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Role of Fish Oil in Paternal Obesity and in Offspring Skeletal Muscle Health

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Abstract

This study investigates the effects of fish oil in father male mice and how it could potentially affect skeletal muscle health of the next generation. Thirty male mice were fed either a low-fat diet (LF), High-Fat Diet (HF), and a High-Fat diet supplemented with fish oil (FO) for 10 weeks to mimic paternal obesity. They were then mated with female mice on chow diet and all mice consumed chow diet during gestation and lactation. Offspring mice were supplemented with chow diet for 16 weeks and half of the mice were sacrificed at 8-weeks and rest at 16-weeks to study the short-term and long-term effect of FO respectively. Results showed that the 8-week offspring born to father fed FO had significant upregulation of genetic markers associated with insulin signaling, and skeletal muscle growth and down regulation of genetic markers of fatty acid synthesis. These findings suggest potential benefits of paternal supplementation of fish oil to compensate for a long-term exposure of HF.

Role of Fish Oil in Paternal Obesity and in Offspring Skeletal Muscle Health

by

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Literature Review

1. Obesity

1.1 Obesity in general (Prevalence, epidemiology data from past to present)

Obesity is defined as an excessive accumulation of adipose tissue throughout the body and is characterized by a higher body mass index (BMI). BMI is defined as the weight in kg divided by height in meter squared. BMI between 18.5-25 is classified as healthy weight. BMI under 18.5 is classified as underweight and BMI between 25-30 is classified as overweight. BMI over 30 is classified as obese and is divided into three classes of obesity according to the US Centers of Disease and Control and Prevention (CDC). Class 1 is between range of 30 – 35, and class 2 is between 35 to 40.[1] Class three is called severe obesity and has a BMI over 40. According to the CDC, the US obesity prevalence has increased from about 30.5% to 42.5% from 1999 to 2017.[2] Rates of severe obesity also increased from 4.7% to 9.2% in the same period. Figure 1 depicts the current obesity rates based on self-reported results retrieved from NHANES. Most states in the US have obesity rates greater than 25% (Fig.1), and obesity similarly affects all age groups and sexes (Fig, 2).

Central obesity is referred to the accumulation of excessive fat in the abdominal or trunk region compared to the hip and lower extremities of the body. It is associated with Type 2 diabetes mellitus, (T2D) cardiovascular disease, multiple types of cancer, and other life-threatening chronic disorders. In US, the prevalence of obesity is consistently rising over the years.[3] Furthermore, risk for cardiovascular disease is greater in populations with high waist circumference regardless of their BMI. For men, the risk significantly increases if the waist circumference is over 102 cm and 88 cm for women respectively.[4]

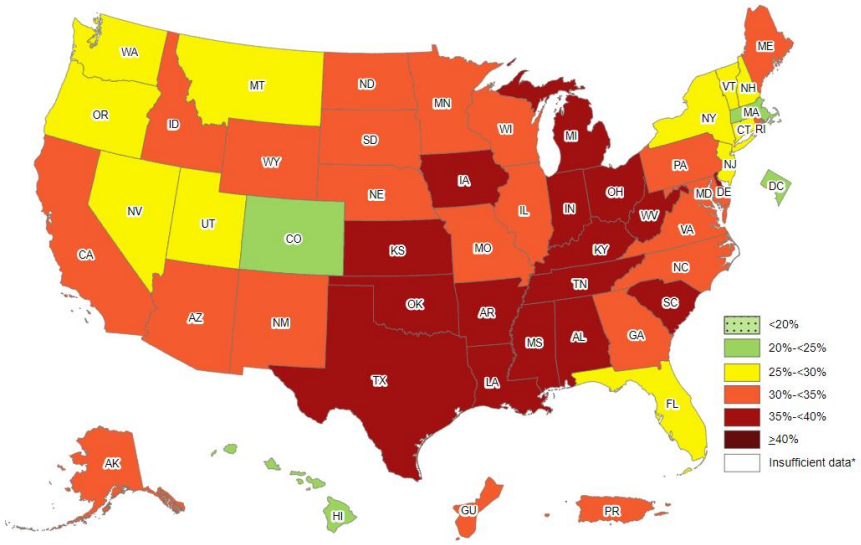


Figure 1: Obesity prevalence map in 2020[5]

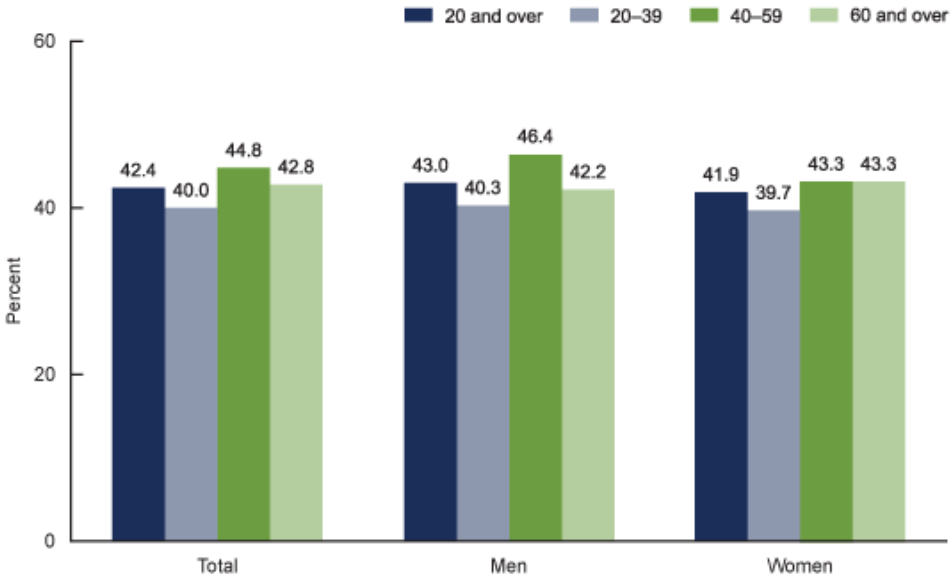


Figure 2: Prevalence of obesity of adults over 20 years old from 2017-2018[2]

1.2 Contributors to obesity

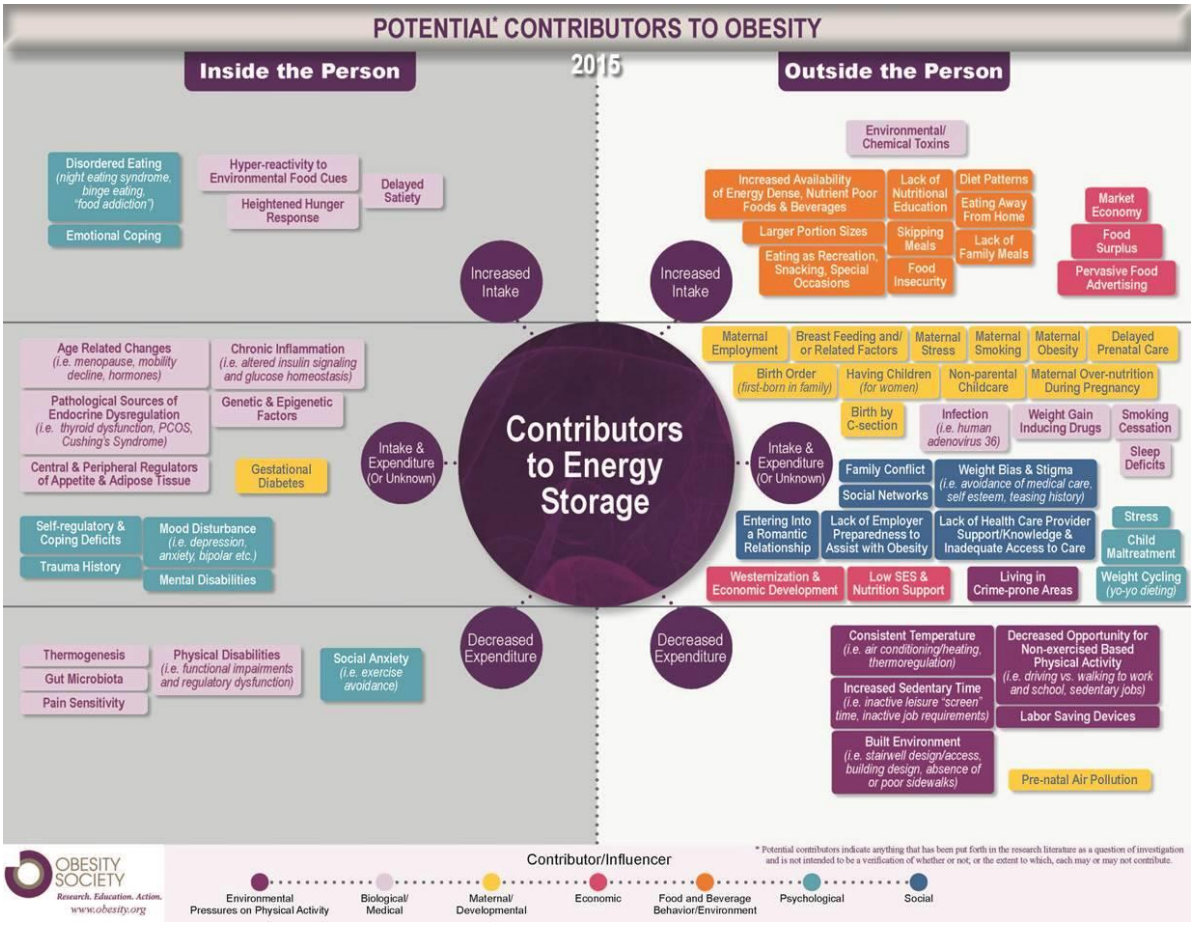


Figure 3: Endogenous and exogenous factors contributing to obesity (increased energy storage).[6]

As illustrated in Fig. 3, there are several contributors to obesity based on both endogenous and exogenous factors. The exogenous factors include environmental exposures, such as availability of energy-dense food, unhealthy food habits (increased snacking behaviors, skipping meals etc.), and food insecurity. These factors are important and should be highlighted from societal and public health perspectives to address obesity. Endogenous factors, on the other hand means

specific processes that lead to obesity inside the body triggering epigenetics, oxidative stress, insulin resistance, and mitochondrial dysfunction. For the thesis, I have focused predominately on genetic influence and the ways in which it contributes to obesity. In the latter sections, insulin resistance, chronic inflammation, oxidative stress, and mitochondria dysfunction are discussed in detail.

1.2.1 Environmental impact during prenatal and neonatal periods.

Obesity is prone to develop in the early stages of life. During prenatal and neonatal periods, metabolic imprinting takes place at epigenetic and genomic levels. Metabolic imprinting refers to the factors that impact low birth weight of infants that leads to potential obesity in children. Birth weight is impacted profoundly by gestational weight of the mother. Based on the study done by Hivert et al., the gestational weight gain, especially during the first trimester is directly associated with childhood obesity.[7] The adiposity in the offspring is more profound if the BMI of the mother is over 30 at pregnancy onset. Further, food consumed by the mother has an impact on the birth weight. According to the study done by Ling-Wei Chen et al., the more carbohydrates the birth mother consumes during pregnancy, the higher the BMI of the offspring.[8] According to the study, BMI increases with increased sugar intake. On the other hand, many studies have revealed that sugar intake is also associated with gestational weight gain, which is another risk factor for infant obesity.[9,10] High sugar intake during pregnancy is also associated with higher risk of childhood obesity and potentially could lead to diseases such as Type 2 Diabetes (T2D) and cardiovascular disease in the offspring. In two animal studies, high fructose intake by the mother resulted in multiple metabolic dysfunctions, including insulin resistance,

hyperlipidemia, increased oxidative stress and increased expression of lipid synthesis genes, FAS (fatty acid synthase) and ACC (acetyl-CoA carboxylase) in adipose tissue.[9,10]

1.3 Obesity and Health Consequences

1.3.1 Obesity

There are two types of fat in the body. One is brown fat, and the other is white fat. Brown fat is located in the upper spine near the scapular region to provide energy. Brown fat is packed with mitochondria and could be used for energy rapidly. The proportion of brown fat decreases with the age[11]. The release of brown fat depends upon triggering sympathetic nervous system to release catecholamines, epinephrine, and norepinephrine. Those hormones then bind to the Beta-3 adrenergic receptors embedded in brown fat, providing energy. As people age, brown tissue generation is decreased due to decreased sensitivity of Beta-3 receptors as well as decreased mitochondrial numbers[11].

Another type of fat tissue is the white adipose tissue, which is more abundant than brown fat in adults. The storage of white adipose tissue is mainly found subcutaneously and in between organs (visceral fat).[12] With obesity, there is hyperplasia in adipose tissue, an increase in cell numbers along with hypertrophy. Adipose hypertrophy refers to the enlargement of adipose cells that results in limiting the ability to generate new adipose cells. Adipose hypertrophy could further lead to rupture of cell walls gradually, causing chronic low-grade inflammation. Rupturing of the cell walls could also lead to release of pro-inflammatory adipokines. These adipokines released from adipose tissue contribute to insulin-resistance as some of the adipokines could interfere with glucose and lipid metabolism [13].

1.3.2 Chronic inflammation in Obesity

As mentioned before, adipose tissue feeds on excessive energy, and uptake of fatty acids is possible through two mechanisms: hyperplasia and hypertrophy. The latter contributes to increased secretions of proinflammatory adipokines, including TNF-alpha, interleukin-1 beta, and interleukin-6. Those adipokines enter the blood flow and result in systemic inflammation. TNF-alpha is produced by macrophages via NFKB pathways. NFKB is triggered by an accumulation of lipid in adipose tissue following hypertrophy. As a result, it triggers the release of proinflammatory cytokines as mentioned above. TNF-alpha could induce lipolysis, following which, free fatty acids and saturated fatty acids are released from adipose tissue. Saturated fatty acids could bind to Toll like Receptors 4 on the surface of adipocytes and trigger the NFkB pathway. The systemic inflammation contributes to many chronic and metabolic diseases, such as T2D, hyperlipidemias, and atherosclerosis. One study has shown that decreased level of inflammation markers including TNF-alpha and interleukins contribute to rebound insulin resistance.[14] The study has demonstrated that mice lacking p55 (TNF receptor) showed improved insulin sensitivity when compared to mice lacking p 75 (another TNF receptor). The study indicated the specific receptor that linked with insulin sensitivity as well as inflammation and highlighted the pathological role TNF potentially played.

1.3.3. Insulin production and action.

Insulin is a hormone that is released when there is an influx of glucose coming into the body, which is also called the fed state. The hormone is released from beta cells of pancreas. During this period, the influx of glucose enters pancreatic beta cells via GLUT2 and thus contributes towards the increased ATP/ADP ratio. The increased ratio inhibits the opening of ATP dependent potassium channel, triggering membrane depolarization that leads to the activation of voltage-gated calcium channel, stimulating the release of insulin-containing vesicles.[15] After the

release, it binds to the insulin receptor embedded across the cell membrane of different tissues, such as muscle, liver, and adipose tissues. The insulin receptor is a tyrosine kinase receptor, which is shaped like a dimer with each one containing two parts: alpha and beta. The alpha region is the extracellular binding site for insulin, and beta site is the part across the cell membrane reaching the intracellular cytoplasm. Upon binding, the beta unit is activated and autophosphorylation takes place. The activated receptor then binds and activates insulin receptor substrate (IRS). Activated IRS recruits phosphatidylinositol 3-kinase (PI3K) and phosphatidylinositol-4,5 -bisphosphate, resulting it to convert into phosphatidylinositol- 3,4,5 – trisphosphate (Ptdlns (3,4,5) P3). This molecule binds to phosphoinositide-dependent protein kinase-1 (PDK1), PDK2, and protein kinase B (AKT), resulting in a cascade of phosphorylation events that will eventually lead to the translocation of GLUT 4 vesicles to cell surfaces. GLUT 4 is a transporter that is important for up taking glucose into tissues.

T2D is the impairment of glucose uptake and utilization. The prevalent clinical diagnose of T2D includes A1C test, glucose tolerance test, and fasting glucose level test. The A1C test is performed by measuring the mean blood glucose level over few months. A measured level over 6.5% indicate T2D.[16] If the measured glucose levels of an individual on an overnight or 7-8 hours fast is over 126 mg/dL, then it is an indication for T2D.[16] Glucose tolerance test measures the blood glucose level before and after 2 hours an individual has consumed carbonated liquid. After the time interval, blood glucose level of over or equal to 200 mg/dL is considered T2D.[16] These are the methods to indirectly measure insulin resistance. Methods such as Hyperinsulinemic euglycemic clamp (HEC) takes more time and might not be cost-effective[17] to measure insulin resistance.

Insulin resistance refers to body's decreased responsiveness to insulin. It is the main symptom for T2D and affects adipose, muscle, and liver due to less responsiveness to insulin. The symptoms of insulin resistance mainly include hyperinsulinemia, hyperlipidemia, and hyperglycemia. The high circulating insulin is due to the reduced sensitivity to insulin. The circulating glucose levels remains high. Consequently, beta cells of pancreas keep secreting insulin to decrease circulating glucose level. The major cause of high circulating resistance is the increased load of energy or, in other words, long-term use of high-fat diet. The beta cells secrete large loads of insulin after digesting a high-energy meal, which gradually leads to insulin resistance of muscle, hepatic, and adipose tissues. The high-energy meals also contributes to beta cell dysfunction.[18] The signaling defect secondary to decreased numbers of insulin receptors account for insulin resistance in skeletal muscle tissues. The IRS1 tyrosine phosphorylation is decreased in insulin-resistant skeletal muscle tissues.[19] Consequently, the downstream kinases activity is decreased, including PI3K and AKT2. The decreased signaling leads to decreased translocation of GLUT4 and contributes to the decreased muscle functioning to generate and store glycogen. The specific mechanism for insulin resistance in skeletal muscle is unclear.

The mechanism for insulin resistance is incomplete, but there are few theories for explanation.
1. The first theory was proposed in 1963 by Philips Randle. The proposed mechanisms emphasized the competition between glucose and fatty acid in muscle and adipose tissue.[20] The theory demonstrated the reciprocal relationship between glucose and fatty acid: an excessive fatty acid supply drives predominant fatty acid oxidation while shutting down the glucose oxidation pathway, and vice versa. In fasted state, where lipolysis is stimulated by glucagon, fatty acid oxidation (beta-oxidation) is increased, which then leads to increased Acetyl CoA level

in the mitochondria of skeletal muscle, liver, and other tissues that could utilize fatty acid for energy.[19] The increased acetyl CoA allosterically inhibit pyruvate dehydrogenase, resulting in accumulation of pyruvate in cytosol. In addition to that, the increased Acetyl CoA is transferred into citrate in cytosol and inhibit phosphofructokinase. Decrease in both PDH and PFK contributes to the accumulation of intracellular glucose level. However, the theory was proven to be irrelevant for insulin resistance in human. It was later discovered that instead of the increased glucose concentration in the cell, it is the glucose concentration that is decreased along with glycogen synthesis due to the impaired GLUT4 translocation. The GLUT4 translocation depends on the cell signal pathway of PI3K and consequently, the distribution of the signal is the main contributor to decreased GLUT4 translocation.

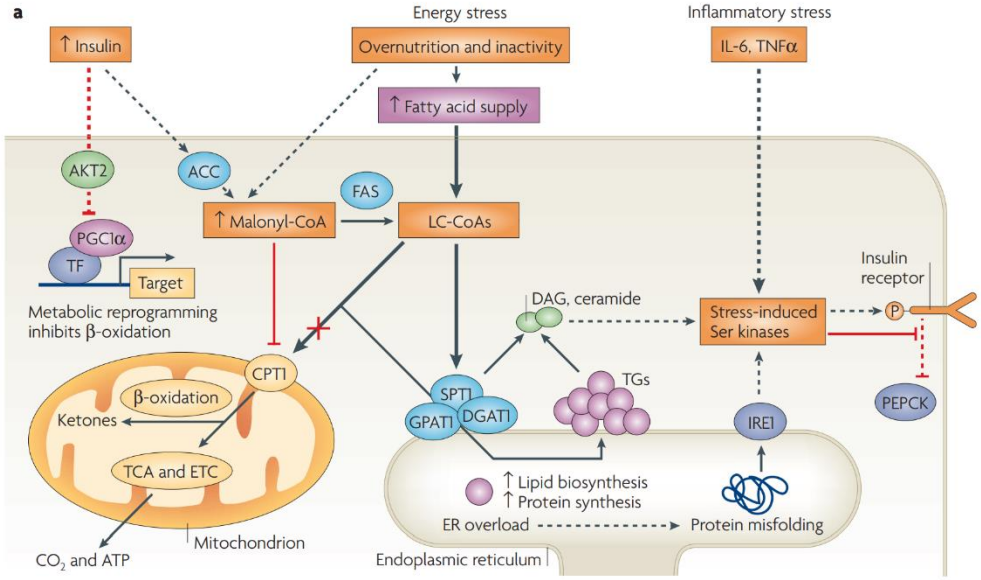


Figure 4: The interrelation of chronic inflammation and obesity on insulin resistance.[21]

2. The second theory focused on the metabolic load on liver due to obesity. The theory proposed that hepatic insulin resistance is induced by accumulation of fatty acid resulting from impaired

fatty acid oxidation.[19] As depicted in figure 4, large influx of glucose upregulates glycolysis and enters the TCA cycle. The rate controlling substance oxaloacetate tends to accumulate, thus the rate of the TCA cycle is decreased. The excessive oxalacetate reacts with acetyl CoA and gets converted to citrate. The increased level of citrate inhibits the phosphofructokinase, thus limiting the TCA cycle by decreasing pyruvate generation. The excess citrate then crosses the cytosol, where it is converted to malonyl-CoA first, and then to fatty acid. Insulin helps activate acetyl CoA carboxylase (ACC) by dephosphorylation. In addition to that, insulin also inhibits beta oxidation by inhibiting PPAR alpha. PPAR alpha is a transcription factor that regulates beta-oxidation. ACC breaks citrate into acetyl CoA and oxalacetate, where oxalacetate is converted to pyruvate and acetyl CoA is converted to make malonyl-CoA, and eventually to fatty acid CoAs. The compound will be further repackaged into triglycerides in the endoplasmic reticulum, and then transferred to different parts of the body in the forms of VLDL, HDL, and LDL. In mitochondria, where the lipid synthesis takes place due to the large influx of fatty acid resulting from overnutrition and obesity, protein misfolding could take place. Therefore, serine kinases are generated. It has been found that serine kinases negatively affect the effectiveness of insulin by inhibiting the AKT, (protein Kinase B) thus affecting the release of GLUT4 transporter to uptake glucose into cells.

1.3.3 Oxidative Stress and obesity

Oxidative stress is characterized as the imbalance of free radicals and antioxidants, creating excess free radicals that are detrimental to the body, including affecting the cell membrane integrity, contributing to protein misfolding, and affecting DNA structure as well. Free radicals include reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, hydroxyl radical, and nitric oxide. Oxidative stress is closely related to obesity as many of the free radicals

are released as the byproduct of macronutrient metabolism. First, fatty acid metabolism in peroxisome tend to induce production of hydrogen peroxide, which leads to oxidative stress. On the other hand, the enzymatic activity of cytochrome P450, which is one of the steps of electron transport chain of ATP synthesis of glucose, could also generate superoxide anion resulting from accidental binding of electrons to oxygen. For obese populations, the increased need for energy triggers a higher activation rate of TCA cycle, which further puts them at a greater risk for generating high oxidative stress. Other than that, the activation of immune system contributes to an increased level of ROS because of macrophage and T cells activation.[22] As previously mentioned, the release of different adipokines manages to generate different cytokines that subsequently contribute to oxidative stress by producing more ROS. Levels of Leptin—a type of hormone that regulate satiety—is found to be at an increased state in obese populations. The effect of leptin includes increase in insulin resistance by promoting lipolysis and inhibiting lipogenesis. It is a hormone that increases satiety and slowly builds up with increased influx of glucose into the body. The increased level of leptin activates macrophages causing it to increase C-reactive protein as well as inflammatory effect and oxidative stress.[23] TNF-alpha is also released from the adipocyte, which contributes to chronic inflammation. TNF-alpha also activates NF-KB, resulting in further inflammation responses, which consequently increases the oxidative stress more. Other cytokines such as interleukin 6, angiotensinogen, and resistin share similar functions. The biomarkers for oxidative stress include malondialdehyde (MDA) and F-2 isoprostanes (F2-IsoPs) that are found to accumulate in high BMI populations in multiple studies.[24,25]

1.3.4 Oxidative stress and muscle function

The production of ROS has long been considered as a cause of affecting skeletal muscle function. It has been revealed that in obese populations, the increased ROS production could be responsible for muscle atrophy, and contractile malfunction that could eventually develop to sarcopenia.[26,27] It has been explained that ROS could affect cell signaling related with calcium release, therefore contributing to calcium release.[27] The production of ROS in skeletal muscle stems from the activity of NADPH and mitochondrial respiration.[28] Some studies have shown that the level of ROS is linked with numbers of mitochondria that is connected with a type of muscle fibers. There are two types of muscle fibers in the body: type I and type II fibers. Type I fiber is resistant to fatigue due to the increased number of mitochondria, and production of energy relies on oxygen. On the other hand, type II fibers are less resistant to fatigue and have less numbers of mitochondria. The energy supply is mainly glycolytic. The further classification of type II fibers includes type IIa and type IIb. The difference is related with fiber shifting that type IIa can transform to type I fiber, but not type IIb. Studies have revealed that type I fiber is more resistant to oxidative stress compared with type II fibers. Such studies have stated that due to the large mitochondria numbers, the endogenous antioxidant system is more efficient to decrease the concentration of ROS in type I dominant muscles.[29,30] In obese population, the number of type I fiber tend to be low in numbers, and thus they are more likely to be affected by high ROS concentration. In addition to that, some research has pointed out that a chronic high-fat diet could contribute to an increased oxidative stress in all types of skeletal muscle due to electron transport chain and lipid peroxidation.[30] Some literature has also cited the importance of exercise to endogenous antioxidant system.[31,32] Although high-intensity exercise for untrained persons have shown to increase the production of ROS from rapid mitochondrial respiration, the same effect was not detected in trained persons.[33] In trained person, ROS production is countered

by improved antioxidant capacity and mitochondrial biosynthesis.[27] TNF-alpha is linked with high ROS production and lipid peroxidation. Skeletal muscle dysfunction is associated with TNF-alpha concentration.[34] It has been found that TNF alpha is related with protein catabolism in muscle and is also linked to insulin resistance in skeletal muscle.[34] Type II fibers is the location where TNF-alpha is mostly secreted in skeletal muscle.[35] Satellite cell is the stem cell that is responsible for the production of skeletal muscle cells. The building up of ROS could potentially affect the number of satellite cells in mice.[36]

1.3.5 Mitochondrial dysfunction in obesity

Mitochondria is a powerhouse of the body that provides energy in the form of ATP. Mitochondria is also important for both production and elimination of ROS. Mitochondrial dysfunction is defined as the limited capability to generate ATP. In addition to that, the term is also used to describe abnormalities in mitochondrial functions related with catabolism, DNA mutations, gene expression, changes in size, ROS production, and apoptosis or mitochondrial fission.[37] Many studies have illustrated that obesity is related to mitochondrial dysfunction.[37,38] Obesity is related to increased nutrient intake, especially saturated fatty acid intake. As mentioned before, insulin blocks beta oxidation to facilitate lipid synthesis, and the decrease in beta oxidation could result in compromised mitochondrial function and vice versa. As some studies have demonstrated, the compromised mitochondria also contributes to the decreased generation of mitochondria as evidenced by decreased mitochondria biogenesis followed by mitochondrial dysfunction[39,40]. The fatty acid pathways are altered, including lipid synthesis, beta oxidation, and adiponectin production due to mitochondrial dysfunction. Some research has observed that in skeletal muscle, obese mice have smaller mitochondria with decreased

mitochondria numbers in muscle tissues.[41] In addition to that, reduced beta oxidation and glucose transport via GLUT4 is also observed in skeletal muscle.[38] The specific mechanism leading to skeletal muscle insulin resistance and mitochondrial dysfunction remain unclear. On the other hand, mitochondrial dysfunction in liver is largely due to a high-fat diet. The oxidation capacity of mitochondria and mitochondria numbers are both decreased in obese diabetic mice.

1.3.6 Mitochondrial dysfunction in skeletal muscle

Many studies have found that obesity is linked with mitochondrial dysfunction, and decrease in mitochondrial size, number, and limited functions have been observed.[37,38,40,41] To explain this phenomenon, two terms are introduced that are related with mitochondrial dysfunction: mitochondrial fission and mitochondrial fusion. Mitochondrial fission is characterized by the process of mitochondria degradation and mitochondrial fusion. On the other hand, mitochondria fusion defined as optimization of mitochondrial functions and structures. Both processes are regulated by a combination of genes. The main proteins that are involved in fusion process are mitofusin 1 and 2 (Mfn1 and 2) and autosomal dominant optic athrophy-1 (Opa 1).[42] Mfn proteins control the fusion of outer mitochondrial membrane and Opa 1 controls the fusion of inner mitochondrial membrane. Mfn2 has additional role in connecting mitochondria and the ER in terms of function and structure. It is possible that the expression of Mfn2 is linked to ER stress and insulin resistance. For Mitochondrial fission processes, dynamin related protein 1 (Drp1) and fission protein 1 (Fis1) are the main proteins that activate the process.[41] Drp1 is in cytosol, and it regulates apoptosis. The balance of both processes determines the mitochondrial functions. Diet is thought to contribute largely on mitochondrial functions. High saturated fat diet is closely associated with mitochondrial functions in skeletal muscle resulting from increased ROS production and imbalance of mitochondrial fusion and fission. The high fat diet contributes to

decrease in Mfn2 and increase in Drp1 and Fis 1 that further leads to decreased numbers of mitochondria.[41] In addition to that, a high fat diet induces fatty acid utilization in mitochondrial, and results in massive production of ROS. The production that could affect the structure and cell membrane integrity of mitochondria and is linked with mitochondrial structure changes in terms of size and function. Those findings are predominately present in skeletal muscle in multiple studies[38–40].

2 Muscle and obesity

2.1 Intramyocellular lipid concentration and insulin resistance

The lipid or fat droplets stored in the muscle cells are called intramyocellular lipid (IMCL). Many research studies have pointed out that IMCL is related with insulin resistance and obesity amongst the insulin-resistant populations or T2D populations.[43,44] The following two subsections details two major IMCL: ceramides; and diacetyl.

2.2 Ceramides

Ceramides are the products of long-chain fatty acyl-CoA palmitoyl-CoA and belongs to part of sphingolipids. Their rate of production depends on the exogenous saturated fatty acid supply, so obese populations tend to have a higher rate of ceramides buildup in the body.[45] The site of de novo production of ceramides is in the ER. Ceramides are also produced in response to increased oxidative stress by triggering the breakdown of sphingomyelin around the myelin sheath. Muscle ceramide content is closely associated with skeletal muscle insulin resistance. Ceramides inhibits or interfere with the insulin signal transduction pathway by inhibiting AKT or PKB pathway. [46] It is a signaling transduction pathway that responsible for release of insulin to cell membrane. As a result, GLUT4 translocation to sarcolemma is largely affected, and insulin resistance persists. Studies have noticed that ceramides could inhibit electron transport chain at Complex III and

contribute to increased ROS production due to increased TNF-alpha production.[47] Exercise is thought to increase insulin sensitivity, and partial reason for that is due to the decreased ceramides levels after exercise[48].

2.3 Diacetyl

Diacetyl functions as the second messengers at sarcolemma and is also one of the intermediates in triglycerides production. It is also the by-product of breakdown of lipid droplets. The elevated diacetyl levels contribute to the increased activity of Protein Kinase C (PKC). The activated PKC phosphorylates the insulin receptor substrate 1 (IRS-1) and inhibit the downstream activation of Akt, which are important signaling molecules for GLUT4 translocation. The research about diacetyl is equivocal as some research have showcased a clear association between the level of diacetyl and insulin resistance and other research demonstrated no association.[49,50] In one study, it has been demonstrated that it could improve insulin-resistance if the insulin-resistance is triggered with a high-unsaturated diet. The reason might be due to the different isomers of diacetyl; and only one diacetyl (1.2-DAG) has been found to be related with insulin resistance.[44(p2)] Although DAG and ceramide both contribute to IR due to ectopic lipid accumulation, the mechanisms differ. For example, one study showed that among athletes, decreased ceramides and increased DAG is associated with decreased insulin sensitivity.[48] There are also studies about increased insulin activities that effect DAG level and not ceramide level. Those studies suggested that degree of saturation or the ratio of saturated fatty acids and unsaturated fatty acids might play a role in distinguishing two different insulin resistance. The specific mechanism of how separate substances could contribute to insulin resistance remains unclear.

3 Paternal Obesity

3.1 Paternal obesity in general

Paternal obesity refers to the obesity in the father of the offspring. Half of the genetic information is provided by the father of the offspring. Maternal obesity, on the other hand, is defined as obesity in birth mother. Compared with paternal obesity, maternal obesity is heavily researched in recent years. Many studies have revealed that maternal obesity and a high-sugar diet during pregnancy negatively contribute to offspring health.[8,10,51,52] However, heavy focus on maternal obesity puts more societal pressure on women as if their health is the sole factor for the health of the next generation. If the offspring health was not ideal, mothers potentially put more blame on themselves, which potentially lead to various psychological disorders. Admittedly, the weight and nutrition status of the mother is largely associated with the health of the next generation. The research of paternal obesity on next generation is needed. As of now, paternal obesity is not well studied and could potentially carry similar negative effects to offspring as well.

Spermatozoa, the male sex cells carry the man's genetic materials. It carries two important pieces of information that pertain to: First, the ability to fertilize the oocytes to create a zygote and second, provide half of the genetic information to the zygote. Epidemiology studies demonstrate that obesity and overweight rate is staggering in men aged over 25 years old.[53] Current research also has confirmed that it could also negatively impact male reproductive health.[54,55]

Seminal vesicle fluid is an important part of seminal plasma. It is a major energy source for spermatozoa. Reduced seminal vesicle fluid could influence sperm motility and cause infertility. It also serves an important role in female reproductive systems, including up-regulation of embryotropic transcription factors and down-regulation of apoptosis inducing factor.[56] Research has established that obese men have a reduced semen volume, thus are more prone to issues

mentioned above. One animal study highlighted the importance of sperm motility and exercise; and concluded that exercise could potentially improve sperm motility in rodents by increasing AMPK levels.[57] AMPK-AMP-activated protein kinase is an important energy modulator to detect the changes in ATP:ADP ratio. Other research have elaborated that AMPK level is linked with sperm motility and quality.[58] Such studies have also revealed decreased FASN and Acaca expression, and both genes are encoded for lipid synthesis and oxidative stress respectively. Paternal obesity could also affect sperm function via high reactive oxygen species production in sperm. It is evident that spermatogenesis requires a high level of oxygen, resulting in increased ROS production. In addition to that, obesity is associated with increased ROS production as evidenced by increased cytokines production released by adipose tissue, such as TNF-alpha. Increased ROS in sperm could damage DNA of sperm and cell membrane, impairing the sperm function.

3.2 Parental obesity and its effect on offspring

Paternal effect is defined as a causal relationship between variation in paternal genotype or phenotype and variation in the offspring's phenotype. Paternal effect on offspring health is specifically based on paternal genetics, DNA methylation and microRNAs. The following sections discusses potential mechanisms of how each factor contribute to offspring health.

3.2.1 Paternal genetics on offspring health

Spermatozoa is affected by paternal obesity in terms of motility and mitochondrial activity. Research has noted increased ROS in obese male spermatozoa. ROS causes a direct increase in oxidative stress, which in turn may damage spermatozoa membranes and its content, namely the DNA information that is transferred to next generation.[59] A high-fat diet consumed by the paternal father could affect the metabolic health of next generation. In one animal study focusing

on the intergenerational effect of a high-fat diet, it has been uncovered that there is an interaction effect for high-fat diet if the offspring is exposed to the same diet patterns as well. It also has been shown in multiple other studies that the motility of spermatozoa decreases due to the paternal obesity, which is associated with blastocyst development. Another study has also reported a low birth weight of progeny from obese rodent fathers. The reported result demonstrated an imbalanced Growth hormone/ Insulin Growth Factor-I (IGF-1) ratio, and decreased growth hormones that lead to the early exit of puberty, and resulting in abnormal muscle and fat development.[60] On the other hand, paternal exercise could endow a protective effect to offspring if rodent father is fed with a high fat diet.[57,61] Krout, et al. have postulated that those progenies have a lower percentage of body fat and a decreased expression of FGF21, which as a gene that controls adipocyte production.[61]

3.2.2 DNA methylation and paternal obesity

DNA methylation is the epigenetic process of transferring methyl groups to part of the DNA, thus changing expression of DNA. DNA methylation only alters the expression of certain DNA regions without affecting the overall DNA sequence. The evidence about paternal obesity and DNA methylation is inconclusive. One of the current theories illustrates that there is a part of the DNA that escapes from the DNA methylation during gametogenesis and embryo development. Following unhealthy or a high-fat diet, one study showed that sperm from overweight male contained lower methylation at certain gene regions[62]. The lower methylation rate from obese father is consequently passed along to the next generation, and one animal study has showed that female offspring of male fed with high-fat diet had significantly increased insulin secretion[63], indicating the potential intergenerational effect of an unhealthy diet that a father was exposed with. DNA methylation could be either intergenerational or transgenerational. Intergenerational

effect is a direct exposure during or before pregnancy that affects the health of the next generation of offspring. Transgenerational effect takes place when the second generation is not prone to the same level exposures but is carried on to the third generation. The famous Dutch winter study is a good example to demonstrate that the third generation is prone to obesity and T2D due to DNA methylation with the second generation skipping the exposures.

3.2.3 microRNAs and paternal obesity on offspring health

MicroRNAs belong to a specific class of non-coding RNAs that are important for posttranslational protein expression. It is also important for modulating DNA methylation by activating DNA methyltransferases and other enzymes that are important for epigenetic regulation.[64] Alteration in microRNA numbers could impact offspring health in terms of obesity phenotype, growth, and cardiac health as indicated by multiple studies.[62,63] It is a part of the spermatozoa; and paternal obesity might lead to fluctuations in numbers of microRNAs. One study has illustrated that paternal obesity significantly contributes to the alteration in microRNA numbers.[64] The proposed mechanism emphasized the dysregulation of post-translational protein expression that ultimately affects the offspring's phenotype.

3.3 Paternal obesity and skeletal muscle function

It has been suggested that paternal obesity could reduce the offspring's skeletal muscle insulin resistance when combined with exercise.[61] It has previously been found that maternal exercise during pregnancy is positively linked with offspring health, especially if the mothers are overweight or obese during that period. It has also been evidenced that children of overweight mothers have a greater abdominal mass as well as greater levels of inflammatory markers.[52] It has been suggested that paternal obesity could contribute to offspring adiposity especially when both father and offspring are exposed to a high-fat environment.[66] However, very few studies

have revealed the relationship between skeletal muscle insulin signaling, paternal diet, and paternal exercise. One study by Krout et al. has demonstrated the improvement of skeletal muscle insulin response combined with paternal exercise and fed a High-fat diet or a Normal-fat diet.[61] The increased insulin signaling is not evident without exercise in both high-fat diet group and normal-fat group. The mechanism that underlies the result is still undetermined. One explanation might be due to exercise inducing sperm epigenetics, which leads to upregulation of offspring's skeletal muscle insulin signaling pathway. Drawing from an animal model, a study has identified growth hormone and Insulin-like growth factor 1 in muscle growth.[60] The specific study has identified that the relative gene expression of growth hormone and IGF-1 was significantly decreased in the offspring of HFD father. GH is a hormone that controls postnatal growth, and IGF-1 is regulated by GH. Deficiency of GH is related to reducing muscle mass in mice.[67] GH is also related to the increased activity of lipolysis,[67] and decreased GH is associated with increased lipid synthesis and transportation as evidenced by an increased gene expression that regulates those actions, such as fatty-acid binding protein 4 (Fabp4), Fatty-acid synthase (Fasn), and sterol response element-binding factor 1 (Srebf1).[60]

4 Fish oil

4.1 Definition of fish oil

Table 1: Adequate intake for n-3 fatty acids across the life span based on NIH[68]

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months*	0.5 g	0.5 g		
7–12 months*	0.5 g	0.5 g		
1–3 years**	0.7 g	0.7 g		
4–8 years**	0.9 g	0.9 g		
9–13 years**	1.2 g	1.0 g		
14–18 years**	1.6 g	1.1 g	1.4 g	1.3 g
19-50 years**	1.6 g	1.1 g	1.4 g	1.3 g
51+ years**	1.6 g	1.1 g		

*As total omega-3s
**As ALA

Natural fish oil obtained from the fatty fish contains poly unsaturated fatty acids (PUFA), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These PUFAs along with alpha-linolenic acid (ALA) also belongs to the omega three fatty acids (n-3) that contains multiple double carbon bonds along the structure. The difference between DHA, EPA and ALA is that ALA is from plant-based sources, such as flaxseed, while the other two are derived from fish. ALA is also shorter in length compared with other two n-3s. Human body cannot synthesize both DHA and EPA, but they are integral fatty acids that constitute the cell membrane. The function of DHA and EPA in the body include generation of eicosanoid-derived hormones and regulation of blood clotting by releasing of cytokines, and in some cases beneficial inflammation. The digestion of those fatty acids resembles other fatty acids. They are being broken down by bile and forms micelles. Those little micelles are then repackaged into chylomicrons and are distributed around the body via lymphatic system. Besides the functions mentioned above, DHA and EPA are beneficial to human health by preventing certain diseases, such as cardiovascular diseases, cancer, and Alzheimer's.[69] On the other hand, maternal digestion of fish oil benefits the health of offspring as well.[70] Currently, there is no

recommended daily guidelines for consumption of fish oil or EPA and DHA across all age groups. According to 2010 Dietary Guidelines, the recommended consumption of fish is about 8 servings per day, which provides about 250 mg of fish oil for general adult population. As illustrated on figure 5, there is adequate intake (AI) guidance for ALA, which is an essential fatty acid across all the age and gender group. So far, no such publication has been made for fish oil.

4.2 Mechanisms of Fish oil

4.2.1 Circulation triglycerides and fish oil

Triglycerides are stored mainly as lipoproteins in the circulation, including VLDL, HDL, and LDL. Each contains different proportions of lipids and protein with VLDL bearing the most concentrated fatty acids. The level of VLDL depicts the level of triglycerides in the circulation, and fish oil could reduce the hepatic VLDL in circulation to reduce plasma triglycerides. One randomized trial has corroborated with decrease in plasma triglyceride levels that matched decrease in VLDL level among 20 healthy subjects consuming about 30 g/day of FO on a 7-day basis.[71] The limitation of the study was that 30 g of fish oil was higher for certain populations, and the subjects in the experiment were healthy to begin with. The same result would be hard to replicate in other populations, such as obese, hyperglycemic, and hyperlipidemia individuals. Aside from reducing the production of VLDL, it was discovered that fish oil could also affect the clearance of plasma triglycerides. It has been demonstrated that fish oil could affect the activity of lipoprotein lipase.[72] The main function of lipoprotein lipase is to break down the triglycerides found in VLDL and dissipate the fatty acids and glycerol either to adipose cells for uptake or to skeletal muscle and heart for energy. The activity of lipoprotein lipase depends on the energy demand of different tissues.

4.2.2 Fish oil and related transcription factors

Numerous transcription factors are regulated by fish oil, including peroxisome proliferator-activated receptors (PPARs), retinoid X receptor alpha (RXR alpha), liver X receptor alpha (LXR alpha), and sterol regulatory element binding proteins (SREBP) The SREBPs regulates lipid synthesis with SREBP-1 being the primary one in the liver. The action of SREBP is regulated by LXR alpha and RXR alpha. Fish oil inhibit the upstream transcription factor of LXR alpha/ RXR alpha, thus contributing to the decrease in triglyceride synthesis and reduced plasma level.[73]

PPARs are found to be the most consistent transcription factors that is linked to fish oil for its action. For example, PPAR alpha increases beta oxidation in adipose, hepatic, and muscle tissues. PPAR gamma contributes to the uptake of fatty acids in adipose tissue. The metabolites of those PUFA could activate various PPARs, including oxylipins. The activation of various PPARs facilitates the reduction of triglycerides circulating levels.

4.2.3 Fish oil and adipose tissue

The source of non-esterified fatty acids (NEFA)is adipose tissue. The release of NEFA from adipose tissue is mediated by hormone sensitive lipase. It is regulated by glucagon and insulin based on a person's fed or fast state. NEFA is inhibited by insulin and stimulated by glucagon. In insulin resistant state, NEFA is dysregulated, potentially resulting in hyperlipidemia. Fish oil could counteract the dysregulation by maintaining the inflammation state. TNF-alpha and interleukin-6 both contribute to inflammation and also induce lipolysis in adipose tissue and inhibit the action of lipoprotein lipase from breaking down the VLDL. On the other hand, the inflammatory cytokines produced from macrophages is the major source of TNF-alpha, contributing to upregulation of hormone sensitive lipase. One study presented that GPR120—the

g protein coupled receptor—is responsible for the increased activity of the macrophages. Fish oil, specifically DHA, could bind with GPR120, thus attenuating its action.[74]

4.3 Fish oil and Skeletal Muscle

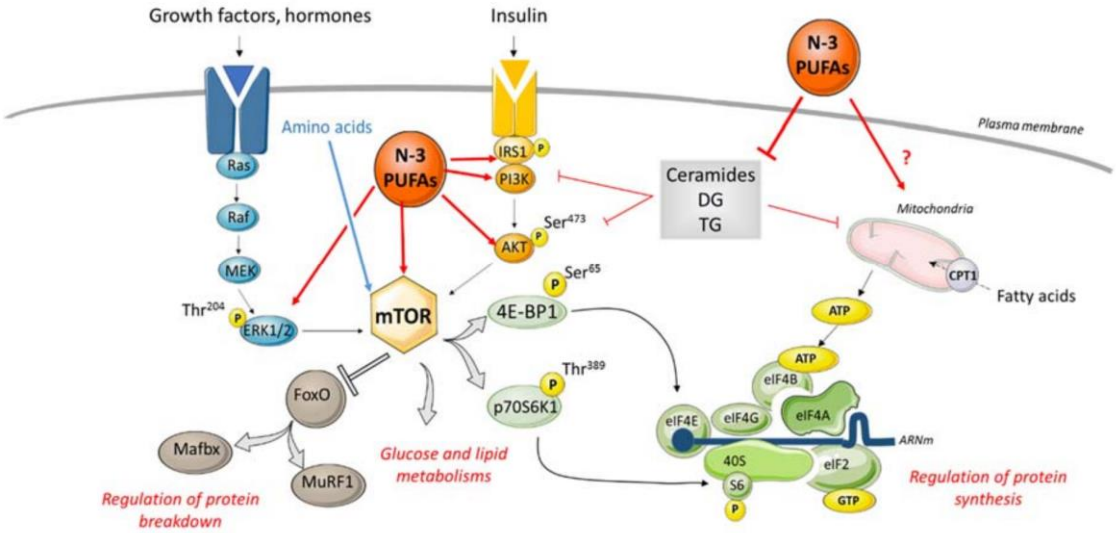


Figure 5: Potential mechanism of Fish oil on skeletal muscle protein synthesis[75]

Skeletal muscle is the primary site for fatty acids utilization. Lipoprotein lipase is relatively active in muscle tissue,[76] and the activity of lipoprotein lipase is increased with supplementation of fish oil.[77] The reduced triglycerides level is because of the increased beta oxidation level with fish oil supplementation favoring the PPAR-alpha expression.[77] Fish oil also contributes to counteract adipokines functions, including the reduced activity of lipoprotein lipase by TNF-alpha in adipose tissue.[77]

In addition to contributing to higher utilization of fatty acids, fish oil could also contribute to skeletal muscle recovery.[78] Liu et al. have demonstrated that fish oil is beneficial in maintaining skeletal muscle protein mass by inhibiting inflammation signaling pathways. Akt is a key protein that assists in signaling transduction pathway. It induces skeletal muscle protein synthesis and inactivates FOXO1. FOXO1 is a transcription factor that is regulated by Akt/PI3 pathway, and activation of FOXO1 leads to potential muscle degeneration as depicted in figure 5. In addition to that, the activation of PI3k/AKT pathway also activates Mammalian target of rapamycin (mTOR), which in turns triggers Ser and Thr[204] of Extracellular Signal-Regulated Kinase (ERK), whose objective is to facilitate protein synthesis. Smith et.al have conducted two studies in human populations, focusing on the effect of fish oil on muscle protein synthesis.[74,75] Although changes in membrane phospholipid composition do not change the rate of muscle protein synthesis, it has been revealed that there is an association between increased mTOR activation, resulting in the subsequent increased skeletal muscle protein synthesis and decreased muscle breakdown.[79,80] One study revealed that the composition of membrane affects skeletal mitochondrial biomechanics, and fish oil supplementation replaces part of the omega-6 on the phospholipid bilayers.[81]Two studies both conducted by Smith et.al. and have focused on young human population and geriatrics respectively. As geriatric population is more prone to muscle loss—given the age-related effect—the study has highlighted the potential importance of fish oil independently on restoring skeletal muscle protein mass in older populations.[79] In younger populations with mean age of 37, Smith et al. revealed an interaction effect of increased muscle protein synthesis combined with sufficient anabolic stimuli (increased insulin and amino acids levels). Another independent study has figured out a similar trend of increasing lean mass and skeletal muscle mass following 6 months of EPA and DHA supplementation.[77] In mice, omega-3

fatty acid alters the protein turnover in C2C12 cells, which controls myotubes and muscle protein formation. Specifically, DHA was found to decrease protein degeneration.[83]

Fish oil supplementation could be used to reduce muscle loss due to disability or difficult moving. In athletes, severe injury could result in protein degeneration during rehabilitation. Thus, it is crucial to find effective ways to preserve muscle protein mass. According to some studies conducted in mice, omega-3 fatty acids could alleviate soleus atrophy after undergoing hindlimb suspension.[84] The mechanism involves potential preservation of Thr^{204} phosphorylation. In one human study focusing on partially immobile women, 6-weeks of fish oil supplementation attenuated the decline in muscle volume and mass.[85]

In terms of mitochondrial functions, fish oil supplementation restores ADP sensitivity. ADP sensitivity refers to the Affinity (Km) of ADP to phosphate during the production of ATP. Decreased ADP sensitivity is associated with increased ROS production, meaning that more ADP and phosphate are needed to produce ATP, resulting in more production of ROS. An increased ADP sensitivity is found in fish oil supplementation group of one study that focused on partially immobile patients.[85] The production of ROS could affect muscle functions, and it is found to be related with diseases that involve muscle-disuse atrophy. On the other hand, fish oil could potentially reduce intracellular lipid levels by upregulating PPAR alpha,[86] which has proven to increase mitochondrial biogenesis and fatty acid oxidation. As PPAR alpha increases mitochondrial biogenesis, the lipid oxidation also increases and could potentially result in the decreased level of intracellular lipid levels.

The major characteristics of chronic diseases such as cancer and T2D, involves the elevation of pro-inflammatory cytokines, indicating the inflammatory state. Both EPA and DHA are anti-inflammatory and contribute to the decreased levels of those cytokines. The phospholipids

membranes consist of both Omega-3 fatty acids and Omega-6 fatty acid arachidonic acid (ARA). Both substrates could be released from the cell membrane by phospholipase, and they release different anti-inflammatory or inflammatory cytokines. Since phospholipase is a specific agonist for either of two omega acids, the type of omega fatty acids released from the body depends on the abundance of each fatty acids. ARA is revealed to a more dominate substrate in skeletal muscle fatty acids.[87] ARA is broken down by cyclooxygenase 2 (COX 2) enzymes to inflammatory agents, including the prostaglandins and leukotrienes. Supplementation of EPA could decrease the inflammatory agents. EPA is also metabolized by COX 2 as well, but the product is lower in affinity and bioavailability with the receptors compared with the ARA.[87] Consequently, less inflammatory cytokines are released with supplementation of EPA or an increased EPA:ARA ratio. As previously discussed, fish oil combined with GPR-120 could suppress expression of pro-inflammatory macrophages by inhibiting TNF-alpha response. In a similar study, it is shown that fish oil could also decrease the level of long chain acetyl CoA levels and ceramides, which are lipid mediators that interfere with insulin signaling. Meanwhile, GLUT4 (Glucose Transporter Type 4) and IRS 1(Insulin Receptor Substrate 1) expressed more compared with other control groups[88]. The increased level of both genes is evident of increased insulin signaling.

Hypothesis

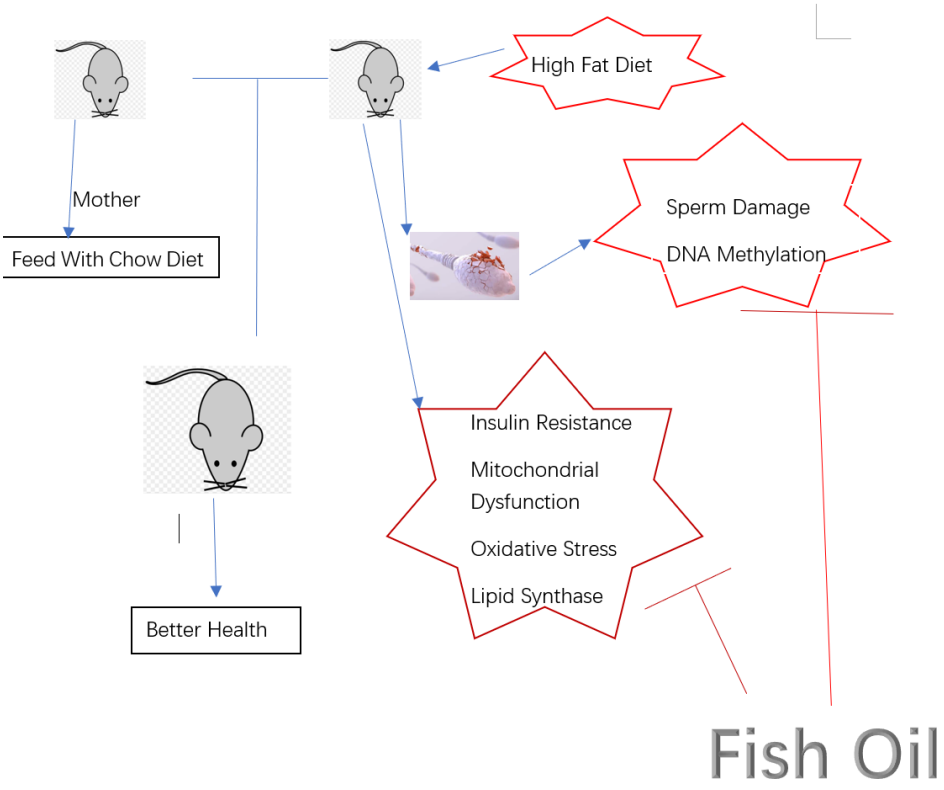


Figure 6: Hypothesis Model

My research focus will be on the potential effect of fish oil in father male mice during preconception and how that will affect the genetic markers for oxidative stress, insulin resistance, muscle growth, lipid synthesis and mitochondria respiration in male and female offspring mice.

Introduction

Obesity is increasing at alarming rates with its prevalence increasing from ~ 30.5% to 42.5% from 1999 to 2017.[2] Obesity is defined as excess accumulation of adipose tissue and is associated with increased secretion of proinflammatory adipokines resulting in systematic inflammation. Further, excess release of free radicals occurs due to higher energy intake resulting in oxidative stress. Both these pathological stresses contribute to co-morbidities of obesity including type II diabetes (T2D) and cardiovascular disorders.

Dysfunction in the skeletal muscle contributes to insulin resistance and T2D, which in turn leads to obesity. Increased reactive oxygen species (ROS) production [89] leads to muscle atrophy and contractile malfunction that eventually develops into sarcopenia and skeletal muscle dysfunction.[26,27,30,31,32] Further, inflammation also contributes to higher ROS production and lipid peroxidation leading to skeletal muscle dysfunction.[89] With obesity, decreased mitochondrial biogenesis occurs and reduced mitochondrial numbers exist with smaller mitochondria contributing to skeletal muscle dysfunction.[37,38] [39–41] This alters fatty acid pathways and glucose transport via GLUT4 in skeletal muscle.[38] Lastly, continuous exposure of high-fat diet contributes to lipid infiltration from adipose tissue, which decreases muscle synthesis and insulin sensitivity contributing to muscle dysfunction. [90]

Well known behavioral, and surgical interventions exist for obesity, but dietary interventions targeting obesity and its symptoms are not limited. We focus on fish oil (FO), which contains polyunsaturated fatty acids (PUFA), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Fish oil has potential benefits in skeletal muscle in part by contributing to higher utilization of fatty acids and improved skeletal muscle recovery.[78] Liu et al. demonstrated that FO is beneficial in maintaining skeletal muscle protein mass by inhibiting

inflammation. Further, FO also reduces intracellular lipid levels by upregulating PPAR alpha,[86] which increases mitochondrial biogenesis and fatty acid oxidation [85].

Obesity in the child is contributed through both the parents. Several studies have revealed that maternal obesity negatively contribute to offspring health, leaving paternal obesity out.[8,10,51,52] Paternal obesity is known to affect sperm function via increased ROS production in sperm, which in turn impairs sperm quality and potentially affects future offspring.[59] Further, obesity decreases motility of spermatozoa,[91] which is associated with blastocyst development. This also affected birth weight of was observed from obese rodent fathers including abnormal muscle and fat development.[60] Corroborating with this, paternal exercise reduces offspring skeletal muscle insulin resistance when combined with a high fat diet.[61] Supplementation of Fish oil (FO) during pregnancy improves the health outcomes of offspring in terms of weight and insulin sensitivity[92]. However, the role of FO in reducing paternal obesity and the impact on offspring metabolic health is not known. Hence, we are focusing on FO in paternal obesity and the impact in improving the skeletal muscle function in offspring using mice models.

Methods

Animal Studies: Thirty C57BL6J male and female mice aged 4-5 weeks were purchased from Jackson labs (Bar Harbor, Maine, USA). Male mice were divided into three groups with different dietary interventions: 8 mice were fed low fat (LF) of 10% fat, 20% protein, and 70% carbohydrates. 11 mice were fed high fat diet (HFD) of 45% aft, 20% protein, and 35% carbohydrates and 11 mice were fed high fat diet supplemented with fish oil (HF-FO) of 45% fat with fish oil, 20% proteins, and 35% carbohydrates for 10 weeks. Food intake and body weight of the mice were recorded weekly. After 10 weeks, male mice were mated with 10 weeks old

female mice fed chow diet. All the female mice were on chow diet throughout the study. During the mating period, all mice were fed chow diet. Once conception was confirmed, pregnant mice were separated from the male mice and was fed chow diet during gestation and lactation. Offspring mice were kept with F1 mice until weaning at 4 weeks.

Offspring mice.

The body weight of offspring mice was measured on day of birth and again at day 7. Three groups of offspring mice were obtained from the three dietary intervention groups of father mice. All the offspring mice were fed chow diet for 16 weeks. Weekly measurement of body weight and food consumption was performed. Half of the offspring mice were sacrificed at 8 weeks and rest at 16 weeks to test the short-term and long-term effect of paternal obesity. On the other hand, the significance of 8-week is that mice reach sexual maturity for both males and females. The first 8-weeks depict the early developmental stages of mice from weaning to exposure of experimental diet. The latter 8-week represents a more stable stage of development in terms of hormones levels, weight, and food consumption. For the reasons above, we decided to divided offspring into 8-week group and 16-week group. Mice were euthanized using isoflurane and then each mouse was dissected for isolation of muscle, tissue was collected and stored in -80 ^{0} Celsius.

Insulin Tolerance Tests (ITT)

F1 male offspring (16 week) were administered with insulin tolerance tests (ITT) at 10 weeks following CD. Blood glucose was measured with a handheld glucometer following five hours of fasting. ITT is carried by injecting 1 IU insulin/kg (Eli Lilly, Indiana, Indianapolis, USA) into those mice with the identical hours of fasting and measurements were taken with the same time points as GTT.

RNA isolation

Skeletal muscle was homogenized with Trizol Solution (Ambion by Life Technologies, Carlsbad, California, USA), using Tissue Lyzer (Qiagen). RNA was then prepared using Zymo Research Quick-RNA MicroPrep Kit (Zymo Research, Irvine, California USA). CDNA was made from the extracted RNA using the Applied biosystems High-Capacity cDNA Reverse Transcription Kit (Applied biosystems by Thermo Fisher Scientific, Baltics, Vilnius, Lithuania). Gene expression was performed with universal PCR Master Mix (Applied biosystems by Thermo Fisher Scientific, Baltics, Vilnius, Lithuania). The analysis was done by Quantstudio™ 3 Real-Time PCR System. Gene expression data was then analyzed on the Thermofisher website with Actin being the control group for relative expression.

Table 2: List of markers used for gene analyses.

Markers	**Role**	**Expected Outcome**
Fatty acid synthase (Fasn)	Fatty acid synthesis	Reduced expression of Fasn in FO in comparison with HF
Fatty Acid Binding Protein 4 (Fabp 4)	Fatty acid synthesis	Reduced expression of Fabp in FO in comparison with HF
Succinate Dehydrogenase Complex, Subunit A (SDHA)	Oxidative Phosphorylation	Increased expression in SDHA in FO with increased energy generation during electron transport chain compared with HF

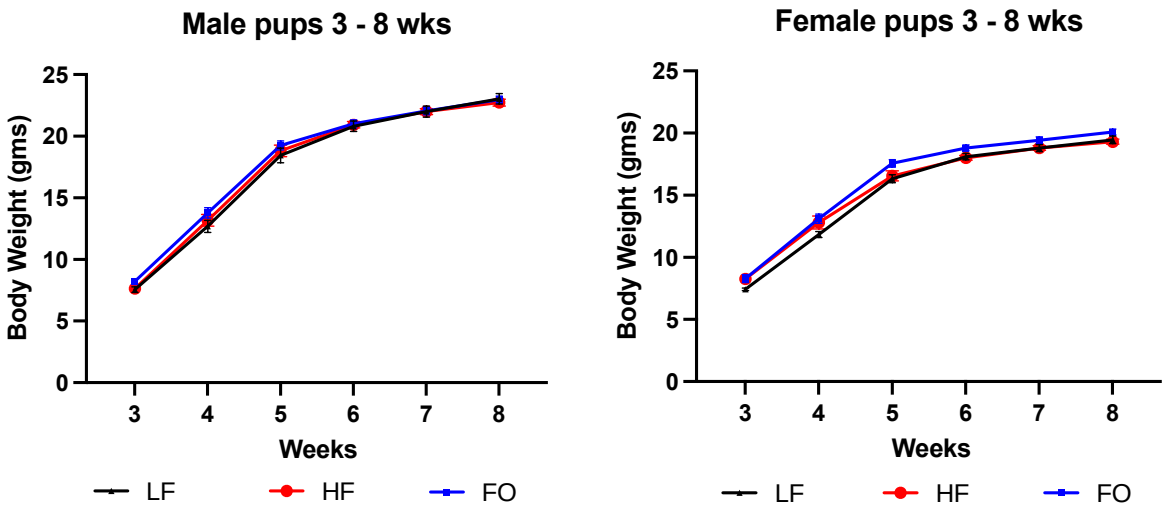
Mitochondrially encoded ATP Synthase Membrane Subunit 8 (mATP 8)	Oxidative Phosphorylation	Increased expression in ATP 8 in FO with increased energy generation during electron transport chain compared with HF
Mitochondrial Cytochrome b (cytb)	Oxidative Phosphorylation	Increased expression in cytb in FO with increased energy generation during electron transport chain compared with HF
Mitochondrially Encoded Cytochrome C Oxidase I. (mtco1)	Oxidative Phosphorylation	Increased expression in mtco1 in FO with increased energy generation during electron transport chain compared with HF
Acetyl-CoA Acetyltransferase (Acat)	Beta Oxidation	Increased expression of Acat indicating increased beta oxidation in FO compared with HF
Cell Death-inducing DNA Fragmentation Factor Alpha-like (CIDEA)	Beta Oxidation	Increased expression of CIDEA indicating increased beta oxidation in FO compared with HF

Peroxisome proliferator-activated receptor alpha (PPAR Alpha)	Beta-oxidation	Increased expression of Acat indicating increased beta oxidation in FO compared with HF
Glucose Transporter Type 4 (Glut 4)	Insulin resistance	Increased expression of Glut 4 is expected in FO compared with HF
Insulin Substrate 1 (mirs 1)	insulin resistance	Increased expression of mirs 1 is expected in FO compared with HF
Phosphatidylinositol-3 Kinase (PI3K)	Insulin resistance	Increased expression of Pi3k is expected in FO compared with HF
Forkhead Box Protein O1 (FOXO 1)	Insulin resistance	Increased expression of FOXO1 is expected in FO compared with HF
Catalase (CAT)	Oxidative Stress	Decreased expression of CAT in FO in comparison with HF
Superoxide Dismutase 2 (SOD 2)	Oxidative Stress	Decreased expression of SOD 2 in FO in comparison with HF
Pyruvate Dehydrogenase Kinase 4 (PDK 4)	Oxidative Stress	Increased expression of PDK 4 indicating increased beta

		oxidation in FO compared with HF
Myogenin (mmyog)	Muscle Growth	Increased expression of mmyog in FO in comparison with HF
Fibroblast Growth Factor 21 (fgf21)	Muscle Growth	Increased expression of fgf21 in FO in comparison with HF.

Statistical Analysis: Relative expressions are analyzed using GraphPad Prism 9. One-way ANOVA test is utilized to compare 3 experiment groups. Each group is processed with a post-hoc test if passed the initial passed ANOVA test. ($p<0.05$) Further, Two-way ANOVA test is carried to compare diet and sex difference across groups.

Results



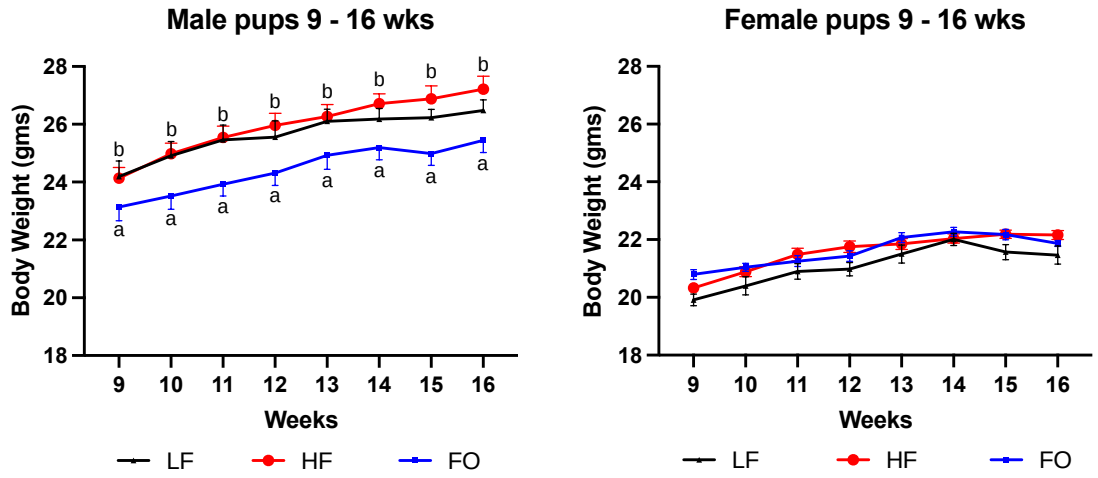


Figure 1: Body weight of male and female offspring born to father with LF, HF, and FO .

As shown in Figure 1, for male offspring of 3-8 weeks, no significant difference of body weight is observed across all groups. On the other hand, the body weight of male offspring with HF father weigh significantly higher compared with male offspring with FO father for male 9–16-week offspring. For all the female offspring, the change for body weight remains consistent throughout all groups.

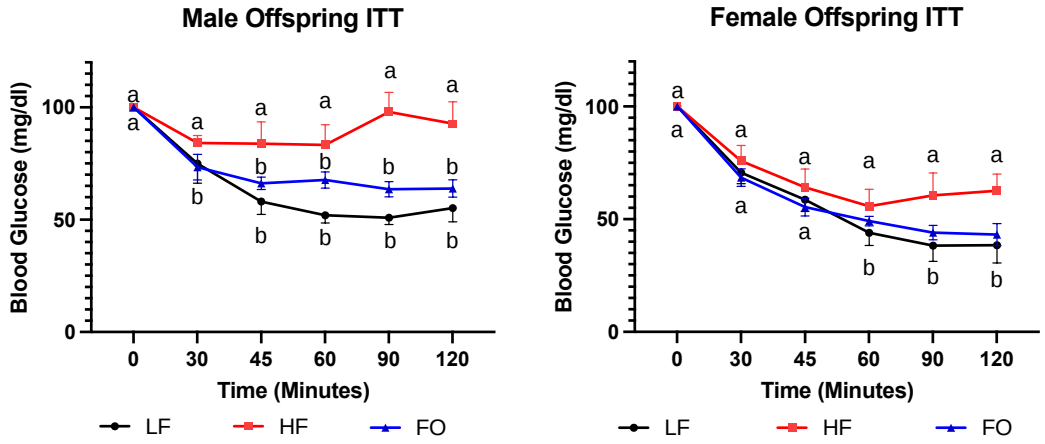


Figure 2: Insulin tolerance test for male and female offspring with HF, FO, and LF fathers.

As shown on figure 2, male offspring born to HF father is significantly more insulin resistant when comparing to both offspring with LF and FO father. On the other hand, for female

offspring, only offspring with LF father showed a significant insulin response compared with offspring born to HF father. However, offspring with FO father showed a similar trend of better insulin response compared to offspring of HF father.

Table 3: Diet and Sex difference across groups.

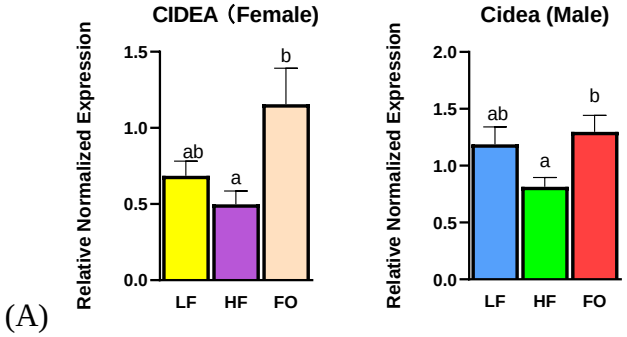
	Sex (S)	Diet (D)	Interactions (S*D)
Acat	0.1298	<0.005	<0.005
Foxo 1	<0.0001	0.2923	0.9385
PDK 4	<0.0001	<0.001	0.1093
CAT	<0.0001	0.0846	<0.05
CIDEA	<0.01	<0.002	0.4777
Cytb	<0.0001	<0.005	<0.05
Fabp 4	<0.05	<0.0005	0.6192
Fasn	<0.01	<0.05	<0.0001
Glut 4	<0.05	<0.0001	<0.05
Fgf21	<0.0002	<0.005	<0.0005
Irs 1	<0.0001	<0.0002	<0.001
Mmyog	0.4625	<0.001	0.9316
ATP 8	<0.01	0.3681	0.3506
Mtco-1	<0.0005	<0.01	<0.005
Pi3k	<0.0001	<0.002	0.1143
PPAR alpha	<0.0001	<0.0001	0.5450
SOD 2	<0.01	<0.0001	0.3644
SDHA	0.1972	0.2093	0.8234

Table 4: Diet and Time difference across groups.

	Time (T)	Diet (D)	Interactions (T*D)
Acat	<0.0001	0.0740	0.3477
Foxo 1	<0.0001	0.1256	0.0796
PDK 4	<0.0001	0.3593	0.5485
CAT	<0.0002	0.1197	<0.05
CIDEA	0.1068	0.6776	0.3226
Cytb	<0.0001	0.8995	0.0914

Fabp 4	0.2840	<0.005	0.0655
Fasn	<0.0001	<0.05	0.3140
Glut 4	<0.0001	<0.005	<0.02
Fgf21	<0.0001	<0.001	<0.05
Irs 1	<0.0001	0.0072	0.2780
Mmyog	0.1737	<0.002	0.9020
ATP 8	0.2923	0.5388	0.1423
Mtco-1	<0.0001	0.4467	0.2364
Pi3k	<0.0001	<0.0001	0.1816
PPAR alpha	0.0949	0.5863	0.2380
SOD 2	<0.05	<0.005	0.02
SDHA	0.0001	0.0402	0.1791

8-Week fatty acid oxidation in male and female offspring



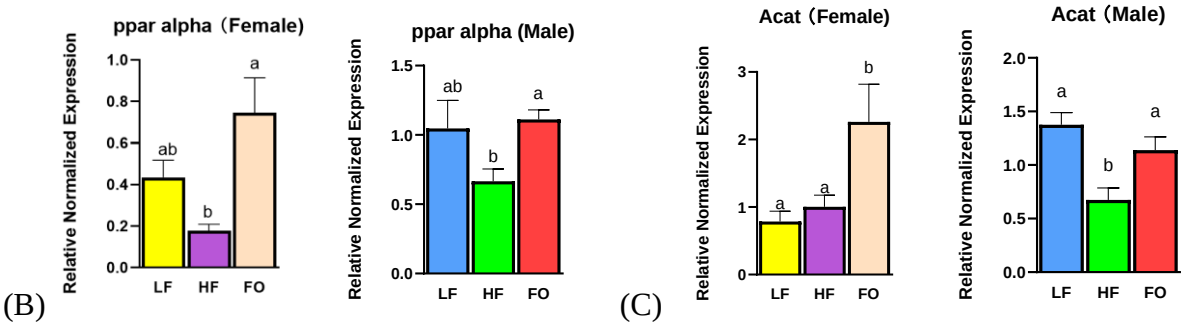


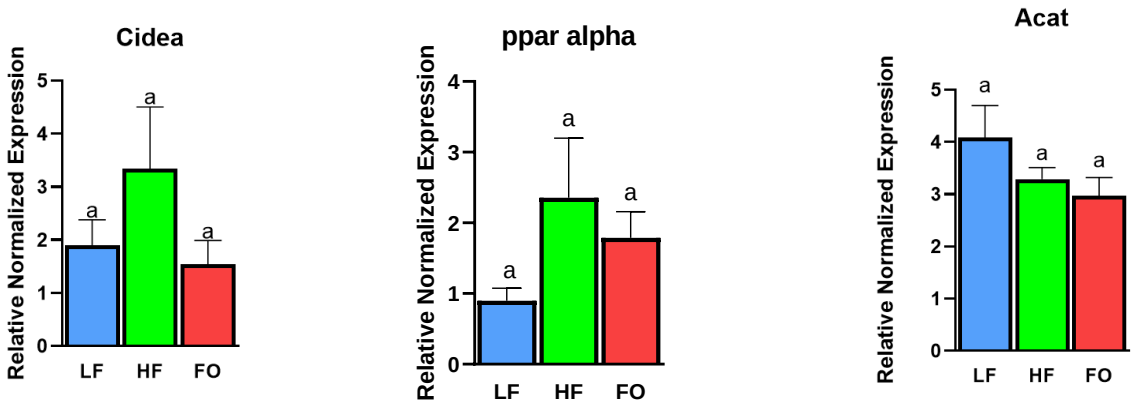
Figure 3: 8-week muscle data in males and females. Relative normalized expression of A) cell death-inducing dna fragmentation factor (Cidea) (B peroxisome proliferator-activated receptor

alpha (ppar alpha) (C) acetyl-CoA acetyltransferase (Acat) Groups with same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.

As presented in Fig 3A, Cell Death-Inducing DNA Fragmentation Alpha-like Effector A (Cidea) was comparable between HF and LF groups in both male and female mice. Further, Cidea was higher in both male and female offspring born to father fed FO compared to offspring born to HF father as shown in Fig 1A. When comparing the male offspring born to father with LF diet, female offspring with HF father had a higher Cidea expression. In addition, female offspring born to father with HF had a significant upregulation of Cidea compared with male with FO father. It's also found that there is a diet difference. No interaction of sex and diet is found for the expression of this genetic marker. No significant difference is found when comparing 8-week male offspring with 16-week male offspring across all groups. For Peroxisome Proliferator-Activated Receptor Alpha (ppar Alpha), no difference was observed between LF and HF offspring among both male and female 8-week groups. However, FO increased levels of PPAR alpha compared to HF in both males and females as shown in Figure 3B similar to Cidea. In addition to that, as shown on Table 3, sex difference was found for PPAR alpha expression with LF and HF female offspring having a higher level of PPAR alpha compared to the male offspring. There is a diet difference for the expression of PPAR alpha. However, no interaction for diet and sex was found. Moreover, no significant difference is found between 8-week offspring and 16-week offspring. As indicated in Fig 3C, Acetyl-CoA Acetyltransferase (Acat) was comparable between HF and LF in females but was lower in HF male offspring compared to male offspring born to LF fathers. However, FO increased levels of Acat in both male and

female offspring compared to offspring born to HF fathers. Female offspring born to FO father had significant upregulation of Acat compared with male offspring born to FO father. Diet difference is detected for the expression of Acat, but no sexual difference. Interestingly, an interaction of sex and diet is found for the expression of Acat.

16-Week fatty acid oxidation in male offspring

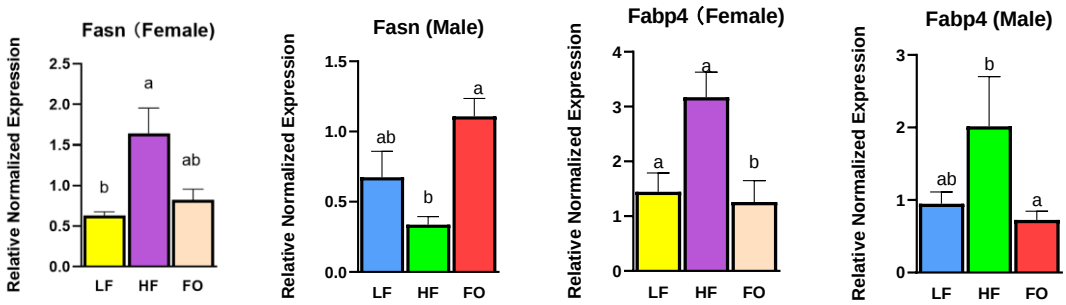


(A) (B) (C)

Figure 4: 16-week muscle data in males. Relative normalized expression of A) cell death-inducing dna fragmentation factor (Cidea) (B peroxisome proliferator-activated receptor alpha (ppar alpha) (C) acetyl-CoA acetyltransferase (Acat). Groups with same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.

Interestingly, none of the fatty acid oxidation markers were significantly different between male offspring groups. Hence, we didn't analyze the 16 weeks females, but focused on 8 weeks data in males and females. However, when comparing 8 and 16 weeks, no difference in CIDEA levels were found at both time points with no interaction between time and diet.

8-Week Fatty Acid Synthesis in Male and Female Offspring



(A) **(B)**

Figure 5: 8-week muscle data in males and females. Relative normalized expression of (A) fatty acid synthase (B) fatty acid binding protein 4. Groups with same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.

We measured fatty acid synthesis using fatty acid synthase (Fasn). As shown in Fig 5A, fasn levels in female offspring born to father fed with HF were higher compared to LF male offspring. Further, FO decreased Fasn levels in female offspring compared to HF but not in males. Sex difference was observed between HF male and female, with female offspring mice having higher levels than male offspring mice. Diet difference is detected for the levels of fasn expression. A sex and diet interaction are found as well. On the other hand, 16-week male offspring born to HF father showed a higher expression of Fasn compared to 8-week male offspring born to HF father. Diet difference is also detected with no interaction of time and diet found. Another fatty acid synthesis marker, fatty acid binding protein 4 (Fabp4) levels were comparable between HF and LF mice both in male and female offspring as shown in Fig 5b. Further, Fabp4 levels were down regulated with FO compared to HF in both male and female offspring. Sex difference is detected when comparing female offspring born to HF father with male offspring born to LF and FO father, with female offspring having higher levels compared to

male offspring with father of LF and FO. Diet difference is found without the interaction of diet and sex. On the other hand, there is no difference for the levels of Fabp4 between 8-week male offspring and 16-week male offspring. However, a diet difference is detected with no interaction of time and diet.

16-week fatty acid synthesis in male offspirng

(A) (B)

Figure 6: *16-week muscle data in males. Relative normalized expression of (A) fatty acid synthase (B) fatty acid binding protein. Groups with the same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.*

No significant difference were detected between groups for both Fasn and Fabp4 as shown in Fig 6A and B.

8-Week Oxidative Phosphorylation in male and female offspring

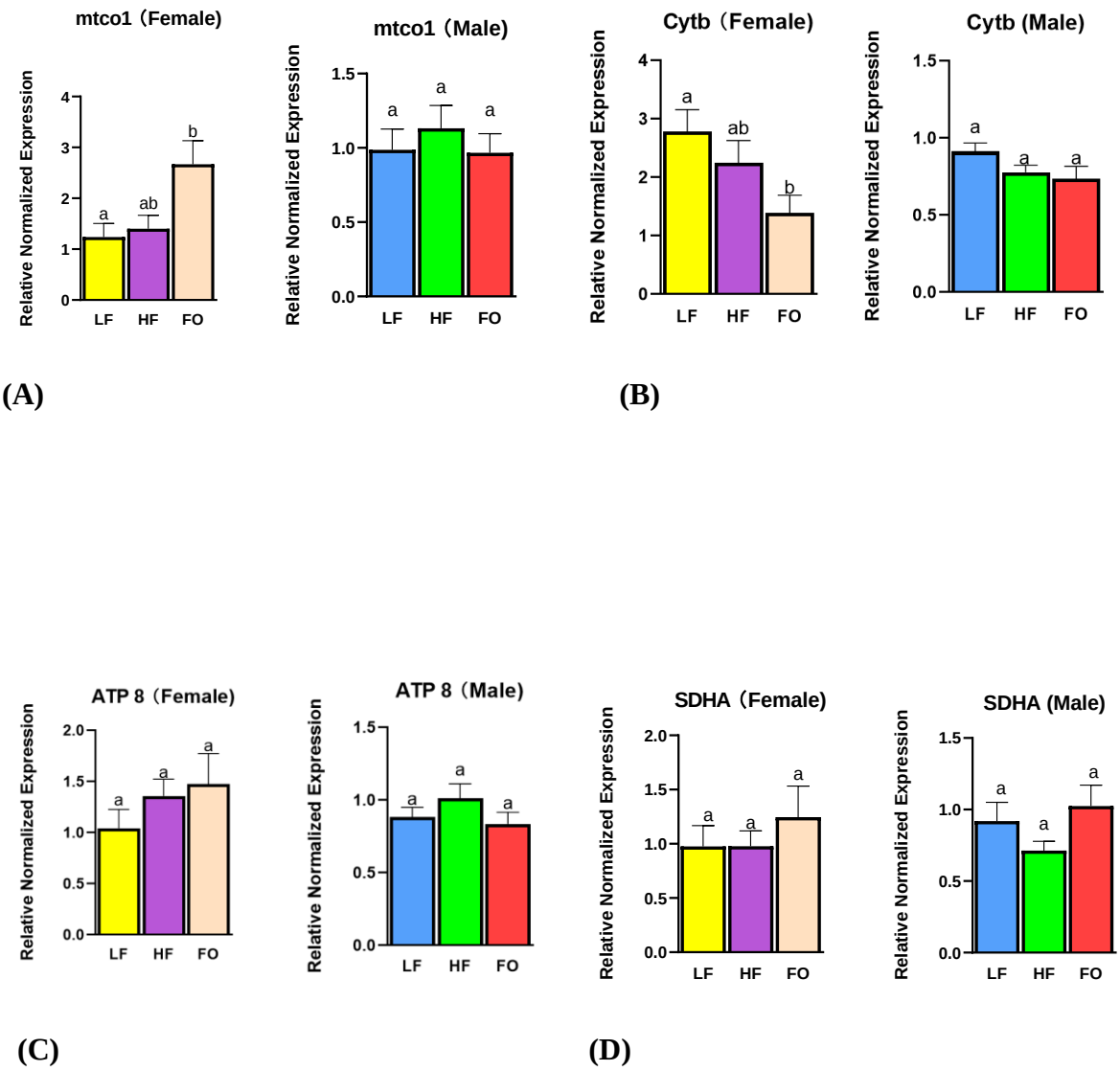
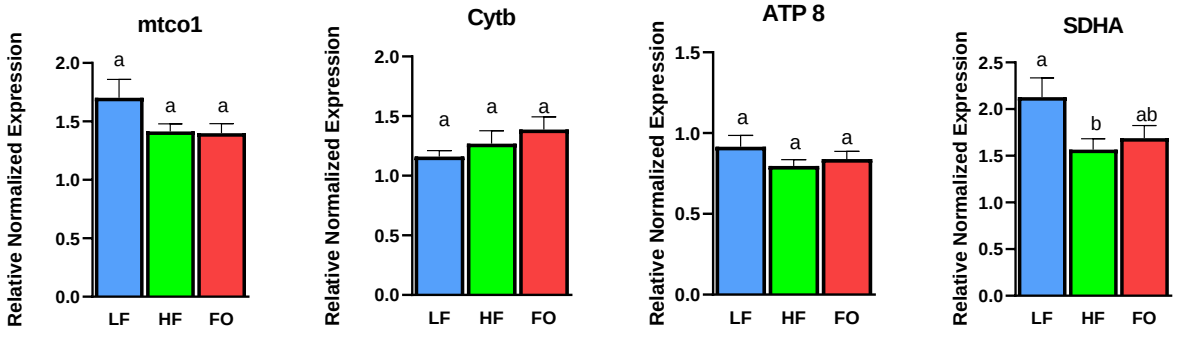


Figure 7: *8-week muscle data in males and females. Relative normalized expression of (A) mitochondrially encoded cytochrome c oxidase I (B) mitochondrial Cytochrome b (C) Mitochondrially encoded ATP Synthase membrane subunit 8 (D) Succinate Dehydrogenase Complex, Subunit A. Groups with same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.*

Oxidative phosphorylation was measured with mitochondrially encoded cytochrome c oxidase I (mtco1). As shown in Fig 7A, female offspring born to FO father exhibited significant increased mtco1 expression compared to female offspring born to LF father. However, levels were comparable in male offspring mice. In terms of sex difference, higher expression of mtco1 was found among FO female offspring compared to male offspring born to FO father. Diet difference is also detected along with an interaction of sex and diet. For time difference, 16-week male offspring born to both FO and LF fathers had higher levels compared to 8-week male offspring with FO and LF fathers respectively. No diet difference is found between those two groups with no interaction of time and diet as well. Another oxidative phosphorylation marker, mitochondrial cytochrome b (Cytb)was measured. Female offspring born to FO father exhibited significant decreased expression compared to female offspring born to LF father, with no difference in male offspring. Female offspring of both LF and HF groups both showed higher expression of Cytb compared to male offspring with LF and HF father respectively. For time difference, 16-week male offspring born to both FO and HF fathers had higher levels compared to 8-week male offspring with FO and HF fathers respectively. No diet difference is found between those two groups with no interaction of time and diet. We also measured succinate dehydrogenase complex, subunit and mitochondrially encoded ATP synthase membrane subunit but found no difference. In addition, no sex, diet, and sex-diet interaction are found between male and female offspring. On the other hand, Time difference is detected with 16-week male offspring expressing higher levels of SDHA compared with 8-week male offspring across all dietary groups. Diet difference is detected as well with no interaction of time and diet. However, for ATP synthase expression, it is comparable between female offspring with FO father and male

offspring with FO father. No difference is found between 8-week male offspring and 16-week offspring for the expression if this genetic marker.

16-Week Oxidative Phosphorylation in Male Offspring

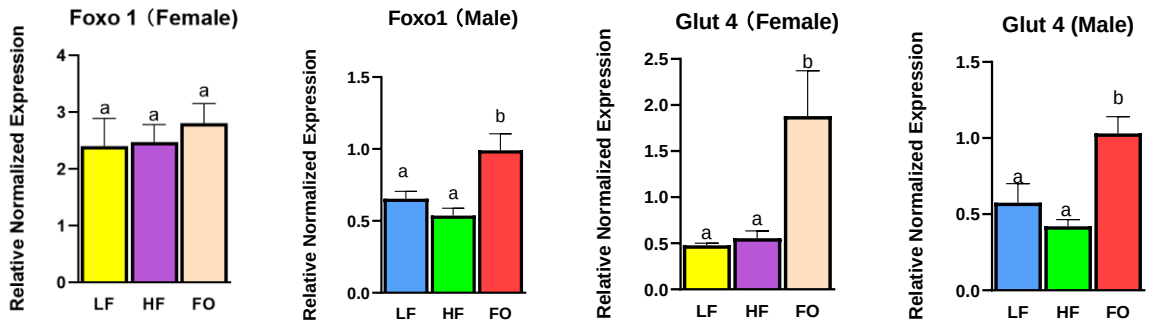


(A) **(B)** **(C)** **(D)**

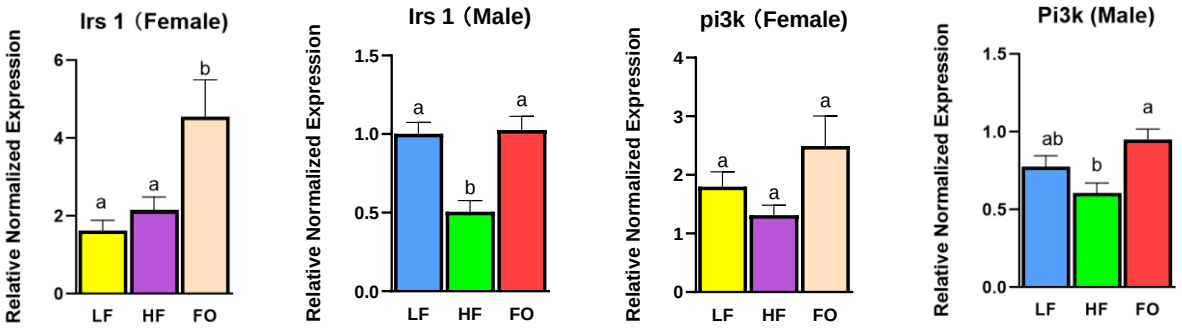
Figure 8: *16-week muscle data in males. Relative normalized expression of (A) mitochondrially encoded cytochrome c oxidase I (B) mitochondrial Cytochrome b (C) mitochondrially encoded ATP Synthase membrane subunit 8 (D) Succinate Dehydrogenase Complex, Subunit A. Groups with same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.*

For the 16-week mice, Mitochondrially Encoded Cytochrome C Oxidase I(mtco1), Mitochondrial Cytochrome b, and Mitochondrially Encoded ATP Synthase Membrane Subunit 8, as indicated in Fig 8A, B, and C, no significant difference is detected between groups. As for SDHA, it is significantly downregulated in male offspring born to HF father compared to male offspring born to father fed with LF, with no difference with FO group.

8-Week Insulin resistance in male and female offspring



(A) (B)

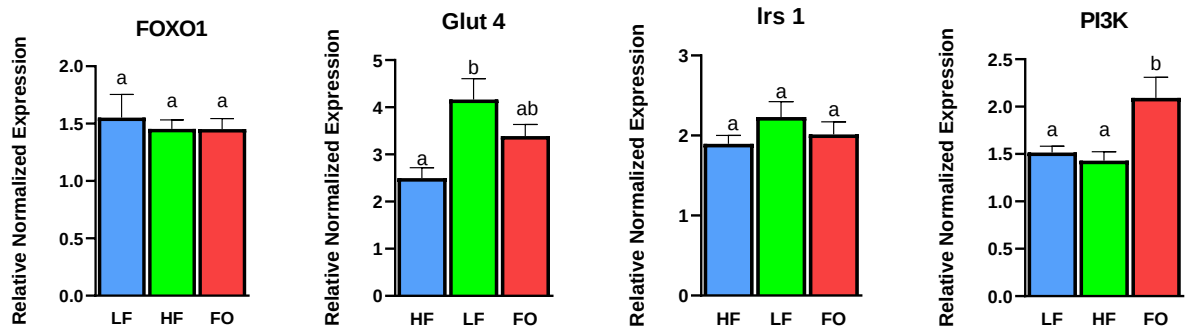


(C) (D)

Figure 9: 8-week muscle data in males and females. Relative Normalized Expression of (A)forkhead box protein O1 (B) glucose transporter type 4. (C) insulin substrate 1 (D) phosphatidylinositol-3 kinase. Groups with same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.

As insulin resistance occurs with obesity, we measured markers on insulin signaling which contributes to insulin resistance. Levels of forkhead box protein O1 (FOXO1) as shown in Fig 9A was not difference between female offspring. However, FO increased FOXO1 in male offspring compared to both LF and HF male offspring. All FOXO1 female groups had higher expression of FOXO1 compared to the respective male offspring group. No diet difference and

sex-diet interaction are found. However, 16-week male offspring had higher levels of FOXO1 compared to 8-week male offspring across all groups. No diet and time-diet interaction are found for the levels of FOXO1. Glucose transporter type 4 (Glut4) levels were higher with FO in both male and female offspring compared to both HF and LF as shown in Fig 9B. In addition, sex difference is detected with female offspring born to FO fathers having higher levels compared to male offspring with FO fathers. Diet difference is found along with an interaction of sex and diet. On the other hand, 16-week male offspring had higher levels compared to 8-week male offspring for all dietary groups. Diet difference is found in addition to a time-diet interaction. In terms of insulin resistance, insulin substrate 1 (Irs1) levels were higher with FO in female offspring compared to female offspring from both LF and HF father. However, male offspring from LF and HF father had comparable levels. Further, similar to females, FO increased Irs1 levels compared to male offspring born to HF father. Higher expression of Irs 1 is found in female offspring born to FO fed father compared to male offspring. Diet difference along with diet and sex interaction is found for the expression of Irs 1. In terms of time difference, 16-week male offspring had an upregulation of Irs 1 compared with 8-week male offspring across all dietary groups. A diet difference is found as well with no interaction of time and diet indicated. On the other hand, Phsphatidylinositol-3 kinase (pi3k) was similar in female offspring, but a significant upregulation of pi3k was observed in FO offspring compared to HF offspring. No difference in diet and diet-sex interaction, but sex was found for PI3K levels. Female offspring have a significant higher expression of Pi3k in all diet groups compared to male offspring. In terms of time difference, 16-week male offspring had an upregulation of Pi3k compared with 8-week male offspring across all dietary groups. Diet difference is indicated without the diet and time interaction.

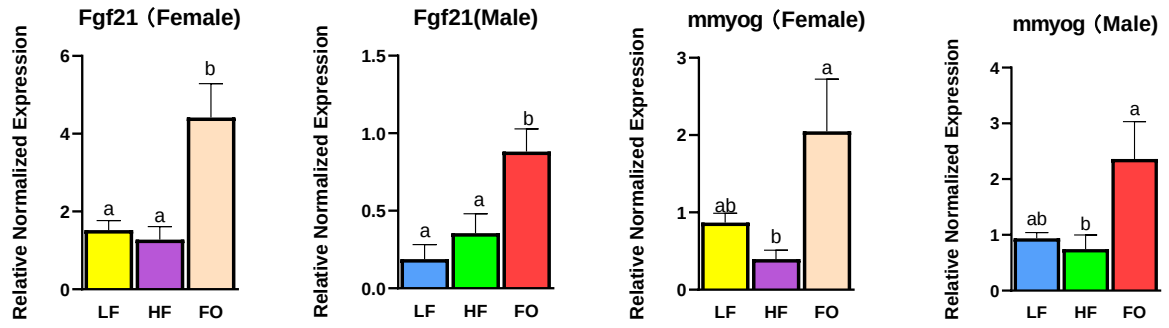


(A) (B) (C) (D)

Figure 10: 16-week muscle data in males and females. Relative Normalized Expression of (A)forkhead box protein O1 (B) glucose transporter type 4. (C) insulin substrate 1 (D) phosphatidylinositol-3 kinase. Groups with the same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.

At 16 weeks, we observed no significant differences between FOXO1 as shown in Fig 10A. As expected, offspring born to HF father showed decreased Glut 4 expression compared to offspring born to LF father as indicated in Fig 10B.On the other hand, no significant difference was detected with FO. Further, there is no difference among any groups for Irs1. As indicated in Fig 10D, FO significant upregulated PI3K compared to both LF and HF offspring.

8-week skeletal muscle growth in male and female offspring



(A) (B)

Figure 11: 8-week muscle data in males and females. relative normalized expression of (A) myogenin (B) fibroblast growth factor 21. Groups with the same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.

As indicated in Fig 11A, FO increased fibroblast growth factor 21 (fgf21) levels in both male and female offspring compared to other groups. On the other hand, female offspring with HF father have a higher expression of fgf21 compared to male offspring with HF father. A diet difference along with diet and sex interaction is found for the levels of fgf21. On the other hand, time difference is found with 16-week male offspring born to LF and FO fathers having higher levels of fgf21 compared to 8-week male offspring with LF and FO fathers. Diet different is found along with a diet and time interaction. In terms of another important genetic markers of myogenin, FO increased myogenin (mmyog) levels in both male and female offspring compared to HF as shown in Fig 9B. No sexual difference is found for mmyog. Diet difference is found for 8-week offspring of male and female and 16-week male offspring. Nevertheless, no time difference is found between 8-week and 16-week male offspring.

16-week muscle growth in males

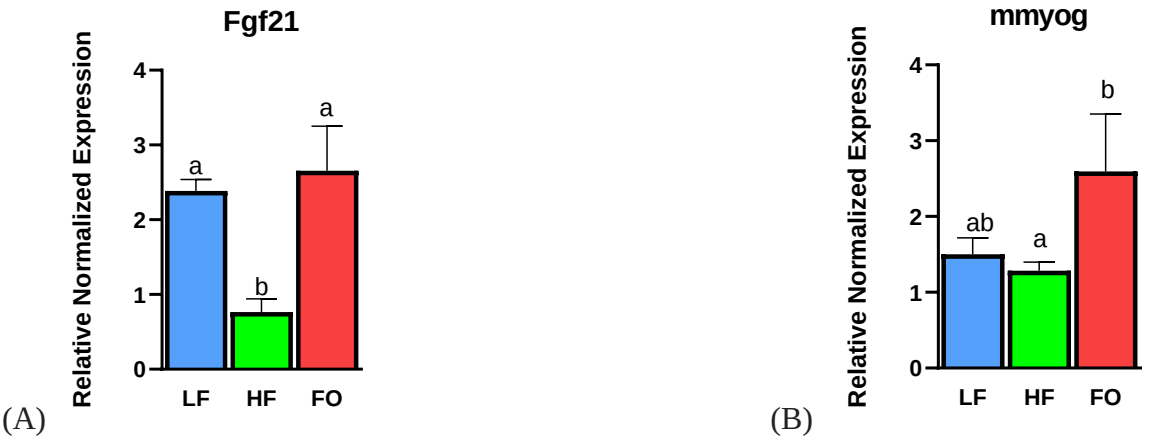
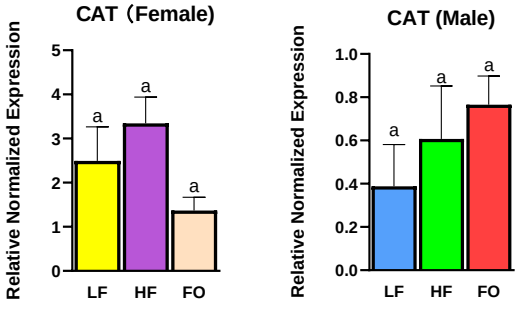


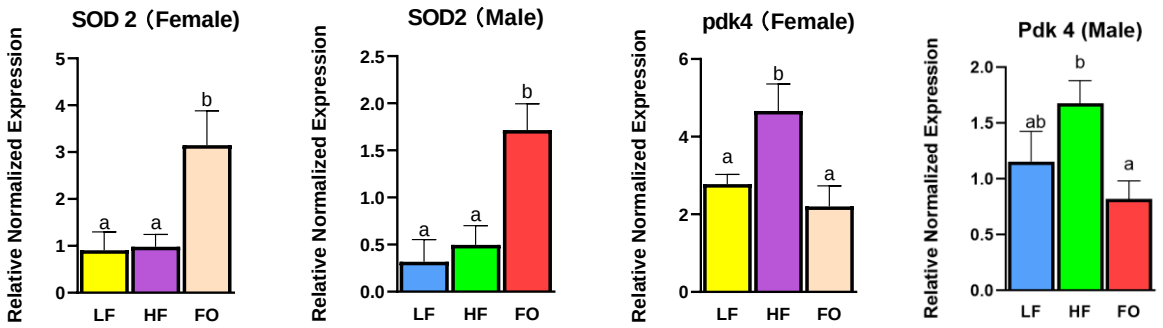
Figure 12: 16-week muscle data in males. Relative normalized expression of (A) myogenin (B) fibroblast growth factor 21. Groups with the same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.

Fgf21 levels were lower in HF offspring compared to LF offspring. Further, FO increased fgf21 at 16 weeks, compared to HF father. As indicated in figure 10B, an increased mmyog expression was detected with FO, compared to offspring born to HF father.

8-Week oxidative stress in male and female offspring



(A)

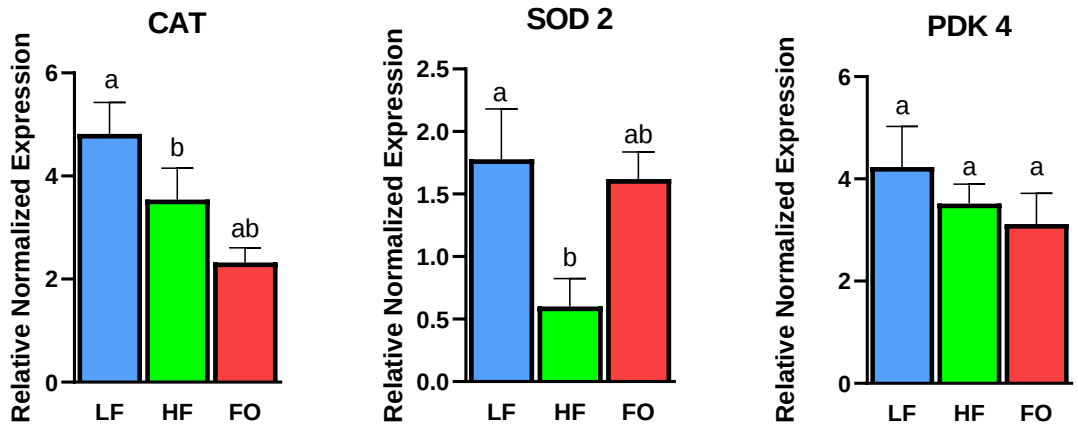


(B) (C)

Figure 13: 8-week muscle data in males and females. Relative normalized expression of (A) catalase (B) superoxide dismutase 2. (C) pyruvate dehydrogenase kinase 4. Groups with same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.

The relative normalized expression of genetic markers of oxidative stress are depicted in figure 13. For catalase as shown in Fig 13A, no significant difference was detected between groups in both male and female offspring. However, female offspring born to both LF father and HF father had higher expression compared to male offspring with LF and HF father respectively. Although no diet difference is found for the expression of Acat, a diet and sex interaction is found. On the other hand, 16-week male offspring with LF and HF father have higher levels in contrast to 8-week male offspring born to LF and HF father respectively. Interestingly, no diet difference is found, but a diet and time interaction is indicated. Further, as indicated on Fig 13B, SOD2 levels were comparable between HF and LF female and male offspring. Interestingly, FO increased SOD2 levels in both male and female offspring compared to offspring born to HF and LF father. For female offspring, higher expression is seen in FO fed father compared to male offspring with

FO fed father. A diet difference is detected with no sex and diet difference. In terms of time difference, 16-week male offspring with LF father had higher levels of SOD2 compared with 8-week male offspring born to LF fed father. Diet difference is found in addition to diet and time interaction. On the other hand, pyruvate dehydrogenase kinase 4 (PDK 4) of Fig 13C was higher in female offspring born to HF father compared to LF female offspring. Further, FO reduced PDK4 levels compared to HF. On the other hand, no difference was observed between male offspring and LF offspring, but FO reduced PDK 4 levels compared to HF father. However, for female offspring with LF fed and HF fed father, PDK 4 showed a significant upregulation compared to male offspring born to LF and HF father respectively. Diet difference is found with no interaction of diet and sex interaction. For time difference, 16-week male offspring with LF and FO father had a higher SOD 2 expression in comparison with 8-week male offspring with LF and FO father respectively. No diet and diet-time interaction is found.



(A) (B) (C)

Figure 14: 16-week muscle data in males. Relative Normalized Expression of (A) catalase (B) superoxide dismutase 2. (C) pyruvate dehydrogenase kinase 4. Groups with same letters indicate no statistical significance and groups with different letters indicate significance at p value less than 0.05.

The genetic markers presented in Fig 14 are correlated with oxidative stress. For CAT and sod2 as shown in Fig 14A-B, a significant downregulation was observed in male offspring born to HF father compared to offspring of LF, but no difference between HF and LF. No significant differences here are detected for PDK4 between all groups.

Discussion

In this study, we examined the specific role of paternal obesity and FO on metabolic health of the next generation. We observed improvements with FO in insulin signaling, oxidative stress, muscle growth and fatty acid utilization as evidenced by upregulation of key genetic markers. This is one of the first studies indicating the beneficial effects of FO supplementation in reducing paternal obesity and improving offspring skeletal muscle health.

Major characteristics of obesity and T2D is chronic inflammatory condition, indicated by elevated pro-inflammatory cytokines[93]. Excess accumulation of lipids in the adipose tissue due to obesity induces pro-inflammatory cytokines including TNF alpha, which then increases protein catabolism leading to skeletal muscle insulin resistance. [34] Further, increased inflammation also leads to oxidative stress in obese populations. This is in line with our data which shows increased SOD2 expression. Further, FO is an anti-inflammatory agent and in part also reduces oxidative stress [94]. One study conducted by Bhaswant et al. in 2017 showed FO supplementation to

improve muscle insulin responsiveness as evidenced by increased glucose uptake and oxidation rate and mitochondria function in skeletal muscle among rats compared to rats fed with HF exclusively. Further, decreased oxidative stress levels were observed as indicated by increased catalase expression. This is in line with our study, where we observed upregulation of genetic markers like super oxide dismutase 2 linked with oxidative stress in mice fed FO.

HF diet also affects skeletal muscle function due to increased oxidative stress. In obese populations, increased ROS production is responsible for muscle atrophy, and contractile malfunction that could eventually develop to sarcopenia[26,27]. On the other hand, FO contributes to skeletal muscle recovery.[78] Liu et al. have demonstrated that FO is beneficial in maintaining skeletal muscle protein mass by inhibiting inflammation signaling pathways. One study showed decreased level of inflammation markers including TNF-alpha and interleukins that contribute to rebound insulin resistance.[14] The study demonstrated that mice lacking p55 (TNF receptor) showed improved insulin sensitivity when compared to mice lacking p 75 (Another TNF receptor). The study indicated the specific receptor that is linked with insulin sensitivity as well as inflammation and highlighted the pathological role TNF potentially played. Akt is a key downstream protein of that assists in insulin signaling transduction pathway. It induces skeletal muscle protein synthesis and inactivates Foxo1. Foxo1 is a transcription factor that is regulated by Akt/PI3 pathway, and activation of Foxo1 leads to potential muscle degeneration. The Akt/PI3 pathway, on the other hand, is also essential for insulin signaling to translocate the Glut 4 vesicles to cell membrane and facilitate glucose uptake. Our study found that FO can stimulate the pathway, leading to a better insulin response. Our study reveals an increase in Glut 4 and Irs 1 expression among FO diet compared to HF diet.

Another defect with obesity is impaired mitochondrial dysfunction.[37,38] As some studies have demonstrated, compromised mitochondria also contributes to decreased generation of mitochondria as evidenced by decreased mitochondria biogenesis followed by mitochondrial dysfunction[39,40] Study conducted by Bhaswant found increased oxygen consumption and AMPK phosphorylation indicative of increased mitochondria functions levels in offspring supplemented with FO. With our study, we found increased expression of Mtco1 in female offspring, indicating a similar increase of mitochondrial function.

Aside from that, another factor that contributes to insulin resistance is accumulation of intramyocellular lipids among the insulin-resistant or T2D populations.[43,44] FO decreased levels of long chain acetyl CoA levels and ceramides, which are lipid mediators that interferes with insulin signaling. Meanwhile, Glut 4 and Irs 1 expressed more compared with in FO group compared with HF group.[52] Insulin provides a pivotal in lipid synthesis and oxidation as well. Insulin facilitates lipid synthesis and inhibits beta oxidation.[95] With HF diet, large influx of lipid entering mitochondria, resulting in decreased rates of beta oxidation and potentially protein misfolding. FO restores the balance of lipid synthesis and oxidation. On the other hand, fish oil could potentially reduce intracellular lipid levels by upregulating beta oxidation via PPAR alpha,[86] which has proven to increase mitochondrial biogenesis. In line with the above studies, our study indicated deceased fatty acid synthesis and increased beta oxidation as evidenced by decreased expression of Fasn and Fabp4 and upregulation of ppar alpha, cidea, and acat with FO supplementation. In addition to that, we also observed better insulin response of more rapid decrease of blood glucose level following insulin injection according to the insulin tolerance test. This could be responsible for partial alleviation of skeletal muscle insulin resistance. Our study

also highlights this potential muscle productive effect as evidenced by increased expression of mmyog and Fgf21.

While most studies address the direct effects of fish oil on skeletal muscle health, the intergeneration effect of fish oil is not fully understood, especially consumption of FO during the pre-conception period by father. Numerous studies showed that paternal obesity negatively affects offspring health[63,96,97]. One study has reported a low birth weight of progeny from obese rodent fathers with imbalanced growth hormone/ insulin growth factor-I (IGF-1) ratio, and decreased growth hormones resulting in abnormal muscle and fat development as evidenced by decreased expression of myogenic differentiation factor 1 and Igf1r expression respectively.[60] On the other hand, paternal exercise endowed a protective effect to offspring if rodent father is fed with a high fat diet.[57,61] Krout, et al. have postulated that those progenies have a lower percentage of body fat and a decreased expression of FGF21, which as a gene that controls adipocyte production.[61] Specific mechanisms on how paternal obesity affects the offspring remains inconclusive, but several research studies have pointed out epigenetics[64,98]. Corroborating with this, one study showed that overweight male sperm contained lower methylation rate at certain gene regions..[62] We haven't looked into the epigenetics and would be in future studies.

In conclusion, our study found that paternal supplementation of FO before pregnancy in fathers is more beneficial to the next generation. However, we found beneficial effects at 8 weeks compared to 16 weeks. It could be because of prolonged exposure of a rather healthy diet. (Chow-Diet) that offsets the negative effect of paternal obesity. Specifically, markers of insulin signaling, and fatty acid synthesis were not altered at 16 weeks with FO. Interestingly, we found

increased mmyog and fgf21 expression at 16 weeks, which are related with long-term muscle development. Short-term health benefits, on the other hand, are prominent. According to the 2-way ANOVA result (Table 4), upregulation of genetic markers related with insulin signaling, fatty acid synthesis, and beta oxidation showed a long-term effect of FO. Recent research by Li et.al showed long-term fish oil supplementation is connected to a decreased all-cause mortality. This is also in line with our finding of lower body weight found in male offspring with FO father. The profound impact we observed here might be related with a relatively higher body weight of male offspring, which one study done by Du et. al has pointed out that over-weight or obese subjects might be benefited the most[99]. Our findings highlighted this beneficial and significant effect of fish oil as well. When we compare males and females, sex difference is depicted based on Table 3. Most of the genetic markers, except Acat, mmyog, and SDHA, showed significant sex-dependent genetic expression variation with female offspring potentially benefitting more from the paternal supplementation of FO with increased mtco1 expression, decreased fasn expression, and increased irs 1 expression.

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EDUCATION

Syracuse University *August 2020 - Now*
M.S: Nutrition Science Falk Collge Overall GPA: 3.9/4.0

University of Iowa *August 2015 - May 2019*
Major: Human Physiology B.S. Psychology B.A. College of Liberal Arts Overall GPA: 3.25/4.0

SKILLS

Genetic Testing

qPCR Analysis with Quantstudio

Lab Animal Management

RESEARCH EXPERIENCE

Research Assistant Semptember 2021 - Now
Ramalingam lab *Syracuse University*

- Assisted research related with paternal obesity, fish oil and mice with a core focus on muscle tissue.
- Manufactured mRNA and CDNA using Zymo Reserach kit and Applied Biosystem kit respectively
- Weekly weighed and recorded food consumption of lab mice using lab laptop.
- Helped professor dissecting mice and extracting liver, brain, muscle, and adipose tissue.
- Sorted academic over 50 articles that are helpful for the research.
- Weekly reported research update

Article Reviewer May 2021 - September 2021
Department of Nutrition Science *Syracuse University*

- Worked in a team of two to review articles related with Parkinson and vitamin deficiency.
- Discussed and made specific inclusion and exclusion criteria.
- Finalized specific articles to include in the study

AWARDS AND ACCOMPLISHMENTS

Nutrition Counseling Certificate of Achievement

Graduation Tuition Scholarship

WORKING EXPERIENCE

Phone Operator January 2017 - May 2017
Crisis Center *Iowa City*

- Discussed with over 20 callers about their concerns
- Summarized, updated and made profile about each call and caller
- Provided minor psychological counseling.