INVESTIGATING THE TRANSFER OF GUT MICROBIOTA FROM EXERCISE-TRAINED ANIMALS

PHYSIOLOGICAL ADAPTATION ASSOCIATED WITH TRANSFER OF MICROBIOTA FROM EXERCISE-TRAINED MICE INTO GERM-FREE MICE

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LAY ABSTRACT

The gut microbiome or microbiota describes the composition of the human gut – remarkably, over 100 trillion bacterial cells live in symbiosis with the cells of the human body. Research from the past decade has elucidated the salient nature of the human gut microbiome on immunity, metabolic homeostasis, and overall health and disease. Transformative research in the field has demonstrated the ability to transfer these bacterial colonies from one individual to another and elicit change, such as altering body mass and adiposity, respective to their donor. The interaction between gut microbiota and other organ systems i.e. brain, liver, adipose tissue has been the focus of several recent investigations, suggesting that lifestyle changes such as diet and exercise can influence communication between the gut and various other organs and contribute to changes in function. Skeletal muscle is the largest muscle in the human body accounting for 40% of total mass and although the main role of skeletal muscle is locomotion and postural stabilization, it is integral for the regulation of blood glucose as well as a reservoir for other macronutrients. Acute and chronic physical exercise cause a myriad of adaptive responses throughout the human body including in skeletal muscle and the gut. Therefore, the existence and influence of a gut-muscle link or 'axis' on human health cannot be ignored. What is unclear exactly, is if exercise-induced adaptations in the gut of an individual can be transferred to elicit change in the gut of a recipient and further induce adaptations at the level of the skeletal muscle.

ABSTRACT

Exercise is known to induce changes in the gut, typically referred to as the 'forgotten organ', and changes in gut microbiota can also occur with exercise possibly imparting systemic benefits. The question remains whether or not microbiota from an exercised animal can independently affect skeletal muscle morphology. Our first objective was to examine whether an endurance exercise program could modify the microbiota in donor mice. Second, we aimed to elucidate if such an endurance-trained microbiota could be transferred to germ-free mice via fecal inoculation. Finally, we sought to determine how the morphology and functional characteristics of skeletal muscle were influenced as a result of fecal inoculation. We hypothesized that germ-free mice recipients inoculated with the microbiota from endurance trained donors would undergo morphological changes in muscle fibre type composition and physiological changes in skeletal muscle function associated with a more oxidative phenotype. Eight-week-old male C57BL/6NCrl donor mice (n = 20) were randomized into two groups: one group completed an endurance exercise training protocol on a treadmill machine 3x/week for 11 weeks (n = 10) while one group remained cage-bound (n = 10). Ten-week-old male (n = 7) and female (n = 9) germ-free mice were colonized with the cecal microbiota of the donor mice in that, equal numbers of germ-free mice were inoculated with exercised-microbiota as sedentary-microbiota. Glucose metabolism and performance measures were evaluated in the donors as well as the recipients post-inoculation. Muscle tissue was extracted for immunohistochemistry and mitochondrial assays. During the intra-peritoneal glucose tolerance test (IPGTT), significant differences in blood glucose were found at 30min

between exercise-inoculated and sedentary-inoculated (23.4 ± 2.2; 29.0 ± 1.9 mmol/L, $p<0.05$).and change in blood glucose relative to baseline (12.04 ± 2.4; 18.3 ± 1.9 mmol/L, $p<0.01$). There were significant sex-based differences in the blood glucose response in inoculated animals such that there were significant differences in blood glucose between the exercise-inoculated females and sedentary-inoculated females at 15mins (28.4 ± 2.4; 30.6 ± 1.1 mmol/L, $p<0.05$) and 30mins (24.7 ± 3.6; 29.9 ± 2.4 mmol/L, $p<0.01$), however no differences between exercise-inoculated males and sedentary-inoculated males. In addition, there were significant differences in the change in blood glucose relative to baseline between the exercise-inoculated females and sedentary-inoculated females at 15mins (12.3 ± 1.9; 20.6 ± 0.8 mmol/L, $p<0.01$) and 30mins (10.2 ± 2.6; 19.9 ± 2.1 mmol/L, $p<0.001$). This novel characterization of a link between gut microbiota and skeletal muscle suggests a transmissible capacity of microbiota to impart properties of 'healthy' muscle into compromised populations.

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Praise be.

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LIST OF ABBREVIATIONS

16S/18S	Svedberg (metric used to distinguish ribosomes)
ANOVA	analysis of variance
AUC	area under the curve
β-HAD	beta-hydroxyacyl dehydrogenase
BMI	body mass index
BSA	bovine serum albumin
C. difficile	Colstridium difficile
C/Fi	capillary-to-fibre ratio
CFPE	capillary-to-fibre perimeter exchange index
COXIV	cytochrome c oxidase subunit 4
CS	citrate synthase
CSA	cross sectional area
DAPI	4', 6-diamidino-2-phenylindole
DSHB	Developmental Studies Hybridoma Bank
ECV	extra-cellular vesicles
FTR	forced treadmill running
GF	germ-free
GI	gastrointestinal
GLUT4	glucose transporter 4
GS	goat serum
GTT	glucose tolerance test
IGPTT	intra-peritoneal glucose tolerance test
MHC	myosin heavy chain
mRNA	messenger ribonucleic acid
OTUs	operational taxonomic units
PBS	phosphate buffered solution
PFA	paraformaldehyde
rRNA	ribosomal ribonucleic acid
SCFA	short-chain fatty acid
SPF	specific pathogen free
TA	tibialis anterior
VO_2max	volume of maximum oxygen consumption
VWR	voluntary wheeling running

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DECLARATION OF ACADEMIC ACHIEVEMENT

N. Saddler was the principal contributor for conceptualizing the research question, research hypothesis, experimental design, data collection, data analysis and data interpretation. G. Parise assisted with research question, research hypothesis, experimental design, and data interpretation. S. Fortino, A. Thomas, and I. Khan assisted with data analysis.

INTRODUCTION

i. Exercise-induced Adaptations in Skeletal Muscle

Skeletal muscle is the largest organ in the human body and integral for metabolic homeostasis in mammalian organisms. In addition, skeletal muscle is one of the largest sites of glucose disposal and assists in regulating fatty acid utilization, attesting to its involvement with energy substrate metabolism[1]. Being fundamental to driving locomotion, this tissue, and its adaptations with training, has the capacity to determine the efficiency at which exercise can be performed. Endurance or aerobic exercise refers to activities that increase the activity of the cardiovascular system i.e. heart rate and breathing, for an extended period of time. The physiological and biochemical demands of endurance exercise disrupt homeostasis and induce responses in the systemic environment and skeletal muscle[2]. In response to aerobic stimuli, the human body has the remarkable ability to undergo cellular and structural adaptations, which gives rise to improvements in mechanical, metabolic, and neuromuscular efficiency.

The practice of endurance exercise over a period of weeks, months, or years is referred to as endurance-training and the extent of systemic adaptations are dependant on the duration, intensity, and type of endurance training performed. Considering skeletal muscle makes up 40% of the human body by weight[3], the changes that occur in these tissues contribute significantly to whole-body metabolic and physiological adaptations. The phenotypic adaptations of skeletal muscle occur at the level of the individual muscle fibres by improving the peripheral and mechanical properties of muscle[3–5]. Adaptations

associated with endurance exercise generally include a more oxidative phenotype or a shift toward type I fibre characteristics[6,7], i.e. increase in mitochondrial density (mitochondrial biogenesis)[5,8,9], increase in capillarization[10,11], upregulation of oxidative metabolic enzymes, and an increase in mitochondrial protein content[7,12–14].

Tighter regulation of oxidative metabolism is achieved through enhanced delivery and utilization of oxygen at the systemic and cellular level. This can be influenced by the pre-existing distribution of muscle fibres and muscle fibre composition in the host. The skeletal muscle fibres of mammals can generally be categorized into slow-twitch (oxidative) and fast-twitch (glycolytic). Slow-twitch muscle fibres contain myosin heavy chain (MHC) type I, the slow MHC isoform, recognized as type I in mammalian muscle. Fast-twitch muscle fibres contain MHC type II, the fast isoform, recognized as type IIa and IIx in humans, while in rodents it includes type IIa, IIx, and IIb[15,16]. Type IIa is the most oxidative of the fast twitch muscle fibres in rodents, therefore often prominent in slow muscles[17]. The soleus is classified as a slow-twitch oxidative muscle containing predominantly type I and type IIa muscle fibres while the tibialis anterior (TA) is classified as a fast-twitch glycolytic muscle containing mainly type IIx and type IIb muscle fibres[18].

Typical macroscopic measures of training adaptations include measures of VO_2 peak, which is predominantly performed on human models, as well as various other performance tests, such as run or swim to exhaustion, which are typically reserved for assessing animal models. VO_2 peak is a measure of maximum oxygen consumption and is an indicator of cardiorespiratory fitness. Following seven weeks of aerobic exercise on

a cycle ergometer, males had markedly higher VO_2 values than females despite both sexes improving post-training. However, when values were made relative to fat-free mass, sex differences did not persist[19]. In rodent models, performance testing in the form of running or swimming to exhaustion is a common practice. In a training study, progressively increasing exercise intensity in rats for three months found that time to exhaustion for the trained group was significantly higher than the untrained group[20]. The result is the same regardless of whether rodents were trained on a treadmill[20] or through voluntary wheel running[21].

At the cellular level, these adaptations typically manifest as changes in vascularization, fibre type conversion, and mitochondrial function. Reported increases in capillary density have ranged from 15-20%[22] and have been noted by Holloszy (1985) to increase up to 30% following an endurance training regime[7]. Standard aerobic training is non-hypertrophic in nature and therefore, in most circumstances, cross-sectional area (CSA) is unchanged[23,24]. Phenotypic shifts in fibre type tend to occur in response to endurance training in order to become more oxidative or possess more oxidative properties. Joanisse and colleagues (2015) observed an increase in the proportion of hybrid fibres concomitantly with a reduction in type II fibres. This finding of fibre type transitioning is supported by studies which have found exercise-induced adaptations in the oxidative morphology of the gastrocnemius and the tibialis anterior muscles of trained mice. More specifically, in the gastrocnemius, there were increases in the proportion of type I and type IIa fibres[22]. In the tibialis anterior muscle, increases in type IIa/IIx fibres and associated reductions in type IIb fibres were exhibited in trained mice[23]. With respect

to energy metabolism following training, there tends to be a reduction in the utilization of carbohydrates which is compensated by a proportional increase in lipid oxidation. Lastly, findings in both rodent and human models have shown that consistent aerobic training upregulates the activity of mitochondrial enzymes, including citrate synthase (CS), complex II-III, and complex IV (COXIV), as well as markers of beta-oxidation, such as beta-hydroxyacyl dehydrogenase (β-HAD).

ii. The 'Forgotten Organ': Eubiosis and Dysbiosis

The mammalian gut contains a dense collection of microbial communities, harbouring approximately 100 trillion microbial cells. Recent findings in the last decade have recognized these gut microbes as the 'forgotten organ'[25], as it has been elucidated that they have a substantial influence on human health. The human gut microbiome is composed of these specialized bacterial microorganisms that inhabit and line the gastrointestinal tract shortly after birth playing a significant role in maintaining health: providing nutrients[26–28], bolstering the immune system, and modulating gastrointestinal development[26,27].

Another function of the gut microbiota is regulating the production of short-chain fatty acids (SCFA), which have a key role in host energy metabolism. Humans are unable to digest dietary fibre due to absence of the proper enzymes, allowing them to simply pass through the primary segments of the gastrointestinal tract. In the cecum and remainder of the large intestine, the microbial communities ferment the leftover non-digested mass, which produces SCFAs among other 'waste' by-products. The most

common SCFAs produced by the resident bacteria are acetate, propionate, and butyrate which are secreted into the gut lumen, absorbed into host circulation eventually landing in specific target organs[25,29,30]. Typically 95% of the produced SCFAs are absorbed and transported in the bloodstream while 5% are excreted in the feces, which is typically measured as a surrogate for comparing group differences in SCFA production[30].

Possessing both human and microbial cells in a 1:1-1:3 ratio, respectively, this coexistence is the reason the human body has come to be referred to as a hybrid organism[31,32]. The gastrointestinal tract is considered sterile until birth after which microbes begin to colonize the body[25], interfering with physiological and immunological development until the host dies. The gut microbiota is highly variable from individual to individual, dependent on maternal influence, as well as cues from the surrounding environment. Environmental factors are able to stimulate the proliferation of specific bacterial colonies while subsequently depleting others. There have been several groupings that have been identified in the mammalian gut including *Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia, Actinobacteria*, *Cyanobacteria,* and *Fusobacteria*[33]. Components of these colonies constituting the microbiome are incorporated into systemic circulation in the host and thus, transported to different organs, all with varying effects[34,35]. In reference to SCFAs, the primary end product fermented and produced by the *Firmicutes* phylum is butyrate while the *Bacteroidetes* phylum commonly is recognized for producing acetate and propionate[30].

Eubiotic status is characterized by a dominating influence of potentially beneficial bacterial species thriving within the gut. This status can be threatened by antibiotic

treatments and pathogens among other factors leading to disharmony and a lack of diversity in bacterial colonies[26]. Dysbiosis describes this microbial imbalance when it is maladaptive to the host in conditions, such as obesity[27,28,36–39], diabetes[37,38], and metabolic syndrome[27,37,38]. Potential therapies have been targeted at this deleterious state including probiotics and prebiotics which may be of importance when considering inflammatory bowel disease and colitis[40]. Although it is also recognized extensively in the literature that diet is influential in shaping the gut microbiome, investigations focusing on the impact of exercise have provided compelling evidence on its effect on bacterial colonies as well as its ability to modulate the deleterious effects of disease on the microbiome.

iii. Exercise and the Microbiome

Physical exertion during endurance exercise induces physiological and biochemical changes in systemic circulation and in the past decade, more focus has shifted toward the impact of exercise on the microbial colonies and conversely the influence of the microbiota on exercise performance. The influence of exercise on microbiota composition was first assessed by Matsumoto et al. (2008), in which they found that there were increases in the concentration of n-butyrate: a short-chain fatty acid synthesized by bacteria in the colon, following 5 weeks of voluntary running in rats. Further analyses found a higher expression of butyrate-producing bacterium, specifically from the *firmicutes* phylum, post-training[41]. In humans, six weeks of moderate to vigorous endurance training increased fecal concentrations of acetate, propionate, and

butyrate but seemed to be associated with pre-intervention body mass index status (BMI), as the change was primarily observed in lean subjects. Of interest, when the individuals returned to their previously sedentary behaviour for six weeks, fecal concentrations of the SCFAs returned to pre-intervention levels, thus suggesting that exercise directly influenced gut microbiota function, particularly in SCFA production[42].

Exercise alone was able to induce significant shifts in the two main bacterial phyla, *Firmicutes* and *Bacteroidetes*, almost to the same extent in directionality and magnitude when compared to the effects of a high-fat diet in a study conducted by Kang et al (2014). Mice performed a forced exercise regime on speed-regulated running wheels causing alterations in the gut microbial community induced by the training. The changes were noted to be orthogonal to those induced by a high-fat diet[43]. Conversely, voluntary exercise as set by an accessible running wheel within the cage showed an increase in microbiota diversity correlated with total distance run. The balance of *bacteriodetes*, *firmicutes*, as well as *actinobacteria* were all altered following twelve weeks of access to the running wheel[39].

It is notable that in the simplest sense, exercise is able to increase the richness and diversity in the microbial communities of the mammalian gut[39,43–46]. However, there is conflicting evidence in the literature regarding what constitutes the 'optimal' combination of bacterial phyla in the gut. With respect to animal models, exercise in mice and rats was associated with higher *bacteroidetes* and lower *firmicutes* in fecal matter[39,44], but cecal microbiota following six weeks of training contained a greater abundance of specific firmicutes species and lower concentrations of the *bacteroides/prevotella* genera[45]. A

similar finding to the latter was found at the phyla level in separate trials – an increase in firmicutes, proteobacteria, and actinobacteria but an exercise-related reduction in *bacteroidetes*[43,46]. Contradictory findings clearly exist when considering animal models, but it is possible that methodological differences in exercise protocols can explain these differences.

The modality and conditions of the endurance exercise is performed can vastly change the resulting effects on the gastrointestinal physiology in animal models. In a recent investigation by Allen and colleagues (2015), exercise-induced adaptations in the bacterial colonies were compared following two different exercise modalities: six weeks of forced treadmill running (FTR) for a set length and intensity or voluntary wheel running (VWR) with an accessible wheel fixated in the home cage. Contrary to the hypothesis of the investigators, VWR was observed to have the least diverse microbiota in the feces and cecal context according to the α-diversity metric. However, another composition metric, the Shannon Index, exhibited that cecal contents of the VWR group trended towards the highest level of diversity in comparison to the FTR group and the sedentary group. The basis of these metrics is analytically unique meaning that although the VWR group was the least rich, the microbial colonies were more evenly distributed. The significant finding of this investigation though was that forced and voluntary exercise induce contrasting effects on the host microbiome in terms of diversity, structure, and taxonomy. This may be attributed to the differences in microbial colonies as mentioned previously in response to exercise training whether performed in a forced[43,45] or voluntary[39,43,44,46] manner.

The relationship between exercise and the microbiota is not restricted to post-aerobic training interventions but influenced by general training status as well. Peak VO_2 was found to be positively correlated with increased bacterial diversity, specifically able to account for more than 20% of the variation in taxonomic richness. The predicted functions included an increase in butyrate-producing bacteria and other aspects associated with improved gut health[42]. When comparing trained athletes to untrained individuals, as Clark et al. (2014) did with elite rugby players, the trained athletes possessed a clear increase in diversity as determined using the Shannon Index. BMI was even accounted for, separating untrained persons into low (<25.2) and high (>26.5) BMI subsets and the difference in microbial diversity between athletes and these two groups persisted. Among the top flux changes in relative abundance, *firmicutes* were revealed to be significantly higher than in either the low or high BMI groups – *lactobacillus*, *bacteroides*, and *lactobacillaceae* were found to constitute the lowest proportion[47]. In a follow-up study, a number of correlations were drawn between metabolic pathways, predicted using the composition of the gut, and dietary characteristics including macronutrients[48]. Although it must be acknowledged that diet was not necessarily controlled for and it could not be confirmed or denied that athletes were eating healthier in either investigation[47,48]. Exploratory analysis from the American Gut Project further elucidated in humans that a shift in performing moderate exercise from never to daily augmented α-diversity with the most significant increase in the *firmicutes* phylum[49]. Although exercise has been shown to influence the gut microbiota and that diet may be a confounding variable, it remains to

be elucidated how the change in gut microbiota may independently affect skeletal muscle health and function.

iv. Use of the Gnotobiotic Mouse Model

A gnotobiotic mouse model is an animal in which only specific known strains of micro-organisms (i.e. bacteria, viruses, parasites, fungi) exist. Therefore, by definition, germ-free mice are an example of a gnotobiotic mouse model. Germ-free mice are void of all microorganisms and can be produced by caesarean section, avoiding bacterial colonization from maternal contact during vaginal birth, or a specialized and sterile embryo-transfer technique. These mouse models allow researchers to examine the symbiotic relationship between specific bacterial colonies and homeostasis, health, and performance[50]. The use of this strain allows for inoculation or colonization of a pre-existing microbiome from a donor to isolate gut and whole-body physiological and functional changes. In the current investigation, the transfer of an 'exercised microbiome' may provide the opportunity to isolate the connection between exercise-trained systemic circulation, skeletal muscle function, and the gut microbiota.

The problem with using germ-free mice is the labour-intensive nature and strict standards of care to protect against contamination and as a result – housing and caring for these animals is extremely costly[51]. Gnotobiotic facilities must have extremely restricted access and filtered ventilation with most containing double-door and fail-safe systems to prevent any chance of contamination. In addition, all bacterial presence in food, water, and bedding must be eradicated through extreme heat (above 100˚) sterilization

procedures[50]. The high cost and degree of maintenance inspired the use of another method to prevent the influence of pre-existing microbial communities and subsequently allow for reconstitution of the mammalian gut. The use of antibiotic drugs such as ampicillin or vancomyosin eradicates pre-existing bacterial colonies within the gut and is a common treatment to prepare a standard mouse model for a microbiota transplant. Although this method of non-competitive colonization has been used[52,53] there are notable caveats that must be addressed.

The depletion of microorganisms using antibiotics is by no means, a 'clean' method. It is simply not possible to control for what bacterial colonies will be eradicated and what colonies will endure or develop antibiotic-resistance through treatment. The impact on the microbial communities is not ubiquitous across all subjects treated – and considering the pre-existence of the microbiota, host physiology has already been exposed and influenced regardless of future eradication. Finally, the effect of the antibiotic treatment on host physiology or subsequent repopulation cannot be ignored as there are systemic effects that can be confounding in later analyses[51]. Although the gnotobiotic mouse model and germ-free models in specific, are by no means perfect, it avoids the aforementioned technical hindrances when superimposing a new microbial profile.

v. Microbiota Transplantation

The concept of transferring healthy gut microbiota from a donor to a recipient is not a novel approach[28,29,36,38,52,54–56]. Gnotobiotic mice have been inoculated with fecal

matter from obese and lean donors in several studies[28,36,54,56] demonstrating a transmissible phenotype driven solely by the influence of gut bacteria.

A mouse to mouse microbiota transfer experiment conducted by Turnbaugh et al. (2006) demonstrated the successful inoculation of gut microbiota from lean and obese donors into germ-free recipients. Remarkably, the transfer induced changes in body mass and microbiota composition reflective of their donors. Further advancing this concept, germ-free mice were colonized with uncultured fecal samples from human twins discordant for obesity intending to 'humanize' the gut of the recipient mice[56]. This served as a proof-of-principle that the fecal transplant technique was viable from a human GI system to a mouse, which indeed elicited microbiota composition changes in the recipients respective to their lean or obese donors. However, the interesting novel finding of this study was seen when obese and lean mice recipients were cohoused for ten days, five days post-inoculation. The recipients of the obese twin microbiota exhibited significantly lower increases in adiposity, similar to their lean counterparts, compared to a group of obese recipients that were not cohoused. Understanding that mice are coprophagic, the authors proposed that the lean mice microbiota was stable and therefore resistant to the obese microbiota and associated obese phenotype[56]. Yet, the obese recipient microbiota was able to be influenced through diversification by particular invasive bacterial colonies in the lean microbiota resulting in the bacterial profiles of the cohoused animals to be indistinguishable.

Microbiota transplants or fecal transplants are not exclusive to animal models – this technique is feasible and currently practiced in humans for treating deleterious

gastrointestinal conditions such as *c. difficile*[57]. The transplant method has been proven to be feasible, effective, and safe in terms of treating gastrointestinal disease in humans but remains unclear whether benefits exist that can be extrapolated beyond the digestive system as has been demonstrated in animal models.

vi. Microbiota Transfer from Exercised Individual

There is only published data from one investigation examining the transfer of exercise-induced changes in microbiota. Allen et al. (2018) colonized gnotobiotic mice with microbiota from recipients that had access to a running wheel for six weeks. Microbial communities clustered and thrived in the germ-free mice in a similar manner to the exercised donor pool – evidence that exercise not only altered the composition of the gut but that these bacterial colonies could be transplanted into another host. The transplanted colonies had functional consequences as shown when comparing exercised-microbiota recipients and sedentary control recipients in terms of distinct inflammatory responses, altered luminal concentrations of microbial-derived metabolites, and attenuated responses to acute colitis insult[58,59].

vii. Sex Differences in Microbiota Transplantation

Conflicting data is prominent in the literature concerning the exact sex-based differences in microbial communities or if any with microbiota transfer. In humans, there does not seem to be any sex-based limitations with fecal transplants as currently performed for amelioration of *c. difficile* – the donor and recipient do not need to be the

same sex[60]. However, there is evidence that there is an influence of sex on the development of microbial communities. *Bacteroidetes* appear to be in larger abundances in males than females[61], lining up with cross-sectional data from European countries (France, Germany, Italy, Sweden) revealing that concentrations of the *bacteroides-prevotella* group were significantly higher in males than females[62]. Although there is no apparent or reported sex-based limitations to fecal transplant treatment, in no circumstance does that mean the microbiota colonize and cluster in the same regard and may differ in lasting effects.

Sexual dimorphism exists in mouse models as well – male mice tended to possess a less diverse microbiota compared to females and a greater abundance of *parabacteroides,* a derivative of *bacteroidetes*[63], aligning with human data[61,62]. Sex-based differences in mouse models can be isolated more accurately using methods such as hormone treatment or gonadectomy, adding or removing the influence of sex hormones. A report using these methods found inconsistencies between 89 common inbred strains citing other factors such as diet, age, and host genetic background as analytic noise. The significance of these findings was that the sex-specificity in the relative abundance of particular microbiota colonies had functional consequences including an effect on bile acid profiles and microbiota response when introduced to a high fat or high sugar diet[64,65].

A common metric for determining sex-based or post-intervention differences in microbial communities is by identifying and comparing the operational taxonomic units (OTUs). OTUs generally refers to clusters of uncultivated or unknown organisms grouped by similar DNA sequences as analyzed by 16S or 18S rRNA sequencing analytic

software[66]. One study assessing the sexual dimorphism in microbiota transfer found there was sex-based differences in colonization at the level of the phyla, the OTUs, and the diversity metric. Male and female gnotobiotic recipients were inoculated with fecal microbiota from a young adult male subject to a seven-day vegetarian diet supplemented with inulin to increase the abundance of potentially beneficial bacteria. The Shannon index revealed that the inoculation stimulated a more diverse microbiota in female recipients while in males, a greater abundance of *bacteroidetes*-related species was discovered[67], following the trends of both animal and human studies[61–63]. The differences in OTUs were sexually distinct: 46 unique groups of sequences in different abundances between males and females post colonization[67]. The final area of interest is comparing the gut composition of the donor to the recipients in which the divergent nature of the colonization can be truly observed. The transfer stimulated colonies to thrive within the female recipients vastly different than the males causing there to be a much more significant deviation in gut composition similarities when assessing females recipients to the donor in comparison to male recipients and the donor[67]. The sex-based differences in microbial communities, especially following microbiota transplantation, remains a novel and thus exploratory field and should be considered when identifying treatments for gastrointestinal disease and gut-directed responses.

viii. Glucose Metabolism

The homeostatic control of glucose, one of the main fuels for oxidative and non-oxidative metabolism, is influenced by a variety of factors including diet and exercise,

and the response of blood glucose concentration is indicative of metabolic health. The ingestion of a meal containing carbohydrates results in hyperglycaemia and in response, insulin is secreted. Following the secretion of insulin, glucose uptake from the blood occurs at the level of the liver, fat and muscle cells. Glucose handling is influenced by a myriad of factors, all of which are related to the concept of insulin secretion or insulin resistance[68,69]. Glucose transport is the rate limiting step in glucose metabolism and is mediated by two separate glucose transporter 4 (GLUT4) signaling pathways in skeletal muscle: insulin-mediated glucose uptake and contraction-mediated glucose uptake. Acute bouts of exercise can sensitize skeletal muscle to insulin through contraction-mediated pathways and chronic exercise can improve whole body glucose homeostasis[70].

A glucose tolerance test is a standard protocol used to assess the homeostatic response to exogenous glucose and identify possible dysfunction. In humans, the test is typically performed after an overnight fast through the oral consumption of a 75g glucose beverage followed by two hours of subsequent blood draws. In mice, this can be performed orally using the gavage technique or through an intra-peritoneal injection following a fast ranging from 3-24 hours[71], dependent on measures of interest. The test is performed typically using bolus concentrations of 10%[72] to 20/25%[39,73,74] glucose solution administered at 1[39,72,73] , 1.5[74,75], or 2g/kg[69,72] of body weight. As mentioned previously, glucose regulation is influenced by environmental factors, specifically diet and exercise, and therefore the response to this test is dependent on a host of factors.

Habitual diet is especially important as identified by Foley and colleagues (2017), as a high-fat diet can induce gut dysbiosis leading to glucose and insulin intolerance. The

composition of the gut microbiota can influence the postprandial response and therefore how blood glucose is regulated. In this investigation dysbiosis occurred following 1-3 days of ingesting a high-fat diet whereas manifestation of insulin and glucose intolerance occurred by 3-4 days[69]. Although it is not entirely clear how the mammalian microbiome contributes to the progression of glucose intolerance, this elucidates the temporal relationship between gut dysbiosis and glucose/insulin dysregulation. These effects at the level of the gut bacteria can be transferred as shown in an investigation by Vrieze and colleagues (2012). The intestinal colonies from the lean donors were transplanted into individuals with metabolic syndrome significantly increasing their sensitivity to insulin as measured by glucose clearance[38]. As this data shows, the functional changes in glucose and insulin sensitivity is transmissible through microbiota transplantation.

Exercise is also a significant modulator of glucose and insulin sensitivity. As assessed by Trembley and colleagues (1985), endurance trained athletes had a reduced insulin response to glucose administration yet similar glucose tolerance when compared to untrained individuals. As the authors discussed, the reduced insulin response alludes to increased insulin sensitivity and therefore less insulin secretion to assist the uptake of a similar amounts of glucose from circulation[76]. A similar response was even observed in plasma insulin of obese adults four hours following a single bout of moderate intensity cycling[77]. Although as Trembley mentions, there are other adaptive mechanisms that contribute to the improvement of insulin sensitivity[76] in endurance trained individuals, it is evident that aspects of glucose metabolism are modulated and improved post-acute and -chronic exercise training.

It must be acknowledged that for individuals with elevated fasting glucose (5.28-6.94mmol/L), the improvements in glucose response following an intervention changing diet and introducing moderate exercise compared to exclusively introducing moderate exercise were not substantially different. Similar improvements were seen between the 6-month interventions of solely moderate-intensity exercise (6.4% improvement, effect size 0.60) and the diet + exercise intervention (8.2% improvement, effect size 0.73)[78]. Consistently, when moderate to moderately high aerobic exercise was combined with a diet compared to diet alone, both interventions had reductions in insulin response but OGTT insulin area-under-the-curve was reduced to a greater extent with an intervention of both diet and exercise [79].

The combination of exercise and diet is the gold standard for improving glucose metabolism but as further analyses have clarified, both the introduction of exercise and a healthier diet separately are effective at improving postprandial insulin and glucose responses[80].

ix. Sex Differences in Glucose Metabolism

Sex differences, if any, are critical in understanding the differences in homeostatic response to glucose administration and subsequent insulin secretion. In humans, females tend to have more fat mass and less fat-free mass in comparison to males. Considering muscle is a major metabolically active tissue involved in glucose uptake, the initial comparison determines that women possess an attenuated ability to metabolize the fixed amount of glucose in a GTT as compared to men[81,82]. However, the high prevalence of

impaired glucose tolerance in females may be artifact as the GTT is not an individualized, relative concentration of glucose but instead is an absolute amount. When males and females were matched for anthropometric measures i.e. height, weight, waist circumference[83,84]or matched for body composition[85], minimal differences in glucose absorption were observed in response to an GTT.

There is evidence seen in various strains of mice subjected to a GTT, that female rodents are more insulin-sensitive than their male counterparts. At multiple timepoints following glucose administration, female mice tended to have lower glucose levels than males as well as a greater fall in blood glucose following insulin administration[86]. The data on various strains suggests that female animals are more insulin sensitive than males and although quite the debate in humans, it must be considered in the current investigation.

x. Gut-Muscle Axis

The relationship between gut microbiota and skeletal muscle, deemed the 'gut-muscle axis', was previously coined by Bindels et al. (2013) in a review discussing gut microbes as therapeutic target for cachectic conditions[87]. A unique approach to examining the gut-muscle axis was conducted by Yan et al. (2016), aiming to determine the transmissible properties of skeletal muscle via fecal microbiota transplantation. The investigation found that when germ-free mice were colonized with fecal transplants from pigs that were either of a lean breed or obese breed, the two groups of mouse recipients developed unique body compositions, muscle fibre characteristics, and muscle fibre type distributions.

Specifically, there was a trend toward higher body fat mass in the obese breed recipient mouse population compared to the lean breed recipient mouse population. The fibre diameter and cross-sectional area in the recipient populations followed trends of the donors in which the lean breed recipients trended towards a larger fibre diameter and cross-sectional area in the gastrocnemius muscle. Lastly, the mRNA expression of myosin heavy chain isoforms reflected the common trends as seen with obesity – higher expression of the slow-twitch isoform and reduced expression of the fast-twitch isoform was expressed in the obese breed recipients, which was opposite to the trends in isoform expression in the lean breed recipients[36]. This novel characterization of a link between gut microbiota and skeletal muscle suggests the transmissible properties of 'healthy' muscle into compromised populations, however it lacks a clear indication of translatable function or performance.

There has only been a single study that has evaluated how exercise performance may be influenced by the gut microbiota. The performance measure used was a swim-to-exhaustion test and was performed in standard specific pathogen-free mice (SPF), gnotobiotic mice (GF), and *bacteroides fragilis* gnotobiotic mice (BF – gnotobiotic mice that had be colonized with *bacteroides fragilis*)[88]. One of the proposed associative functions of the intestinal microbiota involves antioxidant endogenous defense i.e. protecting against oxidative damage, which is crucial during intense exercise when oxidative stress is significant. During exertion, antioxidant enzymes including glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) are responsible for the critical role of preventing oxidative stress and before this trial, it remained to be seen

in what capacity the microbiota was involved in this metabolic pathway and if there was any gut-related regulation of antioxidant enzyme efficiency. The GF mice tested 2.09-fold shorter in time to exhaustion than the SPF mice with the BF mice exhausting in between the two groups. Notably, GF mice also possessed significantly lower amounts of CAT and hepatic GPx in comparison to the SPF and BF cohorts[88]. The absence of microbial communities within the gut reduced antioxidant enzyme activities, which the authors suggest may have had an impact on exercise performance. However, the respective data highlights the extent we know concerning microbiota transfers from exercised individuals or revelations on the gut-muscle axis. There remains a plethora of measures of muscle composition and function that have yet to be explored that could further our understanding in this field.

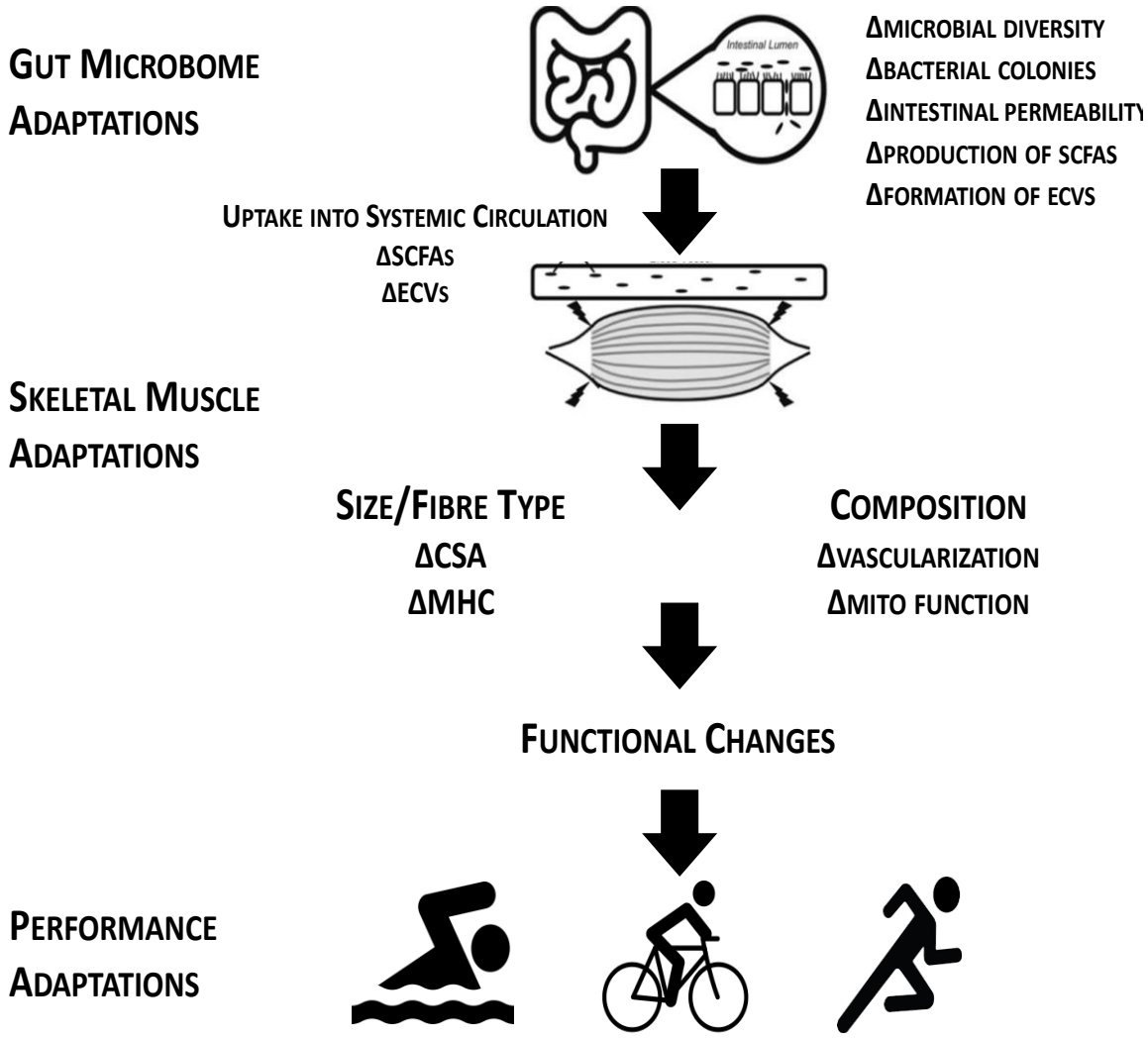


Figure 1: *Gut-Muscle Axis.* Changes induced by diet, exercise, and/or other potent stimuli at the level of the gut microbiota can have resulting effects in other tissues once by-products of bacterial fermentation i.e. SCFAs, ECVs are taken up into systemic circulation. Circulating signalling molecules can have a cascade effect at the skeletal muscle from cellular adaptations to functional and performance adaptations

xi. Objectives and Hypothesis

The purpose of this investigation was to characterize the morphological changes and physiological changes in skeletal muscle function following inoculation from the cecal microbiota of endurance trained donor mice into germ-free recipients. As a proof of concept, we sought to confirm that a forced endurance exercise regime was able to alter the intestinal microbiota composition in addition to supporting the notion that intestinal microbiota influence exercise performance and contribute to skeletal muscle adaptations and the overall health benefits of endurance exercise. We hypothesized that germ-free mice recipients inoculated with the microbiota from endurance trained donors would undergo morphological changes in muscle fibre type composition and physiological changes in skeletal muscle function associated with a more oxidative phenotype. We also hypothesized that metabolic measures and the performance test in germ-free recipients would directionally follow their respective donors.

METHODS

Mice

All experiments were approved by the McMaster University Animal Research Ethics Board (Hamilton, ON, Canada). Eight-week-old male C57BL/6NCrl mice (n=20) were purchased from Charles River Laboratories and maintained on a 12:12 light-dark cycle. Mice were randomly distributed into two groups: one group was subjected to forced endurance exercise training on a motor-driven treadmill (n=10) and the other group was left untrained for the duration of exercise training (n=10). Mice were housed no more than 5 mice per cage and fed a standard chow and water *ad libitum.* Cages containing trained mice and untrained mice were placed on different holding racks inside the housing room and were designated as the "Donor Pool". Germ-free mice (GF) were obtained using the two-stage embryo transfer method in the Farncombe Family Axenic-Gnotobiotic Facility. Male (n=7) and female (n=9) GF mice were aged to 10 weeks and then exported from the Axenic-Gnotobiotic Facility and were colonized by gavaging a cecal-content slurry from the Donor Pool previously mentioned. These GF recipients were colonized every 2 weeks for a total of 4 inoculations, then sacrificed at 8 weeks as shown in **Fig. 2**. They were housed in clean conditions using ventilated racks and all colonized mice were handled exclusively in the level II biosafety hood to prevent bacterial contamination.

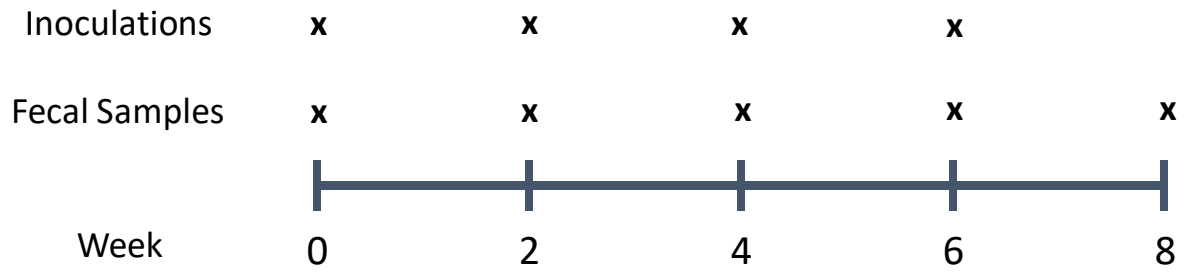


Figure 2: *Time course of inoculations conducted on gnotobiotic mice*. Inoculations were performed on weeks 0, 2, 4, and 6. Fecal samples were taken every two weeks.

Endurance Exercise Training

The endurance exercise regime was conducted on an Exer 6M treadmill (Instruments, Columbus, OH, USA) for 60min (10min warm-up, 45min run, 5min cool-down), 3x a week for 11 consecutive weeks. The warm-up and cool down were performed at a speed of 8m/min. The run started at 12m/min in week 1 and progressed to 20m/min by week 11. Firm wire brushes were used to provide a touch stimulus to encourage running. As a last resort, a light shock using a shock grid at the back of the treadmill was used for motivation when mice ceased to run. Untrained mice were exposed to the treadmill in the 11^{th} week.

Performance Measures

GF recipients were subjected to a run-to-exhaustion protocol, as seen in **Table 1,** adapted from established tests in the literature[89] to assess running performance. Prior to the exhaustion protocol (48h) both inoculated groups were exposed to the treadmill: stationary for 5min, then 10m/min for 10min.

Table 1: Performance Test Protocol – Run to Exhaustion

Duration	Speed (m/min)	Elevation (˚)
0-5 **(WARM UP)**	10	0°
5-7	11	0°
7-9	12	0°
9-11	13	0°
11-13	14	0°
13-15	15	0°
15-17	16	0°
17-19	17	0°
19-21	18	0°
21-23	19	0°
23-25	20	0°
25-27	21	0°
27-29	22	0°
29-31	23	0°
31-33	24	0°
33-43	25	0°
43-53	25	5°
53-63	25	10°
63-65	26	10°
65-67	27	10°
67-69	28	10°
69-71	29	10°
71-73	30	10°

Mice were considered exhausted when they could no longer keep the pace of the treadmill indicated by remaining on the shocker for ≥10 seconds if pedalling or ≥5 seconds if all limbs are on the shocker.

Metabolic Measures

All mice were subject to an intra-peritoneal glucose tolerance test (IPGTT) following the completion of the training program or inoculation schedule. Mice were fasted for 6 hours with food removed and bedding changed before initial blood collection via tail vein and then subsequent blood collections following injection of 2g/kg body mass of 10% d-glucose in 1xPBS at timepoints 15, 30, 60 and 120mins. Blood samples were measured using the OneTouch Ultra 2® (LifeScan Europe, Zug, Switzerland).

Animal Euthanasia

All mice were euthanized via cervical dislocation as to not disrupt biochemical processing or morphology of skeletal muscle. The tibialis anterior (TA), extensor digitorum longus (EDL), soleus, gastrocnemius, and quadriceps were harvested and weighed (**Table 2)** – half were snap-frozen in liquid nitrogen and half were mounted in optimal cutting temperature compound (Tissue-Tek; Sakura Finetek, Torrance, CA, USA), cooled in isopentane, and then frozen in liquid nitrogen for immunohistochemical analysis of fibre type composition, cross-sectional area, and capillarization.

Immunohistochemistry

To determine capillarization, TA and soleus muscle sections were stained with CD31 and laminin as previously described[90]. Slides were treated with paraformaldehyde (PFA) for 10 min, washed in PBS with Tween 20, and blocked in 1% BSA and 10% goat serum for 60 min. Slides were incubated in CD31 primary antibody (1:30; 28364; Abcam) overnight at 4°C. Slides were washed and CD31 was detected by using Alexa Fluor 594 goat anti-rabbit (1:500). Slides were then incubated with laminin primary antibody (1:500; ab11575; Abcam) for 2 h at room temperature. Slides were washed, and laminin was detected by using Alexa Fluor 647 goat anti-rabbit (1:500). Nuclei were detected by using DAPI. Capillary-to-individual fibre ratio (C/Fi) was determined as previously described[91,92] and capillary-to-fibre perimeter exchange index (CFPE) was calculated to act as an estimate of capillary-to-fibre surface area as previously described[93], providing a proxy for muscle fibre perfusion. Images were taken with a

CoolSNAP HQ2 fluorescent camera (Nikon Instruments). Images were analyzed by using Nikon NIS elements AR software. C/Fi and CFPE were determined on an average of 75 fibres/animal.

To determine fibre composition, sections were stained according to previously published methods with minor modifications[18]. Slides were blocked in 1% BSA and 10% goat serum for 60 min. Slides were then incubated for 120min in a primary antibody cocktail including type I (1:50; BA-F8, DSHB), type IIa (1:100, SC-71, DSHB), and type IIx (1:50, 6H1, DSHB) for the soleus muscle and type I (1:50; BA-F8, DSHB), type IIa (1:100, SC-71, DSHB), and type IIb (1:100, BF-F3, DSHB) for the TA muscle. Following several washes, slides were incubated in a secondary cocktail for 60min consisting of Alexa Fluor 488 IgG_{2b} 1:500 (type I), Alexa Fluor 488 IgG_1 1:500 (type IIa), and Alexa Fluor 594 IgM 1:500 (type IIx) for the soleus muscle and Alexa Fluor 350 IgG_{2b} 1:500 (type I), Alexa Fluor 488 IgG_1 1:500 (type IIa), and Alexa Fluor 594 IgM 1:500 (type IIx) for the TA muscle. Following more washes, slides were incubated in wheat germ agglutinin, Alexa Fluor 647 conjugate staining the sarcolemma of muscle fibres.

Fibre-type composition measurements were completed on the entirety of each soleus sample. CSA analyses was completed on the soleus using ~50 muscle fibres each of type I, IIa, and IIx as well as between 10-30 hybrid fibre types – type I/IIa and type IIa/IIx dependent on the number of fibres identified per sample. Muscle fibre types IIx/b and IIb were excluded from CSA analysis in the soleus due to a marginal amount of these fibre types in the soleus muscle.

Fibre-type composition measurements were completed on the entirety of each TA sample. CSA analyses was completed on the TA using ~50 muscle fibres each of type IIa, IIx, and IIb as well as between 10-30 of hybrid fibre types – type IIa/IIx and type IIx/IIb dependent on the number of fibres identified per sample.

Muscle fibres on the periphery of muscle cross sections were not included in the analysis. All immunofluorescence analyses were completed in a blinded fashion after being assigned a code by another lab-member.

Mitochondrial Enzyme Activity

One piece of muscle (~25 mg) was homogenized in Lysing Matrix D tubes (MP Biomedicals, Solon, OH, USA) using the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH, USA) for 5 x 5-second cycles at a speed of 4.0 m/s with samples placed on ice for 5 minutes between cycles. Samples were homogenized in 20 volumes of buffer containing 70 mM sucrose, 220 mM mannitol, 10mM HEPES, 1mM EGTA, supplemented with protease inhibitors (Complete Mini®, Roche Applied Science, Laval, PQ, Canada). The maximal activities of CS was determined with modification to that previously described[19].

For determination of **CS** maximal activity, 15 µl of muscle homogenate was added to cuvette containing: 825µl 0.1M Tris Buffer (pH 8.0), 100µl 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, 0.5mg/mL Tris Buffer) and 10 µl acetyl CoA (6mg/mL Tris Buffer). The cuvette was warmed to 37°C, and 50 µL of oxaloacetate (6.1mg/mL Tris buffer) was added to initiate the reaction. Absorbance was recorded at 412 nm for 120

seconds and the slope between 30 and 90 seconds was recorded.

For **COX activity**, oxidized cytochrome *c* (Sigma C7752) was reduced by sodium dithionite in 0.05 M potassium phosphate buffer (KH_2PO_4, pH 7.4). Twenty microliters of muscle homogenate were added to 955 µL of 0.05 M potassium phosphate buffer and 30 µL of reduced cytochrome *c* in a cuvette that had been warmed to 37°C. The rate of oxidation of reduced cytochrome *c* was measured at 550 nm for 3 min at 37°C.

For the determination of **β-HAD**, the following were added to a cuvette that had been warmed at 30°C: 800 µL of Tris buffer (Tris HCl 1 M, EDTA 200 mM; pH 7.0); 10 µL of Triton (10%) and 35 µL of homogenate was added and mixed. Following this, 10 µL of acetoacetyl CoA was added to initiate the reaction. The absorbance was recorded at 340 nm every 2 min for 4 min. CS, COX and β-HAD activity was expressed in nmol/min/mg protein. All enzyme assays have been previously established in the literature[19,94] and were performed utilizing a spectrophotometer (Cary Bio-300, Varion, Inc., Palo Alto, CA, USA) that had been properly calibrated and zeroed.

Statistical Analysis

To determine physiological changes associated with the transfer microbiota from an exercised animal, 2-way repeated measures ANOVA (group x time) was used to analyze glucose data (0, 15, 30, 60, 120 timepoints) and student's *t* tests were run on all other outcome measures between the groups either colonized with exercise microbiota or sedentary microbiota. Statistically significance was accepted at $p \leq 0.05$. Any significant main effects found with the 2-way ANOVA were analyzed by using Sidak's *post hoc* test

for multiple comparisons. Fibre composition and CSA measures were analyzed using multiple t-tests with a Holm-Sidak correction factor to control for family-wise error. All results are presented as means ± SEM.

RESULTS

Proof of principle: Absolute blood glucose concentration is lower in the exercise group compared to the sedentary group during an IPGTT – **(Fig. 3A-i/ii)** Absolute blood glucose concentrations during an IPGTT were found to be significantly lower in the exercise group compared to the sedentary group using a two-way repeated measures ANOVA (group x time). Following post-hoc analyses using Sidak's test for multiple comparisons, absolute blood glucose concentrations from the exercise group were significantly lower than those who remained sedentary at 15mins (23.9 ± 1.91; 29.6 ± 0.9 mmol/L, $p<0.05$) and 30mins (21.5 ± 2.2; 27.8 ± 1.6 mmol/L, $p<0.05$), and 60mins (14.71 ± 1.5; 21.1 ± 2.4 mmol/L, $p<0.05$). There were no significant differences between the exercise group and sedentary group at baseline (9.6 ± 0.6; 10.6 ± 0.4 mmol/L) or 120mins (9.4 ± 0.6; 13.2 ± 1.1, mmol/L).

Proof of principle: Absolute glucose area-under-the-curve (AUC) is lower in exercise animals compared to sedentary animals – **(Fig. 3A-iii)** Glucose area-under-the-curve is significantly reduced in the exercise group compared to the sedentary group (1829 ± 150; 2493 ± 162, $p<0.01$).

Absolute blood glucose concentration is lower in exercised-inoculated animals compared to sedentary-inoculated animals during IPGTT– **(Fig. 3B-i/ii)** Blood glucose concentrations during an IPGTT were found to be significantly lower in the exercise-

inoculated group compared to the sedentary-inoculated group using a two-way repeated measures ANOVA (group x time). Following post-hoc analyses using Sidak's test for multiple comparisons, blood glucose concentration at 30mins was significantly lower in the exercise-inoculated group as compared to the sedentary-inoculated group (23.4 ± 2.2; 29.0 ± 1.9 mmol/L, $p<0.05$). There were no significant differences between the exercise-inoculated group and sedentary-inoculated group at baseline (10.1 ± 0.5; 10.6 ± 0.6 mmol/L), 15mins (25.2 ± 1.6; 29.4 ± 1.4 mmol/L), 60min (16 ± 1.2; 19.5 ± 1.1 mmol/L), or 120mins (11.2 ± 0.2; 12.0 ± 1.4 mmol/L).

Absolute glucose area-under-the-curve (AUC) is lower in exercise-inoculated animals compared to sedentary-inoculated animals – **(Fig. 3B-iii)** Glucose area-under-the-curve is significantly reduced in the exercise-inoculated group compared to the sedentary-inoculated group (2005 ± 111; 2401 ± 82, $p<0.05$).

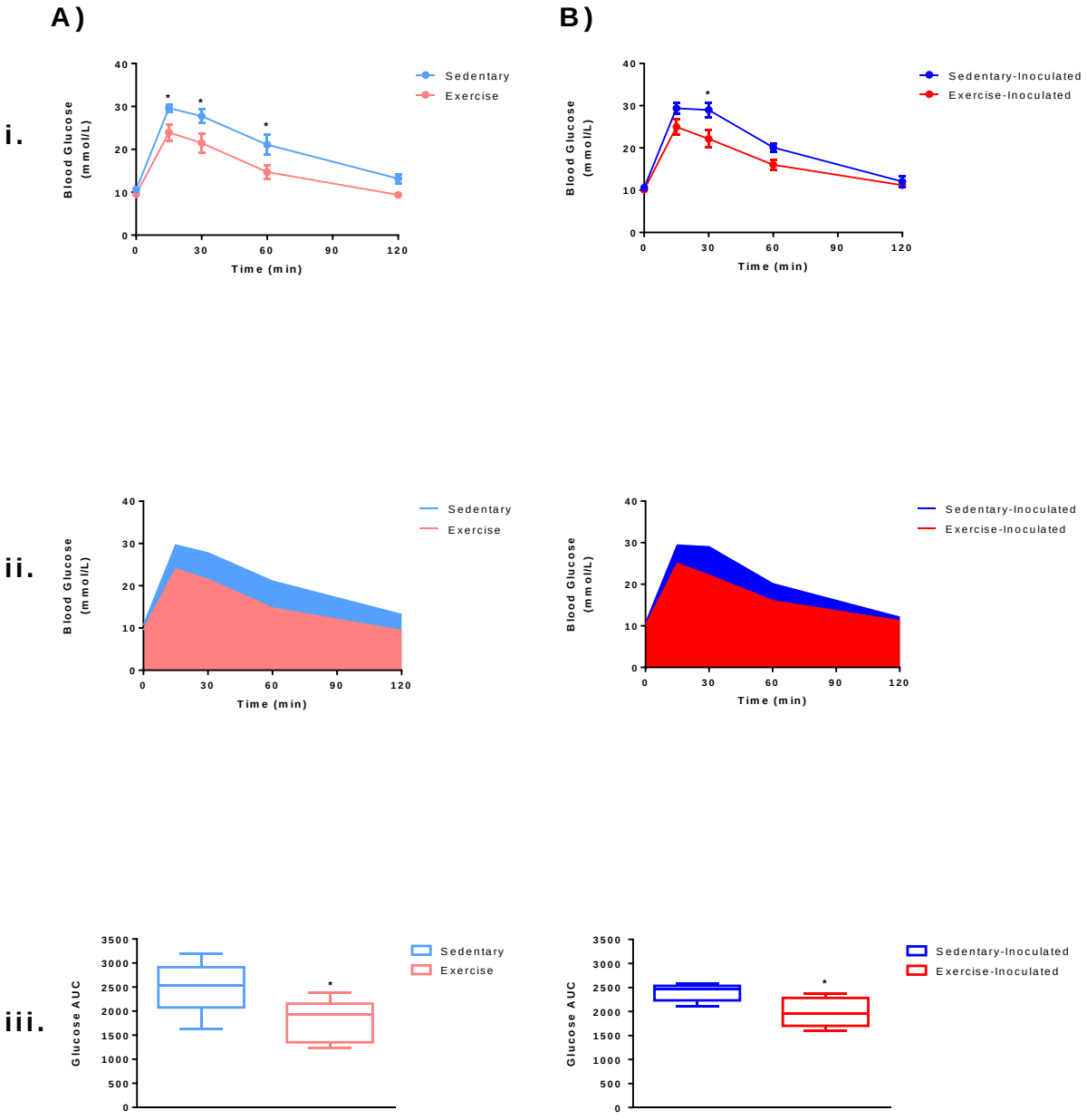


Figure 3. *Blood glucose response, as expressed in absolute values, during an IPGTT.* The following set of graphs denote the absolute blood glucose concentration as measured by tail vein following the administration of a bolus of glucose (2g/kg, 10% glucose solution) at timepoint 0min. (i) glucose values during IPGTT at timepoints 0, 15, 30, 60, 120mins; mean ± SEM (ii) illustration of glucose AUC (iii) box and whisker plot of glucose AUC, box denotes median, 25th and 75th percentiles, whiskers representing the maximum and minimum values. The IPGTT was conducted on the (A) Exercise and sedentary donors (B) exercise-inoculated and sedentary-inoculated groups. *, $p<0.05$ for exercise v.s. sedentary or exercise-inoculated v.s. sedentary-inoculated.

Proof of principle: Changes in blood glucose concentrations from baseline are lower in the exercise group compared to sedentary group during an IPGTT – **(Fig. 4A-i/ii)** The changes in blood glucose concentrations from baseline during an IPGTT were found to be significantly lower in the exercise group compared to the sedentary group using a two-way repeated measures ANOVA (group x time). Following post-hoc analyses using Sidak's test for multiple comparisons, the changes in blood glucose concentrations from baseline were significantly lower in the exercise group compared to the sedentary group at 30mins (11.9 ± 1.9; 17.1 ± 1.4 mmol/L, $p<0.05$) and 60mins (5.3 ± 1.4; 10.5 ± 2.1 mmol/L, $p<0.05$) and close to significance at 15mins (14.4 ± 1.5; 19.0 ± 0.9 mmol/L, $p=0.06$). There were no significant differences between the exercise group and sedentary group at 120mins (-0.1 ± 0.6; 2.6 ± 1.0 mmol/L).

Proof of principle: The change in glucose area-under-the-curve (AUC) was not different between the exercise group and sedentary group – **(Fig. 4A-iii)** The change in glucose area-under-the-curve was not statistically different between the exercise group and sedentary group (695 ± 123; 1217 ± 130, $p<0.01$).

Changes in blood glucose concentrations from baseline are lower in the exercise-inoculated group compared to sedentary-inoculated group during an IPGTT – **(Fig. 4B-i/ii)** The changes in blood glucose concentrations from baseline during an IPGTT were found to be significantly lower in the exercise-inoculated group compared to the sedentary-inoculated group using a two-way repeated measures ANOVA (group x time).

Following post-hoc analyses using Sidak's test for multiple comparisons, blood glucose concentration at 30mins was significantly lower in the exercise-inoculated group compared to the sedentary-inoculated group (12.04 ± 2.4; 18.3 ± 1.9 mmol/L, $p<0.01$). There were no significant differences between the exercise-inoculated group and sedentary-inoculated group at 15mins (15.1 ± 1.5; 18.8 ± 1.7mmol/L), 60mins (5.9 ± 1.5; 8.5 ± 1.4 mmol/L), or 120mins (1.1 ± 0.4; 1.4 ± 0.8 mmol/L).

The change in glucose area-under-the-curve (AUC) was not different between the exercise-inoculated group and sedentary-inoculated group – **(Fig. 4B-iii)** The change in glucose area-under-the-curve was not statistically different between the exercise-inoculated group and sedentary-inoculated group (791 ± 138; 1081 ± 100).

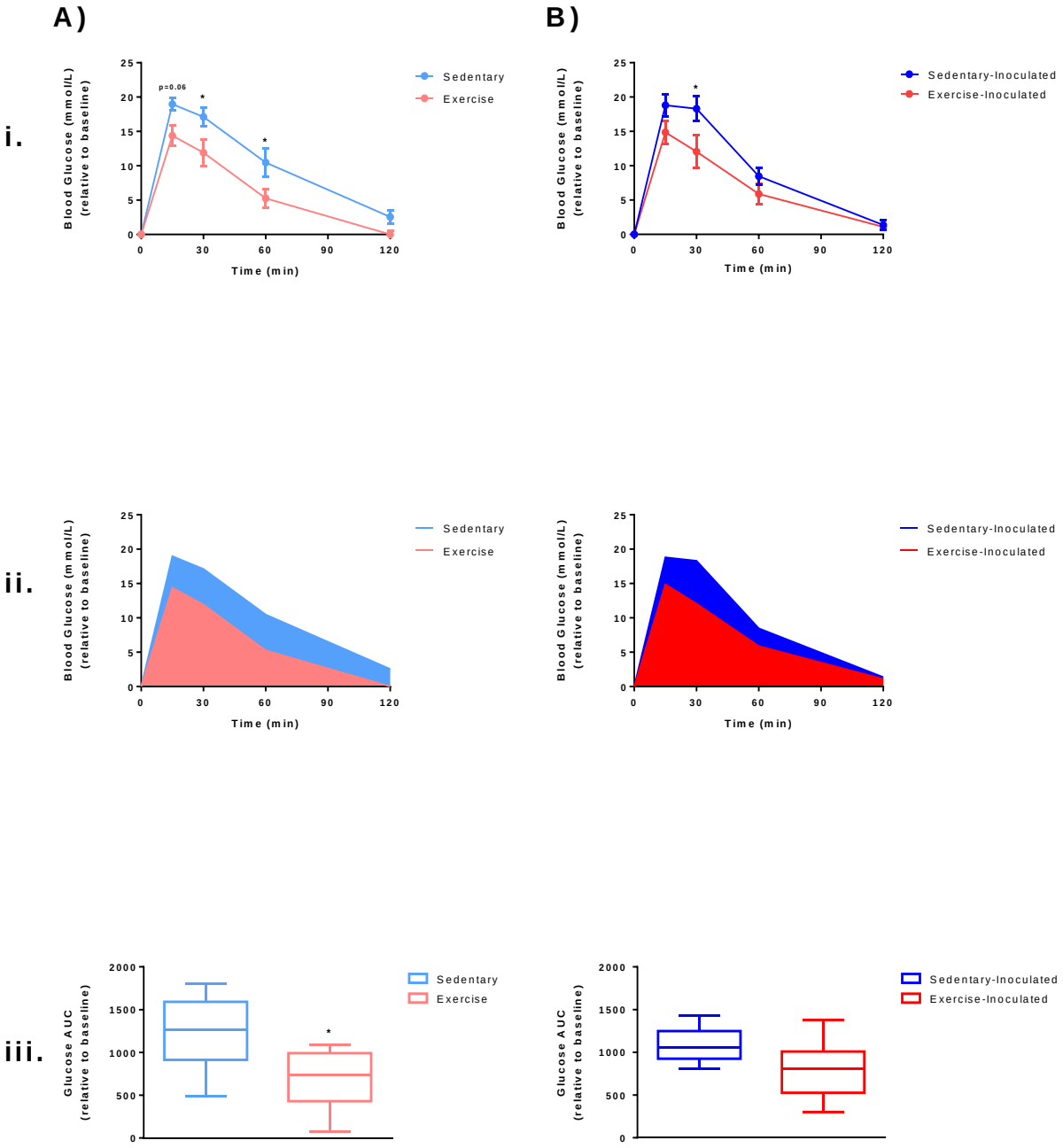


Figure 4. *Blood glucose response, as expressed by change from baseline, during an IPGTT.* The following set of graphs denote the Δ blood glucose concentration relative to baseline (0min). (i) Δ glucose values relative to baseline during IPGTT at timepoints 0, 15, 30, 60, 120mins; mean ± SEM (ii) illustration of Δ glucose AUC relative to baseline, (iii) box and whisker plot of Δ glucose AUC relative to baseline, box denotes median, 25th and 75th percentiles, whiskers representing the maximum and minimum values. The IPGTT was conducted on the (A) Exercise and sedentary donors (B) exercise-inoculated and sedentary-inoculated groups. *, $p<0.05$ for exercise v.s. sedentary or exercise-inoculated v.s. sedentary-inoculated.

Absolute blood glucose concentration is lower in exercised-inoculated female animals compared to sedentary-inoculated female animals during IPGTT – **(Fig. 5A-i/ii, 5B-i/ii)** Blood glucose concentrations during an IPGTT were found to be significantly lower in the female exercise-inoculated animals compared to the sedentary-inoculated animals using a two-way repeated measures ANOVA (group x time). Following post-hoc analyses using Sidak's test for multiple comparisons, blood glucose concentration was significantly lower in the exercise-inoculated group as compared to the sedentary inoculated group at 15mins (28.4 ± 2.4; 30.6 ± 1.1 mmol/L, $p<0.05$) and 30mins (24.7 ± 3.6; 29.9 ± 2.4 mmol/L, $p<0.01$). There were no significant differences between female animals in the exercise-inoculated group and sedentary-inoculated group at baseline (10.1 ± 1.0; 10.0 ± 0.8 mmol/L), 60mins (17.4 ± 2.0; 20.0 ± 3.9 mmol/L) or 120mins (11.4 ± 0.3; 10.9 ± 1.7 mmol/L). No significant differences were found between male animals in the exercise-inoculated group and sedentary-inoculated group at baseline (10.1 ± 1.0; 11.5 ± 0.9 mmol/L), 15mins (28.4 ± 2.4; 27.4 ± 3.2 mmol/L), 30mins (24.7 ± 3.6; 26.8 ± 3.3 mmol/L), 60mins (17.4 ± 2.0; 20.3 ± 1.0 mmol/L) or 120mins (11.37 ± 0.3; 14.8 ± 0 mmol/L).

Changes in blood glucose concentrations from baseline are lower in the exercise-inoculated female group compared to female sedentary-inoculated group during an IPGTT – **(Fig. 5A-iii/iv, 5B-iii/iv)** The change in blood glucose concentrations from baseline during an IPGTT were found to be significantly lower in the female exercise-inoculated group compared to the sedentary-inoculated group using a two-way repeated

measures ANOVA (group x time). Following post-hoc analyses using Sidak's test for multiple comparisons, the change in blood glucose concentration relative to baseline was identified to be significant between the exercise inoculated group and the sedentary inoculated group at 15mins (12.3 ± 1.9; 20.6 ± 0.8 mmol/L, $p<0.01$) and 30mins (10.2 ± 2.6; 19.9 ± 2.1 mmol/L, $p<0.001$). There were no significant differences between female animals in the exercise-inoculated group and sedentary-inoculated group at 60mins (4.9 ± 1.5; 8.8 ± 2.5 mmol/L) or 120mins (1.0 ± 0.5; 0.94 ± 1.1 mmol/L) No significant differences were found between male animals in the exercise-inoculated group and sedentary-inoculated group at 15mins (18.0 ± 1.0; 15.9 ± 4.1 mmol/L), 30mins (16.5 ± 3.8; 15.9 ± 3.9 mmol/L), 60mins (7.3 ± 3.0; 8.0 ± 0.4 mmol/L), 120mins (1.2 ± 0.8; 2.5 ± 0.7 mmol/L).

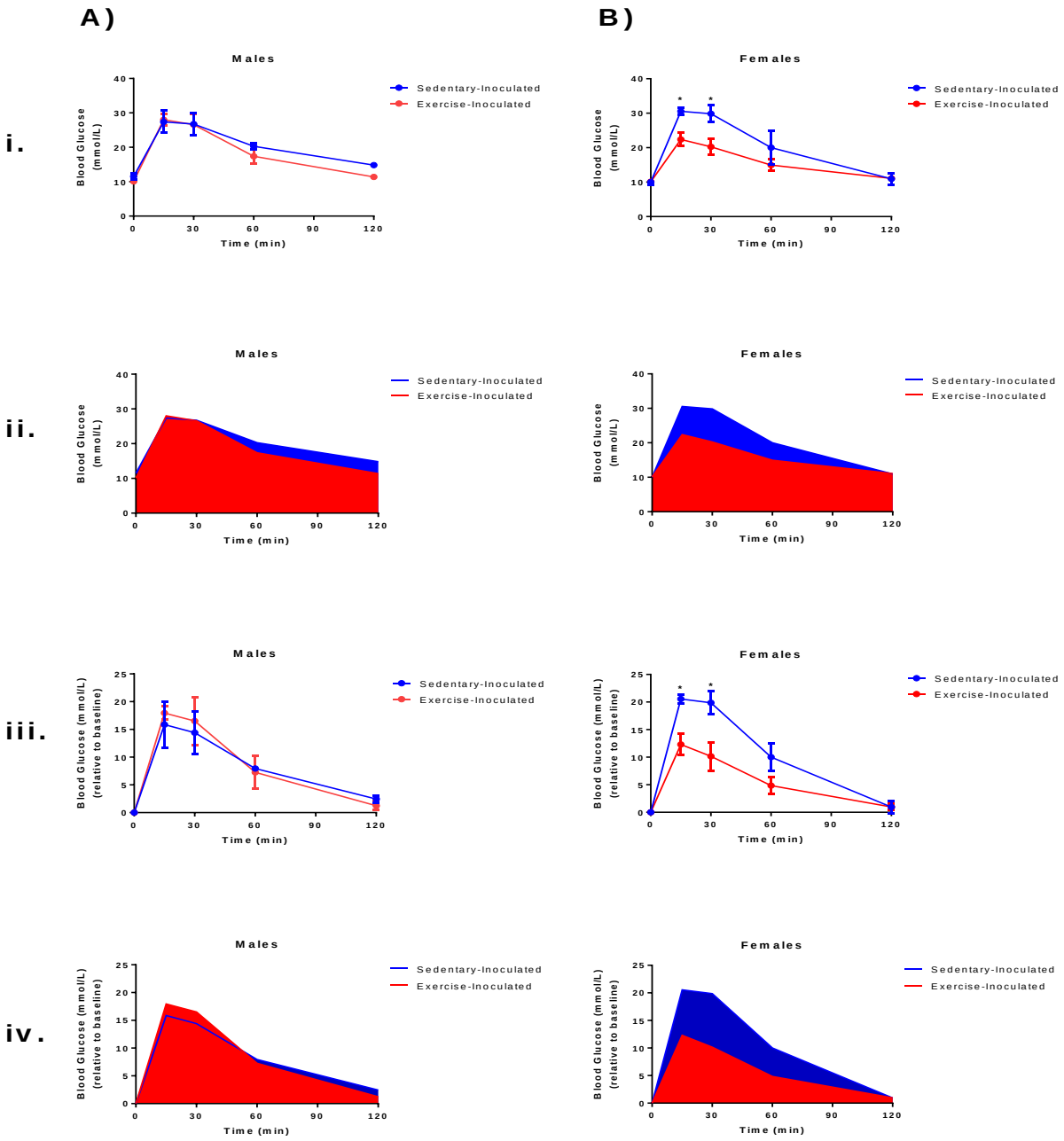


Figure 5. *Sex differences in glucose response to IPGTT.* The following set of graphs denote the sex differences in absolute blood glucose concentration and Δ relative to baseline (0min). (i) absolute glucose values; mean ± SEM (ii) illustration of absolute glucose AUC (iii) Δ glucose relative to baseline; mean ± SEM (iv) illustration of Δ glucose AUC relative to baseline. Graphical representations are separated by sex (A) Male (B) Female. *, $p<0.05$ for exercise inoculated v.s. sedentary inoculated.

No sex-based differences in absolute or relative to baseline values for glucose-area-under-the-curve (AUC) measures **(Fig. 6A-i/ii, 6B-i/ii)**– Absolute glucose-area-under-the-curve was not statistically different between exercise-inoculated and sedentary-inoculated groups in males (2182 ± 117; 2419 ± 56) and females (1872 ± 149; 2219 ± 199). Change in glucose area-under-the-curve was not statistically different between exercise-inoculated and sedentary-inoculated groups in males (1352 ± 422; 937 ± 134) and females (660 ± 156, 1043 ± 161).

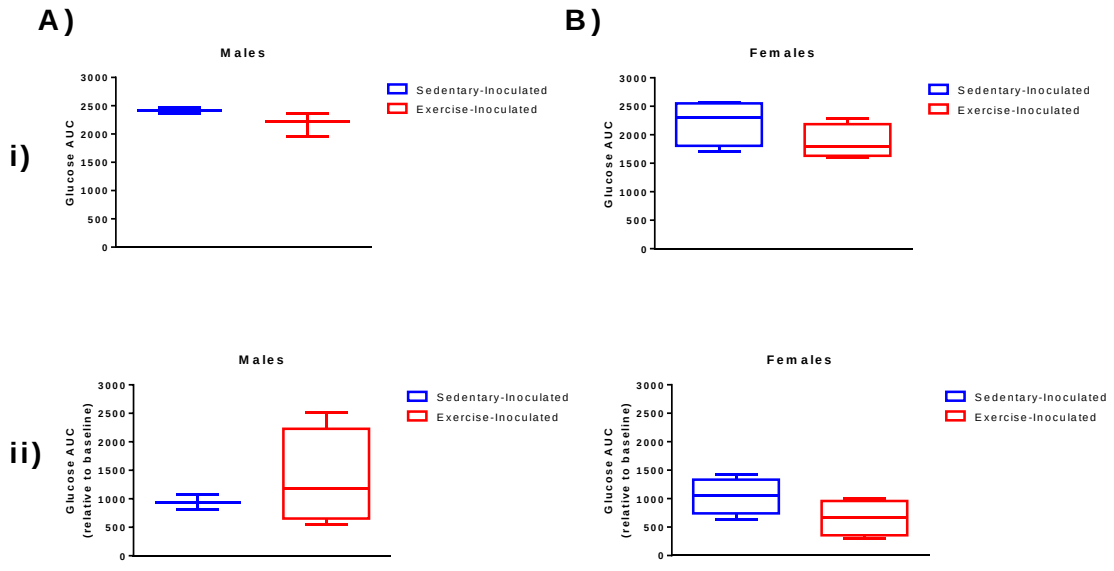


Figure 6. *Sex differences in glucose AUC during IPGTT.* The following set of graphs denote the sex differences in glucose area-under-the-curve during an IPGTT (i) box and whisker plot of glucose AUC, (ii) box and whisker plot of Δ glucose AUC relative to baseline, box and whisker plot of box denotes median, 25th and 75th percentiles, whiskers representing the maximum and minimum values. Graphical representations are separated by sex (A) Male (B) Female.

No differences in proportions of fibre types in the TA or soleus muscles of the exercise inoculated group compared to the sedentary inoculated group – **(Fig. 7A, 7B)** There were no significant differences in the relative proportions of fibre types between the exercise-inoculated group and sedentary-inoculated group in the TA (I (2.5 ± 0.8; 1.8 ± 0.8 per 100 fibres) ↔ I/IIa (3.4 ± 1.0; 1.7 ± 0.6 per 100 fibres) ↔ IIa (10.1 ± 1.5; 9.3 ± 1.4 per 100 fibres) ↔ IIa/x (7.2 ± 0.7; 8.9 ± 1.3 per 100 fibres) ↔ IIx (21.9 ± 3.6; 24.9 ± 2.1 per 100 fibres) ↔ IIx/IIb (6.8 ± 1.7; 3.4 ± 1.7 per 100 fibres) ↔ IIb (53.9 ± 3.3; 55.9 ± 3.4 per 100 fibres)) the soleus (I (52.2 ± 2.6; 58.8 ± 3.4 per 100 fibres) ↔ I/IIa (0.8 ± 0.2; 0.6 ± 0.1 per 100 fibres) ↔ IIa (37.0 ± 2.4; 31.1 ± 3.3 per 100 fibres) ↔ IIa/x ↔ IIx (5.8 ± 1.8; 8.3 ± 0.7 per 100 fibres) ↔ IIx/IIb (0.3 ± 0.2; 0.4 ± 0.03 per 100 fibres) ↔ IIb (0.1 ± 0.06; 0.1 ± 0.05 per 100 fibres)).

No differences in CSA between groups in the TA or soleus muscles– **(Fig. 7C, 7D)** There were no significant differences in cross-sectional area (CSA) when comparing the exercise-inoculated group to the sedentary-inoculated group in the TA (IIa (869.8 ± 61.2; 908.4 ± 31.5 μm^2) ↔ IIa/x (1231.7 ± 83.0; 1208.9 ± 117.3 μm^2) ↔ IIx (1595.9 ± 111.5; 1503.9 ± 63.7 μm^2) ↔ IIx/b (1770.2 ± 76.7; 1616.3 ± 181.6μm^2) ↔ IIb (3014.1 ± 112.7; 2988.8 ± 105.6μm^2) or soleus (I (1342.9 ± 92.4; 1353.0 ± 119.1 μm^2) ↔ I/IIa (1042.0 ± 115.5; 924.5 ± 81.7 μm^2) ↔ IIa (1209.1 ± 68.4; 1237.7 ± 105.6μm^2) ↔ IIa/x (1497.8 ± 145.3; 1399.7 ± 147.1 μm^2) ↔ IIx (1640.5 ± 142.4; 1587.1 ± 218.0 μm^2).

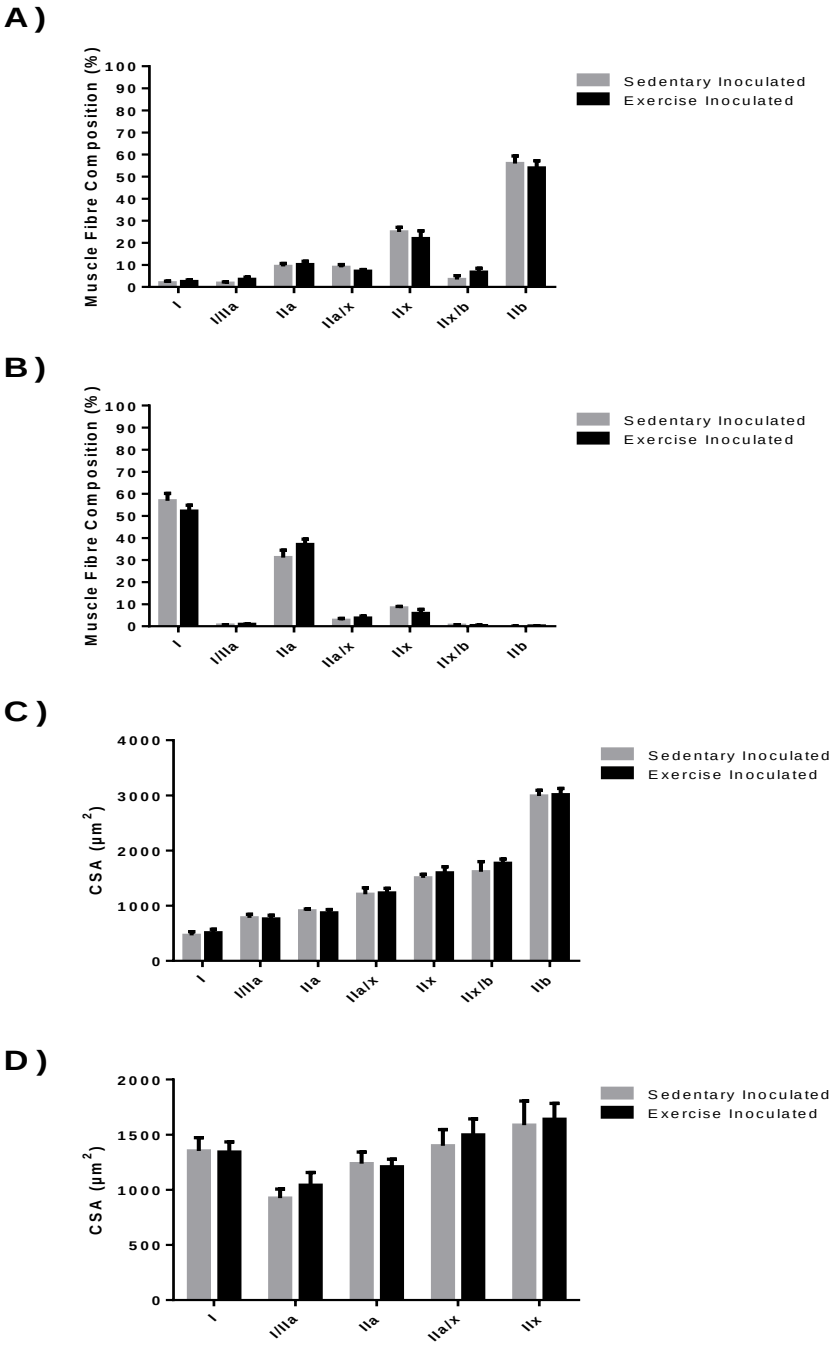


Figure 7. *Fibre Type Composition and CSA Measures.* The composition of muscle fibres in terms of fibre type and cross-sectional area was analyzed using immunohistochemical staining. Fibre proportions across spectrum of muscle fibre types present in mouse muscle tissue were measured in the (A) TA (B) Soleus. Cross-sectional area was measured in the (C) TA (D) Soleus. Data are shown as mean ± SEM.

No differences in capillary density between groups – **(Fig. 8A-i/ii, 8B-i/ii)** There was no significant differences in capillary density as assessed by capillary-to-fibre ratio (C/Fi) when comparing the exercise-inoculated group to the sedentary-inoculated group in the TA (1.69 ± 0.14; 1.48 ± 0.07) or the soleus (1.55 ± 0.07; 1.46 ± 0.08). There were no differences between the inoculated groups in the TA specific to males (1.86 ± 0.21; 1.64 ± 0.08) or females (1.52 ± 0.18; 1.38 ± 0.08) and no differences in the soleus specific to males (1.59 ± 0.13; 1.39 ± 0.11) or females (1.51 ± 0.06; 1.51 ± 0.10).

No differences in a proxy measure of muscle perfusion between groups – **(Fig. 8A-iii/iv, 8B-iii/iv)** There was no significant differences in capillary-to-fibre perimeter exchange ratio (CFPE) when comparing the exercise-inoculated group to the sedentary-inoculated group in the TA (6.02 ± 1.3; 4.63 ± 0.95) or the soleus (11.24 ± 0.57; 9.79 ± 0.66). There were no differences between inoculated groups in the TA specific to males (10.47 ± 1.2; 8.62 ± 0.41) or females (9.14 ± 1.17; 8.39 ± 0.45) and no differences in the soleus specific to males (11.85 ± 0.99; 8.66 ± 1.19) or females (10.55 ± 0.41; 10.64 ± 0.63).

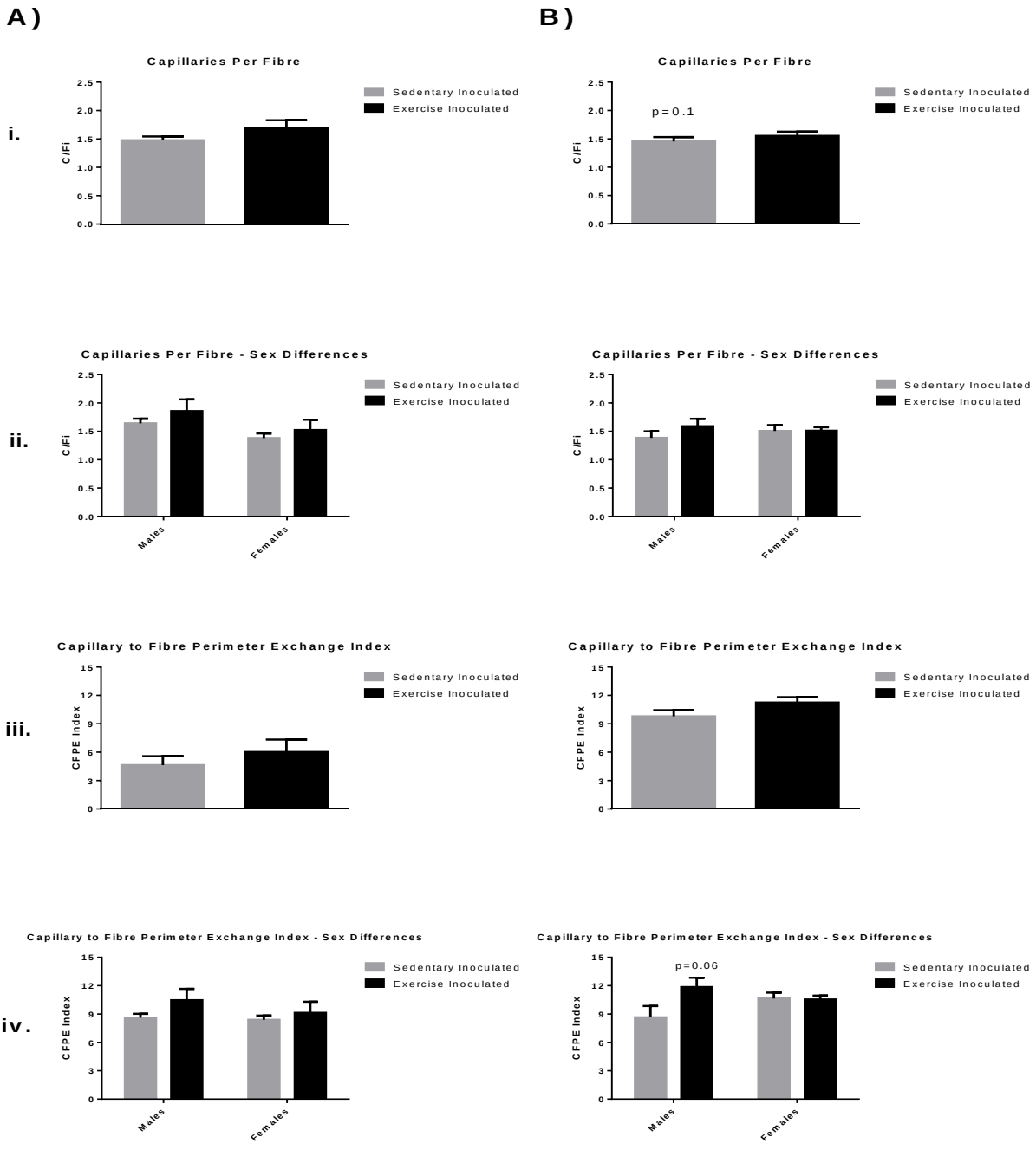


Figure 8. *Capillarization Measures.* Capillary density was measured using capillaries-per-fibre (C/Fi). A proxy measure of skeletal muscle perfusion was measured using capillary density in respect to the perimeter of respective skeletal muscle fibres known as the capillary to fibre exchange index (CFPE). (i) and (iv) denote the sex-collapsed data. (ii) and (iii) denote the results separated by sex. These analyses were conducted in (A) TA (B) Soleus. Data are shown as mean ± SEM.

Higher enzymatic activity of COXIV in the TA muscles of the exercise inoculated group exclusively in males – **(Fig. 9)** COXIV activity was significantly higher in the TA of the males inoculated with exercised-microbiota compared to males inoculated with sedentary-microbiota (223.7 ± 28.06; 107.2 ± 24.94 nmol/min/mg, $p<0.05$). There were no significant differences between exercise-inoculated and sedentary-inoculated animals in the TA (136.7 ± 35.72; 74.25 ± 14.07 nmol/min/mg) or the soleus (287.3 ± 37.64; 341.4 ± 38.47 nmol/min/mg). There were also no significant differences between groups in the TA in females (49.82 ± 11.42; 54.51 ± 10.38 nmol/min/mg) and no significant differences in the soleus specific to males (332.1 ± 34.52; 369.0 ± 48.99 nmol/min/mg) or females (242.5 ± 63.88; 324.8 ± 56.93 nmol/min/mg).

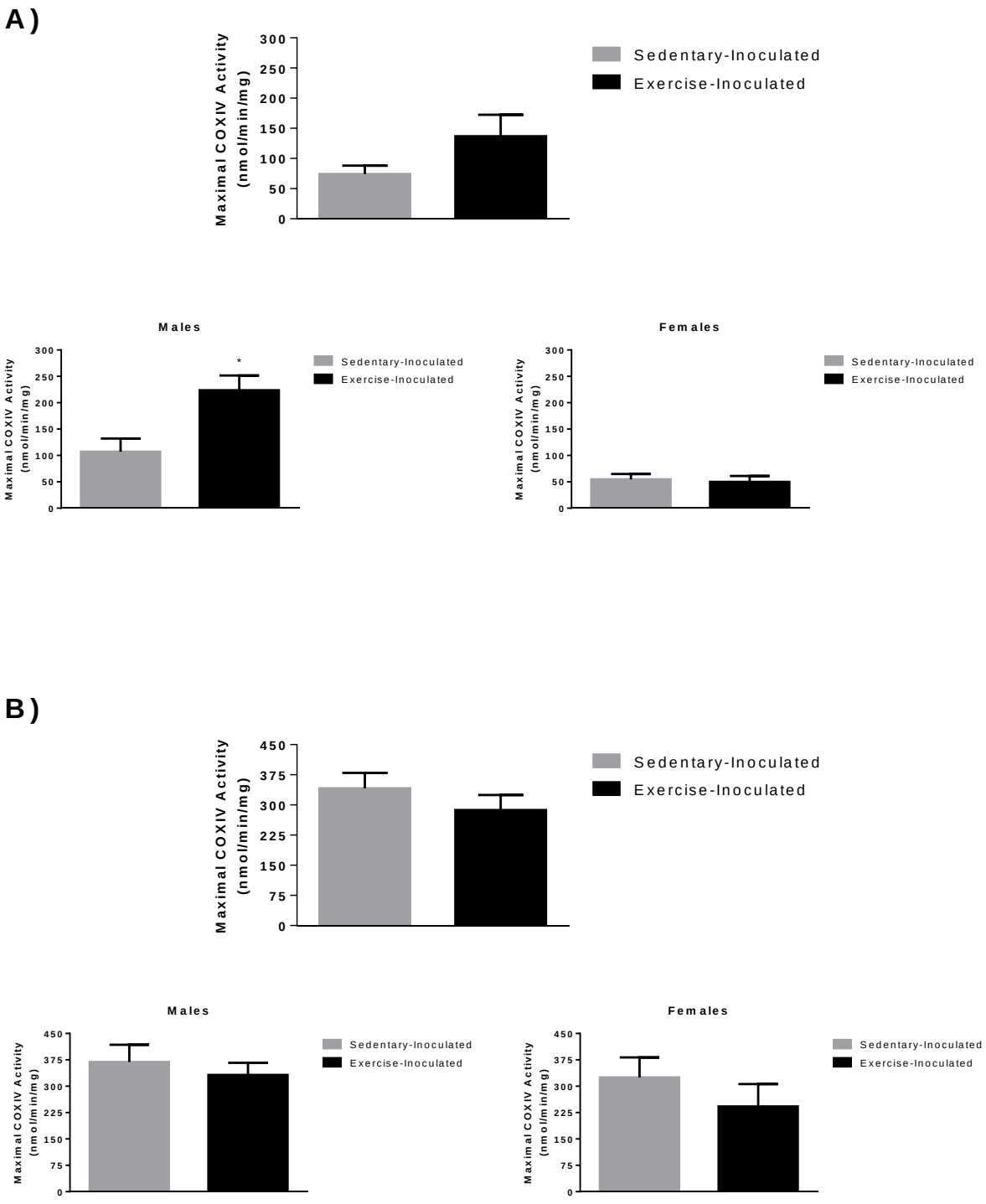


Figure 9. *Maximal activity of mitochondrial enzymes – COXIV.* For each muscle data has been shown with the entire cohort and separated by sex (A) Activity in the TA (B) Activity in the soleus. *, $p<0.05$ exercise inoculated v.s. sedentary inoculated. Data are shown as mean ± SEM.

No differences in maximal CS activity between groups – **(Fig. 10)** There were no significant differences in the maximal activity of citrate synthase between the exercise-inoculated group or sedentary-inoculated group in the TA (339.9 ± 18.51; 310.9 ± 14.06 nmol/min/mg or the soleus (422.0 ± 35.29; 508.3 ± 52.00 nmol/min/mg). There were also no significant differences between groups in the TA specific to males (354.6 ± 33.52, 325.0 ± 22.46 nmol/min/mg) or females (325.3 ± 18.23; 302.5 ± 18.84 nmol/min/mg) and no significant differences in the soleus specific to males (421.3 ± 62.63; 563.4 ± 85.51 nmol/min/mg) or females (422.8 ± 43.48; 475.2 ± 68.19 nmol/min/mg).

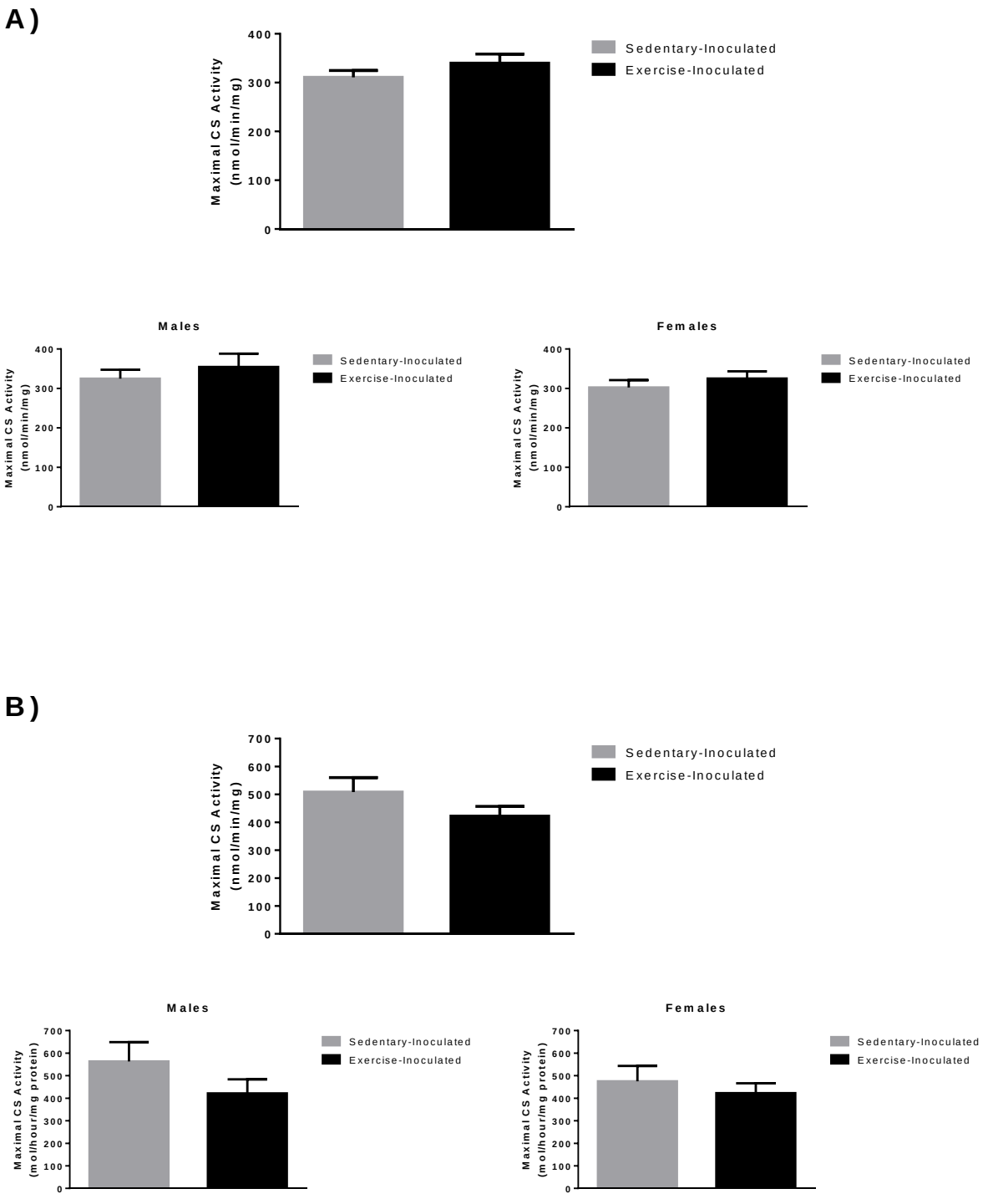


Figure 10. *Maximal activity of mitochondrial enzymes – CS.* For each muscle data has been shown with the entire cohort and separated by sex (A) Activity in the TA (B) Activity in the soleus. *, $p<0.05$ exercise inoculated v.s. sedentary inoculated. Data are shown as mean ± SEM.

No differences in maximal β-HAD activity between groups – **(Fig. 11)** There were no significant differences in the maximal activity of β-HAD between the exercise-inoculated group or sedentary-inoculated group in the TA (70.03 ± 3.04; 62.90 ± 2.40 nmol/min/mg). There were also no significant differences between groups in the TA specific to males (73.88 ± 4.40; 74.03 ± 6.64 nmol/min/mg) or females (66.18 ± 3.72; 62.15 ± 3.36 nmol/min/mg).

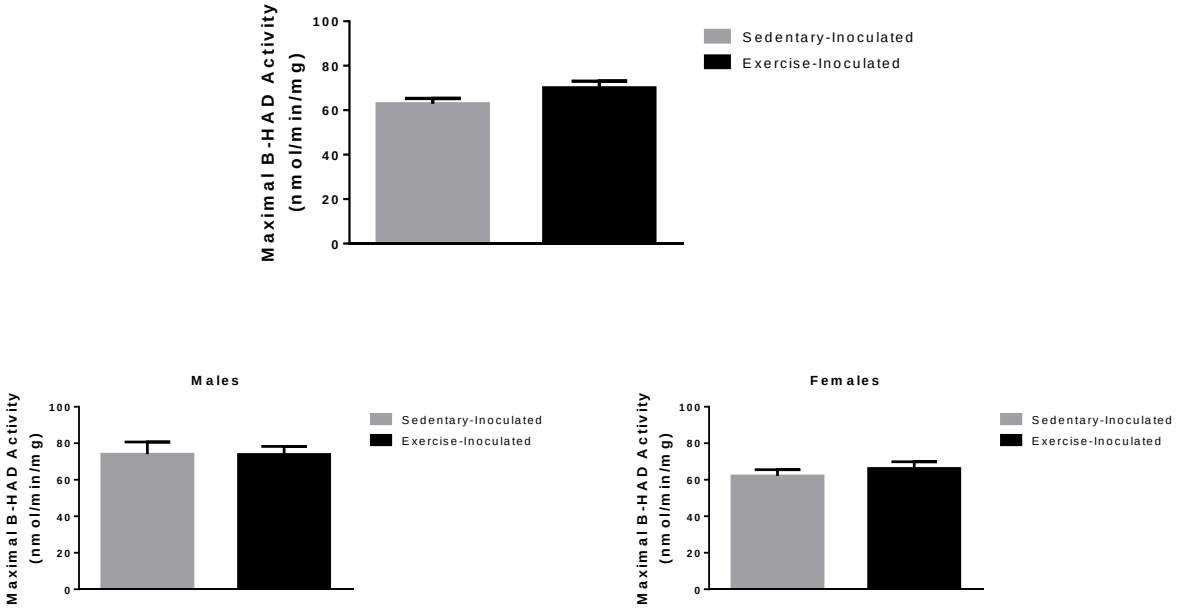


Figure 11. *Maximal activity of mitochondrial enzymes – β-HAD.* Muscle data has been shown with the entire cohort and separated by sex (A) Activity in the TA. *, $p<0.05$ exercise inoculated v.s. sedentary inoculated.

No performance differences between exercise inoculated animals and sedentary inoculated animals – **(Fig. 12)** There were no differences between the exercise-inoculated group and the sedentary-inoculated group (47.6 ± 4.7; 50.1 ± 4.6 mins) when subject to a run-to-exhaustion performance test.

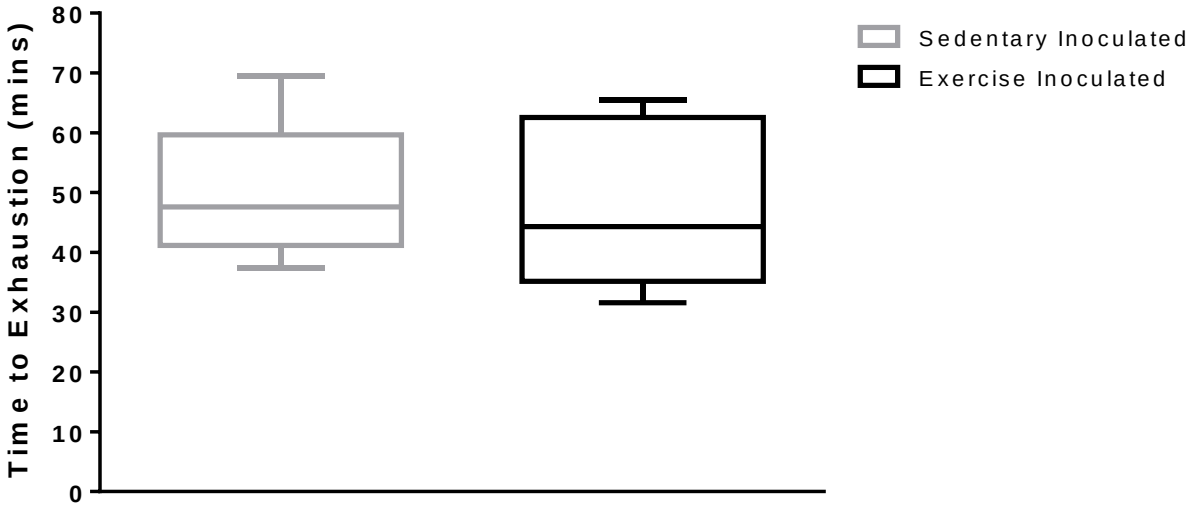


Figure 12. *Performance Test – Run to Exhaustion*. Performance test values in a run-to-exhaustion challenge on a rodent treadmill recorded during the 8th week. Box and whisker plot denoting the median, 25th and 75th percentiles, whiskers representing the minimum and maximum values of the respective datasets.

DISCUSSION

The current investigation is the first to assess metabolic, physiological, and functional changes in skeletal muscle following a transfer of microbiota from an exercised animal. Exercise is a potent stimulus for a myriad of responses at the level of skeletal muscle and more recently revealed, the gastrointestinal tract. We aimed to isolate the influence of endurance training on the gut microbiome and subsequent impact on whole-body physiology through transfer of cecal microbiota content from endurance-trained and untrained donors into germ-free recipients. The current findings offer a unique approach to further elucidating the relationship between skeletal muscle and the gut, coined as the gut-muscle axis[87]. Furthermore, this study is the first to identify sex-dependent adaptations following the transfer of microbiota from an exercised animal. To our knowledge, only one other investigation has conducted microbiota transfers from exercise individuals; this study did not assess any changes in substrate metabolism or physiological changes in skeletal muscle[95]. Therefore, analyses of mammalian glucose metabolism, skeletal muscle fibre morphology, vascularization, and mitochondrial enzyme activity between exercised-inoculated and sedentary inoculated groups are all novel research contributions.

The Influence of Microbiota Transfers on Glucose Metabolism

Differences in glucose metabolism were assessed in the inoculated groups as well as the donor groups by subjecting animals to an IPGTT. Prior to IPGTT, animals were

weighed and as expected due to training, the exercise group had a lower mass than the sedentary group. The test results were not caused by this, as the exercise-inoculated group had a higher mass than the sedentary-inoculated group specifically in the females.

It has been well established that both acute and chronic exercise sensitize the muscle, optimizing insulin-dependent and -independent pathways and subsequently improving glucose uptake[70]. Therefore, as expected, endurance training resulted in improved glucose clearance compared to sedentary counterparts whether measured in absolute blood glucose concentration or as a change relative to baseline values. Remarkably, when the cecal contents of these rodents were extracted and used to colonize two distinct germ-free sets of rodents, the improvements in glucose handling persisted. Although the improvements are isolated to the 30min timepoint in the exercise-inoculated group, this is consistent with the significant timepoints in the donors. Moreover, the 30min timepoint, whether measured in absolute blood glucose or change relative to baseline values, was significantly reduced indicating better glucose clearance in both the exercise-trained and in the exercise-inoculated animals.

The influence of the gut microbiota on glucose regulation has been extensively studied in humans and mouse models. Methods such as 16S/18S/OTU functional sequencing and microbiota transplantation have been utilized to investigate the effect of gut bacteria on glucose uptake. Bacterial colonies are restricted to the confines of the gastrointestinal tract; however, Choi and colleagues (2015) investigated extra-cellular vesicles secreted by the microbiota that penetrate the intestinal barrier and are transported via systemic circulation to various organs and tissues. Specifically of interest, were

insulin-responsive target organs such as skeletal muscle and adipose tissue. It was revealed that the gut microbe-derived extra-cellular vesicles directly interfered with insulin-dependent pathways and thus overall glucose metabolism[96]. By extension, bacterial communities used to colonize the recipient groups may have influenced glucose clearance through the secretion of extra-cellular vesicles. This may suggest that microbiota-induced modulation of the insulin-dependent pathway in skeletal muscle and adipose tissue were related to insulin sensitivity and/or insulin production and secretion.

Aside from extra-cellular vesicles, glucose homeostasis could also be related to one of the innate functions of the gut microbiota: the production of SCFAs that are delivered to various organs and tissues via systemic circulation[25,29,30]. Knowing that exercise elicits beneficial changes in the gut microbiota leading to increased SCFA production[42], it can be proposed that SCFA may interfere with glucose metabolism due to increased availability of short chain fatty acids. This concept of interference has been proposed by several researchers[97,98] and would support the notion of the isolated impact of gut microbiota on glucose metabolism especially following a microbiota transplant.

Glucose AUC is lower in the exercise group as well as the exercise inoculated group in absolute glucose concentration compared to their respective sedentary counterparts. However, the exercise inoculated group fails to reach statistical significance for AUC when values are expressed as the change relative to baseline. This may be explained by the fact that an N of 8 may simply be underpowered to achieve statistical significance. To achieve a statistical power of 0.8, a sample size of 72 is required for this particular relationship.

Sex dependent analyses on the same data revealed that female recipients drove the benefit of exercise microbiota on glucose clearance. Exercise-inoculated females had lower absolute blood concentrations at 15, 30, and 60mins compared to the sedentary-inoculated females, and these lower values persisted at the 15 and 30mins timepoints when values were made relative to baseline. The difference between exercise-inoculated males and females was close to statistical significance at 30min ($p=0.07$) so it can be suggested that with an increase in N, the difference would be significant. The rationale for this sex-based discrepancy may reside in notable sex-based differences in insulin sensitivity[86]. Although it has been mentioned that acute exercise can improve glucose metabolism through insulin-independent pathways i.e. contraction stimulated GLUT4 translocation, it is also well established that acute and chronic exercise can modulate insulin-dependent pathways as well. If females naturally have higher insulin sensitivity, the addition of transplanted microbiota from an exercised individual could hypothetically lead to a synergistic increase in insulin sensitivity and an improved glucose response. This finding is an example of how critical it is to account for possible dichotomous responses due to sex-based physiological differences.

The Influence of Microbiota Transfers on Vascularization and Mitochondrial Enzyme Activity

No statistically significant differences were found between exercise inoculated and sedentary inoculated animals in measures of capillarization or select mitochondrial enzymes (CS, COXIV, β-HAD). There was a significantly higher rate of activity in

COXIV exclusively in the TA of the exercise inoculated group, however caution must be used when interpreting this finding. The absence of similar findings in the other mitochondrial enzymes measured restrict plausible explanations for this isolated finding and it may be suggested that this lone significant difference is due to type I error.

The Influence of Microbiota Transfers on Exercise Performance

No significant differences were found between the exercise-inoculated group and sedentary-inoculated group during the performance run-to-exhaustion test. Despite differences in muscle physiology between the two inoculated groups, there was no functional benefit as depicted from the chosen performance test. Run-to-exhaustion tests are used widely through the literature as an indicator of functional performance[89,99–102], however, it must be addressed that several of these investigations include compromised mouse models[99,101,102] that innately restrict exercise capacity. This changes the nature of these investigations from enhancements in performance to recovery or rescue in performance respective to a healthy model. Our investigation does not involve the amelioration of a pathology or other condition directly causing a performance decrement; the animals used in this study are naïve to bacterial colonization but are otherwise healthy. As shown in a study comparing colonized and uncolonized rodents[88], animals inoculated with even a single bacterial strain perform better than germ-free animals. Based on this observation, we assume that both inoculated groups would improve performance with inoculation as compared to in the germ-free condition. Therefore, any subtle performance benefit due to the microbiota transplanted may not have been realized

in this study due to the relatively small number of mice tested. Additionally, the performance protocol for the run-to-exhaustion performance test may not have been optimal. Protocols in the literature vary in time, speed and/or incline adjustments, and objective indication of fatigue among other issues generate conflict and discrepancy when attempting to compare results from various studies. In addition to the absence of a standardized protocol, the genetic variability between different mouse strains is a unavoidable issue[103], all of which impact performance.

Modifications to the Current Investigation

Upon completion of this phase of the project, there are future considerations to be made with follow-up or associated work. The breeding and production of gnotobiotic mice takes exceedingly longer than originally was anticipated and as a result, within the confined timeline of experiments, a sample size of 16 divided between exercised inoculated and sedentary inoculated was achieved. In hindsight, the breeding should have occurred concomitant with the exercise training of the donors so to reduce lapse time (which equated to several months).

Inoculum material were prepared through diluting frozen cecal contents from the donors with phosphate-buffered solution. As demonstrated by Turnbaugh and colleagues (2009), the integrity of the transfer is not compromised through freezing the sample[104]. It must be acknowledged that the bacterial colonies extracted from the donors and transplanted into the recipients were exclusively from the cecum. However, fecal samples were taken every two weeks, as illustrated in **Fig. 1**, and based off observations by Denou

et al. (2016), measures of phylum and bacterial diversity in the feces are highly representative of those in the cecum and distal colon, so changes can be tracked through sequencing. Exercise had similar effects in the distal colon and feces in the aforementioned study[105] and therefore it is justified that the cecal content would be representative of the exercise-induced effects in the donors in the current investigation. Although there is confidence in this facet of the microbiota transfer procedure, we did not control for the number of bacteria and/or number of live bacteria in the inoculum. To the credit of this investigation, the mass of cecal content diluted and inoculum dosages every two weeks were consistent and as mentioned, the changes in bacterial composition following inoculation can still be tracked by sequencing the fecal samples of the gnotobiotic recipients.

The performance measure used in the current investigation has been critiqued, however there is two unique and reliable approaches that could be utilized in a future investigation: a swim to exhaustion test[106–110] and wire hang test[110–112]. As mentioned previously, there are inconsistencies in the literature concerning exhaustion and thus termination of a treadmill test. Cessation of the test may be due to inability to keep pace with the treadmill[99,101], consistent "pedalling" (sitting on the shocker using only forelimbs)[100], a specific time spent on the shocker i.e. 5 seconds[89] or an otherwise ambiguous statement denoting exhaustion when mice do not stay on the treadmill[102]. The use of shock grids is inconsistent as well with some studies opting out for more innocuous stimuli such as tongue depressors[100], bristles[101], or aerosol sprays[99]. Although, the problem arises in which mice may climb on the first two listed tools and compromise

the test. The swim to exhaustion test and wire hang test remove the ambiguity associated with complete fatigue during a run to exhaustion test. The threat of drowning is a potent motivator and as such, complete fatigue is exceedingly more reliable when a mouse swims for survival. Once mice are acclimated to the methods of the hang test as well, the end point is binary. If the mouse lets go of the wire cage and falls, the cessation of the test is unequivocal.

Regardless, the remainder of the technical methods in this paper were reliable measures analysing what was intended to be analysed. As indicated by the proof of principle measures in the donors during IPGTT, the exercise training was a potent stimulus and induced distinct responses between trained and untrained donor groups.

Future Directions

The objective of the current investigation was to delve into the novel concept of the gut-muscle axis, intending to assess the skeletal muscle physiological adaptations induced by a transfer of microbiota from exercised mice into germ-free mice. Observations suggest that proliferation of specific beneficial bacterial colonies during exercise can be transferred to another host through a cecal transplant resulting in improvements in glucose clearance during an IPGTT.

The future direction of this research should aim to focus on glucose metabolism and performance/functional aspects of this field. The use of fecal transplants in humans is currently a viable and safe medical practice and the breadth of the technique in medicine can be expanded dependent to this research. Glucose metabolism is especially important

to those suffering from conditions such as type II diabetes, and to suggest the involvement of the microbiota in attenuating the pathology or improving glucose homeostasis bears high relevance. Further research is merited into transferring microbiota from trained individuals and examining local and systemic effects. In summary, it was evident that following a transfer of microbiota from exercised animals, there were improvements in glucose homeostasis, the significance of which was driven by the glucose response of female animals.

APPENDIX I: SUPPLEMENTARY TABLES, FIGURES, IMAGES

Table 2: Mass of Harvested Muscles

	Exercise Donors	Sedentary Donors	Exercise Inoculated	Sedentary Inoculated
Heart	157.2 ± 9.8 mg	153.2 ± 6.3 mg	156 ± 9.4 mg **M:** 167.4 ± 12.1 mg **F:** 147.4 ± 13.4 mg	144.8 ± 10.7 mg **M:** 155.9 ± 19.7 mg **F:** 136.4 ± 12.2 mg
TA	44.3 ± 2.3 mg	44.3 ± 1.7 mg	45.4 ± 1.7 mg **M:** 44.8 ± 2.4 mg **F:** 46.0 ± 2.6 mg*	39.9 ± 3.4 mg **M:** 50.1 ± 3.8 mg **F:** 32.2 ± 3.3 mg*
EDL	16.2 ± 1.7 mg	13.8 ± 1.2 mg	16.7 ± 2.3 mg **M:** 17.5 ± 2.9 mg **F:** 15.9 ± 3.6 mg	14.1 ± 2.2 mg **M:** 18.9 ± 3.8 mg **F:** 10.5 ± 1.8 mg
Soleus	10.4 ± 0.6 mg	12.2 ± 1.1 mg	9.4 ± 0.6 mg **M:** 9.0 ± 1.0 mg **F:** 10.1 ± 1.3 mg*	8.1 ± 0.8 mg **M:** 9.9 ± 0.8 mg **F:** 6.6 ± 0.4 mg*
Quad	174.5 ± 8.0 mg	186.6 ± 7.5 mg	159.6 ± 3.8 mg **M:** 158.7 ± 6.0 mg **F:** 160.5 ± 5.2 mg*	151.7 ± 7.9 mg **M:** 172.2 ± 11.5 mg **F:** 136.3 ± 7.3 mg*
Gastroc	162.8 ± 10.9 mg	178.9 ± 12.4 mg	162.9 ± 7.3 mg **M:** 160.1 ± 11.8 mg **F:** 170.3 ± 20.2 mg*	146.8 ± 10.8 mg **M:** 165.8 ± 9.3 mg **F:** 129.2 ± 7.4 mg*

Values are means ± SEM. Exercise Donors N=9, Sedentary Donors N=10, Exercise Inoculated N=8, Sedentary Inoculated N=8. TA refers to tibialis anterior, EDL refers to extensor digitorum longus, Quad refers to quadriceps, Gastroc refers to gastrocnemius.
No statistically differences were found between the exercise and sedentary donors.
*, $p<0.05$ exercise inoculated v.s. sedentary inoculated within sex

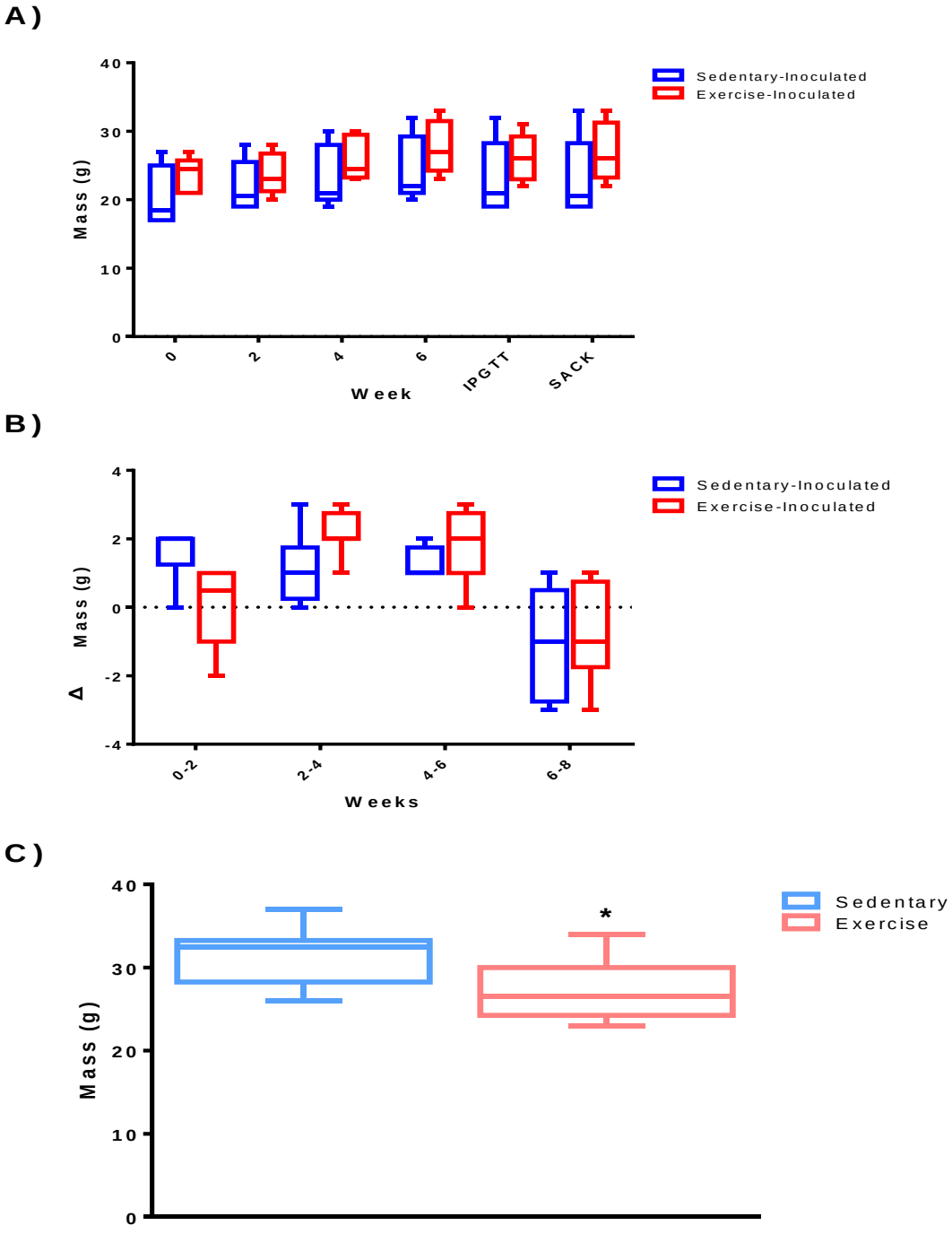


Figure 13. *Body mass*. (A) Absolute body mass at weeks 0, 2, 4, 6 as well as before the IPGTT and sack (B) Change in body mass every 2 weeks (C) Absolute body mass in exercise and sedentary donors prior to IPGTT. Data are presented in box and whisker plots – the box denoting the median, 25th and 75th percentiles, whiskers representing the minimum and maximum values of the respective datasets. $p<0.05$ exercise inoculated v.s. sedentary inoculated.

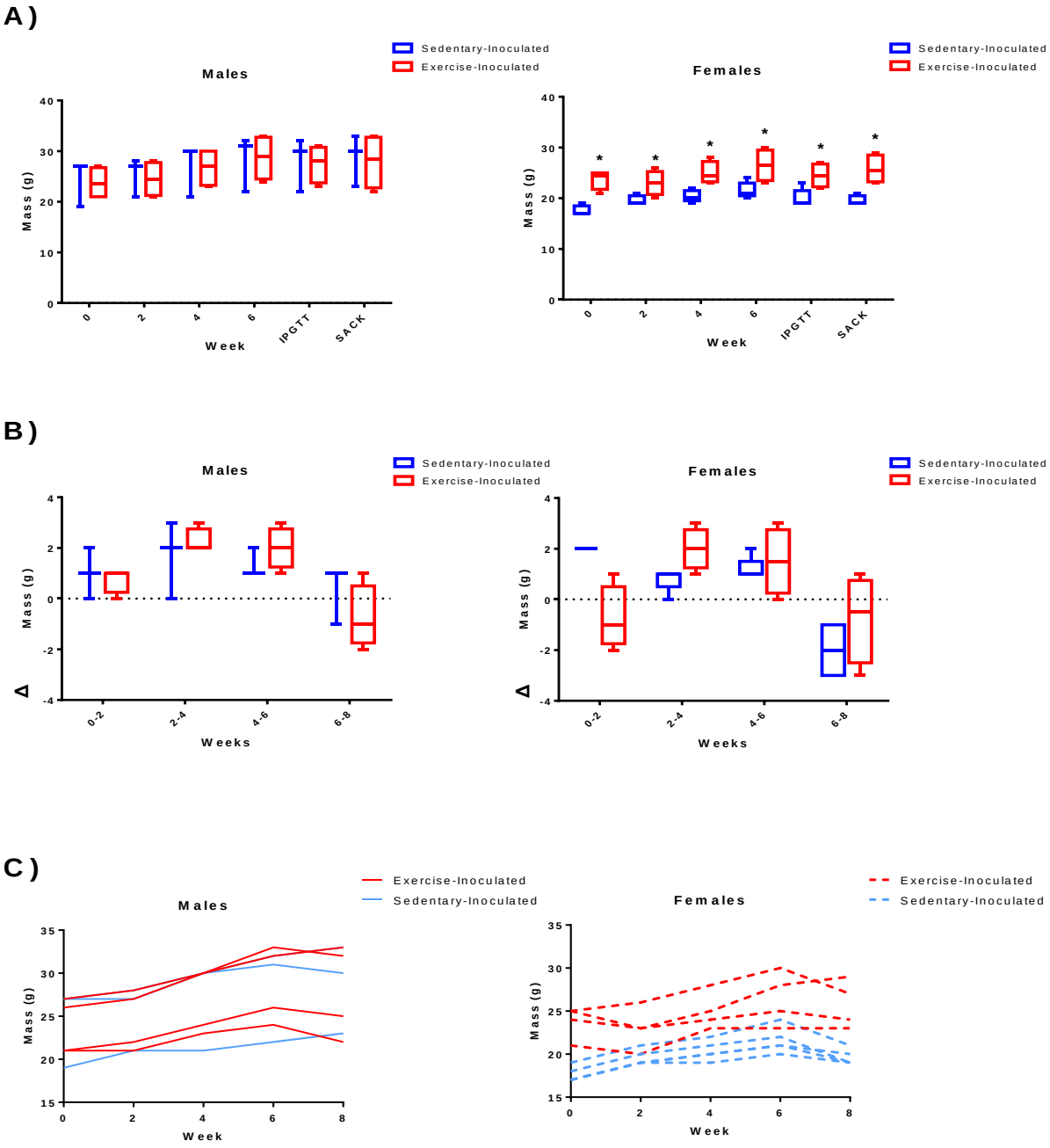


Figure 14. *Body Mass – Sex-Specific*. (A) Absolute body mass in males and females at weeks 0, 2, 4, 6 as well as before the IPGTT and sack(B) Change in body mass every 2 weeks in males and females (C) Individualized absolute body mass in males and females at weeks 0, 2 ,4 ,6 and Sack. Data in (A) and (B) are presented in box and whisker plots – the box denoting the median, 25th and 75th percentiles, whiskers representing the minimum and maximum values of the respective datasets. *, $p<0.05$ exercise inoculated v.s. sedentary inoculated within sex.

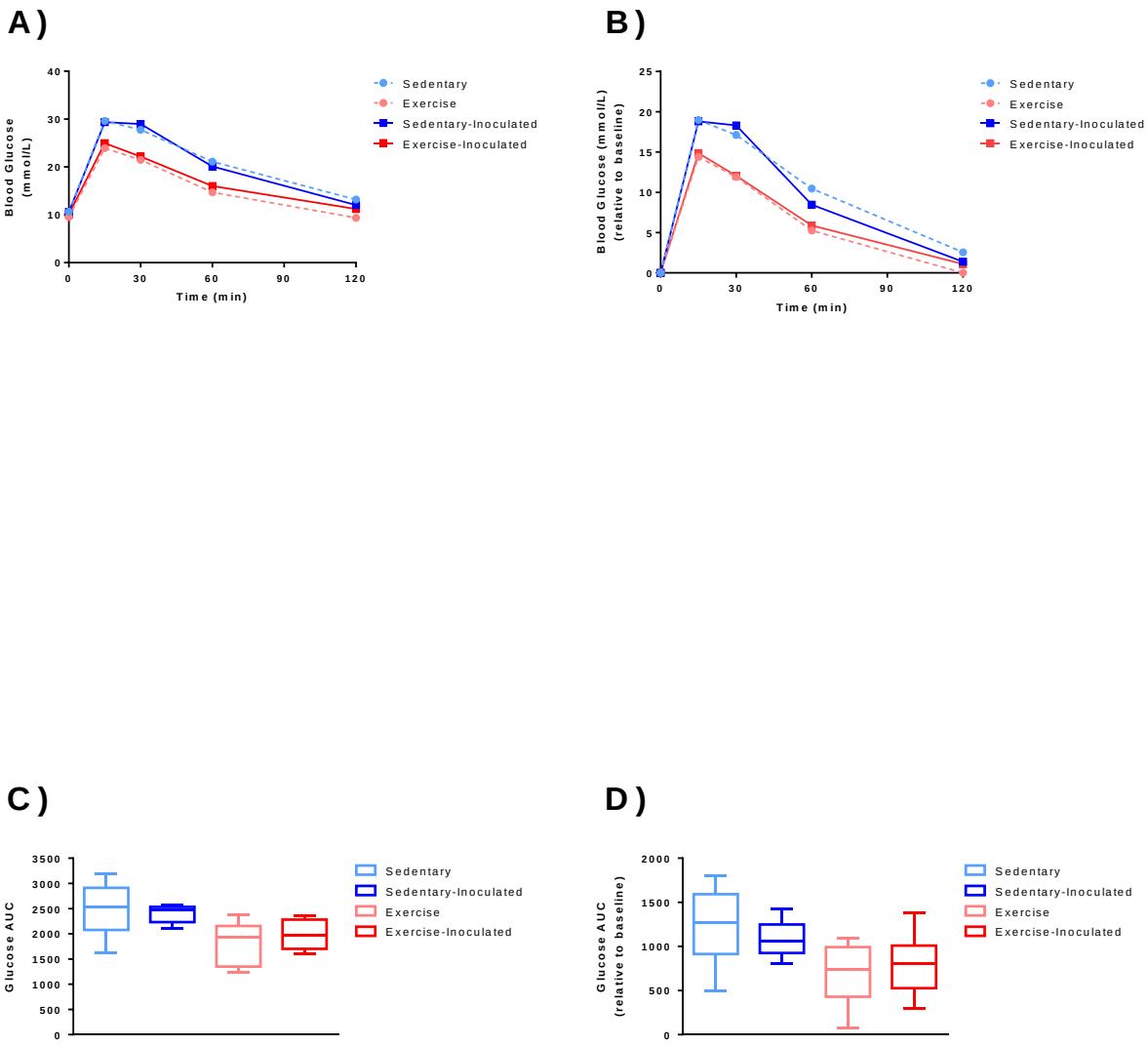


Figure 15. *Collapsed data of donor groups and recipient groups.* The following set of graphs denote blood glucose responses to an IPGTT and glucose area-under-the-curve during an IPGTT in both donor groups and both inoculated groups, as expressed in absolute concentrations and concentrations relative to baseline. (A) absolute glucose values during IPGTT at timepoints 0, 15, 30, 60, 120mins; mean (B) Δ glucose values relative to baseline during IPGTT at timepoints 0, 15, 30, 60, 120mins; mean (C) box and whisker plot of absolute glucose AUC during IPGTT (D) box and whisker plot of change in glucose AUC relative to baseline. Box denotes median, 25th and 75th percentiles, whiskers representing the maximum and minimum values.

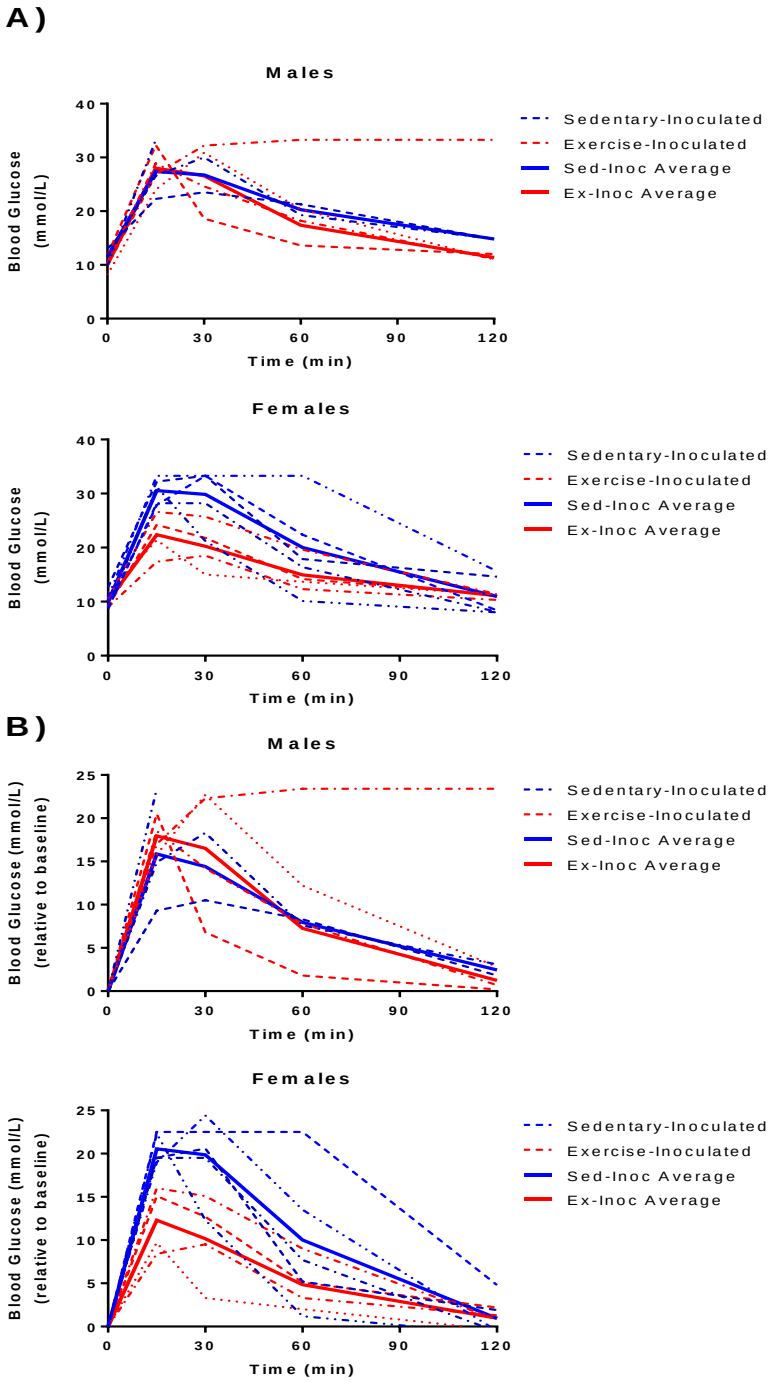


Figure 16. *Individualized glucose values during IPGTT.* The following set of graphs denote individual blood glucose responses to an IPGTT in both inoculated groups plotted with the average blood glucose response of each inoculated group respectively. (A) Individual absolute glucose in males and females (B) Individual Δ glucose relative to baseline values in males and females.

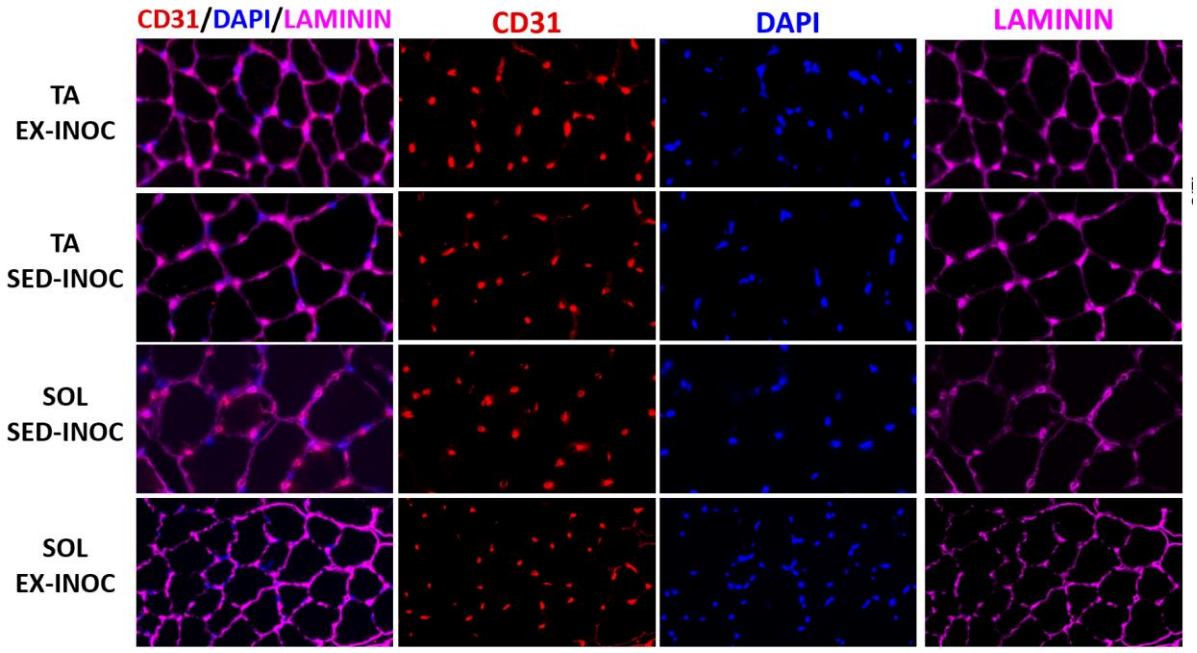


Figure 17. *Representative Image of Immunohistochemical Stain for Capillaries*. IHC staining was performed on muscle cross sections of the TA and soleus. CD31 in TRITC, Laminin in Cy5.

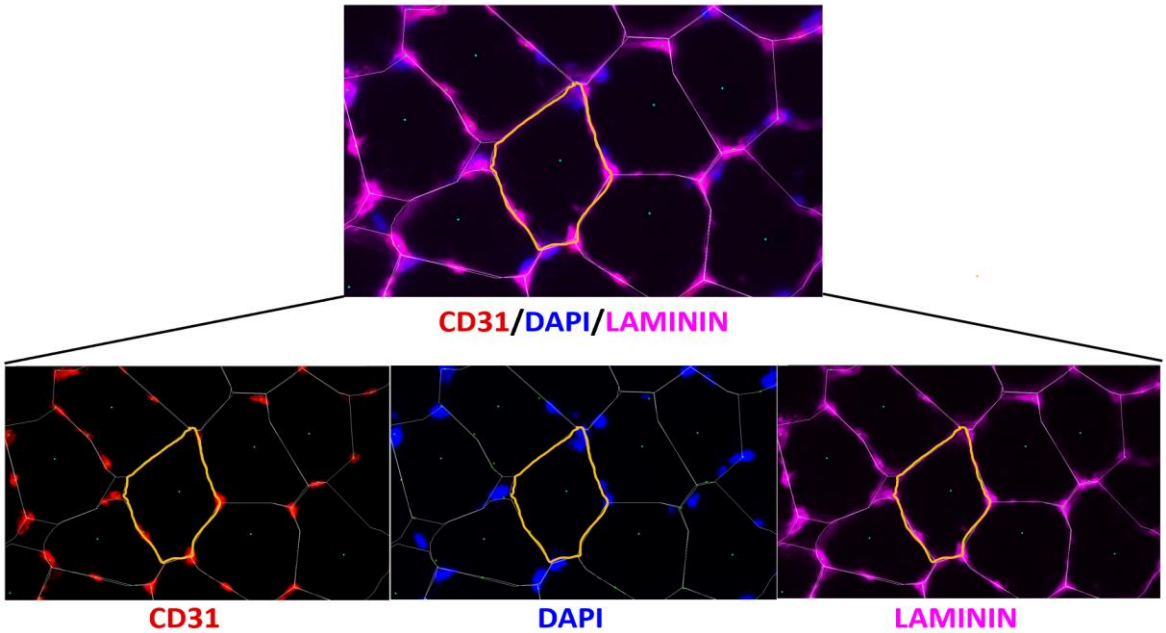
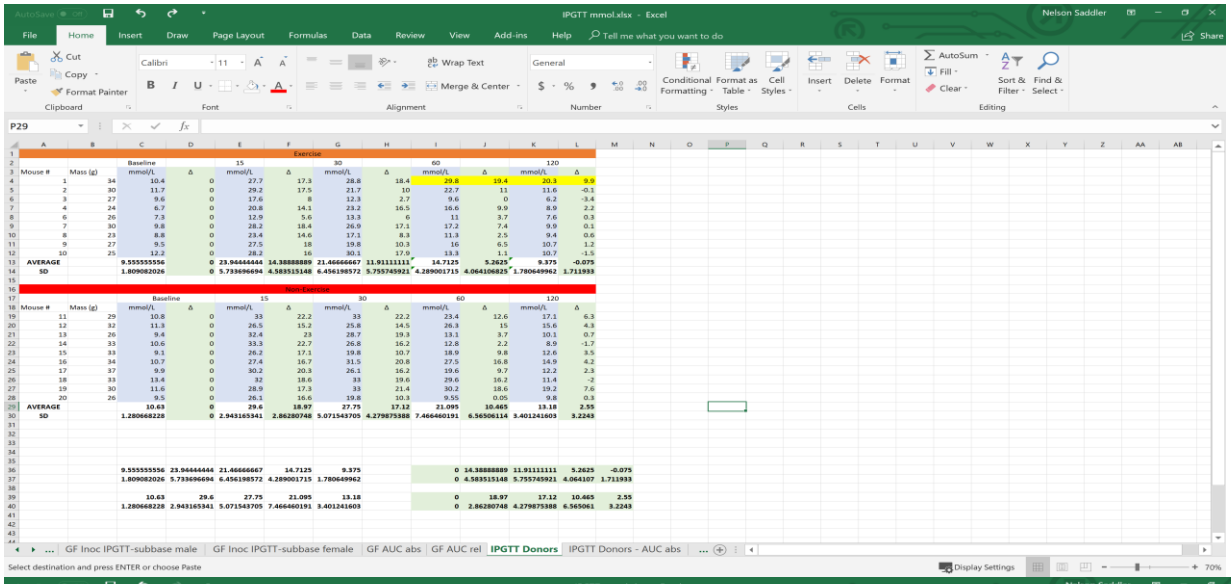
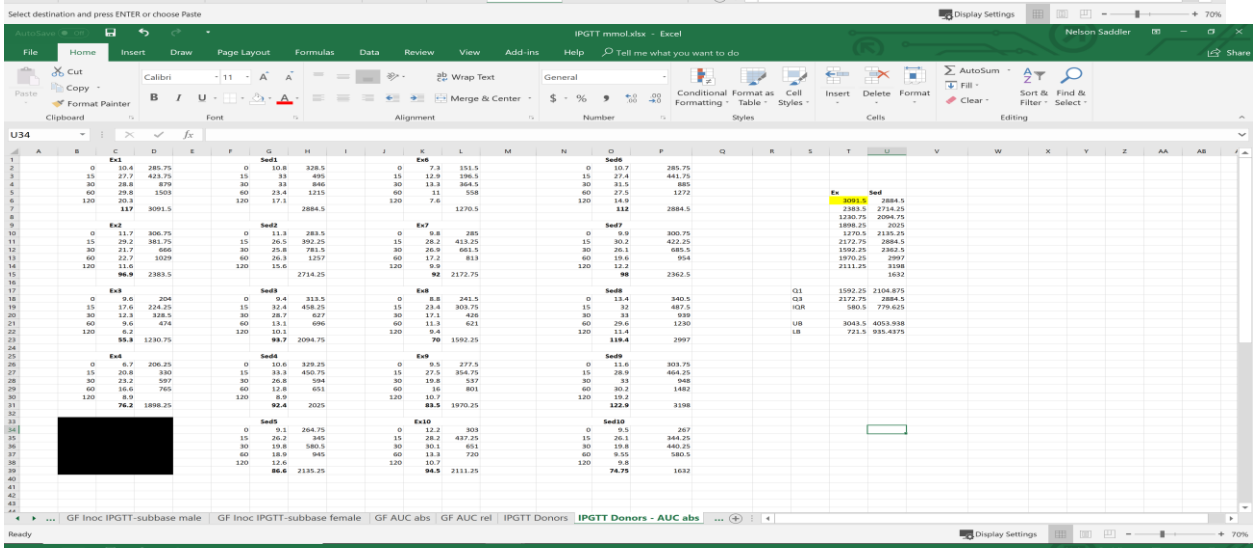


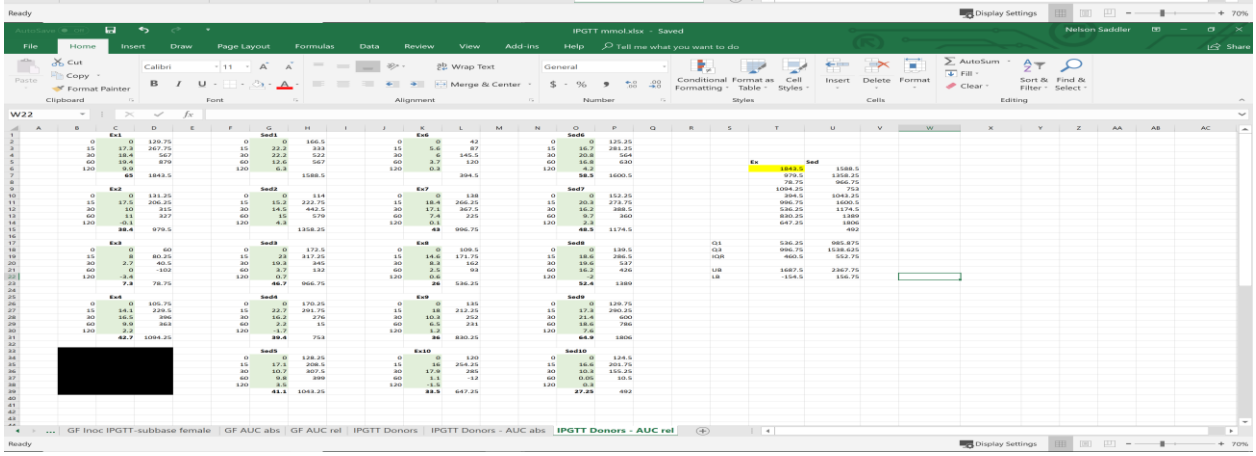
Figure 18. *Representative Image of Sarcolemma Tracing for CFPE*. IHC staining was performed on muscle cross sections of the TA and soleus. CD31 in TRITC, Laminin in Cy5. Example of outlining muscle fibres through sarcolemma tracing in yellow.

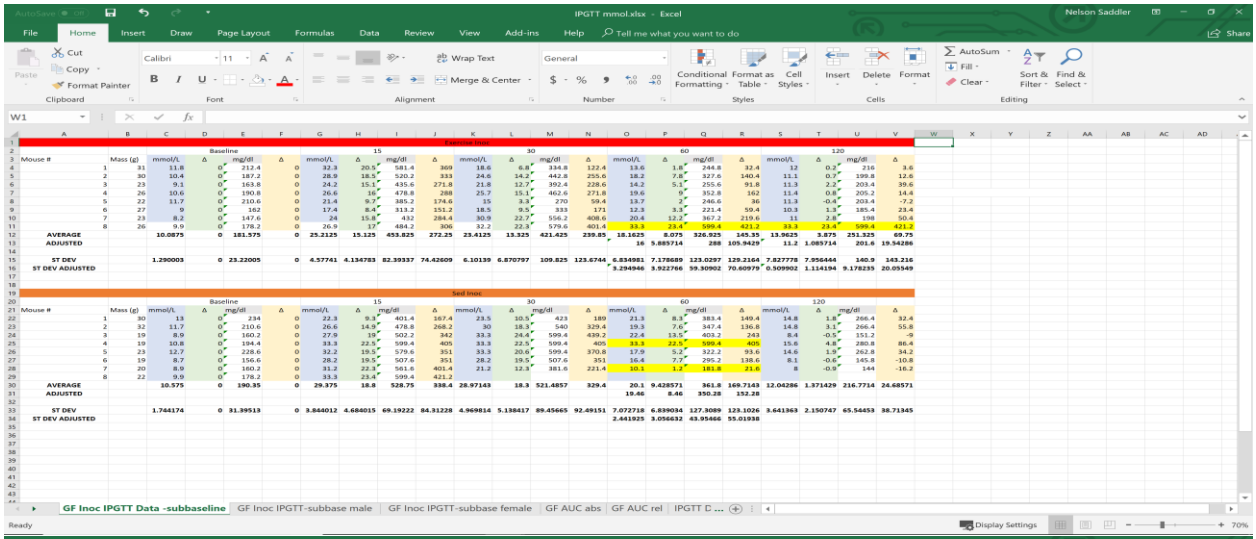
APPENDIX II: RAW DATA AND STATISTICS

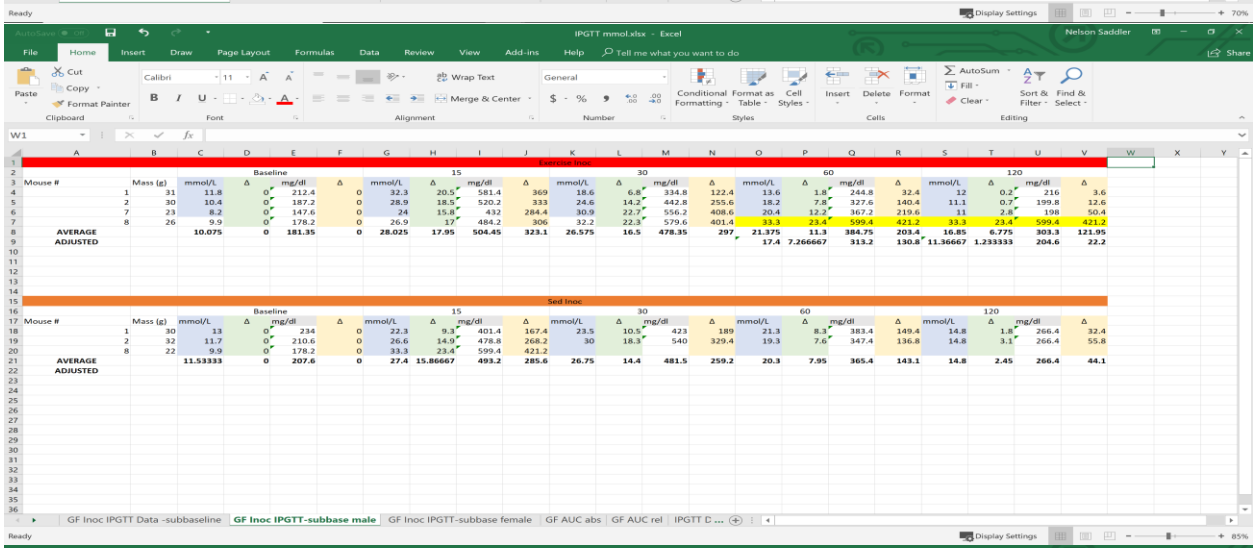
IPGTT RAW DATA:

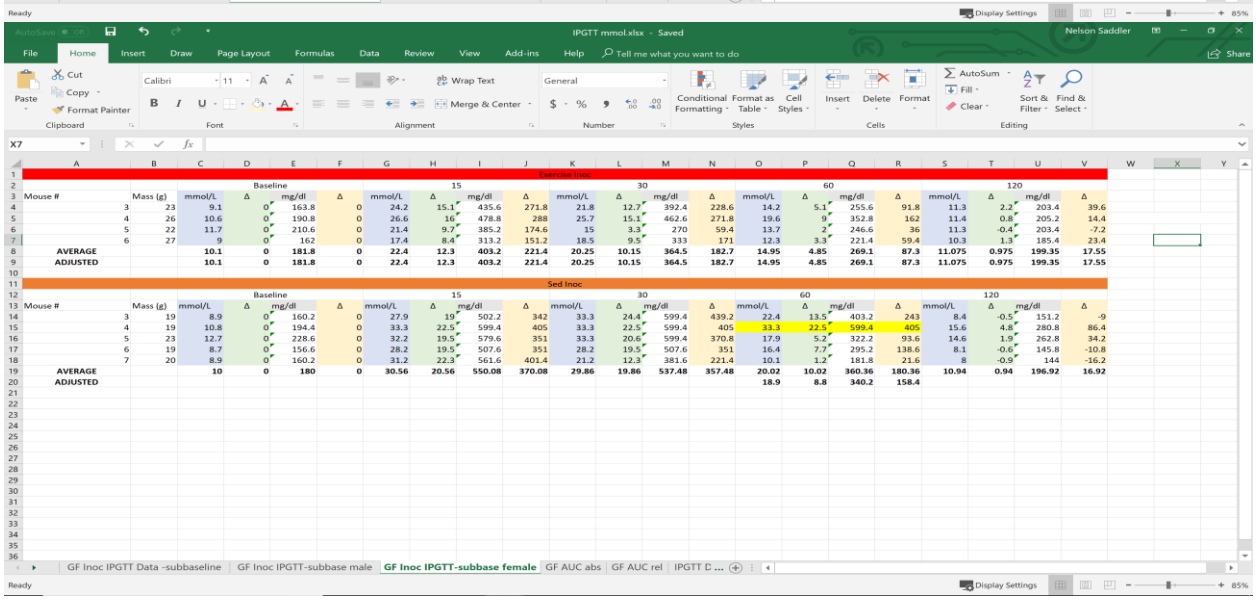


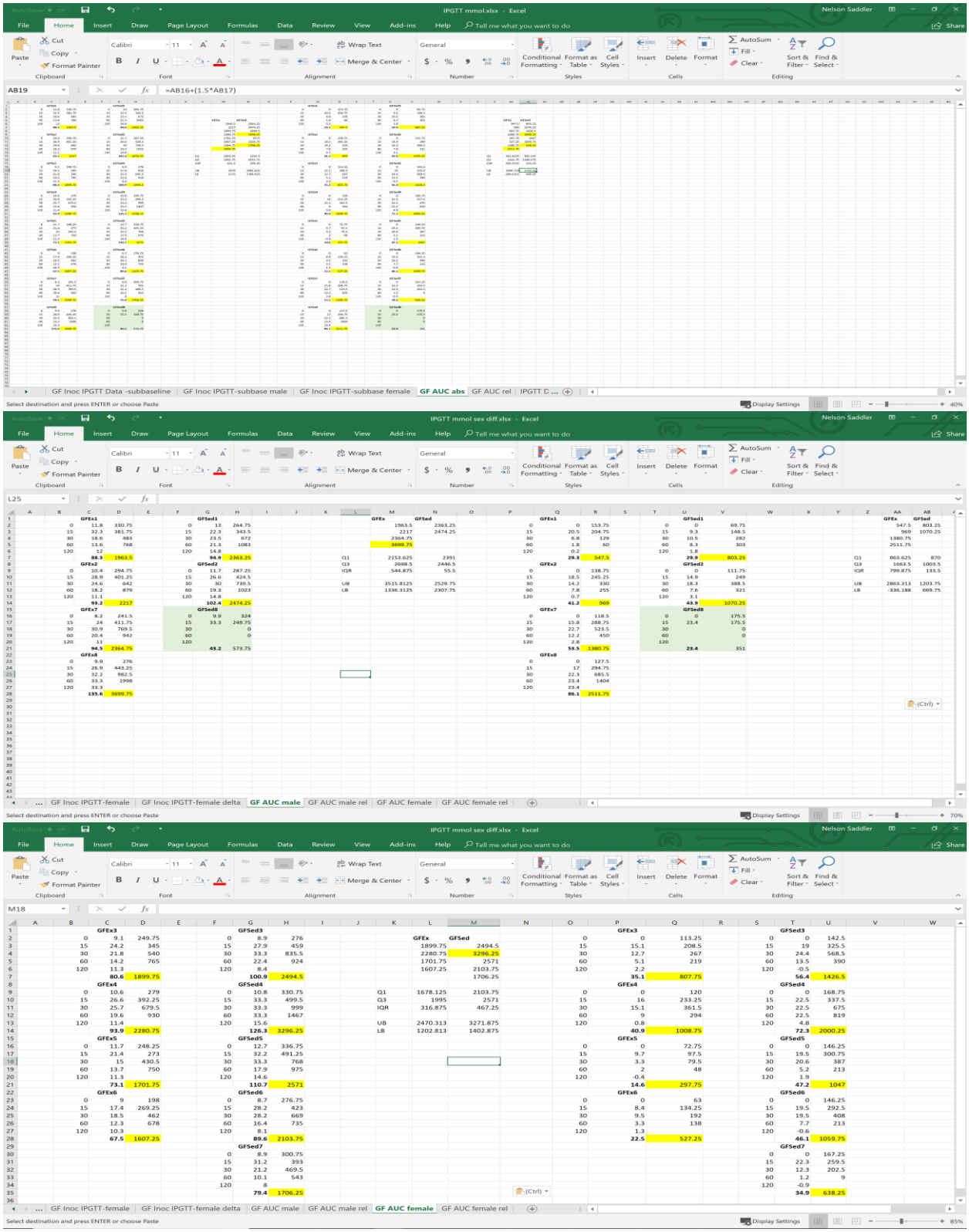












IPGTT: AUC T-TESTS

t test	
Table Analyzed	AUC Donors
Column B	Sedentary
vs.	vs.
Column A	Exercise
Unpaired t test	
P value	0.0096
P value summary	**
Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.941 df=16
How big is the difference?	
Mean ± SEM of column A	1829 ± 149.8 N=8
Mean ± SEM of column B	2493 ± 162.3 N=10
Difference between means	664.1 ± 225.8
95% confidence interval	185.4 to 1143
R square	0.3509
F test to compare variances	
F,DFn, Dfd	1.468, 9, 7
P value	0.6267
P value summary	ns
Significantly different? (P < 0.05)	No

t test	
Table Analyzed	AUC - subtract from baseline Donors
Column B	Sedentary
vs.	vs.
Column A	Exercise
Unpaired t test	
P value	0.0112
P value summary	*
Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.865 df=16
How big is the difference?	
Mean ± SEM of column A	694.7 ± 123.0 N=8
Mean ± SEM of column B	1217 ± 129.8 N=10
Difference between means	522.5 ± 182.3
95% confidence interval	135.9 to 909.0
R square	0.3391
F test to compare variances	
F,DFn, Dfd	1.393, 9, 7
P value	0.6772
P value summary	ns
Significantly different? (P < 0.05)	No

t test	
Table Analyzed	AUC Inoc - abs
Column B	Sedentary-Inoculated
vs.	vs.
Column A	Exercise-Inoculated
Unpaired t test	
P value	0.0238
P value summary	*
Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.663 df=10
How big is the difference?	
Mean ± SEM of column A	2005 ± 110.6 N=7
Mean ± SEM of column B	2401 ± 81.48 N=5
Difference between means	396.4 ± 148.8
95% confidence interval	64.75 to 728.0
R square	0.4149
F test to compare variances	
F,DFn, Dfd	2.577, 6, 4
P value	0.3786
P value summary	ns
Significantly different? (P < 0.05)	No

t test	
Table Analyzed	AUC Inoc - rel
Column B	Sedentary-Inoculated
vs.	vs.
Column A	Exercise-Inoculated
Unpaired t test	
P value	0.1478
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.569 df=10
How big is the difference?	
Mean ± SEM of column A	791.3 ± 138.0 N=7
Mean ± SEM of column B	1081 ± 99.56 N=5
Difference between means	290.1 ± 184.9
95% confidence interval	-122.0 to 702.2
R square	0.1975
F test to compare variances	
F,DFn, Dfd	2.688, 6, 4
P value	0.3577
P value summary	ns
Significantly different? (P < 0.05)	No

IPGTT: 2-WAY REPEATED MEASURES ANOVA & SIDAK CORRECTION *POST HOC*

2way ANOVA Tabular results

	A	B	C	D	E
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
	Y	Y	Y	Y	Y
Table Analyzed	Donor abs				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	1.427	0.1663	ns	No	
Time	64.55	< 0.0001	****	Yes	
Subject	7.611	0.0031	**	Yes	
Subjects (matching)	10.93	0.0006	***	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	95.58	4	23.90	F (4, 68) = 1.673	P = 0.1663
Time	4325	4	1081	F (4, 68) = 75.71	P < 0.0001
Subject	509.9	1	509.9	F (1, 17) = 11.83	P = 0.0031
Subjects (matching)	732.5	17	43.09	F (17, 68) = 3.017	P = 0.0006
Residual	971.1	68	14.28		

2way ANOVA Multiple comparisons

	A	B	C	D	E	F	G	H
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G	Data Set-H
	Y	Y	Y	Y	Y	Y	Y	Y
Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	5							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary				
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	-1.074	-6.479 to 4.330	No	ns				
Row 2	-5.656	-11.06 to -0.2509	Yes	*				
Row 3	-6.283	-11.69 to -0.8787	Yes	*				
Row 4	-6.383	-11.79 to -0.9779	Yes	*				
Row 5	-3.805	-9.210 to 1.600	No	ns				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	9.556	10.63	-1.074	2.057	9	10	0.5223	85
Row 2	23.94	29.60	-5.656	2.057	9	10	2.749	85
Row 3	21.47	27.75	-6.283	2.057	9	10	3.055	85
Row 4	14.71	21.10	-6.383	2.057	9	10	3.103	85
Row 5	9.375	13.18	-3.805	2.057	9	10	1.850	85

2way ANOVA Tabular results

	A	B	C	D	E
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
	Y	Y	Y	Y	Y
Table Analyzed	Donor rel				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	1.566	0.1495	ns	No	
Time	71.19	< 0.0001	****	Yes	
Subject	4.852	0.0018	**	Yes	
Subjects (matching)	6.068	0.0903	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	94.81	4	23.70	F (4, 68) = 1.748	P = 0.1495
Time	4310	4	1077	F (4, 68) = 79.47	P < 0.0001
Subject	293.8	1	293.8	F (1, 17) = 13.59	P = 0.0018
Subjects (matching)	367.4	17	21.61	F (17, 68) = 1.594	P = 0.0903
Residual	921.9	68	13.56		

2way ANOVA Multiple comparisons

	A	B	C	D	E	F	G	H
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G	Data Set-H
	Y	Y	Y	Y	Y	Y	Y	Y
Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	5							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary				
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	0.0	-4.702 to 4.702	No	ns				
Row 2	-4.581	-9.283 to 0.1206	No	ns				
Row 3	-5.209	-9.911 to -0.5072	Yes	*				
Row 4	-5.203	-9.904 to -0.5008	Yes	*				
Row 5	-2.617	-7.318 to 2.085	No	ns				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	0.0	0.0	0.0	1.789	9	10	0.0	85
Row 2	14.39	18.97	-4.581	1.789	9	10	2.560	85
Row 3	11.91	17.12	-5.209	1.789	9	10	2.911	85
Row 4	5.263	10.47	-5.203	1.789	9	10	2.907	85
Row 5	-0.06667	2.550	-2.617	1.789	9	10	1.462	85

2way ANOVA Tabular results

	A	B	C	D	E
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
	Y	Y	Y	Y	Y
Table Analyzed	Inoc rel				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	2.153	0.0311	*	Yes	
Time	78.33	< 0.0001	****	Yes	
Subject	2.646	0.0303	*	Yes	
Subjects (matching)	6.376	0.0097	**	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	106.6	4	26.64	F (4, 56) = 2.872	P = 0.0311
Time	3876	4	968.9	F (4, 56) = 104.5	P < 0.0001
Subject	130.9	1	130.9	F (1, 14) = 5.809	P = 0.0303
Subjects (matching)	315.5	14	22.54	F (14, 56) = 2.429	P = 0.0097
Residual	519.5	56	9.277		

2way ANOVA Multiple comparisons

	A	B	C	D	E	F	G	H
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G	Data Set-H
	Y	Y	Y	Y	Y	Y	Y	Y
Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	5							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary				
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	0.0	-4.559 to 4.559	No	ns				
Row 2	-3.675	-8.234 to 0.8844	No	ns				
Row 3	-8.257	-10.82 to -1.698	Yes	**				
Row 4	-2.574	-7.134 to 1.985	No	ns				
Row 5	-0.2857	-4.845 to 4.274	No	ns				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	0.0	0.0	0.0	1.727	8	8	0.0	70
Row 2	15.13	18.80	-3.675	1.727	8	8	2.128	70
Row 3	12.04	16.30	-8.257	1.727	8	8	3.823	70
Row 4	5.886	8.460	-2.574	1.727	8	8	1.491	70
Row 5	1.086	1.371	-0.2857	1.727	8	8	0.1655	70

2way ANOVA Tabular results

	A	B	C	D	E
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
	Y	Y	Y	Y	Y
Table Analyzed	Inoc abs				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	1.509	0.1492	ns	No	
Time	78.27	< 0.0001	****	Yes	
Subject	3.395	0.0073	**	Yes	
Subjects (matching)	4.844	0.1029	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	77.37	4	19.34	F (4, 56) = 1.763	P = 0.1492
Time	4013	4	1003	F (4, 56) = 91.46	P < 0.0001
Subject	174.1	1	174.1	F (1, 14) = 9.812	P = 0.0073
Subjects (matching)	248.4	14	17.74	F (14, 56) = 1.617	P = 0.1029
Residual	614.3	56	10.97		

2way ANOVA Multiple comparisons

	A	B	C	D	E	F	G	H
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G	Data Set-H
	Y	Y	Y	Y	Y	Y	Y	Y
Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	5							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary				
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	-0.4875	-5.122 to 4.147	No	ns				
Row 2	-4.163	-8.797 to 0.4720	No	ns				
Row 3	-5.559	-10.19 to -0.9244	Yes	*				
Row 4	-3.700	-8.335 to 0.9345	No	ns				
Row 5	-0.8429	-5.477 to 3.792	No	ns				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	10.09	10.58	-0.4875	1.755	8	8	0.2777	70
Row 2	25.21	29.38	-4.163	1.755	8	8	2.371	70
Row 3	23.41	28.97	-5.559	1.755	8	8	3.167	70
Row 4	16.00	19.70	-3.700	1.755	8	8	2.108	70
Row 5	11.20	12.04	-0.8429	1.755	8	8	0.4802	70

IPGTT: AUC T-TESTS – *Sex Differences*

t test	
Table Analyzed	Males abs
Column B	Sedentary-Inoculated
vs.	vs.
Column A	Exercise-Inoculated
Unpaired t test	
P value	0.2279
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.511 df=3
How big is the difference?	
Mean ± SEM of column A	2182 ± 117.2 N=3
Mean ± SEM of column B	2419 ± 55.50 N=2
Difference between means	237.0 ± 156.8
95% confidence interval	-262.1 to 736.1
R square	0.4323
F test to compare variances	
F,DFn, Dfd	
P value	
P value summary	
Significantly different? (P < 0.05)	

t test	
Table Analyzed	Males rel
Column B	Sedentary-Inoculated
vs.	vs.
Column A	Exercise-Inoculated
Unpaired t test	
P value	0.5508
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.6506 df=4
How big is the difference?	
Mean ± SEM of column A	1352 ± 422.3 N=4
Mean ± SEM of column B	936.8 ± 133.5 N=2
Difference between means	-415.5 ± 638.7
95% confidence interval	-2189 to 1358
R square	0.09569
F test to compare variances	
F,DFn, Dfd	
P value	
P value summary	
Significantly different? (P < 0.05)	

t test	
Table Analyzed	Females abs
Column B	Sedentary-Inoculated
vs.	vs.
Column A	Exercise-Inoculated
Unpaired t test	
P value	0.2132
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.393 df=6
How big is the difference?	
Mean ± SEM of column A	1872 ± 149.1 N=4
Mean ± SEM of column B	2219 ± 199.2 N=4
Difference between means	346.5 ± 248.8
95% confidence interval	-262.3 to 955.3
R square	0.2443
F test to compare variances	
F,DFn, Dfd	1.783, 3, 3
P value	0.6465
P value summary	ns
Significantly different? (P < 0.05)	No

t test	
Table Analyzed	Females rel
Column B	Sedentary-Inoculated
vs.	vs.
Column A	Exercise-Inoculated
Unpaired t test	
P value	0.1390
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.706 df=6
How big is the difference?	
Mean ± SEM of column A	660.4 ± 156.1 N=4
Mean ± SEM of column B	1043 ± 161.0 N=4
Difference between means	382.5 ± 224.3
95% confidence interval	-166.2 to 931.2
R square	0.3265
F test to compare variances	
F,DFn, Dfd	1.065, 3, 3
P value	0.9602
P value summary	ns
Significantly different? (P < 0.05)	No

IPGTT: 2-WAY REPEATED MEASURES ANOVA & SIDAK CORRECTION *POST HOC – Sex Differences*

2way ANOVA Tabular results

	A	B	C	D	E
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
	Y	Y	Y	Y	Y
Table Analyzed	Males abs 2way				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	1.100	0.8526	ns	No	
Time	83.27	< 0.0001	****	Yes	
Column Factor	1.423	0.0701	ns	No	
Subjects (matching)	0.9443	0.8836	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	17.90	4	4.474	F (4, 16) = 0.3317	P = 0.8526
Time	1355	4	338.7	F (4, 16) = 25.11	P < 0.0001
Column Factor	23.14	1	23.14	F (1, 4) = 6.026	P = 0.0701
Subjects (matching)	15.36	4	3.841	F (4, 16) = 0.2847	P = 0.8836
Residual	215.8	16	13.49		

2way ANOVA Multiple comparisons

	A	B	C	D	E	F	G	H
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G	Data Set-H
	Y	Y	Y	Y	Y	Y	Y	Y
Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	5							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary				
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	-1.400	-9.273 to 6.473	No	ns				
Row 2	1.000	-6.873 to 8.873	No	ns				
Row 3	-2.050	-9.923 to 5.823	No	ns				
Row 4	-2.900	-10.77 to 4.973	No	ns				
Row 5	-3.433	-11.31 to 4.440	No	ns				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	10.13	11.53	-1.400	2.776	3	3	0.5043	20
Row 2	28.40	27.40	1.000	2.776	3	3	0.3602	20
Row 3	24.70	26.75	-2.050	2.776	3	3	0.7385	20
Row 4	17.40	20.30	-2.900	2.776	3	3	1.045	20
Row 5	11.37	14.80	-3.433	2.776	3	3	1.237	20

2way ANOVA Tabular results

	A	B	C	D	E
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
	Y	Y	Y	Y	Y
Table Analyzed	Males rel 2way				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	0.7926	0.8724	ns	No	
Time	77.59	< 0.0001	****	Yes	
Column Factor	0.08560	0.7786	ns	No	
Subjects (matching)	4.859	0.2383	ns	No	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	16.55	4	4.138	F (4, 20) = 0.3032	P = 0.8724
Time	1620	4	405.1	F (4, 20) = 29.68	P < 0.0001
Column Factor	1.788	1	1.788	F (1, 5) = 0.08808	P = 0.7786
Subjects (matching)	101.5	5	20.29	F (5, 20) = 1.487	P = 0.2383
Residual	273.0	20	13.65		

2way ANOVA Multiple comparisons

	A	B	C	D	E	F	G	H
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G	Data Set-H
	Y	Y	Y	Y	Y	Y	Y	Y
Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	5							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary				
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	0.0	-8.213 to 8.213	No	ns				
Row 2	2.083	-6.130 to 10.30	No	ns				
Row 3	2.100	-6.113 to 10.31	No	ns				
Row 4	-0.6833	-8.897 to 7.530	No	ns				
Row 5	-1.217	-9.430 to 6.997	No	ns				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	0.0	0.0	0.0	2.956	4	3	0.0	25
Row 2	17.95	15.87	2.083	2.956	4	3	0.7048	25
Row 3	16.50	14.40	2.100	2.956	4	3	0.7105	25
Row 4	7.267	7.950	-0.6833	2.956	4	3	0.2312	25
Row 5	1.233	2.450	-1.217	2.956	4	3	0.4116	25

2way ANOVA Tabular results

	A	B	C	D	E
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
	Y	Y	Y	Y	Y
Table Analyzed	Females rel 2way				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	6.151	0.0006	***	Yes	
Time	70.29	< 0.0001	****	Yes	
Column Factor	7.501	0.0096	**	Yes	
Subjects (matching)	4.208	0.0300	*	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	182.4	4	45.61	F (4, 28) = 6.831	P = 0.0006
Time	2085	4	521.2	F (4, 28) = 78.06	P < 0.0001
Column Factor	222.5	1	222.5	F (1, 7) = 12.48	P = 0.0096
Subjects (matching)	124.8	7	17.83	F (7, 28) = 2.671	P = 0.0300
Residual	186.9	28	6.677		

2way ANOVA Multiple comparisons

	A	B	C	D	E	F	G	H
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G	Data Set-H
	Y	Y	Y	Y	Y	Y	Y	Y
Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	5							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary				
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	0.0	-5.437 to 5.437	No	ns				
Row 2	-8.280	-13.70 to -2.823	Yes	**				
Row 3	-9.710	-15.15 to -4.273	Yes	***				
Row 4	-4.438	-9.875 to 0.9990	No	ns				
Row 5	0.03500	-5.402 to 5.472	No	ns				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	0.0	0.0	0.0	2.002	4	5	0.0	35
Row 2	12.30	20.58	-8.280	2.002	4	5	4.126	35
Row 3	10.15	19.86	-9.710	2.002	4	5	4.850	35
Row 4	4.850	9.288	-4.438	2.002	4	5	2.217	35
Row 5	0.9750	0.9400	0.03500	2.002	4	5	0.01748	35

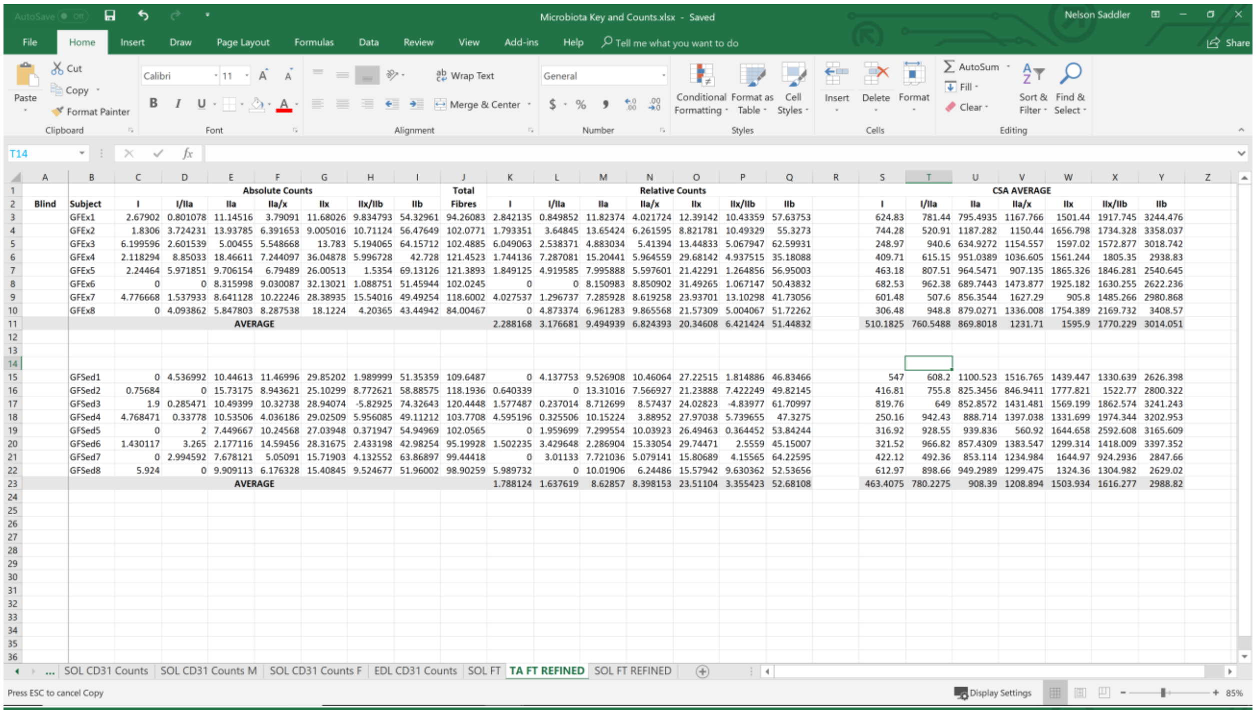
2way ANOVA Tabular results

	A	B	C	D	E
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
	Y	Y	Y	Y	Y
Table Analyzed	Females abs 2way				
Two-way RM ANOVA	Matching: Stacked				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	5.544	0.0089	**	Yes	
Time	62.93	< 0.0001	****	Yes	
Column Factor	6.870	0.0687	ns	No	
Subjects (matching)	10.41	0.0019	**	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	183.3	4	45.82	F (4, 28) = 4.182	P = 0.0089
Time	2080	4	520.1	F (4, 28) = 47.47	P < 0.0001
Column Factor	227.1	1	227.1	F (1, 7) = 4.618	P = 0.0687
Subjects (matching)	344.3	7	49.18	F (7, 28) = 4.489	P = 0.0019
Residual	306.8	28	10.96		

2way ANOVA Multiple comparisons

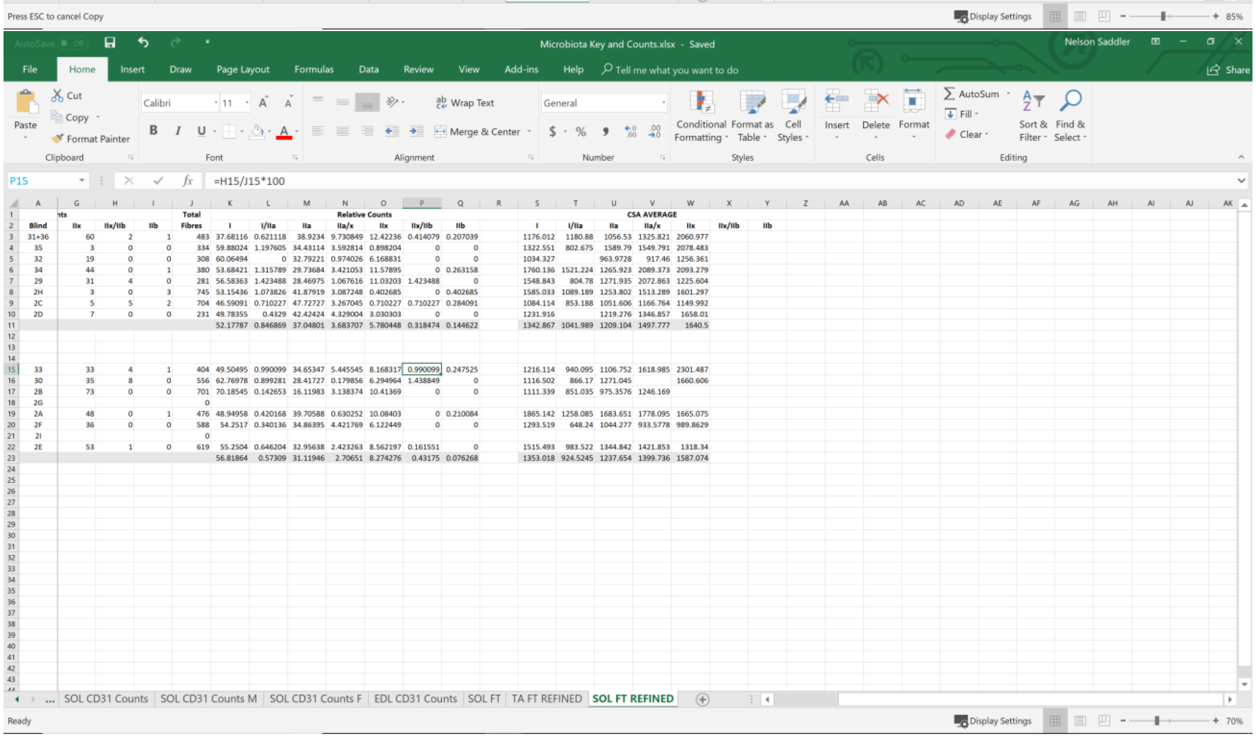
	A	B	C	D	E	F	G	H
	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F	Data Set-G	Data Set-H
	Y	Y	Y	Y	Y	Y	Y	Y
Compare each cell mean with the other cell mean in that row								
Number of families	1							
Number of comparisons per family	5							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary				
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	0.1000	-7.757 to 7.957	No	ns				
Row 2	-8.180	-16.02 to -0.3032	Yes	*				
Row 3	-8.610	-17.47 to -1.753	Yes	*				
Row 4	-5.070	-12.93 to 2.787	No	ns				
Row 5	0.1350	-7.722 to 7.992	No	ns				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	t	DF
Exercise-Inoculated - Sedentary-Inoculated								
Row 1	10.10	10.00	0.1000	2.893	4	5	0.03456	35
Row 2	22.40	30.58	-8.180	2.893	4	5	2.820	35
Row 3	20.25	29.86	-9.610	2.893	4	5	3.322	35
Row 4	14.95	20.02	-5.070	2.893	4	5	1.752	35
Row 5	11.08	10.94	0.1350	2.893	4	5	0.04666	35

MUSCLE COMPOSITION AND CSA: RAW DATA



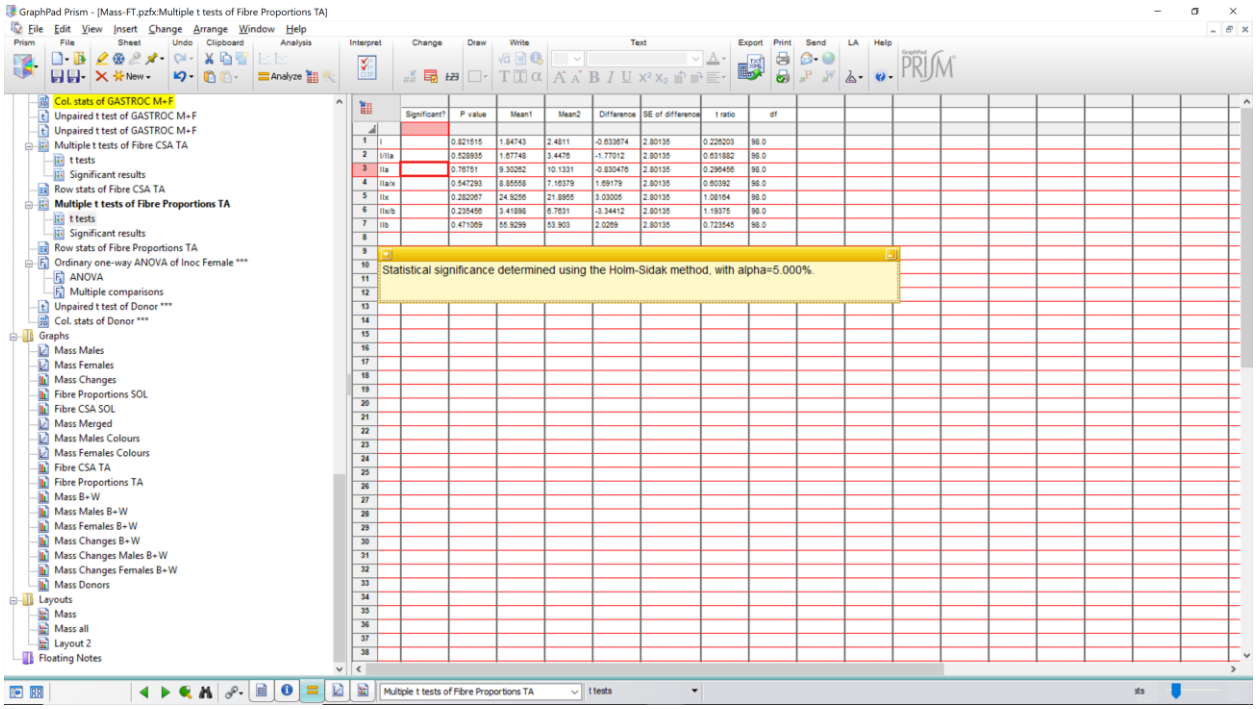
Blind	Subject	Absolute Counts I	I/IIa	IIa	IIa/x	IIx	IIx/IIb	IIb	Total Fibres
	GFEx1	2.67902	0.801078	11.14516	3.79091	11.68026	9.834793	54.32961	94.26083
	GFEx2	1.8306	3.724231	13.93785	6.391653	9.005016	10.71124	56.47649	102.0771
	GFEx3	6.199596	2.601539	5.00455	5.548668	13.783	5.194065	64.15712	102.4885
	GFEx4	2.118294	8.85033	18.46611	7.244097	36.04878	5.996728	42.728	121.4523
	GFEx5	2.24464	5.971851	9.706154	6.79489	26.00513	1.5354	69.13126	121.3893
	GFEx6	0	0	8.315998	9.030087	32.13021	1.088751	51.45944	102.0245
	GFEx7	4.776668	1.537933	8.641128	10.22246	28.38935	15.54016	49.49254	118.6002
	GFEx8	0	4.093862	5.847803	8.287538	18.1224	4.20365	43.44942	84.00467
	AVERAGE								
	GFSed1	0	4.536992	10.44613	11.46996	29.85202	1.989999	51.35359	109.6487
	GFSed2	0.75684	0	15.73175	8.943621	25.10299	8.772621	58.88575	118.1936
	GFSed3	1.9	0.285471	10.49399	10.32738	28.94074	-5.82925	74.32643	120.4448
	GFSed4	4.768471	0.33778	10.53506	4.036186	29.02509	5.956085	49.11212	103.7708
	GFSed5	0	2	7.449667	10.24568	27.03948	0.371947	54.94969	102.0565
	GFSed6	1.430117	3.265	2.177116	14.59456	28.31675	2.433198	42.98254	95.19928
	GFSed7	0	2.994592	7.678121	5.05091	15.71903	4.132552	63.86897	99.44418
	GFSed8	5.924	0	9.909113	6.176328	15.40845	9.524677	51.96002	98.90259
	AVERAGE								

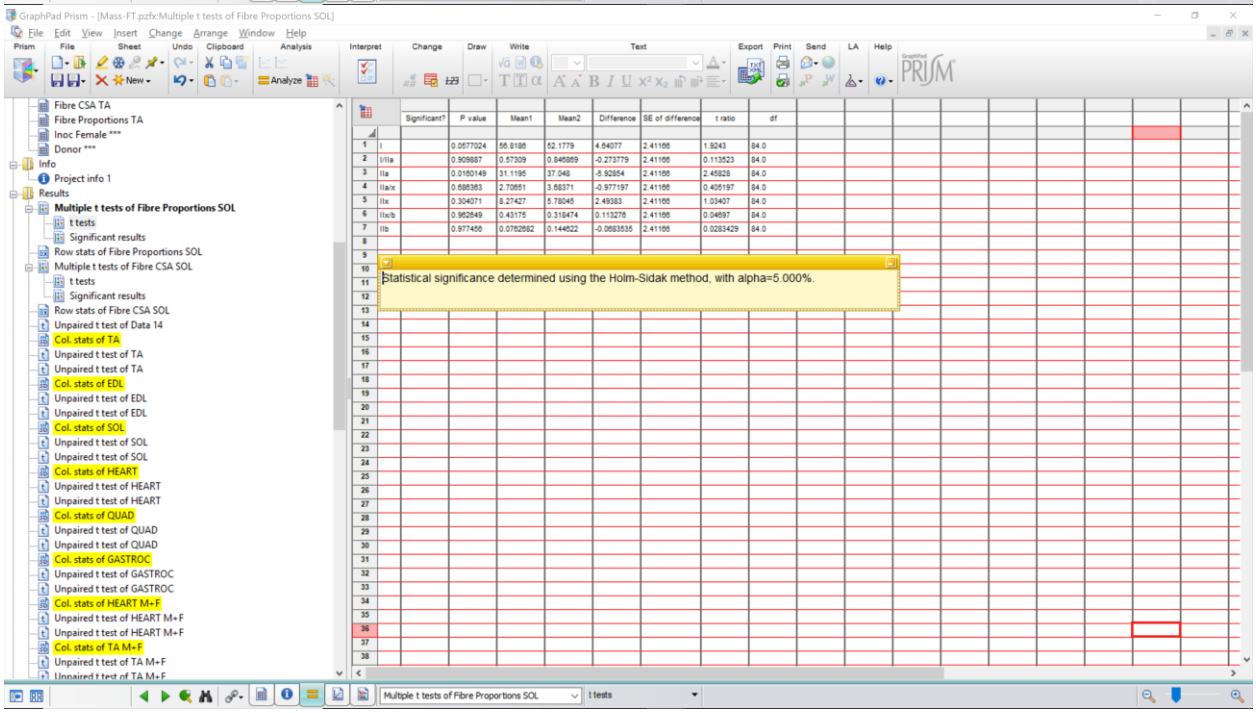
Subject	Relative Counts I	I/IIa	IIa	IIa/x	IIx	IIx/IIb	IIb	CSA AVERAGE I	I/IIa	IIa	IIa/x	IIx	IIx/IIb	IIb
GFEx1	2.842135	0.849852	11.82374	4.021724	12.39142	10.43359	57.63753	624.83	781.44	795.4935	1167.766	1501.44	1917.745	3244.476
GFEx2	1.793351	3.64845	13.65424	6.261595	8.821781	10.49329	55.3273	744.28	520.91	1187.282	1150.44	1656.798	1734.328	3358.037
GFEx3	6.049063	2.538371	4.883034	5.41394	13.44833	5.067947	62.59931	248.97	940.6	634.9272	1154.557	1597.02	1572.877	3018.742
GFEx4	1.744136	7.287081	15.20441	5.964559	29.68142	4.937515	35.18088	409.71	615.15	951.0389	1036.605	1561.244	1805.35	2938.83
GFEx5	1.849125	4.919585	7.995888	5.597601	21.42291	1.264856	56.95003	463.18	807.51	964.5471	907.135	1865.326	1846.281	2540.645
GFEx6	0	0	8.150983	8.850902	31.49265	1.067147	50.43832	682.53	962.38	689.7443	1473.877	1925.182	1630.255	2622.236
GFEx7	4.027537	1.296737	7.285928	8.619258	23.93701	13.10298	41.73056	601.48	507.6	856.3544	1627.29	905.8	1485.266	2980.868
GFEx8	0	4.873374	6.961283	9.865568	21.57309	5.004067	51.72262	306.48	948.8	879.0271	1336.008	1754.389	2169.732	3408.57
AVERAGE	2.288168	3.176681	9.494939	6.824393	20.34608	6.421424	51.44832	510.1825	760.5488	869.8018	1231.71	1595.9	1770.229	3014.051
GFSed1	0	4.137753	9.526908	10.46064	27.22515	1.814886	46.83466	547	608.2	1100.523	1516.765	1439.447	1330.639	2626.398
GFSed2	0.640339	0	13.31016	7.566927	21.23888	7.422249	49.82145	416.81	755.8	825.3456	846.9411	1777.821	1522.77	2800.322
GFSed3	1.577487	0.237014	8.712699	8.57437	24.02823	-4.83977	61.70997	819.76	649	852.8572	1431.481	1569.199	1862.574	3241.243
GFSed4	4.595196	0.325506	10.15224	3.88952	27.97038	5.739655	47.3275	250.16	942.43	888.714	1397.038	1331.699	1974.344	3202.953
GFSed5	0	1.959699	7.299554	10.03923	26.49463	0.364452	53.84244	316.92	928.55	939.836	560.92	1644.658	2592.608	3165.609
GFSed6	1.502235	3.429648	2.286904	15.33054	29.74471	2.5559	45.15007	321.52	966.82	857.4309	1383.547	1299.314	1418.009	3397.352
GFSed7	0	3.01133	7.721036	5.079141	15.80689	4.15565	64.22595	422.12	492.36	853.114	1234.984	1644.97	924.2936	2847.66
GFSed8	5.989732	0	10.01906	6.24486	15.57942	9.630362	52.53656	612.97	898.66	949.2989	1299.475	1324.36	1304.982	2629.02
AVERAGE	1.788124	1.637619	8.62857	8.398153	23.51104	3.355423	52.68108	463.4075	780.2275	908.39	1208.894	1503.934	1616.277	2988.82

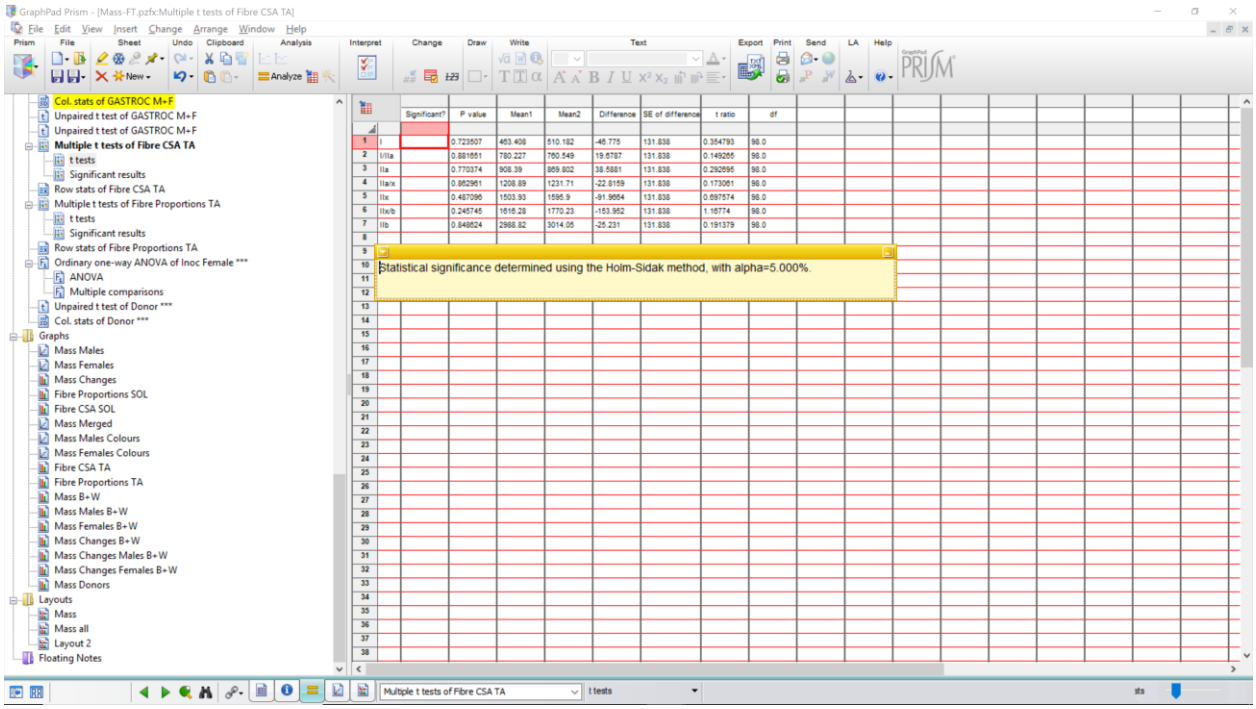


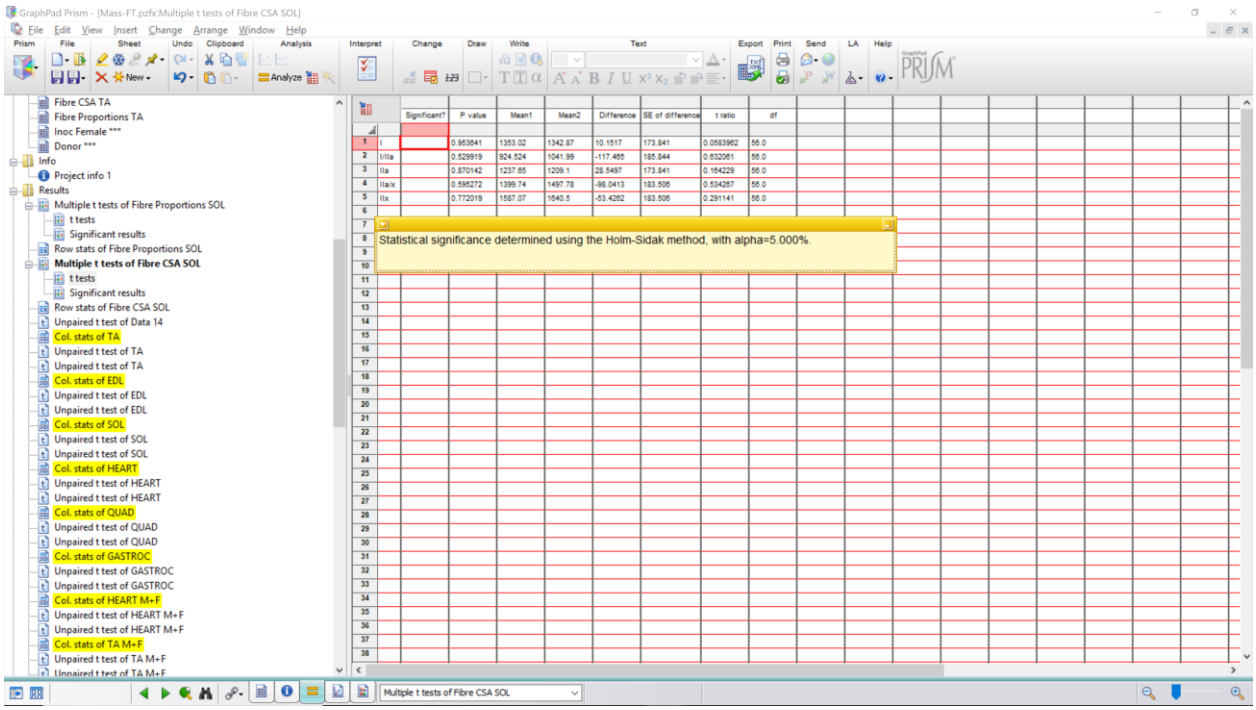
Blind	IIx	IIx/IIb	IIb	Total Fibres	Relative Counts I	I/IIa	IIa	IIa/x	IIx	IIx/IIb	IIb	CSA AVERAGE I	I/IIa	IIa	IIa/x	IIx	IIx/IIb	IIb
31+36	60	2	1	483	37.68116	0.621118	38.9234	9.730849	12.42236	0.414079	0.207039	1176.012	1180.88	1056.53	1325.821	2060.977		
35	3	0	0	334	59.88024	1.197605	34.43114	3.592814	0.898204	0	0	1322.551	802.675	1589.79	1549.791	2078.483		
32	19	0	0	308	60.06494	0	32.79221	0.974026	6.168831	0	0	1034.327		963.9728	917.46	1256.361		
34	44	0	1	380	53.68421	1.315789	29.73684	3.421053	11.57895	0	0.263158	1760.136	1521.224	1265.923	2089.373	2093.279		
29	31	4	0	281	56.58363	1.423488	28.46975	1.067616	11.03203	1.423488	0	1548.843	804.78	1271.935	2072.863	1225.604		
2H	3	0	3	745	53.15436	1.073826	41.87919	3.087248	0.402685	0	0.402685	1585.033	1089.189	1253.802	1513.289	1601.297		
2C	5	5	2	704	46.59091	0.710227	47.72727	3.267045	0.710227	0.710227	0.284091	1084.114	853.188	1051.606	1166.764	1149.992		
2D	7	0	0	231	49.78355	0.4329	42.42424	4.329004	3.030303	0	0	1231.916		1219.276	1346.857	1658.01		
					52.17787	0.846869	37.04801	3.683707	5.780448	0.318474	0.144622	1342.867	1041.989	1209.104	1497.777	1640.5		
33	33	4	1	404	49.50495	0.990099	34.65347	5.445545	8.168317	0.990099	0.247525	1216.114	940.095	1106.752	1618.985	2301.487		
30	35	8	0	556	62.76978	0.899281	28.41727	0.179856	6.294964	1.438849	0	1116.502	866.17	1271.045		1660.606		
2B	73	0	0	701	70.18545	0.142653	16.11983	3.138374	10.41369	0	0	1111.339	851.035	975.3576	1246.169			
2G				0														
2A	48	0	1	476	48.94958	0.420168	39.70588	0.630252	10.08403	0	0.210084	1865.142	1258.085	1683.651	1778.095	1665.075		
2F	36	0	0	588	54.2517	0.340136	34.86395	4.421769	6.122449	0	0	1293.519	648.24	1044.277	933.5778	989.8629		
2I				0														
2E	53	1	0	619	55.2504	0.646204	32.95638	2.423263	8.562197	0.161551	0	1515.493	983.522	1344.842	1421.853	1318.34		
					56.81864	0.57309	31.11946	2.70651	8.274276	0.43175	0.076268	1353.018	924.5245	1237.654	1399.736	1587.074		

MUSCLE COMPOSITION AND CSA: MULTIPLE T-TESTS, HOLM-SIDAK CORRECTION

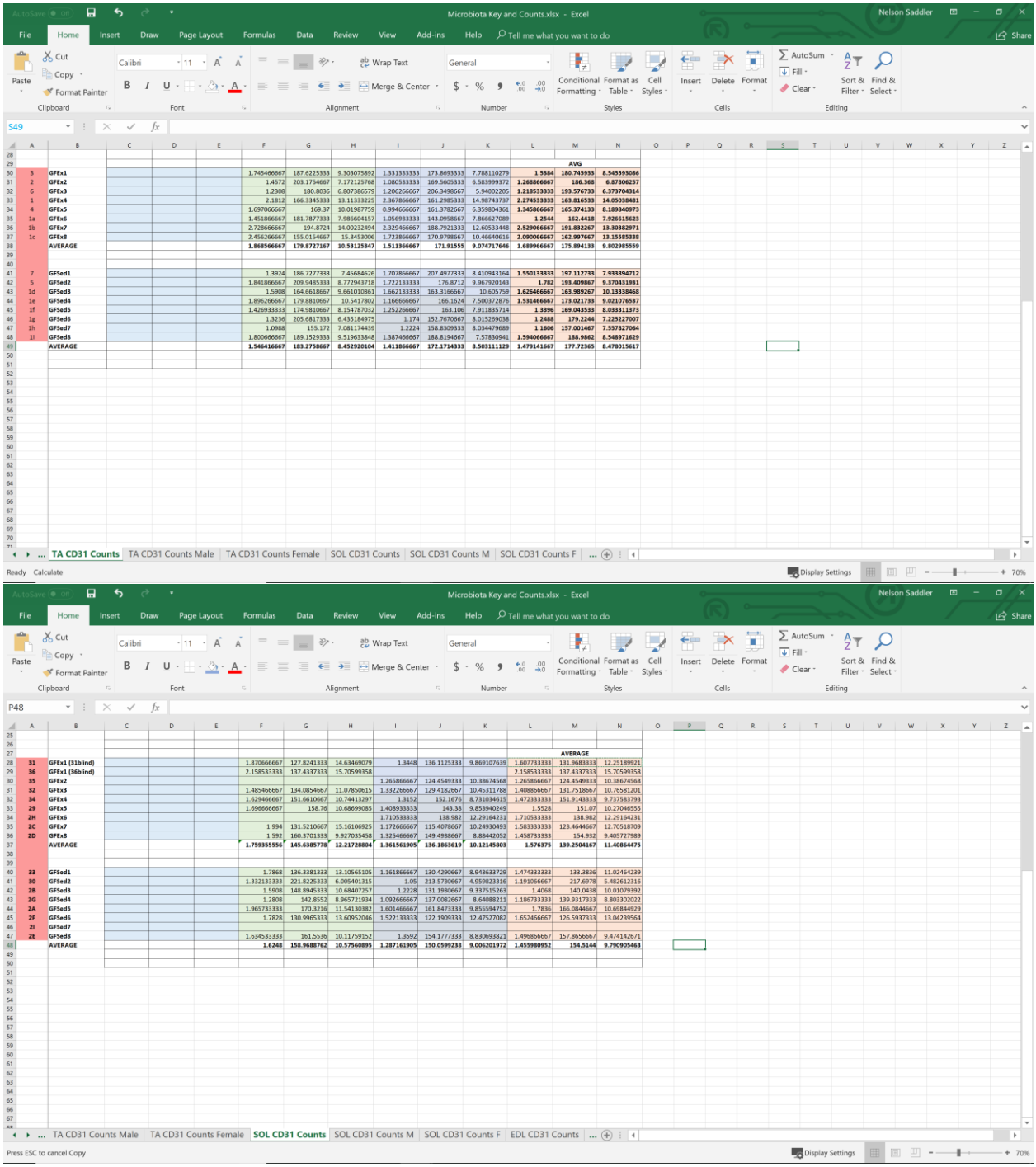





GraphPad Prism - [Mass-FT.pzfx:Multiple t tests of Fibre CSA TA]
Col. stats of GASTROC M+F
Unpaired t test of GASTROC M+F
Unpaired t test of GASTROC M+F
Multiple t tests of Fibre CSA TA
t tests
Significant results
Row stats of Fibre CSA TA
Multiple t tests of Fibre Proportions TA
t tests
Significant results
Row stats of Fibre Proportions TA
Ordinary one-way ANOVA of Inoc Female ***
ANOVA
Multiple comparisons
Unpaired t test of Donor ***
Col. stats of Donor ***
Graphs
Mass Males
Mass Females
Mass Changes
Fibre Proportions SOL
Fibre CSA SOL
Mass Merged
Mass Males Colours
Mass Females Colours
Fibre CSA TA
Fibre Proportions TA
Mass B+W
Mass Males B+W
Mass Females B+W
Mass Changes B+W
Mass Changes Males B+W
Mass Changes Females B+W
Mass Donors
Layouts
Mass
Mass all
Layout 2
Floating Notes
Significant? P value Mean1 Mean2 Difference SE of difference t ratio df
I 0.723507 463.408 510.182 -46.775 131.838 0.354793 98.0
I/IIa 0.881651 780.227 760.549 19.6787 131.838 0.149255 98.0
IIa 0.770374 908.39 869.802 38.5881 131.838 0.292695 98.0
IIa/x 0.962961 1208.89 1231.71 -22.8159 131.838 0.173061 98.0
IIx 0.487096 1503.93 1595.9 -91.9664 131.838 0.697574 98.0
IIx/b 0.245745 1616.28 1770.23 -153.952 131.838 1.16774 98.0
IIb 0.849624 2988.82 3014.05 -25.231 131.838 0.191379 98.0
Statistical significance determined using the Holm-Sidak method, with alpha=5.000%.
Multiple t tests of Fibre CSA TA
t tests


GraphPad Prism - [Mass-FT.pzfx:Multiple t tests of Fibre CSA SOL]
Fibre CSA TA
Fibre Proportions TA
Inoc Female ***
Donor ***
Info
Project info 1
Results
Multiple t tests of Fibre Proportions SOL
t tests
Significant results
Row stats of Fibre Proportions SOL
Multiple t tests of Fibre CSA SOL
t tests
Significant results
Row stats of Fibre CSA SOL
Unpaired t test of Data 14
Col. stats of TA
Unpaired t test of TA
Unpaired t test of TA
Col. stats of EDL
Unpaired t test of EDL
Unpaired t test of EDL
Col. stats of SOL
Unpaired t test of SOL
Unpaired t test of SOL
Col. stats of HEART
Unpaired t test of HEART
Unpaired t test of HEART
Col. stats of QUAD
Unpaired t test of QUAD
Unpaired t test of QUAD
Col. stats of GASTROC
Unpaired t test of GASTROC
Unpaired t test of GASTROC
Col. stats of HEART M+F
Unpaired t test of HEART M+F
Unpaired t test of HEART M+F
Col. stats of TA M+F
Unpaired t test of TA M+F
Significant? P value Mean1 Mean2 Difference SE of difference t ratio df
I 0.953641 1353.02 1342.87 10.1517 173.841 0.0583962 56.0
I/IIa 0.529919 924.524 1041.99 -117.465 185.844 0.632061 56.0
IIa 0.870142 1237.65 1209.1 28.5497 173.841 0.164229 56.0
IIa/x 0.596272 1399.74 1497.78 -98.0413 183.506 0.534267 56.0
IIx 0.772019 1587.07 1640.5 -53.4262 183.506 0.291141 56.0
Statistical significance determined using the Holm-Sidak method, with alpha=5.000%.
Multiple t tests of Fibre CSA SOL

CAPILLARIZATION MEASURES: RAW DATA



												AVG	
3	GFEx1				1.74546667	187.6225333	9.303075892	1.331333333	173.8693333	7.788110279	1.5384	180.745933	8.545593086
2	GFEx2				1.4572	203.1754667	7.172125768	1.080533333	169.5605333	6.583999372	1.268866667	186.368	6.87806257
6	GFEx3				1.2308	180.8036	6.807386579	1.206266667	206.3498667	5.94002205	1.218533333	193.576733	6.373704314
1	GFEx4				2.1812	166.3345333	13.11333225	2.367866667	161.2985333	14.98743737	2.274533333	163.816533	14.05038481
4	GFEx5				1.697066667	169.37	10.01987759	0.994666667	161.3782667	6.359804361	1.345866667	165.374133	8.189840973
1a	GFEx6				1.451866667	181.7877333	7.986604157	1.056933333	143.0958667	7.866627089	1.2544	162.4418	7.926615623
1b	GFEx7				2.728666667	194.8724	14.00232494	2.329466667	188.7921333	12.60533448	2.529066667	191.832267	13.30382971
1c	GFEx8				2.456266667	155.0154667	15.8453006	1.723866667	170.9798667	10.46640616	2.090066667	162.997667	13.15585338
	AVERAGE				**1.868566667**	**179.8727167**	**10.53125347**	**1.511366667**	**171.91555**	**9.074717646**	**1.689966667**	**175.894133**	**9.802985559**
7	GFSed1				1.3924	186.7277333	7.45684626	1.707866667	207.4977333	8.410943164	1.550133333	197.112733	7.933894712
5	GFSed2				1.841866667	209.9485333	8.772943718	1.722133333	176.8712	9.967920143	1.782	193.409867	9.370431931
1d	GFSed3				1.5908	164.6618667	9.661010361	1.662133333	163.3166667	10.605759	1.626466667	163.989267	10.13338468
1e	GFSed4				1.896266667	179.8810667	10.5417802	1.166666667	166.1624	7.500372876	1.531466667	173.021733	9.021076537
1f	GFSed5				1.426933333	174.9810667	8.154787032	1.252266667	163.106	7.911835714	1.3396	169.043533	8.033311373
1g	GFSed6				1.3236	205.6817333	6.435184975	1.174	152.7670667	8.015269038	1.2488	179.2244	7.225227007
1h	GFSed7				1.0988	155.172	7.081174439	1.2224	158.8309333	8.034479689	1.1606	157.001467	7.557827064
1i	GFSed8				1.800666667	189.1529333	9.519633848	1.387466667	188.8194667	7.57830941	1.594066667	188.9862	8.548971629
	AVERAGE				**1.546416667**	**183.2758667**	**8.452920104**	**1.411866667**	**172.1714333**	**8.503111129**	**1.479141667**	**177.72365**	**8.478015617**

												AVERAGE	
31	GFEx1 (31blind)				1.870666667	127.8241333	14.63469079	1.3448	136.1125333	9.869107639	1.607733333	131.9683333	12.25189921
36	GFEx1 (36blind)				2.158533333	137.4337333	15.70599358				2.158533333	137.4337333	15.70599358
35	GFEx2							1.265866667	124.4549333	10.38674568	1.265866667	124.4549333	10.38674568
32	GFEx3				1.485466667	134.0854667	11.07850615	1.332266667	129.4182667	10.45311788	1.408866667	131.7518667	10.76581201
34	GFEx4				1.629466667	151.6610667	10.74413297	1.3152	152.1676	8.731034615	1.472333333	151.9143333	9.737583793
29	GFEx5				1.696666667	158.76	10.68699085	1.408933333	143.38	9.853940249	1.5528	151.07	10.27046555
2H	GFEx6							1.710533333	138.982	12.29164231	1.710533333	138.982	12.29164231
2C	GFEx7				1.994	131.5210667	15.16106925	1.172666667	115.4078667	10.24930493	1.583333333	123.4644667	12.70518709
2D	GFEx8				1.592	160.3701333	9.927035458	1.325466667	149.4938667	8.88442052	1.458733333	154.932	9.405727989
	AVERAGE				**1.759355556**	**145.6385778**	**12.21728804**	**1.361561905**	**136.1863619**	**10.12145803**	**1.576375**	**139.2504167**	**11.40864475**
33	GFSed1				1.7868	136.3381333	13.10565105	1.161866667	130.4290667	8.943633729	1.474333333	133.3836	11.02464239
30	GFSed2				1.332133333	221.8225333	6.005401315	1.05	213.5730667	4.959823316	1.191066667	217.6978	5.482612316
2B	GFSed3				1.5908	148.8945333	10.68407257	1.2228	131.1930667	9.337515263	1.4068	140.0438	10.01079392
2G	GFSed4				1.2808	142.8552	8.965721934	1.092666667	137.0082667	8.64088211	1.186733333	139.9317333	8.803020222
2A	GFSed5				1.965733333	170.3216	11.54130382	1.601466667	161.8473333	9.855594752	1.7836	166.0844667	10.69844929
2F	GFSed6				1.7828	130.9965333	13.60952046	1.522133333	122.1909333	12.47527082	1.652466667	126.5937333	13.04239564
2I	GFSed7												
2E	GFSed8				1.634533333	161.5536	10.11759152	1.3592	154.1777333	8.830693821	1.496866667	157.8656667	9.474142671
	AVERAGE				**1.6248**	**158.9688762**	**10.57560895**	**1.287161905**	**150.0599238**	**9.006201972**	**1.455980952**	**154.5144**	**9.790905463**

CAPILLARIZATION DATA: t-tests

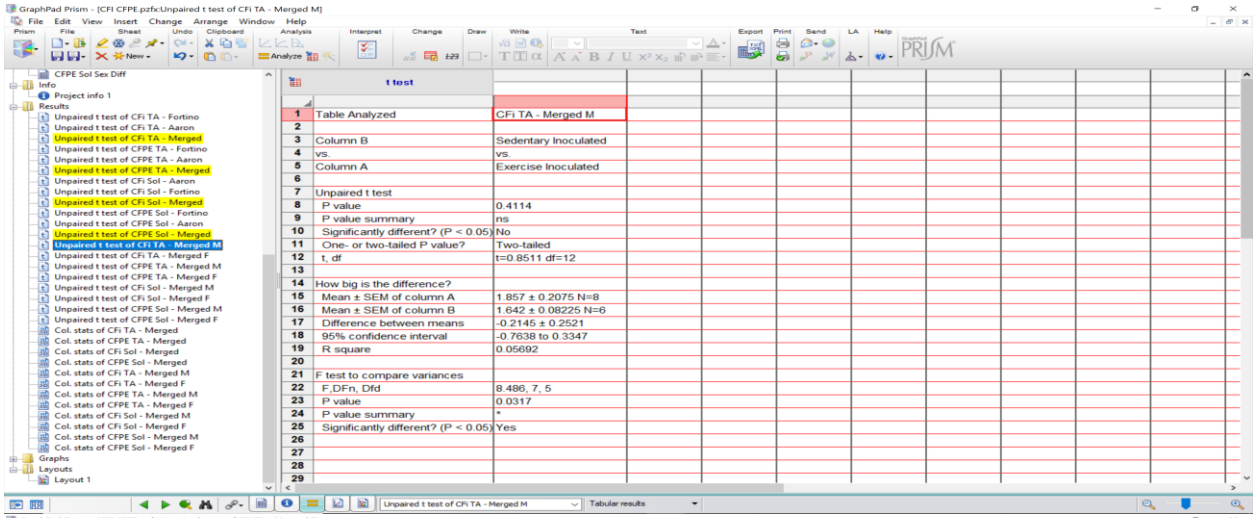
t test	
Table Analyzed	CFi TA - Merged
Column B	Sedentary Inoculated
vs.	vs.
Column A	Exercise Inoculated
Unpaired t test	
P value	0.1845
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.358 df=30
How big is the difference?	
Mean ± SEM of column A	1.690 ± 0.1402 N=16
Mean ± SEM of column B	1.479 ± 0.06652 N=16
Difference between means	-0.2108 ± 0.1552
95% confidence interval	-0.5278 to 0.1062
R square	0.05793
F test to compare variances	
F,DFn, Dfd	4.445, 15, 15
P value	0.0064
P value summary	**
Significantly different? (P < 0.05)	Yes

t test	
Table Analyzed	CFPE TA - Merged
Column B	Sedentary Inoculated
vs.	vs.
Column A	Exercise Inoculated
Unpaired t test	
P value	0.3999
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.8543 df=29
How big is the difference?	
Mean ± SEM of column A	6.021 ± 1.304 N=16
Mean ± SEM of column B	4.627 ± 0.9521 N=15
Difference between means	-1.395 ± 1.633
95% confidence interval	-4.734 to 1.944
R square	0.02455
F test to compare variances	
F,DFn, Dfd	2.000, 15, 14
P value	0.2029
P value summary	ns
Significantly different? (P < 0.05)	No

t test	
Table Analyzed	CFi Sol - Merged
Column B	Sedentary Inoculated
vs.	vs.
Column A	Exercise Inoculated
Unpaired t test	
P value	0.3674
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.9168 df=27
How big is the difference?	
Mean ± SEM of column A	1.554 ± 0.07457 N=15
Mean ± SEM of column B	1.456 ± 0.07577 N=14
Difference between means	-0.09752 ± 0.1064
95% confidence interval	-0.3158 to 0.1207
R square	0.03019
F test to compare variances	
F,DFn, Dfd	1.038, 14, 13
P value	0.9521
P value summary	ns
Significantly different? (P < 0.05)	No

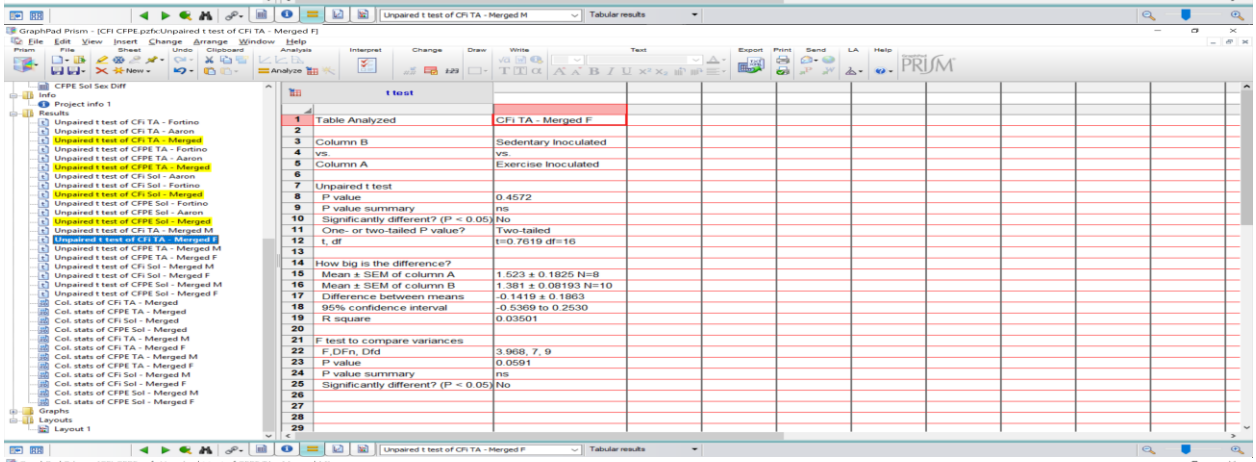
t test	
Table Analyzed	CFPE Sol - Merged
Column B	Sedentary Inoculated
vs.	vs.
Column A	Exercise Inoculated
Unpaired t test	
P value	0.1048
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.679 df=27
How big is the difference?	
Mean ± SEM of column A	11.24 ± 0.5700 N=15
Mean ± SEM of column B	9.791 ± 0.6558 N=14
Difference between means	-1.453 ± 0.8656
95% confidence interval	-3.229 to 0.3231
R square	0.09449
F test to compare variances	
F,DFn, Dfd	1.235, 13, 14
P value	0.6980
P value summary	ns
Significantly different? (P < 0.05)	No

CAPILLARIZATION MEASURES: t-tests – *Sex Differences*



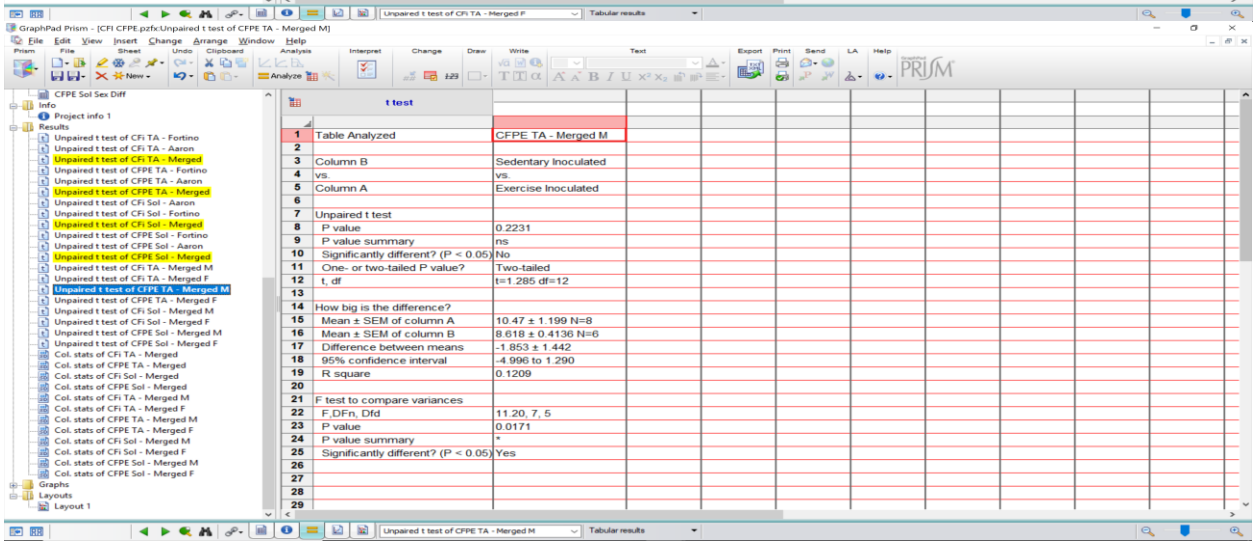
t test

1	Table Analyzed	CFi TA - Merged M
2		
3	Column B	Sedentary Inoculated
4	vs.	vs.
5	Column A	Exercise Inoculated
6		
7	Unpaired t test	
8	P value	0.4114
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.8511 df=12
13		
14	How big is the difference?	
15	Mean ± SEM of column A	1.857 ± 0.2075 N=8
16	Mean ± SEM of column B	1.642 ± 0.08225 N=6
17	Difference between means	0.2145 ± 0.2521
18	95% confidence interval	-0.7638 to 0.3347
19	R square	0.05692
20		
21	F test to compare variances	
22	F,DFn, Dfd	8.486, 7, 5
23	P value	0.0317
24	P value summary	*
25	Significantly different? (P < 0.05)	Yes



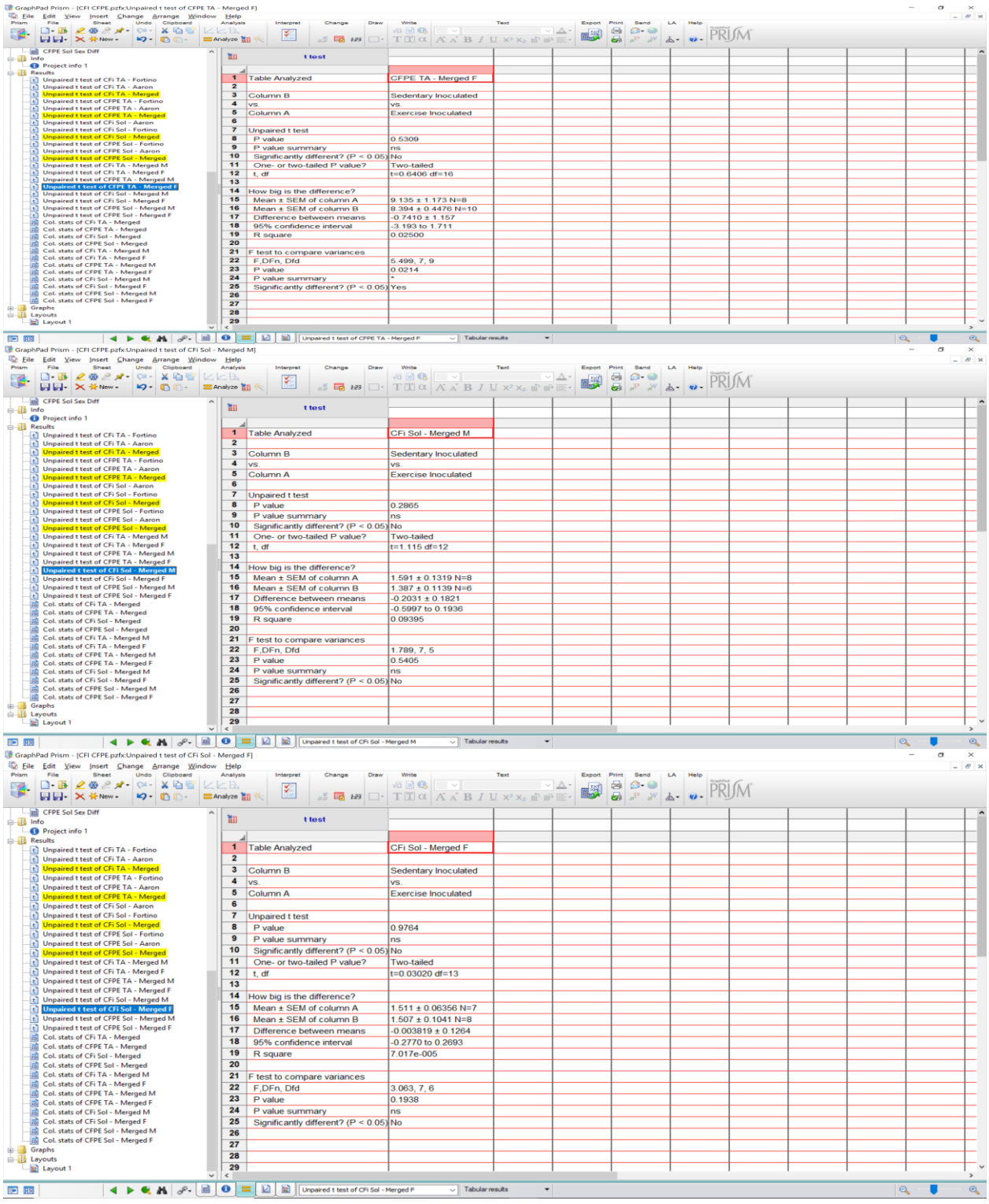
t test

1	Table Analyzed	CFi TA - Merged F
2		
3	Column B	Sedentary Inoculated
4	vs.	vs.
5	Column A	Exercise Inoculated
6		
7	Unpaired t test	
8	P value	0.4572
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.7619 df=16
13		
14	How big is the difference?	
15	Mean ± SEM of column A	1.523 ± 0.1825 N=8
16	Mean ± SEM of column B	1.381 ± 0.08193 N=10
17	Difference between means	0.1419 ± 0.1863
18	95% confidence interval	-0.5369 to 0.2530
19	R square	0.03501
20		
21	F test to compare variances	
22	F,DFn, Dfd	3.968, 7, 9
23	P value	0.0591
24	P value summary	ns
25	Significantly different? (P < 0.05)	No



t test

1	Table Analyzed	CFPE TA - Merged M
2		
3	Column B	Sedentary Inoculated
4	vs.	vs.
5	Column A	Exercise Inoculated
6		
7	Unpaired t test	
8	P value	0.2231
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=1.285 df=12
13		
14	How big is the difference?	
15	Mean ± SEM of column A	10.47 ± 1.199 N=8
16	Mean ± SEM of column B	8.618 ± 0.4136 N=6
17	Difference between means	1.853 ± 1.442
18	95% confidence interval	-4.996 to 1.290
19	R square	0.1209
20		
21	F test to compare variances	
22	F,DFn, Dfd	11.20, 7, 5
23	P value	0.0171
24	P value summary	*
25	Significantly different? (P < 0.05)	Yes



t test

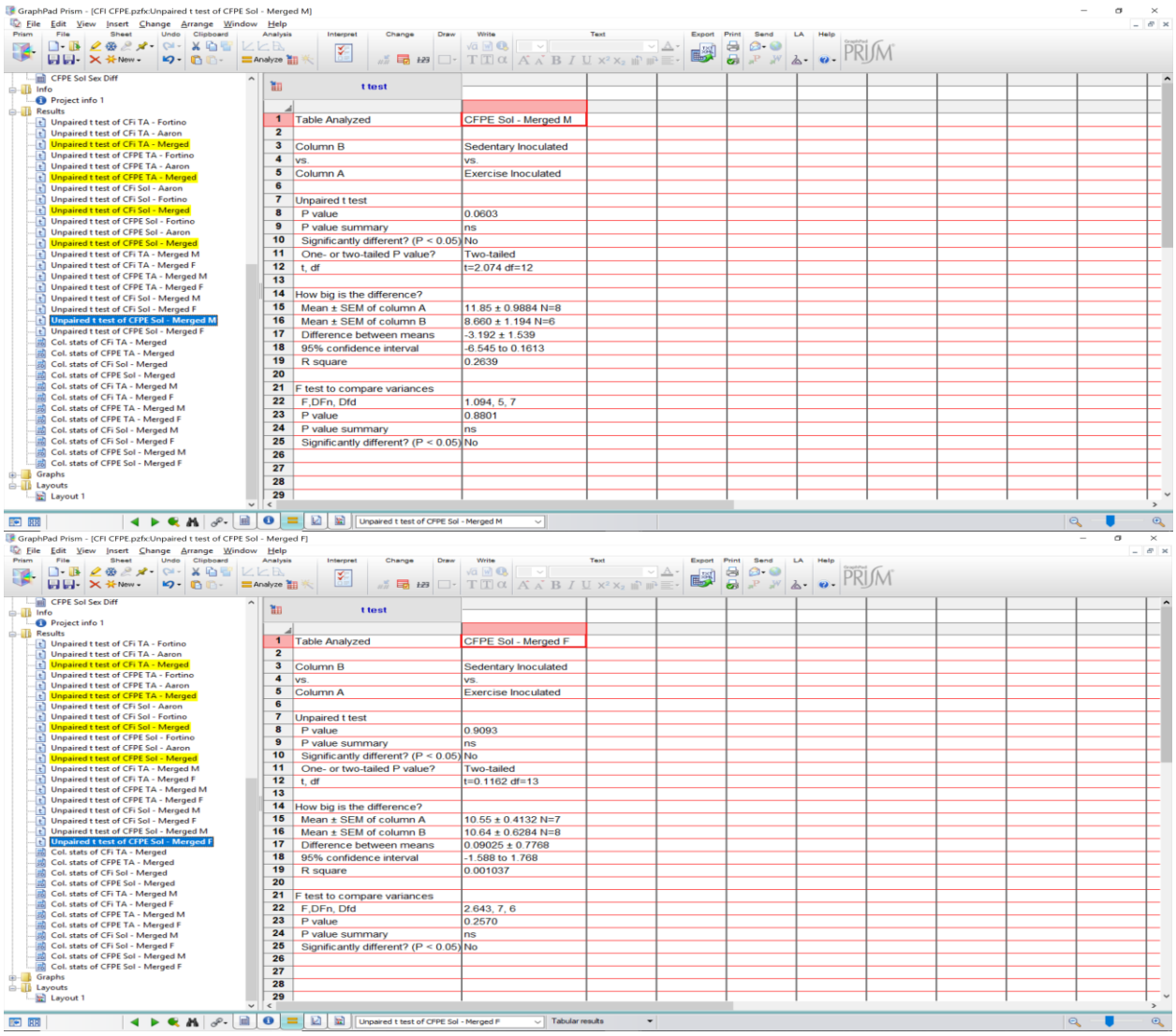
1	Table Analyzed	CFPE TA - Merged F
2		
3	Column B	Sedentary Inoculated
4	vs.	vs.
5	Column A	Exercise Inoculated
6		
7	Unpaired t test	
8	P value	0.5309
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.6406 df=16
13		
14	How big is the difference?	
15	Mean ± SEM of column A	9.135 ± 1.173 N=8
16	Mean ± SEM of column B	8.394 ± 0.4476 N=10
17	Difference between means	-0.7410 ± 1.157
18	95% confidence interval	-3.193 to 1.711
19	R square	0.02500
20		
21	F test to compare variances	
22	F, DFn, Dfd	5.499, 7, 9
23	P value	0.0214
24	P value summary	*
25	Significantly different? (P < 0.05)	Yes

t test

1	Table Analyzed	CFi Sol - Merged M
2		
3	Column B	Sedentary Inoculated
4	vs.	vs.
5	Column A	Exercise Inoculated
6		
7	Unpaired t test	
8	P value	0.2865
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=1.115 df=12
13		
14	How big is the difference?	
15	Mean ± SEM of column A	1.591 ± 0.1319 N=8
16	Mean ± SEM of column B	1.387 ± 0.1139 N=6
17	Difference between means	-0.2031 ± 0.1821
18	95% confidence interval	-0.5997 to 0.1936
19	R square	0.09395
20		
21	F test to compare variances	
22	F, DFn, Dfd	1.789, 7, 5
23	P value	0.5405
24	P value summary	ns
25	Significantly different? (P < 0.05)	No

t test

1	Table Analyzed	CFi Sol - Merged F
2		
3	Column B	Sedentary Inoculated
4	vs.	vs.
5	Column A	Exercise Inoculated
6		
7	Unpaired t test	
8	P value	0.9764
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.03020 df=13
13		
14	How big is the difference?	
15	Mean ± SEM of column A	1.511 ± 0.06356 N=7
16	Mean ± SEM of column B	1.507 ± 0.1041 N=8
17	Difference between means	-0.003819 ± 0.1264
18	95% confidence interval	-0.2770 to 0.2693
19	R square	7.017e-005
20		
21	F test to compare variances	
22	F, DFn, Dfd	3.063, 7, 6
23	P value	0.1938
24	P value summary	ns
25	Significantly different? (P < 0.05)	No



GraphPad Prism - [CFI CFPE.pzfx:Unpaired t test of CFPE Sol - Merged M]

t test

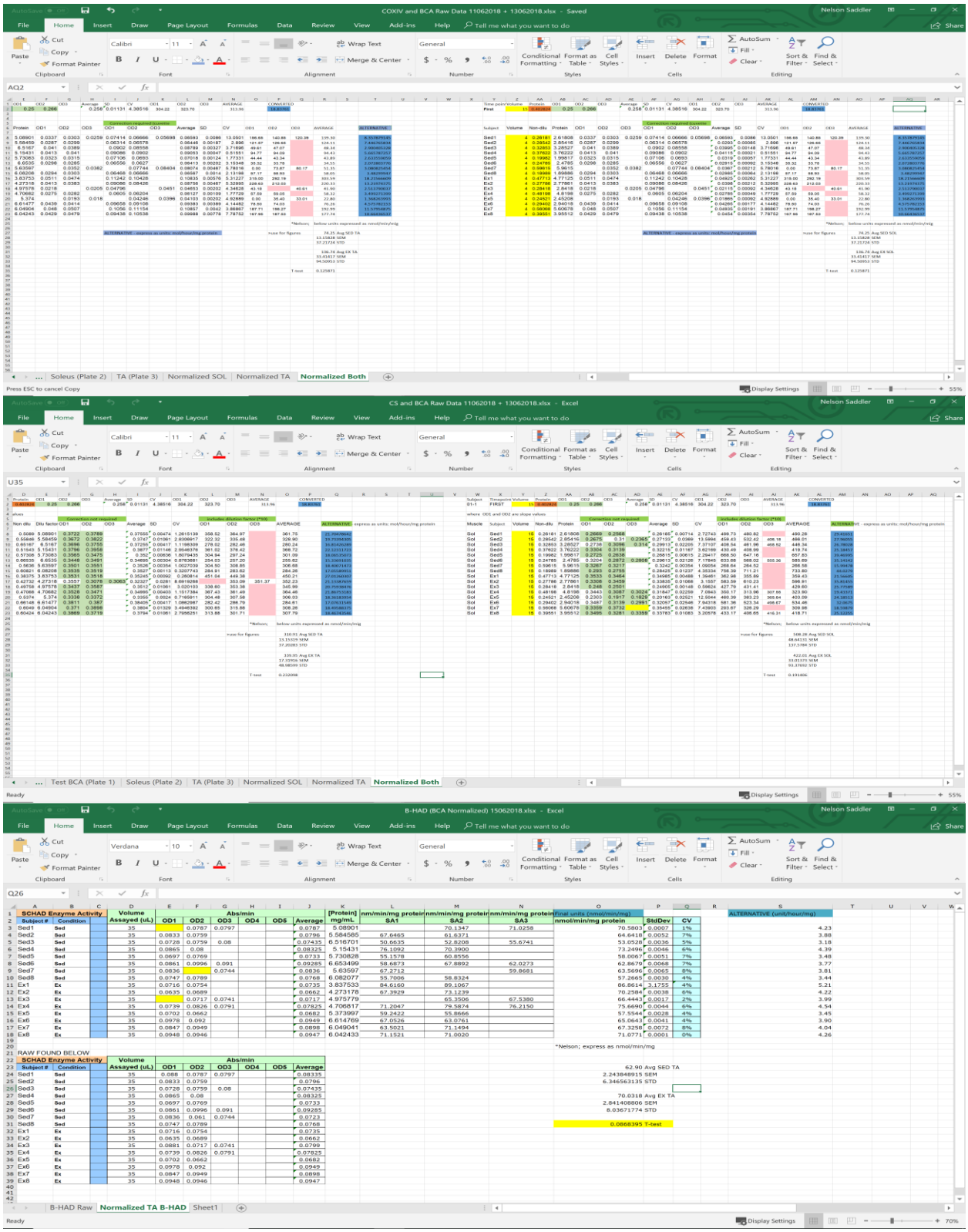
1	Table Analyzed	CFPE Sol - Merged M
2		
3	Column B	Sedentary Inoculated
4	vs.	vs.
5	Column A	Exercise Inoculated
6		
7	Unpaired t test	
8	P value	0.0603
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=2.074 df=12
13		
14	How big is the difference?	
15	Mean ± SEM of column A	11.85 ± 0.9884 N=8
16	Mean ± SEM of column B	8.660 ± 1.194 N=6
17	Difference between means	-3.192 ± 1.539
18	95% confidence interval	-6.545 to 0.1613
19	R square	0.2639
20		
21	F test to compare variances	
22	F,DFn, Dfd	1.094, 5, 7
23	P value	0.8801
24	P value summary	ns
25	Significantly different? (P < 0.05)	No

GraphPad Prism - [CFI CFPE.pzfx:Unpaired t test of CFPE Sol - Merged F]

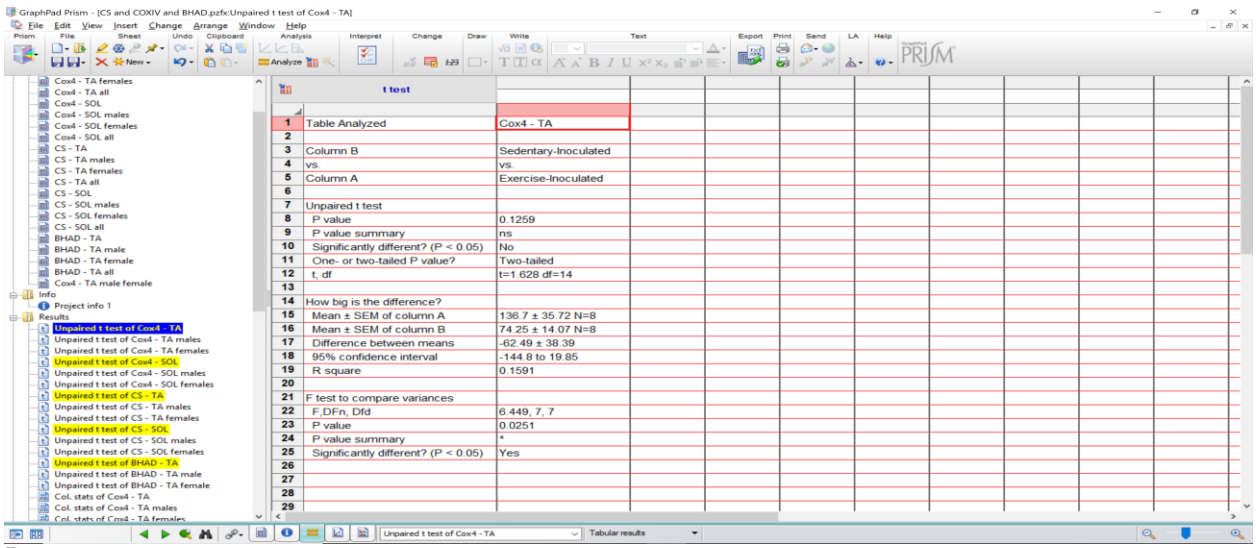
t test

1	Table Analyzed	CFPE Sol - Merged F
2		
3	Column B	Sedentary Inoculated
4	vs.	vs.
5	Column A	Exercise Inoculated
6		
7	Unpaired t test	
8	P value	0.9093
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.1162 df=13
13		
14	How big is the difference?	
15	Mean ± SEM of column A	10.55 ± 0.4132 N=7
16	Mean ± SEM of column B	10.64 ± 0.6284 N=8
17	Difference between means	0.09025 ± 0.7768
18	95% confidence interval	-1.588 to 1.768
19	R square	0.001037
20		
21	F test to compare variances	
22	F,DFn, Dfd	2.643, 7, 6
23	P value	0.2570
24	P value summary	ns
25	Significantly different? (P < 0.05)	No

MITOCHONDRIAL MEASURES: RAW DATA

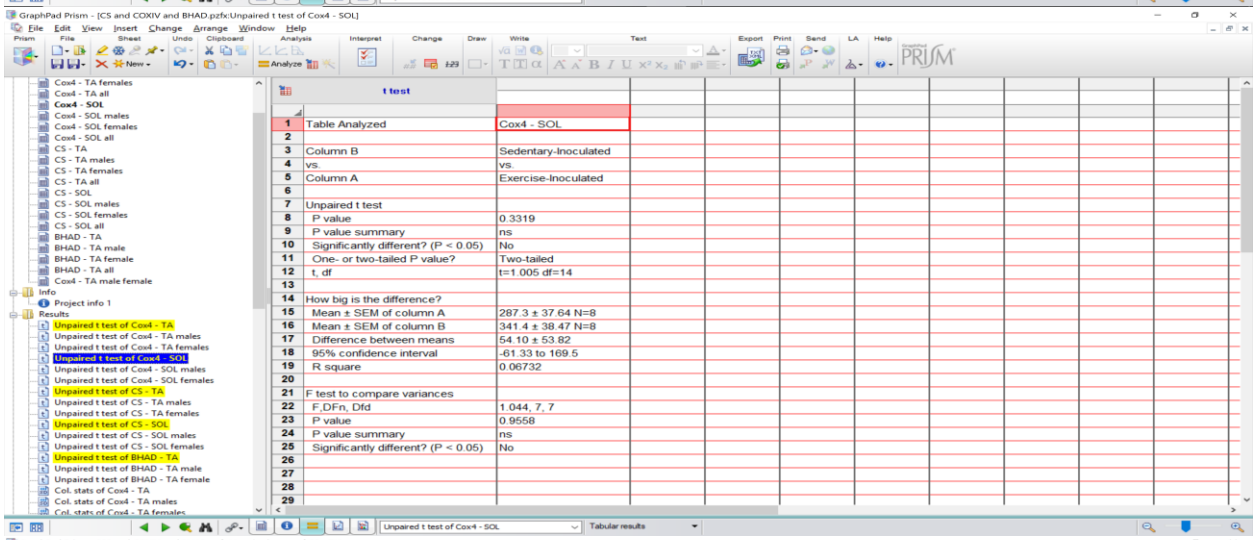


MITOCHONDRIAL MEASURES: t-tests



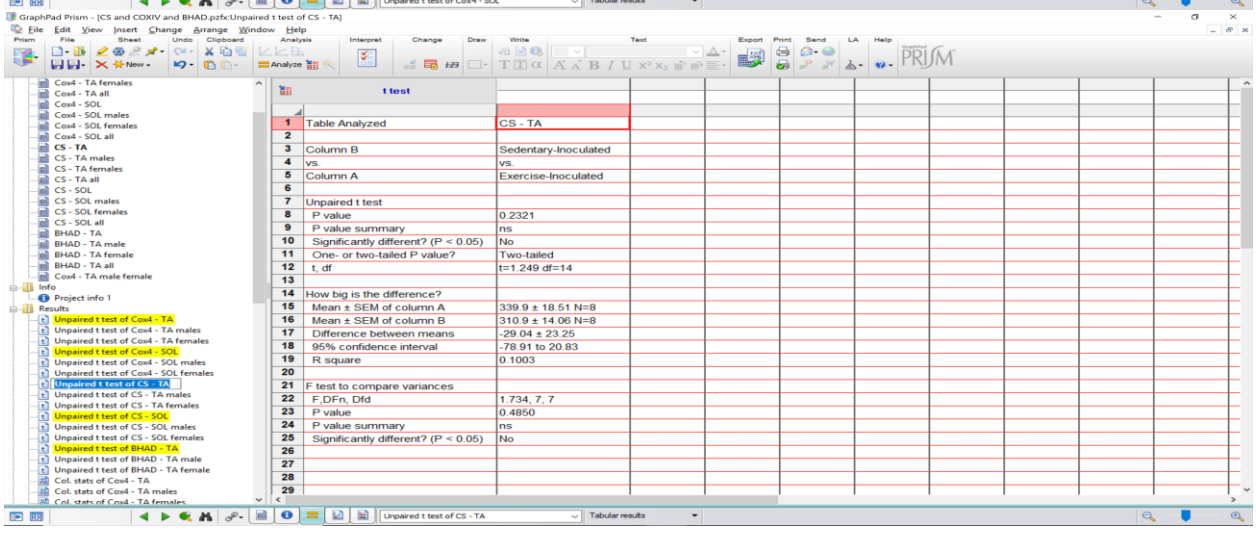
t test

1	Table Analyzed	Cox4 - TA
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.1259
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=1.628 df=14
13		
14	How big is the difference?	
15	Mean ± SEM of column A	136.7 ± 35.72 N=8
16	Mean ± SEM of column B	74.25 ± 14.07 N=8
17	Difference between means	-62.49 ± 38.39
18	95% confidence interval	-144.8 to 19.85
19	R square	0.1591
20		
21	F test to compare variances	
22	F,DFn, Dfd	6.449, 7, 7
23	P value	0.0251
24	P value summary	*
25	Significantly different? (P < 0.05)	Yes
26		
27		
28		
29		



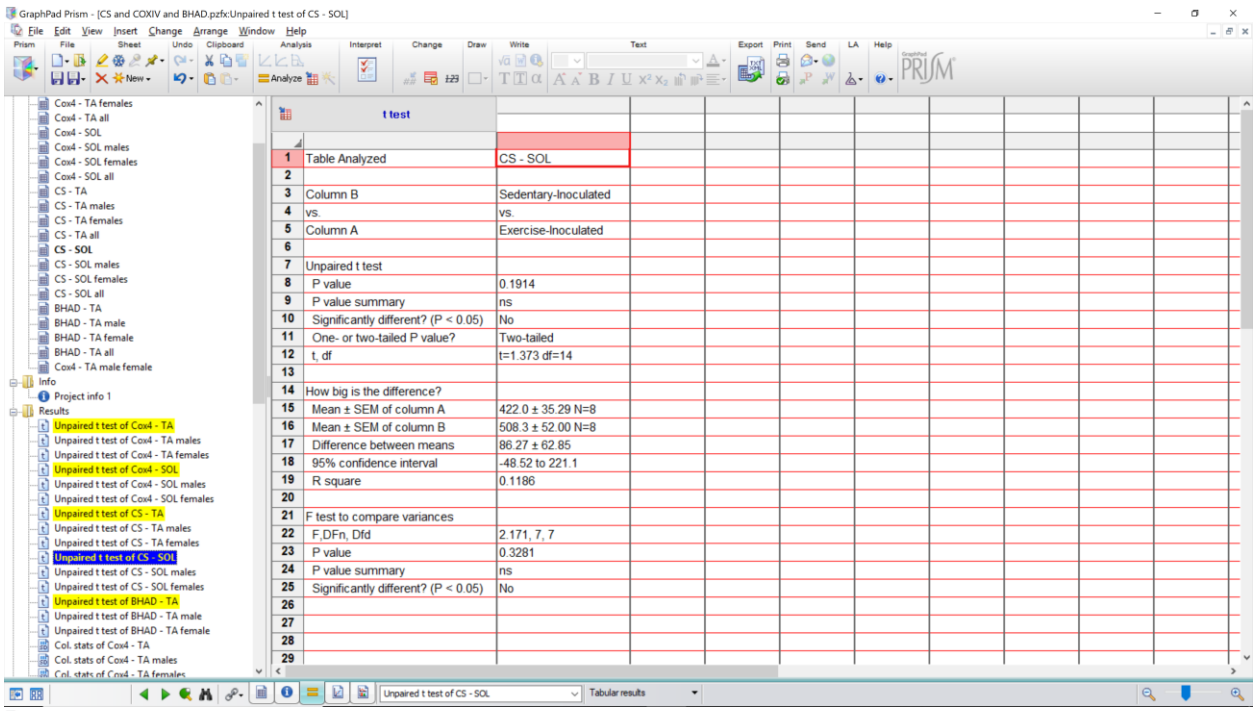
t test

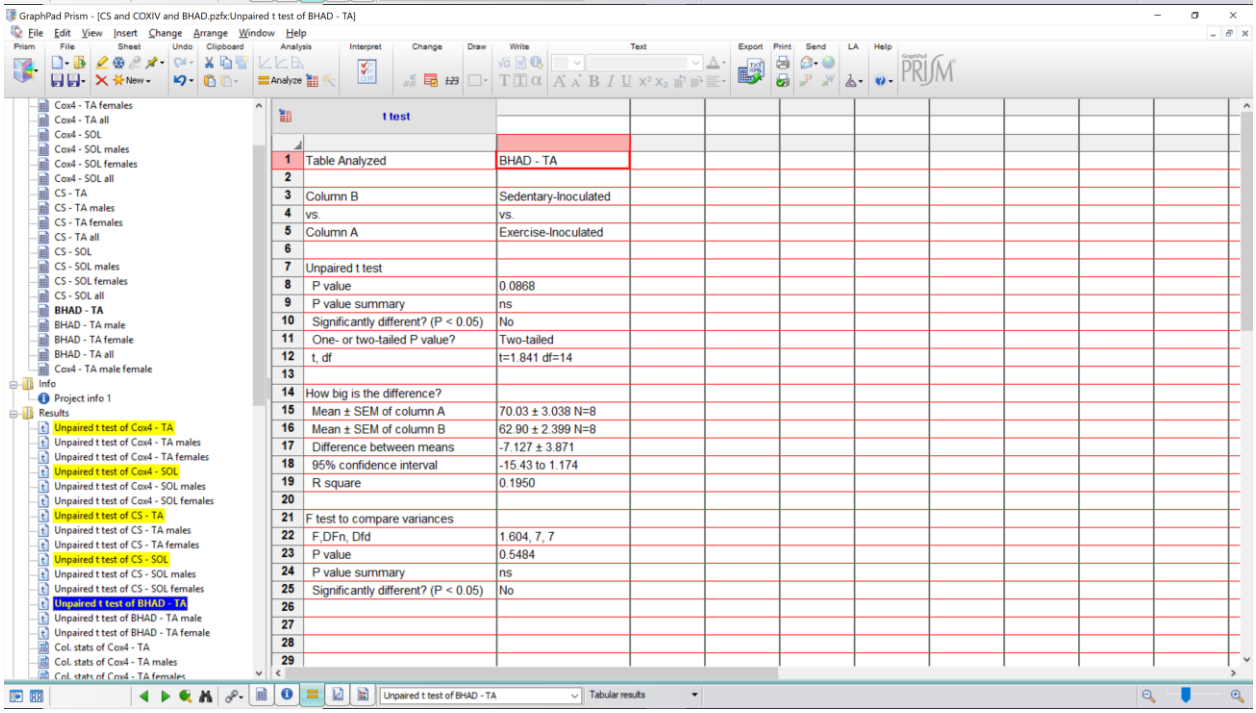
1	Table Analyzed	Cox4 - SOL
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.3319
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=1.005 df=14
13		
14	How big is the difference?	
15	Mean ± SEM of column A	287.3 ± 37.64 N=8
16	Mean ± SEM of column B	341.4 ± 38.47 N=8
17	Difference between means	54.10 ± 53.82
18	95% confidence interval	-61.33 to 169.5
19	R square	0.06732
20		
21	F test to compare variances	
22	F,DFn, Dfd	1.044, 7, 7
23	P value	0.9558
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
28		
29		



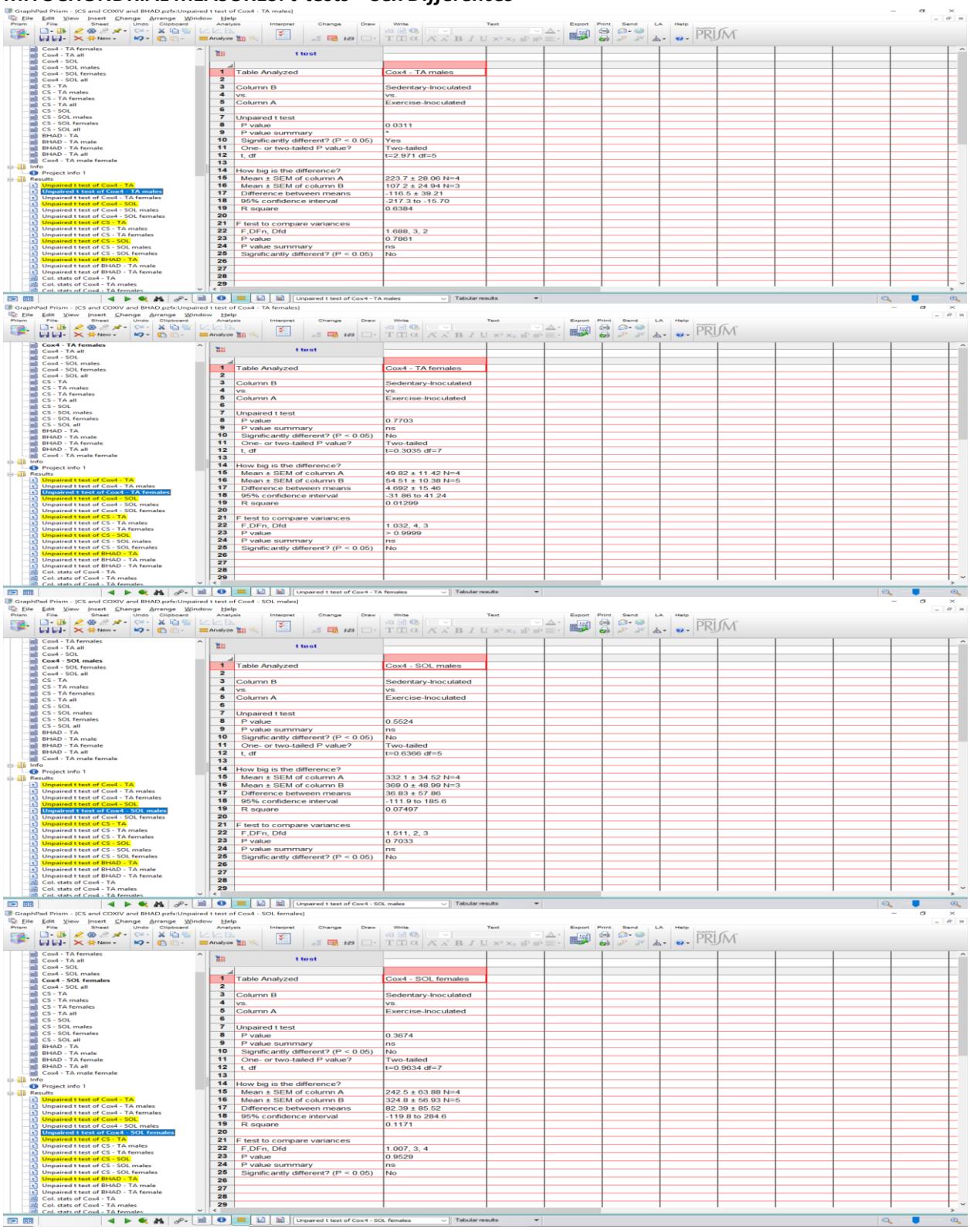
t test

1	Table Analyzed	CS - TA
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.2321
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=1.249 df=14
13		
14	How big is the difference?	
15	Mean ± SEM of column A	339.9 ± 18.51 N=8
16	Mean ± SEM of column B	310.9 ± 14.06 N=8
17	Difference between means	-29.04 ± 23.25
18	95% confidence interval	-78.91 to 20.83
19	R square	0.1003
20		
21	F test to compare variances	
22	F,DFn, Dfd	1.734, 7, 7
23	P value	0.4850
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
28		
29		


GraphPad Prism - [CS and COXIV and BHAD.pzfx:Unpaired t test of CS - SOL]
File Edit View Insert Change Arrange Window Help
Cox4 - TA females
Cox4 - TA all
Cox4 - SOL
Cox4 - SOL males
Cox4 - SOL females
Cox4 - SOL all
CS - TA
CS - TA males
CS - TA females
CS - TA all
CS - SOL
CS - SOL males
CS - SOL females
CS - SOL all
BHAD - TA
BHAD - TA male
BHAD - TA female
BHAD - TA all
Cox4 - TA male female
Info
Project info 1
Results
Unpaired t test of Cox4 - TA
Unpaired t test of Cox4 - TA males
Unpaired t test of Cox4 - TA females
Unpaired t test of Cox4 - SOL
Unpaired t test of Cox4 - SOL males
Unpaired t test of Cox4 - SOL females
Unpaired t test of CS - TA
Unpaired t test of CS - TA males
Unpaired t test of CS - TA females
Unpaired t test of CS - SOL
Unpaired t test of CS - SOL males
Unpaired t test of CS - SOL females
Unpaired t test of BHAD - TA
Unpaired t test of BHAD - TA male
Unpaired t test of BHAD - TA female
Col. stats of Cox4 - TA
Col. stats of Cox4 - TA males
t test
1 Table Analyzed CS - SOL
3 Column B Sedentary-Inoculated
4 vs. vs.
5 Column A Exercise-Inoculated
7 Unpaired t test
8 P value 0.1914
9 P value summary ns
10 Significantly different? (P < 0.05) No
11 One- or two-tailed P value? Two-tailed
12 t, df t=1.373 df=14
14 How big is the difference?
15 Mean ± SEM of column A 422.0 ± 35.29 N=8
16 Mean ± SEM of column B 508.3 ± 52.00 N=8
17 Difference between means 86.27 ± 62.85
18 95% confidence interval -48.52 to 221.1
19 R square 0.1186
21 F test to compare variances
22 F, DFn, Dfd 2.171, 7, 7
23 P value 0.3281
24 P value summary ns
25 Significantly different? (P < 0.05) No
Unpaired t test of CS - SOL
Tabular results


GraphPad Prism - [CS and COXIV and BHAD.pzfx:Unpaired t test of BHAD - TA]
File Edit View Insert Change Arrange Window Help
Cox4 - TA females
Cox4 - TA all
Cox4 - SOL
Cox4 - SOL males
Cox4 - SOL females
Cox4 - SOL all
CS - TA
CS - TA males
CS - TA females
CS - TA all
CS - SOL
CS - SOL males
CS - SOL females
CS - SOL all
BHAD - TA
BHAD - TA male
BHAD - TA female
BHAD - TA all
Cox4 - TA male female
Info
Project info 1
Results
Unpaired t test of Cox4 - TA
Unpaired t test of Cox4 - TA males
Unpaired t test of Cox4 - TA females
Unpaired t test of Cox4 - SOL
Unpaired t test of Cox4 - SOL males
Unpaired t test of Cox4 - SOL females
Unpaired t test of CS - TA
Unpaired t test of CS - TA males
Unpaired t test of CS - TA females
Unpaired t test of CS - SOL
Unpaired t test of CS - SOL males
Unpaired t test of CS - SOL females
Unpaired t test of BHAD - TA
Unpaired t test of BHAD - TA male
Unpaired t test of BHAD - TA female
Col. stats of Cox4 - TA
Col. stats of Cox4 - TA males
t test
1 Table Analyzed BHAD - TA
3 Column B Sedentary-Inoculated
4 vs. vs.
5 Column A Exercise-Inoculated
7 Unpaired t test
8 P value 0.0868
9 P value summary ns
10 Significantly different? (P < 0.05) No
11 One- or two-tailed P value? Two-tailed
12 t, df t=1.841 df=14
14 How big is the difference?
15 Mean ± SEM of column A 70.03 ± 3.038 N=8
16 Mean ± SEM of column B 62.90 ± 2.399 N=8
17 Difference between means -7.127 ± 3.871
18 95% confidence interval -15.43 to 1.174
19 R square 0.1950
21 F test to compare variances
22 F, DFn, Dfd 1.604, 7, 7
23 P value 0.5484
24 P value summary ns
25 Significantly different? (P < 0.05) No
Unpaired t test of BHAD - TA
Tabular results

MITOCHONDRIAL MEASURES: t-tests – *Sex Differences*



t test

1	Table Analyzed	Cox4 - TA males
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.0311
9	P value summary	*
10	Significantly different? (P < 0.05)	Yes
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=2.971 df=5
13		
14	How big is the difference?	
15	Mean ± SEM of column A	223.7 ± 28.06 N=4
16	Mean ± SEM of column B	107.2 ± 24.94 N=3
17	Difference between means	-116.5 ± 39.21
18	95% confidence interval	-217.3 to -15.70
19	R square	0.6384
20		
21	F test to compare variances	
22	F, DFn, Dfd	1.688, 3, 2
23	P value	0.7861
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
28		
29		

t test

1	Table Analyzed	Cox4 - TA females
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.7703
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.3035 df=7
13		
14	How big is the difference?	
15	Mean ± SEM of column A	49.82 ± 11.42 N=4
16	Mean ± SEM of column B	54.51 ± 10.38 N=5
17	Difference between means	4.692 ± 15.46
18	95% confidence interval	-31.86 to 41.24
19	R square	0.01299
20		
21	F test to compare variances	
22	F, DFn, Dfd	1.032, 4, 3
23	P value	> 0.9999
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
28		
29		

t test

1	Table Analyzed	Cox4 - SOL males
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.5524
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.6366 df=5
13		
14	How big is the difference?	
15	Mean ± SEM of column A	332.1 ± 34.52 N=4
16	Mean ± SEM of column B	369.0 ± 48.99 N=3
17	Difference between means	36.83 ± 57.86
18	95% confidence interval	-111.9 to 185.6
19	R square	0.07497
20		
21	F test to compare variances	
22	F, DFn, Dfd	1.511, 2, 3
23	P value	0.7033
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
28		
29		

t test

1	Table Analyzed	Cox4 - SOL females
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.3674
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.9634 df=7
13		
14	How big is the difference?	
15	Mean ± SEM of column A	242.5 ± 63.88 N=4
16	Mean ± SEM of column B	324.8 ± 56.93 N=5
17	Difference between means	82.39 ± 85.52
18	95% confidence interval	-119.8 to 284.6
19	R square	0.1171
20		
21	F test to compare variances	
22	F, DFn, Dfd	1.007, 3, 4
23	P value	0.9529
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
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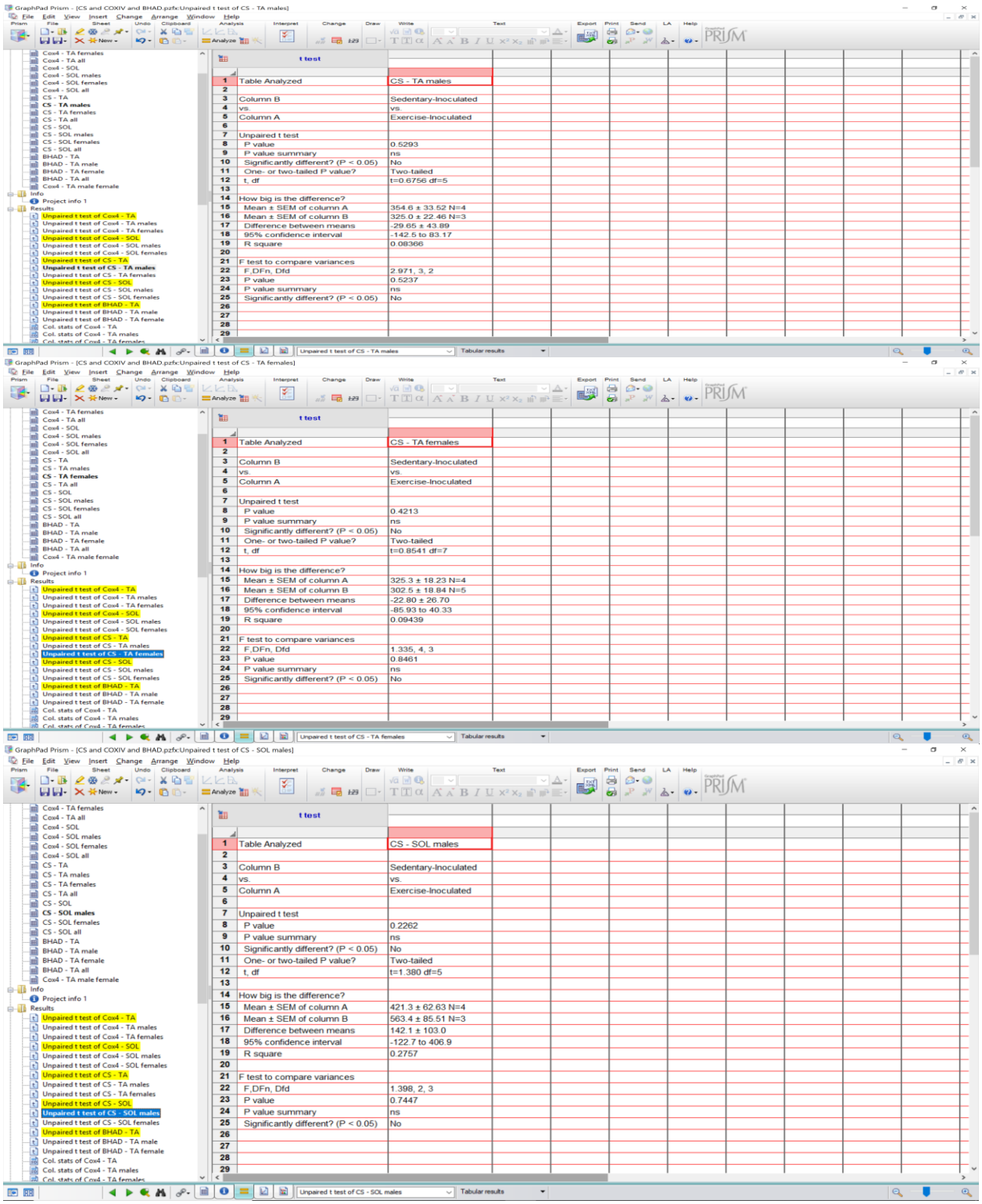
29		



t test

1	Table Analyzed	CS - TA males
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.5293
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.6756 df=5
13		
14	How big is the difference?	
15	Mean ± SEM of column A	354.6 ± 33.52 N=4
16	Mean ± SEM of column B	325.0 ± 22.46 N=3
17	Difference between means	-29.65 ± 43.89
18	95% confidence interval	-142.5 to 83.17
19	R square	0.08366
20		
21	F test to compare variances	
22	F, DFn, Dfd	2.971, 3, 2
23	P value	0.5237
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
28		
29		

t test

1	Table Analyzed	CS - TA females
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.4213
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.8541 df=7
13		
14	How big is the difference?	
15	Mean ± SEM of column A	325.3 ± 18.23 N=4
16	Mean ± SEM of column B	302.5 ± 18.84 N=5
17	Difference between means	-22.80 ± 26.70
18	95% confidence interval	-85.93 to 40.33
19	R square	0.09439
20		
21	F test to compare variances	
22	F, DFn, Dfd	1.335, 4, 3
23	P value	0.8461
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
28		
29		

t test

1	Table Analyzed	CS - SOL males
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.2262
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=1.380 df=5
13		
14	How big is the difference?	
15	Mean ± SEM of column A	421.3 ± 62.63 N=4
16	Mean ± SEM of column B	563.4 ± 85.51 N=3
17	Difference between means	142.1 ± 103.0
18	95% confidence interval	-122.7 to 406.9
19	R square	0.2757
20		
21	F test to compare variances	
22	F, DFn, Dfd	1.398, 2, 3
23	P value	0.7447
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
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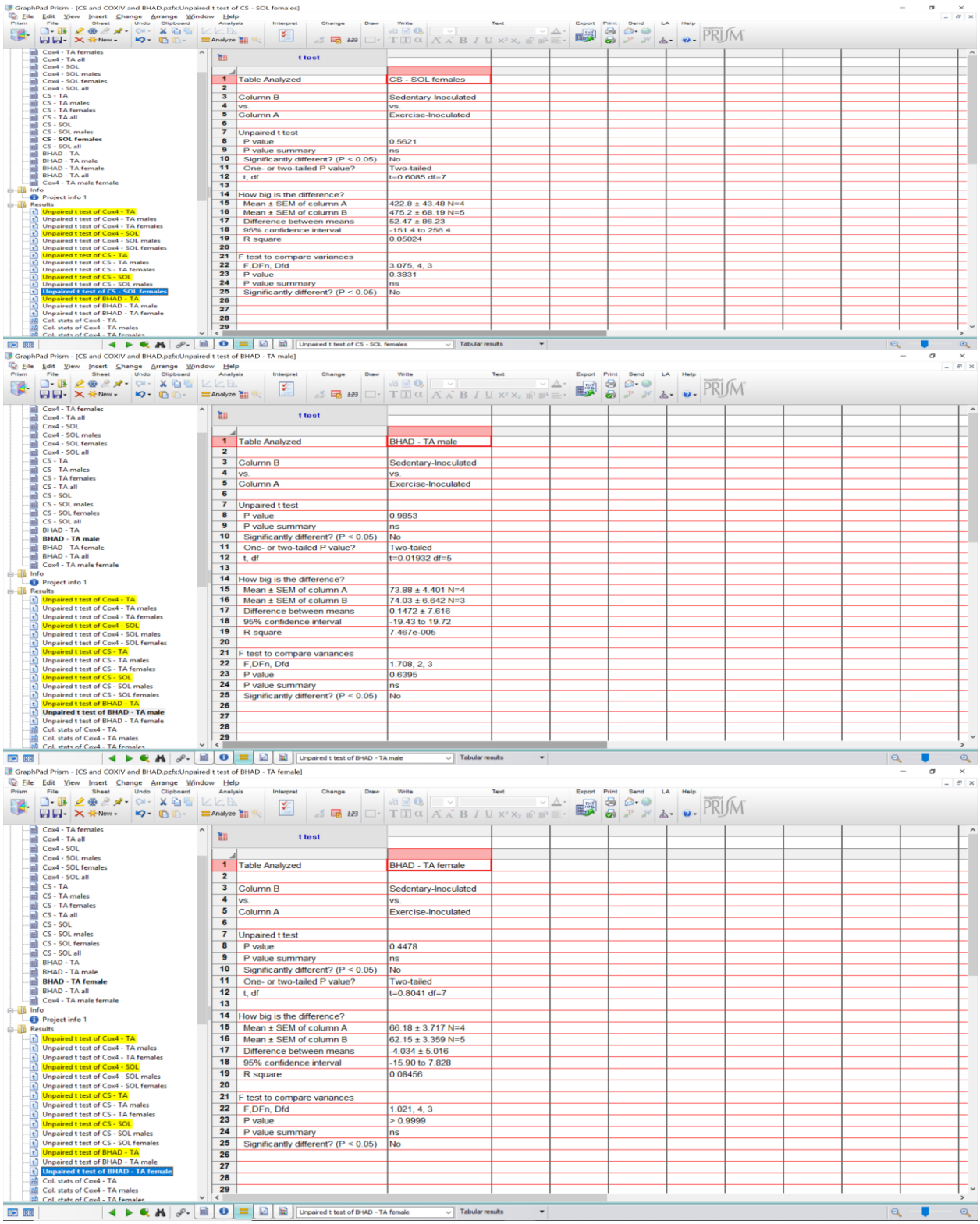

t test

1	Table Analyzed	CS - SOL females
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.5621
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.6085 df=7
13		
14	How big is the difference?	
15	Mean ± SEM of column A	422.8 ± 43.48 N=4
16	Mean ± SEM of column B	475.2 ± 68.19 N=5
17	Difference between means	52.47 ± 86.23
18	95% confidence interval	-151.4 to 256.4
19	R square	0.05024
20		
21	F test to compare variances	
22	F,DFn, Dfd	3.075, 4, 3
23	P value	0.3831
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
28		
29		

t test

1	Table Analyzed	BHAD - TA male
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.9853
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.01932 df=5
13		
14	How big is the difference?	
15	Mean ± SEM of column A	73.88 ± 4.401 N=4
16	Mean ± SEM of column B	74.03 ± 6.642 N=3
17	Difference between means	0.1472 ± 7.616
18	95% confidence interval	-19.43 to 19.72
19	R square	7.467e-005
20		
21	F test to compare variances	
22	F,DFn, Dfd	1.708, 2, 3
23	P value	0.6395
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
27		
28		
29		

t test

1	Table Analyzed	BHAD - TA female
2		
3	Column B	Sedentary-Inoculated
4	vs.	vs.
5	Column A	Exercise-Inoculated
6		
7	Unpaired t test	
8	P value	0.4478
9	P value summary	ns
10	Significantly different? (P < 0.05)	No
11	One- or two-tailed P value?	Two-tailed
12	t, df	t=0.8041 df=7
13		
14	How big is the difference?	
15	Mean ± SEM of column A	66.18 ± 3.717 N=4
16	Mean ± SEM of column B	62.15 ± 3.359 N=5
17	Difference between means	-4.034 ± 5.016
18	95% confidence interval	-15.90 to 7.828
19	R square	0.08456
20		
21	F test to compare variances	
22	F,DFn, Dfd	1.021, 4, 3
23	P value	> 0.9999
24	P value summary	ns
25	Significantly different? (P < 0.05)	No
26		
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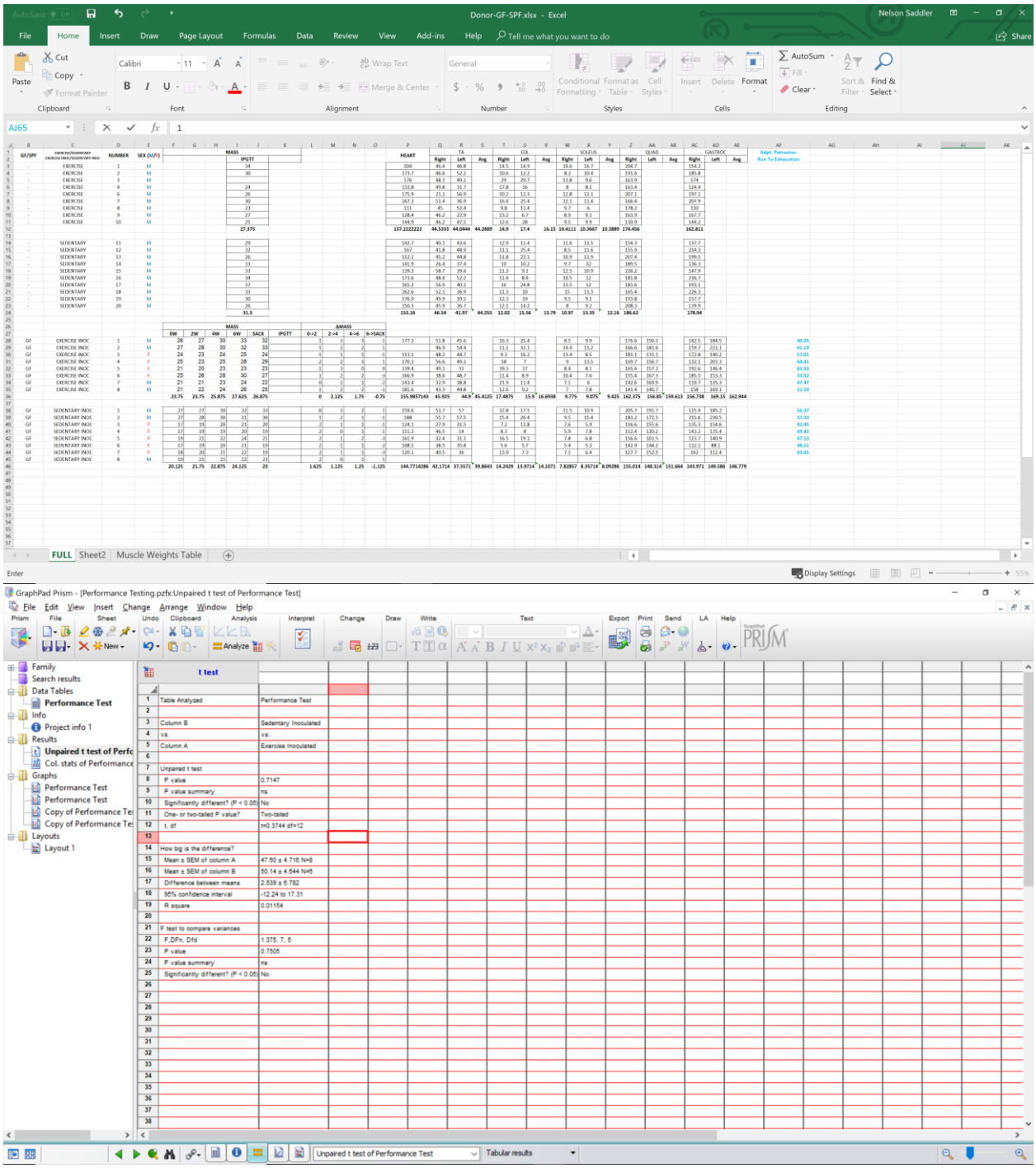
28		
29		

MASS AND PERFORMANCE TEST RECORDINGS



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