oxidative stress; however, the reversibility of these phenotypes has never been studied. Therefore the aim of this study was to assess the reversibility of obesity and its associated sperm physiology and function in mice in response to weight loss through diet and exercise. C57BL6 male mice (n=40) were fed either a control diet (CD) (6% fat) or a high fat diet (HFD) (21% fat) for a period of 10 weeks before allocation to either diet and/or swimming exercise interventions for a period of 8 weeks. Diet alone reduced adiposity (1.6 fold) and serum cholesterol levels (1.7 fold, p<0.05), while exercise alone did not alter these, with exercise and diet also improved glucose tolerance (1.3 fold, p<0.05). Diet and/or exercise improved sperm motility (1.2 fold) and morphology (1.1 fold, p<0.05), reduced sperm DNA damage (1.5 fold), reactive oxygen species (1.1 fold) and mitochondrial membrane potential (1.2 fold, p<0.05) and increased sperm binding (1.4 fold) (p<0.05). Sperm parameters were highly correlated with measures of glycaemia, insulin action and serum cholesterol (all p<0.05), regardless of adiposity or intervention, suggesting a link between systemic metabolic status and sperm function. This is the first study to show that the abnormal sperm physiology resulting from obesity can be reversed through diet and exercise, even in the presence of ongoing obesity, suggesting that diet and lifestyle interventions could be a combined approach to target sub fertility in overweight and obese men.

**Keywords**

Lifestyle interventions, fertility and metabolism
INTRODUCTION

Obesity is a global health problem that is reaching epidemic proportions with 1.6 billion adults classified as overweight and 400 million adults classified as obese worldwide (60). Consequently, the rates of male obesity in reproductive aged men has nearly tripled since the 1970s (33). Recent studies in both humans and other species shows that male obesity impairs male reproductive potential, affecting both the molecular and physical structure of sperm (1, 10, 16, 20, 21, 34, 35, 38, 45, 47, 50, 63, 75, 79), and health of the developing fetus and subsequent offspring (9, 24, 42, 55, 59).

Although fertility is not always impaired in obese men, almost 80% of men that present to fertility clinics are classified as either overweight or obese suggestive of an interaction between obesity and sperm function (9). There is a negative impact of male obesity on standard semen parameters, including motility, count and hormone abundance, such as testosterone and inhibin B (1, 5, 16, 18, 21, 22, 32, 35, 37, 45, 47, 52, 62, 69, 85). Further studies have reported reduced pregnancy success and live birth outcomes after IVF treatment in overweight and obese men (9, 42).

Obesity modifies the molecular makeup of sperm often in conjunction with diabetes, with a number of key proteins regulating motility, capacitation and cell integrity altered in obese men (48). Further, proteins regulating acetylation and DNA damage repair systems are also altered in obese mice (63). High levels of reactive oxygen
species and DNA damage in sperm have also been associated with increased patient body mass index (BMI) in humans (16, 47, 79) with increased levels of IgA antisperm antibodies also reported in obese men (34). However the reversibility of the effects to the molecular and physical structure of sperm resultant from obesity has never been studied.

Unlike many pathologies, obesity can be reversed, by changes to lifestyle, such as increased exercise and decreased calorie intake reducing fat stores and improving metabolic health outcomes (81). To date, few studies have examined the effects of weight loss on reproductive capacity in obese men and many have been limited to hormone profiles, finding increased serum testosterone and inhibin B (12, 30, 40, 41, 78). Currently only one study has looked at weight loss and sperm function in severely obese men, finding decreased adiposity improved sperm counts, motility and morphology (29).

We have therefore used our established mouse model of diet induced obesity (10, 55, 63), to assess whether diet and or exercise can reverse the adverse phenotype seen in sperm from obese males. Using this model, we found that diet and/or exercise in obese mice improved sperm function, in close association with markers of glycaemia, insulin action and in particular cholesterol abundance, suggesting that diet and lifestyle interventions could be effective approaches for the treatment of subfertility in overweight and obese men.
METHODS

Animals and Diet

Five week old male C57BL6 mice (n=40) were randomly assigned to one of two diets for an initial period of 10 weeks: 1) control diet (CD) low in fat (Table 1) (SF04-057; Specialty Feeds, Perth, Australia); or 2) a high fat diet (HFD) high in fat and nutrient matched (Table 1) (SF00-219; Specialty Feeds, Perth, Australia). Diets used in the study have been previously shown to increase adiposity (10, 13, 55, 63). After the initial feeding period, males allocated to the HFD were further allocated to one of the following interventions for a period of 8 weeks: 1) continuation of a HFD (HH) (n=7); 2) change to a CD (HC) (n=8); 3) continuation of a HFD with exercise (HE) (n=8); 4) change to a CD with exercise (HCE) (n=8). Mice allocated to the CD during the initial feeding period were also fed a CD during the intervention period to be used as a baseline control (CC) (n=8). Animals were individually housed for the entire study and fed ad lib. The use and care of all animals used in the study was approved by the Animal Ethics Committee of The University of Adelaide.

Exercise Intervention (Swimming)

Male mice were placed into tank containing warm water at a constant temperature of $32^\circ C \pm 1^\circ C$ to swim freely for the set time period. For the first 2 weeks mice swam for 3x15min periods over 7 days (7). This allowed time for the mice to become accustomed to exercise regime and the swimming tank. For the remainder of the intervention (6 weeks) mice swam for 3x30 min training sessions each week to simulate light exercise. This imposes light exercise in comparison with a moderate training program in which mice freely swam for 60 min, 5 days a week, for 18weeks
Swimming sessions were supervised and the number of mice per swim session (n=4) was kept low to avoid ‘gang swimming’.

**Body Composition**

Individual body weights were recorded weekly during both initial and post intervention periods. At 23 weeks of age peri-renal fat, retroperitoneal fat, dorsal fat, omental fat, gonadal fat, testes, seminal vesicles, liver, kidneys and pancreas were collected and weighed post mortem blinded and performed by the same individual. At 8 weeks post intervention whole body composition of adiposity, lean mass (including muscle and soft tissues) and bone mass were measured by a dual-emission X-ray absorptiometry machine (DEXA) (Piximus, Ge Lunar, Wisconsin, USA) as previously described in (57).

**Metabolites, Corticosterone and Testosterone analysis**

Six hour fasted glucose samples pre-intervention and post-intervention were obtained via a tail bleed and measured using a glucometer (Hemocue, Angelholm, Sweden). After the 8 week intervention period, a blood sample was obtained via cardiac puncture under anaesthetic with 2% Isoflurane (1-chloro-2,2,2-trifluoroethyldifluoromethylether) (Veterinary Companies of Australia, Kings Park, Australia) for measurement of serum testosterone, cholesterol, free fatty acids (FFA), triglycerides and corticosterone. Testosterone was measured by a stable-isotope dilution liquid chromatography coupled with tandem mass spectrometry method using an API 5000 instrument using atmospheric pressure photoionization as described previously (10, 53). Cholesterol, FFA and triglycerides were measured on a Hitachi 912 automated sample system previously described (26) and corticosterone was
measured using a commercially available ELISA kit (KA0468, Abnova, Taipei City, Taiwan) as per the manufactures instructions.

**Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT)**

GTT was performed at 7 weeks post intervention after 6 h of fasting by intra-peritoneal (IP) injection of 2 g/kg of 25% D-glucose solution. ITT was performed at 8 weeks post intervention during a fed state by IP injection of 0.75 IU of human insulin (Actapid®, Novo Nordisk, Bagsvaerd, Denmark). Tail blood glucose concentrations were measured using a glucometer (Hemocue, Angelholm, Sweden) at time points 0 (pre-bolus basal), 15, 30, 60 and 120 min. Data were expressed as mean blood glucose concentration per group as area under curve (AUC).

**Collection of Mouse Sperm**

Sperm were collected post mortem from the cauda epididymidis and ductus deferens and expressed into 1 ml of G-IVF medium (Vitrolife, Gothenberg, Sweden) and incubated for at least 10 min in 6% CO₂ and 5% O₂ at 37°C (10).

**Sperm count, motility and morphology**

Sperm count, motility and morphology were assessed in accordance with WHO guidelines (86) (at least 200 sperm counted for each sample). Sperm count was determined by counting on a haemocytometer. Sperm motility was assessed blinded under a light microscope classifying 200 sperm per animal as either progressive motile, non progressive motile or immotile. Motility was then expressed as percentage total motile (progressive motile and non progressive motile sperm). Sperm morphology was assessed blinded on samples fixed with methanol:acetone
(3:1) and stained with haematoxylin and eosin. For identifying sperm morphology individual sperm were classified as normal, tail defect (bent tails and twisted tails) or head defect (large heads, small heads and deformed heads) (Fig 1). Morphology is expressed as percentage normal forms.

**Sperm Binding**

Sperm binding to the zona pellucida were performed as described in Bakos et al (10). Briefly mature cumulus-enclosed oocytes (COCs) were collected from 4-week old Swiss female mice 12 hr following super ovulation with intraperitoneal injections of PMSG and hCG administered 48 hr apart. Sperm (30x10^4/ml) were co-incubated with COCs in G-IVF for 1 hr at 37°C, 6% CO₂ and 5% O₂. At 1 hr post insemination, sperm binding was determined by counting the number of bound sperm to the oocyte under phase contrast microscopy. At least 10 oocytes were analysed per sperm sample.

**Capacitation and Acrosome Reaction**

Capacitation and acrosome reaction were measured using *Arachis Hypogaea* (peanut) agglutinin (Lectin PNA; Molecular Probes, Eugene, USA) as previously described (8). Sperm were allowed to capacitate in G-IVF for 1 hr at 37°C, 6% CO₂ and 5% O₂ washed in PBS and incubated in Lectin PNA Alexa 594 antibody (1:100) in PBS for 45 min. Samples were stained with Hoechst to identify sperm nuclei. A minimum of 200 sperm were counted per sample (10).

**TUNEL**
Levels of sperm DNA damage were assessed using an in situ Cell Detection Kit (Roche, Mannhein, Germany) (10). A minimum of 200 sperm were counted per sample (10).

**MitoSOX Red**

The intracellular generation of mitochondrial ROS was determined using MitoSox Red (MSR; Molecular Probes, Eugene, USA) as per Koppers et al (46). Sperm (10^6/ml) were incubated with 0.05µM of MSR and 2µM of SytoxGreen (vitality stain) for 30 min 37°C, 6% CO₂ and 5% O₂. A negative control where sperm were only incubated in SytoxGreen was included. MSR and SytoxGreen fluorescence was measured on a FACSCanto flow cytometer (BD Bioscience, North Ryde, Australia). Non specific sperm events were gated out and 20,000 cells were examined per sample. Results were expressed as percentage of live sperm positive for MSR.

**RedoxSensor Red CC-1**

Cytosolic redox potential of sperm was determined by RedoxSensor Red (RSR; Molecular Probes, Eugene, USA). Sperm (10^6/ml) were incubated with 1µM of RSR and 2µM of SytoxGreen (vitality stain) for 45 min at 37°C, 6% CO₂ and 5% O₂. A negative control where sperm were only incubated in SytoxGreen was included. RSR and SytoxGreen fluorescence measured on a FACSCanto flow cytometer (BD Bioscience, North Ryde, Australia). Non specific sperm events were gated out and 20,000 cells were examined per sample. Results were expressed as mean RSR fluorescence per sperm for each intervention group.
Mitochondrial Membrane Potential (MMP) (JC-1)

Sperm mitochondrial membrane potential was determined by using the ratiometric dye JC-1 (Molecular Probes, Eugene, USA) as previously described by Koppers et al (46). Sperm (10^6/ml) were incubated with 2µM of JC-1 for 30 min at 37°C, 6% CO₂ and 5% O₂. Samples were then washed and counterstained with 0.25mg/ml of propidium iodide (PI) (to exclude dead cells from the analysis). A negative control was also included in which sperm were incubated in 10µM of carbonyl cyanide 3-chlorophenylhydrazone to dissipate membrane potential before incubation in JC-1. JC-1 and PI fluorescence were measured on a FACSCanto flow cytometer (BD Bioscience, North Ryde, Australia). Non specific sperm events were gated out and 20,000 cells were examined per sample. Results were expressed as percentage of live sperm positive for high JC-1.

Glucose and Fructose Uptake

To determine the levels of glucose and fructose uptake per sample, 2x10^6/ml sperm were incubated in G-IVF modified to contain 1mM glucose and 1mM fructose for 1 hr at 37°C, 6% CO₂ and 5% O₂. Levels of glucose and fructose uptake were assessed using ultramicrofluorometric analysis (25) utilising the conversion of the non-fluorescent NADP⁺ to the fluorescent NADPH (64). The regression co-efficient (r²) for all standard curves was >0.99. A medium only control was used to establish the starting levels of glucose and fructose. Glucose and fructose uptake were expressed as the amount of glucose and fructose taken up from the media and expressed as µmol/10^6 sperm/hr.

Statistics
All data were expressed as mean ± SEM and checked for normality using a Kolmogorov-Smirnov test and equal variance using a Levene’s test and log transformed as needed. For weight gain data, body composition and DEXA body composition a one way ANOVA was performed. Sperm JC1, sperm capacitation and sperm MSR were determined by a Chi square test. For all other statistical analysis a univariate general linear model or a Mann-Whitney test for non parametric data was performed. Replicate, cohort of animals and bodyweight were fitted as a covariate where appropriate. Correlation data were determined by a Pearson’s Rho. All statistical analysis was performed in SPSS (SPSS Version 18, SPSS Inc., Chicago, USA). A p value <0.05 was considered to be significant.
RESULTS

**Effect of diet and exercise on whole body physiology**

**Before intervention**

The HFD treatment increased body weight after two weeks on the diet compared with males fed the CD (23.7 ± 0.4g vs. 21.6 ± 0.4g) (p<0.01). At the end of the feeding period, HFD males were heavier (32.1 ± 0.7g vs. 25.1 ± 0.7g) (p<0.001) having gained significantly more weight (13.7 ± 0.5g vs. 7.7 ± 0.4g) (p<0.001, Fig 2A) than those on the CD.

The HFD did not alter fasted serum glucose levels after 10 weeks (10.3 ± 0.2 mmol/L vs 10.2 ± 0.4 mmol/L; HFD and CD males respectively, p>0.05).

**After intervention**

Diet intervention alone (HC) reduced body weight by 7.8% (-2.65 ± 0.99g), while diet and exercise interventions combined (HCE) had a reduction in body weight by 13.9% (-4.82 ± 0.73g) (Fig 2B, 2C and Table 2). Exercise intervention alone (HE) did not alter body weight (-0.03 ± 0.74g), while a continued HFD (HH) led to further weight gain of 10.7% (+3.489 ± 0.33) (Fig 2B, 2C and Table 2). Males fed a CD (CC) continued to also gain weight increasing overall weight by 10.0% (+2.637 ± 0.34g) (Fig 2B, 2C and Table 2).
The HH treatment increased summed individual fat stores (gonadal, peri renal, retroperitoneal, dorsal and omental adiposity) compared with CC, both as a total and as a percentage of total body weight (p<0.01, Table 2). Diet alone and in combination with exercise (HC and HCE) reduced gonadal, retroperitoneal and omental adiposity in absolute terms (p<0.05) and in percentage of total body weight (p<0.05), as well as total body adiposity as measured by DEXA (p<0.05) compared with HH and to a similar extent to CC (Table 2). Exercise alone (HE) did not alter adiposity compared with HH (p>0.05, Table 2). HH decreased the percentage of both lean mass and bone mass as a percentage of body weight compared with CC (p<0.01, Table 2), with interventions using diet alone and in combination with exercise (HC and HCE) significantly improving these masses compared with HH (p<0.05, Table 2). Exercise alone (HE) did not alter lean mass or bone mass as a percentage of body weight compared with HH (p>0.05, Table 2). HH increased liver weights in absolute terms compared with CC (p<0.01, Table 2) however this normalised when expressed as a percentage of body weight. All interventions (HC, HE and HCE) did not alter liver, pancreas kidney or testes weights compared with HH (Table 2). Interestingly, exercise interventions (HE and HCE) reduced seminal vesicle weights in absolute terms compared to HH (p<0.05, Table 2) with this reduction continuing when expressed as a percentage of body weight compared with CC and HC for exercise alone (HE) (p<0.05, Table 2).

Effect of diet and exercise on fasting blood glucose, glucose tolerance and insulin tolerance

After the 8 week intervention, fasting blood glucose was similar in CC, HH, HC and HCE groups, although reduced in diet and exercise combined (HCE) (Table 3).
However, exercise alone (HE) did reduce fasting blood glucose (p<0.05, Table 3). HH did not alter glucose tolerance compared with CC males (p>0.05, Fig 3D) although the clearance of glucose was prolonged in HH males (60mins vs. 30mins). Diet and/or exercise interventions (HC, HE and HCE) improved glucose tolerance, compared with HH (p<0.05, Fig 3A, 3B, 3C and 3D). There was no difference between insulin sensitivity between HH, HC, HCE and CC males (p>0.05, Fig 3E, 3G and 3H). Exercise alone (HE) reduced the response to insulin compared with CC (p<0.05, Fig 3F and 3H), however their starting glucose levels were significantly lower than CC (p<0.05, Fig 3F).

**Effect of diet and exercise on blood lipids**

HH increased serum cholesterol compared with CC (p<0.05, Table 3). Interventions involving a diet component (HC and HCE) reduced serum cholesterol levels compared with HH (p<0.05) with HCE achieving similar levels to that of CC (Table 3). HH did not alter serum FFA compared with CC (Table 3), but these were reduced by interventions involving an exercise component (HE and HCE) with those receiving combined diet and exercise interventions (HCE) significantly lower than HH (p<0.05, Table 3). Serum triglyceride levels were increased by HH compared with CC (p<0.05, Table 3), but normalised by interventions involving a diet component (HC and HCE) (p<0.05, Table 3).

**Effect of diet and exercise on serum corticosterone**

There was no difference in serum corticosterone levels between HH and CC (p>0.05, Table 3). All interventions (HC, HE and HCE) significantly increased serum corticosterone levels compared with CC (p<0.05, Table 3).
Effect of diet and exercise on serum testosterone

HH decreased serum testosterone compared with CC (p<0.05, Table 3) and this was restored by diet intervention alone (HC) to those of CC (Table 3). In contrast, exercise interventions (HE or HCE) did not restore serum testosterone to those of CC, which remained similar to that of HH (p>0.05) and lower than HC (p<0.05, Table 3). Seminal vesicle size was correlated positively with serum testosterone ($R^2=0.31$, p<0.05).

Effect of diet and exercise on basic sperm parameters

HH decreased sperm motility and increased abnormal sperm tail morphology compared with CC (p<0.05, Table 4). Both diet and/or exercise interventions (HC, HE and HCE) increased sperm motility and decreased abnormal sperm tail morphology compared with HH (p<0.05, Table 4). Percentage of tail defects correlated negatively with sperm motility ($R^2=0.41$, p<0.01). Although there was no significant change in sperm count among interventions, HH did decrease sperm count compared with CC, with diet alone (HC) improving this and increasing sperm count similar with CC (Table 4). Sperm concentration also positively correlated with serum testosterone ($R^2=0.29$, p<0.05).

Effect of diet and exercise on sperm capacitation and oocyte binding

Sperm capacitation is required for fertilisation and premature acrosome reactions without initiation by an oocyte cumulus complex are associated with decreased sperm binding and fertilisation rates in humans (51). HH decreased sperm capacitation compared with CC (p<0.01, Fig 4A). Both diet and/or exercise
interventions (HC, HE and HCE) increased the percentage of sperm that had undergone capacitation after a 1hr incubation compared with HH (p<0.01, Fig 4A). HCE also reduced the percentage of spontaneous acrosome reacted sperm compared with HH (p<0.05, Fig 4A). HH reduced sperm binding to the zona pellucida of metaphase two oocytes compared with CC (p<0.01, Fig 4B). Diet and/or exercise interventions (HC, HE and HCE) increased sperm binding compared with HH (p<0.01, Fig 4B) and comparable to those in CC (Fig 4B). Sperm binding also correlated positively with sperm motility (R^2=0.29, p<0.05) and negatively with percentage of sperm with tail defects (R^2=0.30, p<0.05).

The effect of diet and exercise on sperm oxidative stress

HH increased the number of sperm positive for MSR compared with CC (p<0.01, Fig 5A). HC and HE each reduced the number of sperm that displayed MSR compared with HH males (p<0.01) to levels similar to CC (Fig 5A). Diet and exercise combined (HCE) reduced MSR compared with HH (p<0.05), however not to the same extent as did the other interventions (Fig 5A). Diet induced obesity and subsequent interventions did not alter sperm RSR (Fig 5B), although the percentage of MSR positive sperm correlated positively with RSR (R^2=0.74, p<0.001).

The effect of diet and exercise on sperm DNA integrity

HH increased the percentage of sperm with DNA damage compared with CC males (p<0.05, Fig 5C). All interventions (HC, HE and HCE) reduced sperm DNA damage compared with HH (p<0.01) to levels similar to CC (Fig 5C). Furthermore, sperm DNA damage was correlated positively with MSR (R^2=0.49, p<0.01).
The effect of diet and exercise on sperm metabolism

Patients with increasing BMI are likely to display reduced sperm motility (32, 47, 52) we therefore established if this was due to a change in mitochondrial function. MMP is commonly used for determining the health of the mitochondria with increased MMP in oocytes and early embryos being associated with increased ROS and impaired oocyte and embryo development (56, 83, 84). HH increased the numbers of sperm positive for high MMP, as determined by JC-1, compared with CC (p<0.001, Fig 6A). Diet alone and exercise alone (HC and HE) reduced the percentage of sperm with high membrane potential compared with HH (p<0.01, Fig 6A). Diet and exercise combined (HCE) reduced the percentage of sperm with high membrane potential compared with HH (p<0.05, Fig 6A) however not to the same extent did the other interventions. Interesting, the percentage of high JC1 stained sperm correlated positively with sperm MSR (R²= 0.39, p<0.01) and negatively with sperm motility (R²=0.28, p<0.05). When sperm were cultured in a medium containing both fructose and glucose all groups preferentially used fructose as their ATP source compared with glucose (p<0.001, Fig 6B). Nevertheless CC and those subject to diet and exercise combined (HCE) utilised glucose to a higher rate than all other groups (p<0.05, Fig 6C). All groups had similar rates of total carbohydrate metabolism when fructose and glucose were combined (data not shown). Total carbohydrate uptake correlated negatively with percentage of capacitated sperm (R²=0.43, p<0.01).

Physiology and sperm function

Because exercise alone did not normalise adiposity but was able to improve sperm function, we further examined their associations with various metabolic and lipid measures to try and identify any influential factors. Percentage of DNA damaged
sperm ($R^2=0.30$, $p<0.05$, Fig 7A) and percentage of non capacitated sperm ($R^2=0.32$, $p<0.05$, Fig 7B) correlated positively with glucose intolerance as measured by AUC from the GTT, while percentage of normal morphological sperm correlated negatively ($R^2=0.36$, $p<0.05$, Fig 7C). Percentage of sperm positive for high JC1 ($R^2=0.57$, $p<0.01$, Fig 7D) and percentage of sperm with tail defects ($R^2=0.32$, $p<0.05$, Fig 7E) was correlated negatively with insulin sensitivity as measured by AUC from IITT. Sperm motility ($R^2=0.43$, $p<0.01$, Fig 7F) and number of sperm bound to an oocyte ($R^2=0.35$, $p<0.05$, Fig 7G) correlated negatively with serum cholesterol levels, with percentage of DNA damaged sperm correlated positively ($R^2=0.30$, $p<0.05$, Fig 7H). While the percentage of sperm with head defects ($R^2=0.36$, $p<0.05$, Fig 7I) correlated positively with serum triglyceride levels.
DISCUSSION

Here we show for the first time that diet and/or exercise in obese mice can improve sperm function, even in the absence of normalisation of their adiposity and in close association with lipid abundance and other metabolic measures. There is increasing evidence for the deleterious effects of male obesity on circulating sex steroids and sperm parameters (reviewed in 18, 31, 49) with recent rodent models of obesity also demonstrating similar adverse effects (10, 23, 63). However, the extent of reversibility of these changes has had limited studies. Diet and exercise may be a likely approach to reduce adiposity in humans and perhaps normalise semen parameters, with currently only one study showing improved semen parameters via weight loss in severely obese men (29). We therefore used our established mouse model of obesity, to assess the impact of diet and exercise interventions to reduce body weight on sperm function (10, 54, 55, 63). This study for the first time showed that the adverse sperm physiology seen in male obesity such as reduced sperm motility, morphology and increased sperm DNA damage and ROS levels can be reversed through diet and/or exercise. More importantly it showed that the metabolic status of a male including cholesterol abundance and glucose and insulin action is closely associated with sperm function.

Diet and/or exercise interventions in obese mice improved all sperm parameters and functional tests to some extent, with particular improvements in basic sperm parameters of motility, morphology, sperm DNA damage and mitochondrial ROS, parameters commonly altered in obese males (18, 31, 49). In human sperm motility, morphology and concentration are standard parameters for assessing male fertility
Levels of sperm DNA damage and ROS have more recently been used as a marker of sperm health with increased levels in humans correlating with reduced fertilisation, embryonic development and pregnancy loss (4, 11, 17, 27, 80, 88). This suggests that diet and/or exercise could significantly improve fertilisation, blastocyst development and pregnancy rates in obese males by improving sperm motility, morphology, levels of sperm DNA damage and sperm mitochondrial ROS.

For successful fertilisation to occur sperm must first undergo capacitation a process that increases hyperactivity and receptivity to an oocyte and then an acrosome reaction a process that allows penetration to the oocyte through the zona pellucida (39). Obese males are more likely to have reduced sperm binding (10, 85), and reduced fertilisation (9, 55) suggesting a dysfunctional capacity of the sperm to undergo capacitation and an acrosome reaction. In this study all interventions improved the level of sperm that had undergone capacitation and in the percentage of motile sperm, which in turn lead to increased sperm binding suggesting increased fertilisation potential. Therefore, diet and/or exercise could significantly improve sperm binding and fertilisation rates in obese males through improvements to sperm capacitation.

Our study has also for the first time assessed the metabolic profile of HFD sperm by examining MMP and glucose and fructose uptake. All interventions reduced MMP in sperm of obese mice compared with no intervention, with high levels of MMP correlating negatively with sperm motility and increased ROS. This implies that diet and exercise could improve sperm motility in obese individuals by changes to
mitochondrial activity in sperm. The significant but small reductions in both sperm
MMP and ROS levels found in males receiving both diet and exercise intervention
(HCE) may be explained by the double intervention where both decreased caloric
intake and exercise may have increased metabolism and contributed to more ROS
production. Even though the total carbohydrate uptake in sperm was not different
between groups, the increased utilisation of glucose by both lean control and males
receiving diet and exercise interventions combined (HCE) was of interest. Mouse
sperm have GLUT transporters (GLUTs1-3, 5,8-9) on their plasma membrane which
allows glucose and fructose to be transported into the cell and to be used as an
energy source (15). It has been found that glucose, not fructose is needed for
fertilisation and is specifically required during sperm oocyte binding and embryo
viability in the mouse (72). Therefore, the higher rates of glucose utilisation by sperm
of HCE males compared with that of obese controls could be contributing to their
improved sperm binding.

Serum testosterone is commonly reduced in obese men and highly correlated with
sperm count (32, 49). Surprisingly, in this study diet intervention alone (HC) was the
only treatment that improved serum testosterone levels compared with obese
controls, with exercise (HE) or diet and exercise combined (HCE) not doing so.
Weight loss by caloric restriction has been previously shown to improve serum
testosterone levels and sexual desire in obese men (43). This suggests that in our
study exercise may possibly have a negative effect on testosterone production and
why our combined diet and exercise group (HCE) displayed no improvements
compared with obese controls. Previous studies investigating the effects of exercise
on fertility found that exercise training in humans was negatively correlated to serum
testosterone levels (reviewed in 14, 68) and reduced seminal vesicle size in mice (28, 77). However, no relationship was found between physical activity and sperm function in humans (87) suggesting that exercise and male infertility is controversial and maybe dependent on the level of exercise undertaken. In the current study, obese male mice were only subjected to a light exercise regime, displayed no change to testes weights compared with controls and actually displayed improved sperm parameters (suggestive of increased fertility). This indicates that reduced serum testosterone levels may only explain their reduced seminal vesicle size. In this study serum testosterone levels were also correlated positively with sperm count, with exercised males (HE and HCE) showing no improvements to sperm counts compared with lean controls. This indicates that testosterone is likely an indicator of sperm count but not necessarily sperm function.

Increased scrotal heat due to obesity has been proposed as a potential mechanism for the adverse sperm parameters described, with improvements to sperm function and an increased incident of pregnancy after scrotal lipectomies in obese men (74). However, our findings suggest that heat is unlikely to be a plausible mechanism in mice. Males receiving only exercise intervention (HE) displayed no reductions in adiposity compared with HFD controls, however, exhibited improved sperm function with reduced sperm DNA damage, a common sperm parameter elevated by heat exposure (65, 66, 76). This therefore, suggests that heat is likely not the predominant mechanism for the adverse changes to sperm in obese mice from this study.
Our study suggests that adverse sperm parameters due to obesity may not be resultant from testosterone levels or increased adiposity but rather resultant from whole body metabolite changes. The use of diet and exercise interventions in obese male mice resulted in three groups of males with very different metabolic profiles. Diet interventions in obese mice (HC and HCE) resulted in males with reduced levels of adiposity, improved serum cholesterol and glucose tolerance. On the other hand males receiving exercise intervention only (HE) maintained both their level of adiposity, serum cholesterol, and response to insulin similar to findings in the human (82). Interventions including exercise (HE and HCE) reduced fasting serum glucose with an increased clearance of glucose after a GTT and reduced serum FFA. This may have occurred as a result of increased glucose utilisation by skeletal muscle (reviewed by 36) and enhancement of hepatic fatty acid oxidation due to exercise (67). All interventions (HC, HE and HCE) displayed a mild increase in corticosterone levels compared with our lean controls, indicating that a change to diet and/or introduction of exercise could pose as an additional stress to the body. Previous studies using genetically modified rodents found that increased corticosterone was associated with increased weight loss (61, 73).

The environment in which sperm are exposed to during spermatogenesis is vital for sperm function, with particular emphasis on exposure during the epididymal transport. Due to lack of cytoplasmic scavenging enzymes and high levels of polyunsaturated fatty acids in their plasma membrane sperm are highly susceptible to oxidative stress and damage during this transit (2, 3, 6). Exposure to increasing levels of both cholesterol and fatty acids of human and animal sperm in vitro causes increased mitochondrial reactive oxygen species (46) reduced sperm motility,
reduced capacitation and reduced sperm fertilization abilities (70, 71). More importantly, in the current study these lipids showed the highest correlations with sperm parameters with circulating serum cholesterol correlated negatively with sperm motility and sperm binding but positively with sperm DNA damage, while serum triglyceride levels correlated positively with percentage of sperm with abnormal sperm head morphology. Changes to seminal fatty acid content due to fatty acid dietary composition in rabbits suggests that transport ion channels are open between circulating lipids and the epididymal lumen (46). This suggests that changes to structure and dynamics of sperm plasma membrane due to dietary free fatty acids, cholesterol and other metabolite levels during this transit or secretions by epididymal endothelium can change membrane fluidity and therefore increase or reduce susceptibility to damage. This in itself could help explain our improvements to sperm oxidative stress and DNA damage in all intervention animals. In contrast, sperm cholesterol levels are highly important for membrane fluidity and motility with increased cholesterol incorporated into the lipid bi-layer having a negative effect on sperm membrane integrity (19). This could explain why our HFD controls displayed reduced motility compared with all other groups and why exercise alone males (HE) displayed a small reduction in motility compared with the other intervention groups. These findings provide evidence that altered systemic cholesterol, Trigs and FFA abundance and their causes in vivo are associated with changes to membrane dynamics and altered sperm motility, morphology and susceptibility to DNA damage.

Our study also suggests that glucose tolerance was an important indicator of sperm function, with this correlating positively with percentage of non capacitated sperm and percentage of DNA damaged sperm while negatively correlating with percentage
of normal morphological sperm. Further, insulin sensitivity negatively correlated with percentage of sperm positive for high JC1 and percentage of sperm with tail defects. A previous study examining glucose transporter expression of GLUT8 and GLUT9 in sperm and testes of two different genetically modified diabetic mice found that mice lacking GLUT9 protein had low sperm motility and decreased fertilisation rates (44). The authors suggested that lack of insulin or hyperglycemia impaired GLUT9 transcription and concluded that insulin and glucose were important in sperm maturation and had important roles in the sugar movement in sperm which indirectly controlled motility during capacitation and fertilisation (44). Furthermore when these same mice were treated with insulin sperm motility and concentration was significantly increased suggesting that insulin signalling improves sperm quality (44). Therefore the improvements to sperm capacitation, morphology and JC1 status in diet and exercise treated males may be explained by improvements to glucose metabolism and insulin action, possibly at the cellular level for sperm. These data are further evidence that the metabolic status of a male may affect sperm health and that diet and exercise may improve sperm function and fertilisation rates in obese males, in part by improving glucose metabolism.

This study has shown for first time that changes to sperm parameters resultant from male obesity can be reversed by improvements to metabolic status through diet and exercise. This study also provides the first evidence that the metabolic status of obese individuals may be a better indicator of sperm function other than serum testosterone and adiposity levels alone. These findings could potentially help explain the controversy in effects of male obesity on sperm function in the current literature (49), as to date that we are aware of no study looking at male obesity and sub fertility
have taken into account the metabolic status of the individuals. This study therefore shows that diet and lifestyle interventions could be a combined approach to target sub fertility in overweight and obese men by changing their metabolite profile and so improving sperm function. Further studies in the human are needed to determine if similar outcomes result.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Professor David Handelsman and Reena Desai from the ANZAC Research Institute for testosterone measurements and Anne McPherson from the School of Paediatrics and Reproductive Health, University of Adelaide for cholesterol, FFA and triglyceride measurements. This work was supported by NHMRC program grant awarded to ML. ML is the recipient of an NHMRC Senior Research Fellowship. NOP is the recipient of an Australian Postgraduate award and a Prospective Lodge Freemason Medical Scholarship.
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modifies sperm and impairs the metabolic and reproductive health of two subsequent generations. *Cell* (under review), 2011.


Figure legends

Figure 1: Abnormal mouse sperm morphology classifications
A Arrows represent abnormal sperm head morphology. B Arrows represent abnormal sperm tail morphology.

Figure 2: Weight gained/lost post and pre intervention periods
A Total weight gained pre intervention. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. B Total weight gained/lost post intervention. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. C DEXA body composition photos of male's representative of each intervention group at 8 weeks intervention.

Figure 3: The effect of diet and exercise on glucose and insulin tolerance
A Glucose tolerance test of diet intervention alone (HC) compared with HH and CC. Closed circle represent CC, open circles represent HH and closed triangles represent HC. B Glucose tolerance test of exercise intervention alone (HE) compared with HH and CC. Closed circle represent CC, open circles represent HH and closed triangles represent HE. C Glucose tolerance test of diet and exercise intervention combined (HCE) compared with HH and CC. Closed circle represent CC, open circles represent HH and closed triangles represent HCE. D Glucose tolerance test AUC for each intervention. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. Data is representative of 7 HH and 8 HC, HE, HCE and CC males per intervention performed at 7 weeks post intervention. E Insulin tolerance test of diet intervention alone (HC) compared with HH and CC. Closed circle represent CC, open circles represent HH and closed triangles represent HC. F Insulin tolerance test of exercise intervention alone (HE) compared with HH and CC. Closed circle represent CC, open circles represent HH and closed triangles represent HE. G Insulin tolerance test of diet and exercise intervention combined (HCE) compared with HH and CC. Closed circle represent CC, open circles represent HH and closed triangles represent HCE. H Insulin tolerance test AUC for each treatment. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. Data is representative of 7 HH and 8 HC, HE, HCE and CC males per intervention performed at 8 weeks intervention.

Figure 4: The effect of diet and exercise on sperm capacitation and binding
A Percentage of sperm that were either non capacitated, capacitated or acrosome reacted after 1 hr incubation in G-IVF. Black bars represent CC males, light gray bars represent HH males, grey bars represent HC males, white bars represent HE males and dark grey bars represent HCE males. Capacitation and acrosome reaction status equates from 1600 sperm counted representative of 7 males from HH and HCE and 8 males from HC, HE, HCE and CC group. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. B Number of sperm bound to an MII oocyte after sperm and oocytes were incubated together for 1 hr in G-IVF. Number of sperm bound to MII
oocyte equates to at least 50 oocytes representative of at least 7 males per intervention. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05.

Figure 5: The effect of diet and exercise on sperm DNA damage and oxidative stress
A Percentage of sperm positive for MSR for each treatment group. MSR percentage was assessed from at least 140,000 sperm representative of 7 males from HH and HCE and 8 males from HC, HE, and CC groups. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. B Mean fluorescent intensity of sperm for RSR for each treatment group. RSR was assessed from at least 140,000 sperm representative of 7 males from HH and HCE and 8 males from HC, HE and CC group. Data is expressed as mean ± SEM. C Percentage of sperm positive for DNA damage for each treatment group. DNA damaged was assessed from at least 2300 sperm representative of 7 males from HH and HCE and 8 males from HC, HE and CC group. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05.

Figure 6: The effect of diet and exercise on sperm metabolism
A Percentage of sperm positive for high JC1 per treatment group. CCCP negative control for JC1 had 1.9 ± 0.05 positive sperm for high JC1. Mitochondrial membrane potential was assessed from 140,000 sperm representative of 7 males from HH and HCE and 8 males from HC, HE and CC group. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. B Differences between glucose and fructose utilisation in sperm. Differences between glucose and fructose were from 20 male mice. Data is expressed as mean ± SEM. Different letters denote significance at p<0.001. C Carbohydrate metabolism of glucose in sperm from male mice from each treatment group. Glucose metabolism was assessed from 8x10^6 sperm representative of 4 males per intervention. Data is expressed as mean ± SEM. Different letters denote significance at p<0.05.

Figure 7: Metabolic and lipid status and sperm function
A Positive correlation between percentage of DNA damage sperm and glucose AUC (R^2=0.30, p=0.04, n=37). B Positive correlation between percentage of non capacitated sperm and glucose AUC (R^2=0.32, p=0.023, n=38). C Negative correlation between percentage of normal morphological sperm and glucose AUC (R^2=0.34, p=0.02, n=38). D Negative correlation between percentage of sperm positive for high JC1 and insulin AUC (R^2=0.57, p=0.00, n=38). E Negative correlation between percentage of sperm with tail defects and insulin AUC (R^2=0.32, p=0.03, n=38). F Negative correlation between sperm motility and serum cholesterol (R^2=0.43, p=0.01, n=35). G Negative correlation between sperm binding and serum cholesterol (R^2=0.38, p=0.03, n=36). H Positive correlation between sperm DNA damage and serum cholesterol (R^2=0.30, p=0.008, n=36). I Positive correlation between percentage of sperm with head defects and serum triglycerides (R^2=0.36, p=0.02, n=36).
## Table 1: Composition of animal diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CD (SF04-057) Low Fat control</th>
<th>HFD (SF00-219) Harlan Teklad TD88137 Equival</th>
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<tr>
<td>Sucrose (g/100g)</td>
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<td>34.1</td>
</tr>
<tr>
<td>Casein (Acid) (g/100g)</td>
<td>19.5</td>
<td>19.5</td>
</tr>
<tr>
<td>Canola Oil (g/100g)</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td>Clarified Butter (g/100g)</td>
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<tr>
<td>Cellulose (g/100g)</td>
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<td>5.0</td>
</tr>
<tr>
<td>Wheat starch (g/100g)</td>
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<td>15.5</td>
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<tr>
<td>Minerals (g/100g)</td>
<td>4.9</td>
<td>4.9</td>
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<tr>
<td>Digestible energy (MJ/kg)</td>
<td>16.1</td>
<td>19.4</td>
</tr>
<tr>
<td>Digestible energy from lipids (%)</td>
<td>21.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Digestible energy from protein (%)</td>
<td>14.0</td>
<td>17.0</td>
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Table 2: The effect of diet and exercise on body composition

<table>
<thead>
<tr>
<th>Diet/Intervention</th>
<th>CC</th>
<th>HH</th>
<th>HC</th>
<th>HE</th>
<th>HCE</th>
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<tbody>
<tr>
<td><strong>Weight (g)</strong></td>
<td></td>
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<tr>
<td>Total body weight</td>
<td>28.3 ± 0.71^a</td>
<td>34.2 ± 1.23^b</td>
<td>28.4 ± 1.36^a</td>
<td>32.2 ± 1.33^b</td>
<td>29.1 ± 0.91^a^c</td>
</tr>
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<td><strong>Adiposity</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Gonadal</td>
<td>0.66 ± 0.04^a</td>
<td>1.67 ± 0.13^b</td>
<td>0.71 ± 0.16^a</td>
<td>1.34 ± 0.20^b</td>
<td>0.76 ± 0.07^a</td>
</tr>
<tr>
<td>Dorsal</td>
<td>0.31 ± 0.02^a</td>
<td>0.40 ± 0.07^b</td>
<td>0.25 ± 0.05^a</td>
<td>0.38 ± 0.06^bc</td>
<td>0.28 ± 0.04^ac</td>
</tr>
<tr>
<td>Peri-renal</td>
<td>0.06 ± 0.01^a</td>
<td>0.13 ± 0.01^b</td>
<td>0.10 ± 0.02^ab</td>
<td>0.10 ± 0.02^ab</td>
<td>0.07 ± 0.01^a</td>
</tr>
<tr>
<td>Retroperitoneal</td>
<td>0.18 ± 0.02^a</td>
<td>0.40 ± 0.03^b</td>
<td>0.17 ± 0.04^a</td>
<td>0.38 ± 0.07^b</td>
<td>0.20 ± 0.03^a</td>
</tr>
<tr>
<td>Omental</td>
<td>0.15 ± 0.02^a</td>
<td>0.38 ± 0.05^b</td>
<td>0.15 ± 0.04^a</td>
<td>0.27 ± 0.05^bc</td>
<td>0.17 ± 0.03^ac</td>
</tr>
<tr>
<td>Total</td>
<td>1.28 ± 0.11^a</td>
<td>2.98 ± 0.27^b</td>
<td>1.37 ± 0.30^a</td>
<td>2.40 ± 0.39^b</td>
<td>1.48 ± 0.16^a</td>
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<td><strong>Organs</strong></td>
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<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.14 ± 0.05^a</td>
<td>1.53 ± 0.14^b</td>
<td>1.23 ± 0.11^ab</td>
<td>1.34 ± 0.19^ab</td>
<td>1.16 ± 0.07^ab</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.12 ± 0.00^a</td>
<td>0.14 ± 0.01^ab</td>
<td>0.12 ± 0.00^a</td>
<td>0.15 ± 0.01^b</td>
<td>0.13 ± 0.01^a</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.37 ± 0.01^a</td>
<td>0.40 ± 0.03^a</td>
<td>0.40 ± 0.02^a</td>
<td>0.38 ± 0.01^a</td>
<td>0.39 ± 0.02^a</td>
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<tr>
<td><strong>Reproductive Organs</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Testes</td>
<td>0.17 ± 0.01^a</td>
<td>0.17 ± 0.00^a</td>
<td>0.16 ± 0.00^a</td>
<td>0.16 ± 0.01^a</td>
<td>0.17 ± 0.01^a</td>
</tr>
<tr>
<td>Seminal Vesicles</td>
<td>0.34 ± 0.01^ab</td>
<td>0.38 ± 0.01^a</td>
<td>0.33 ± 0.02^ab</td>
<td>0.30 ± 0.04^b</td>
<td>0.31 ± 0.01^b</td>
</tr>
<tr>
<td><strong>% of body weight</strong></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Adiposity</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gonadal</td>
<td>2.31 ± 0.12^a</td>
<td>4.85 ± 0.23^b</td>
<td>2.36 ± 0.46^a</td>
<td>4.04 ± 0.52^b</td>
<td>2.62 ± 0.26^a</td>
</tr>
<tr>
<td>Dorsal</td>
<td>0.86 ± 0.12^a</td>
<td>1.14 ± 0.18^a</td>
<td>0.84 ± 0.13^a</td>
<td>1.17 ± 0.18^a</td>
<td>0.98 ± 0.15^a</td>
</tr>
<tr>
<td>Peri-renal</td>
<td>0.22 ± 0.02^a</td>
<td>0.38 ± 0.03^b</td>
<td>0.34 ± 0.08^ab</td>
<td>0.31 ± 0.05^ab</td>
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<tr>
<td>Retroperitoneal</td>
<td>0.63 ± 0.07^a</td>
<td>1.17 ± 0.07^b</td>
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<td>1.13 ± 0.18^a</td>
<td>0.67 ± 0.10^a</td>
</tr>
<tr>
<td>Omental</td>
<td>0.52 ± 0.07^a</td>
<td>1.09 ± 0.11^b</td>
<td>0.50 ± 0.13^a</td>
<td>0.80 ± 0.15^ab</td>
<td>0.58 ± 0.10^a</td>
</tr>
<tr>
<td>Total</td>
<td>4.55 ± 0.36^a</td>
<td>8.62 ± 0.54^b</td>
<td>4.61 ± 0.83^a</td>
<td>7.21 ± 1.02^b</td>
<td>5.11 ± 0.59^a</td>
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<tr>
<td>Organs</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Liver</td>
<td>4.04 ± 0.12^a</td>
<td>4.52 ± 0.44^a</td>
<td>4.28 ± 0.21^a</td>
<td>4.11 ± 0.48^a</td>
<td>4.00 ± 0.23^a</td>
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<tr>
<td>Pancreas</td>
<td>0.42 ± 0.02^ab</td>
<td>0.40 ± 0.03^a</td>
<td>0.43 ± 0.02^ab</td>
<td>0.48 ± 0.03^b</td>
<td>0.44 ± 0.04^ab</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.32 ± 0.05^ac</td>
<td>1.15 ± 0.05^b</td>
<td>1.40 ± 0.03^a</td>
<td>1.21 ± 0.07^bc</td>
<td>1.36 ± 0.07^ac</td>
</tr>
<tr>
<td>Reproductive Organs</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testes</td>
<td>0.60 ± 0.03^a</td>
<td>0.55 ± 0.02^b</td>
<td>0.59 ± 0.02^ab</td>
<td>0.51 ± 0.03^b</td>
<td>0.60 ± 0.03^a</td>
</tr>
<tr>
<td>Seminal Vesicles</td>
<td>1.18 ± 0.03^a</td>
<td>1.10 ± 0.06^ab</td>
<td>1.15 ± 0.05^a</td>
<td>0.93 ± 0.11^a</td>
<td>1.08 ± 0.03^ab</td>
</tr>
<tr>
<td><strong>% of body weight</strong></td>
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<tr>
<td>Adiposity</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Gonadal</td>
<td>15.3 ± 0.82^a</td>
<td>24.7 ± 0.98^b</td>
<td>13.0 ± 2.52^a</td>
<td>25.2 ± 2.38^b</td>
<td>15.6 ± 1.00^a</td>
</tr>
<tr>
<td>Dorsal</td>
<td>79.1 ± 0.96^a</td>
<td>72.0 ± 1.16^b</td>
<td>83.0 ± 2.56^a</td>
<td>70.1 ± 2.82^b</td>
<td>80.3 ± 2.02^a</td>
</tr>
<tr>
<td>Total Bone</td>
<td>1.36 ± 0.01^a</td>
<td>1.07 ± 0.05^b</td>
<td>1.33 ± 0.05^ac</td>
<td>1.15 ± 0.14^bc</td>
<td>1.29 ± 0.04^ac</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. ^denotes difference to HE at p=0.06. Different letters denote difference at p=0.07 for dorsal fat (g). *denotes difference to HH at p=0.07. Adiposity and organs data is representative of 7 HH and 8 CC, HC, HE and HCE males. DEXA body composition is representative of 3 HH and 4 CC, HC, HE and HCE males.
Table 3: The effect of diet and exercise on blood metabolites, corticosterone and testosterone levels post intervention

<table>
<thead>
<tr>
<th>Diet/Intervention</th>
<th>Glucose (mmol/L⁻¹)</th>
<th>Cholesterol (mmol/L⁻¹)</th>
<th>FFA (mmol/L⁻¹)</th>
<th>Triglycerides (mmol/L⁻¹)</th>
<th>Corticosterone (nmol/L⁻¹)</th>
<th>Testosterone (nmol/L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>10.4 ± 0.53abc</td>
<td>2.79 ± 0.26a</td>
<td>1.01 ± 0.04ab</td>
<td>0.60 ± 0.04a</td>
<td>535.8 ± 10.6a</td>
<td>1.73 ± 0.48abc</td>
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<tr>
<td>HH</td>
<td>11.2 ± 0.56a</td>
<td>4.53 ± 0.28b</td>
<td>1.07 ± 0.04b</td>
<td>0.76 ± 0.04b</td>
<td>559.0 ± 20.0ab</td>
<td>0.17 ± 0.47b</td>
</tr>
<tr>
<td>HC</td>
<td>10.7 ± 0.52ac</td>
<td>3.27 ± 0.26a</td>
<td>1.04 ± 0.08ab</td>
<td>0.63 ± 0.04a</td>
<td>601.7 ± 25.4b</td>
<td>1.28 ± 0.44a</td>
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<tr>
<td>HE</td>
<td>9.2 ± 0.50b</td>
<td>4.34 ± 0.31b</td>
<td>0.95 ± 0.05ab</td>
<td>0.75 ± 0.05b</td>
<td>618.4 ± 16.0b</td>
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<tr>
<td>HCE</td>
<td>9.7 ± 0.52bc^</td>
<td>2.81 ± 0.28a</td>
<td>0.89 ± 0.04b</td>
<td>0.59 ± 0.04a</td>
<td>596.3 ± 7.7b</td>
<td>0.40 ± 0.48bc</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. ^different to HH p=0.06. Data is representative of 5 males per treatment group for corticosterone and at least 7 males for glucose, cholesterol, FFA, trigs and testosterone.
Table 4: The effect of diet and exercise on simple sperm parameters

<table>
<thead>
<tr>
<th>Diet/Intervention</th>
<th>Count (x10^6/ml)</th>
<th>Motility (%)</th>
<th>Normal forms (%)</th>
<th>Abnormal tails (%)</th>
<th>Abnormal heads (%)</th>
</tr>
</thead>
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<tr>
<td>CC</td>
<td>18.4 ± 2.24 a</td>
<td>79.8 ± 3.29 a</td>
<td>61.9 ± 1.82 a</td>
<td>23.8 ± 1.43 a</td>
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<td>HH</td>
<td>14.7 ± 2.40 a</td>
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<td>30.3 ± 1.53 b</td>
<td>14.4 ± 1.68 ab</td>
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<td>18.4 ± 2.24 a</td>
<td>79.1 ± 3.29 a</td>
<td>59.6 ± 1.83 a</td>
<td>24.4 ± 1.43 a</td>
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<td>75.0 ± 3.29 a</td>
<td>63.0 ± 1.82 a</td>
<td>25.0 ± 1.44 a</td>
<td>12.1 ± 1.57 a</td>
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<tr>
<td>HCE</td>
<td>15.2 ± 2.40 a</td>
<td>76.6 ± 3.53 a</td>
<td>62.4 ± 1.95 a</td>
<td>24.3 ± 1.53 a</td>
<td>14.2 ± 1.58 ab</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SEM. Different letters denote significance at p<0.05. Motility represents both progressive motility and non progressive motility. Data is representative of ≥1400 sperm counted from 7 males from HH and HCE and 8 males from CC, HC and HE.