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Effect of Obesity on Gait Symmetry Following Anterior Cruciate Ligament Transection

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Obesity is considered a risk factor for both the onset and progression of osteoarthritis (OA). Obesity, OA and mechanical instability have all been identified individually to increase gait asymmetry. The purpose of this study was to assess the effect of obesity on the progression of gait asymmetry as a component of a diet induced obesity (DIO) OA model in the presence of mechanical instability. Methods: 17 Sprague Dawley Rats were assigned to a high fat, high sucrose diet or a low fat diet group (LFD). Twelve weeks post diet assignment, groups receive an anterior cruciate ligament transection (ACL-X), or SHAM surgery. Pre-surgery, 1-week, 8-week, and 16-weeks post-surgery, kinetic data were collected by 3-D force plate analysis. Peak vertical ground reaction force (pVGRF), vertical impulse, and stance times were quantified then compared between limbs to quantify an asymmetry index (AI). Results: There were no differences in normalized pVGRF AI between hind limbs in the DIO or LFD group animals. Stance times decreased for both hind limbs in both DIO group animals. DIO ACL-X group animals had a greater AI for vertical impulse compared to LFD group animals at 1-week post-surgery, and both DIO group animals had greater AI at 8 and 16 weeks post-surgery, compared to LFD group animals. Conclusion: DIO group animals exhibited gait patterns with increased asymmetries compared to LFD group animals, regardless of presence or absence of ACL-X.

Introduction

Osteoarthritis (OA) is defined as a progressive disease characterized by degradation of cartilage and bone leading to joint pain and disability [1]. There are several identified risk factors for the onset and progression of OA, including joint trauma, age, gender and obesity [2]. Investigation into the relationship between obesity and OA is essential because unlike other risk factors, obesity can be modified through diet and exercise [3]. Obesity has been suggested to increase the progression and severity of OA due to the increase in body mass and associated increase in force through load bearing joints [4]. However evidence is emerging that obesity influences OA through mechanisms beyond mechanical joint loading, as obese individuals are found to have 5-8 times more OA in non-load-bearing joints of the hand [5].

Diet induced obesity (DIO) is associated with chronic inflammation, which is thought to exacerbate OA [6]. In a study by Mooney et al. [7], DIO accelerated the progression of OA with no correlation to weight gain, which suggests that intrinsic factors

due to diet are more important factors than the mechanical loading. Brunner et al. [8] and Louer et al. [9], found that DIO increases the progression and severity of OA. Griffin et al. [3] fed mice a high fat diet and found that these mice had elevated OA compared to controls [3]. These findings suggest that diet induced obesity accelerates the progression of OA.

Experimental models of OA have been developed and used in animals such as dogs, rabbits, cats, and rats [10,11,12,13]. In these models, joint instability following an injury or surgical intervention was shown to decrease the time from injury to disease onset, and contribute to the progression of OA. One such surgical intervention is the transection of the anterior cruciate ligament (ACL-X), which results in knee joint instability [10]. The ACL-X is a validated model of OA onset and progression [11,13]. These surgical models of mechanical intervention are helpful because the time from intervention to disease onset and progression is controlled [9].

Gait analysis, or movement analysis, is a method used to examine the functional deficits associated with OA in human patients and pre-clinical animal models of OA [10]. Commonly, gait data is used to understand the effects of OA on joint loading and unloading, and possible compensation when painful joints are favoured relative to healthy joints. Previous work by Herzog et al [13] and Suter et al [14] in ACL-deficient cats showed differences in vertical ground reaction forces between the experimental and contralateral limb for up to 12 weeks post-surgery. These differences in ground reaction forces demonstrate an asymmetric gait. After this time point, ground reaction forces had become about equally shared between hind limbs suggesting a trend towards a recovery of gait symmetry. Contrary to these results, it was previously found that dogs and rats did not recover gait symmetry over time; following induced mechanical instability [10,15]. These studies suggest that different species exhibit different gait compensation strategies following an induced mechanical instability procedure.

To our knowledge, Brady et al. [16] were the first to study the effects of diet on gait variables. They found that obese dogs exhibited a greater range of motion for the shoulder, elbow, hip and tarsal joints during the stance phase than lean control group dogs [16]. Furthermore, obese animals had greater vertical ground reaction forces than the lean control animals. However, this latter result is to be expected as vertical ground reaction forces are strongly correlated with body mass [16], and values were not normalised in this study. Brady et al. determined gait differences in lean and obese animals, but the study did not address how diet affects gait following a mechanical instability intervention.

Male Sprague-Dawley (SD) rats respond well to diet induced obesity [17] by gaining mass shortly after diet initiation. They tend to gain fat mass viscerally and abdominally, similar to humans [10], validating their use as an obesity model that may be representative of human obesity. Osteoarthritis is usually diagnosed late in the disease process, often resulting in delayed treatment [18]. To our knowledge, the combined effects of obesity and knee instability produced by anterior cruciate ligament transection (ACL-X) have yet to be evaluated to understand functional gait adaptions in the presence of these two primary risk factors for osteoarthritis. The purpose of this study was to determine the effects of obesity on gait with and without ACL-X in rats. It has been shown that OA is exacerbated by ACL-X and by obesity in isolation [13,14,15,16,19], so we are interested in the combined effect. With gait analysis as a means to assess the functional deficits of OA, we speculate that greater gait asymmetry is associated with more severe OA. We hypothesized that (1) DIO animals will have greater gait asymmetries than LFD animals, and that (2) gait asymmetry will increase and be sustained over time for the DIO animals, regardless of surgical intervention.

Methods

Twenty-eight male Sprague-Dawley rats (8-12 weeks old) were randomly assigned to either a high fat diet (DIO) or a low fat diet (LFD) group. The DIO group (n=18) received high fat sucrose food (40% fat, Diet 102412, Dyets, Inc) and the LFD group (n=10) received lean chow (13.5% fat, LabDiet 5001). Twelve weeks post obesity induction, ground reaction forces were measured during locomotion using a 1-meter custom runway with two embedded side-by-side 7.5 x 30 cm 3-dimensional force plates (Bertec, Columbus, OH). The force plates had a sampling rate of 500Hz. Sagittal plane kinematics were recorded using a

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high-speed camera filming at 200Hz. Animals were acclimatized to the runway prior to measurements, and a dark hiding area was positioned at the end of the runway to entice the rats to walk towards the hiding area across the force platforms. A trial was deemed successful if the animal walked over the force plates at a uniform speed, with no stopping or pausing, with only one hind limb landing on each plate. Speed was determined in video analysis by comparing the time it took the rat to cross a known distance. A minimum of two successful trials was needed for a rat to be included in the final analysis. Following baseline testing, all animals were assigned to receive a unilateral ACL-X (DIO n=12, LFD n=5) or a unilateral SHAM surgery (DIO n=6, LFD n=5). The surgical limb (ACL-X or SHAM) was randomly assigned. The same individual conducted all surgeries. ACL-X surgery was initiated by creating an incision on the lateral aspect of the knee. The joint capsule was then opened, the anterior cruciate ligament cut, and the incision closed. The SHAM surgery consisted of opening the capsule, spraying the knee joint with saline, then sealing the incision. Kinetics and kinematics were measured 1-week post-surgery, 8-weeks post-surgery, and 16-weeks post-surgery.

The primary kinetic outcome measures were the peak vertical ground reaction force (pVGRF) and the vertical impulse. Stance times for hind limbs were determined from the high-speed video. Similar to the study by Brady et al., a linear relationship was found between mass and ground reaction forces and impulses, so values were normalized to body weight. Hind limb asymmetry was determined using an Asymmetry Index (AI) based on the normalized vertical impulses of the experimental and contralateral limbs:

$$AI = \frac{Impulse_{CL} - Impulse_{EX}}{Impulse_{CL}} * 100\% \quad (1)$$

Larger AI values indicate a greater difference in the vertical impulse between limbs. Data will be normalized to the individual animal body mass. A non-parametric Kruskal-Wallis statistical test was used to compare the main factors diet (DIO and LFD), intervention (ACL-X and SHAM), and time (Pre-surgery, 1 week, 8 weeks and 16 weeks post-intervention). A Kruskal-Wallis test was also done to compare the mass and stance time variables. The level of significance was set a priori at =0.05.

Measure	DIO	LFD	p-value
Δ Stance EXP	-68.8 (±12.8)	3.04 (±15.2)	0.001
Δ Stance CON	-66.4 (±12)	16 (±15.2)	0.002

Table 1: *The stance change in stance times comparing the stance times at baseline and 16 weeks for both the DIO and LFD groups. The p-value is for the change in stance times for the DIO.*

Ethics was obtained from the Life and Environmental Sciences Animal Care Committee at the University of Calgary.

Results

After exclusion of rats because of an insufficient number of acceptable trials, 8 DIO ACL-X, 4 DIO SHAM, 5 LFD ACL-X, and 0 LFD SHAM (total n=17) group animals were analyzed. At 16 weeks post intervention, the mean body mass of DIO ACL-X, DIO SHAM, and LFD ACL-X group animals was 828 ± 51.93g, 762 ± 69.53g, and 611 ± 39.93g respectively. Both DIO group animals were significantly heavier than the LFD group animals ($p<0.05$). All animals were relatively the same weight prior to diet intervention, ranging between 400-500g. There was no statistical difference in mass between the DIO ACL-X and the DIO SHAM group animals, although the DIO ACL-X animals had a higher average mass.

The body mass normalized pVGRFs were similar for all three groups and at all time points. Walking speeds remained consistent within all groups at all time points. Stance times ranged from 188 ms to 470 ms. Stance times in DIO group animals decreased from baseline to 16 weeks post intervention by 69 ± 13 ms and 66 ± 12 ms for the experimental and contralateral limb respectively despite consistent walking speeds. Stance times for the LFD group animals did not change over the 16-week experimental period for either hind limb as shown in Table 1.

The mean AI for the normalized vertical impulses from DIO ACL-X animals at baseline, 1-week, 8-week, and 16-week post surgery was 17 ± 13%, 37 ± 5% ($p<0.05$), 44 ± 10% ($p<0.05$), and 45 ± 14% ($p<0.05$) respectively (Fig. 1). The DIO SHAM group had AIs of 16 ± 17%, 34 ± 7%, 44 ± 13% ($p<0.05$), and 45 ± 9% ($p<0.05$) respectively. The LFD ACL-X group had AIs of 12 ± 15%, 20 ± 7%, 17 ± 6%, and 25 ±

13% respectively. Significant difference between DIO and LFD groups indicated by ($p<0.05$). AIs increased in all groups at 1-week post-surgery, with the DIO ACL-X group animals having a significantly higher AI than the LFD ACL-X group animals ($p<0.05$). Both DIO group animals demonstrated a greater normalized AI vertical impulse when compared with the LFD ACL-X group animals at 8-weeks and 16 weeks post-surgery ($p<0.05$). No differences exist between the AIs of the DIO ACL-X group animals and the DIO SHAM group animals. There was a main effect of diet, with the DIO animals exhibiting greater asymmetries than the LFD group. There was no observable effect of diet between the DIO ACL-X and the DIO SHAM group animals. There was a main effect of time for the DIO group animals, with both groups experiencing an increased AI at 16-weeks compared to baseline values.

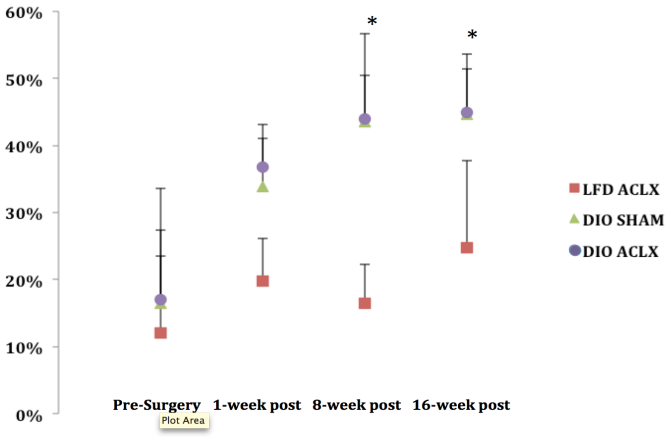

Figure 1:
Normalized AI vertical impulse demonstrated over time for four time points: pre-surgery, 1-week post surgery, 8-week post-surgery, and 16-weeks post surgery. Bars demonstrate SE, * Indicates differences between LFD and DIO, $p<0.05$.

Discussion

Kinetic data from this cohort of DIO rats was highly variable between subjects with large standard errors. Large gait variability could be attributed to the DIO, because similar variability has been seen in obese human populations [20,21]. This variability may explain why no differences were found in the normalized pVGRF between groups. However, when evaluating vertical impulses, which is a measure of cumulative load over the stance time, we found differences between groups of animals. The vertical impulse represents a measure of force over the entire stance time, rather than just an isolated value in time, and thus may be a better indicator of gait than pVGRF alone. The literature suggests that analysis of pVGRF and vertical impulse typically show similar trends, but the vertical impulse appears to be less variable, and thus may be the more meaningful and valid measure of ground reaction force than pVGRF [10].

Stance time on contralateral and experimental limbs was decreased in the DIO animals from baseline to 16-weeks. This decrease in stance times occurred while the average walking speed remained the same, thus we speculate that stride length decreased, but stride frequency increased at 16 weeks following intervention compared to baseline. Our data support the finding by Brady et al. (2013) who found that stride length decreased in obese dogs compared to lean controls. Further investigation into stride length and stride frequency would be valuable in future studies. There was no change in stance times for the LFD group animals in the present study, and no difference in stance times between the experimental and contralateral hind limbs. This result where the stance times are similar between limbs, is contrary to those found by Allen et al (2012) where lean Lewis rats were found to have shorter stance times on the experimental compared to the contralateral limb 9, 16 and 23 days post-surgery. These results suggest that rats do not show the limping gait following ACL transection that other animals often exhibit and that obesity in rats is associated with a distinct change in stance time patterns during walking.

Rats received limited training on the walkway prior to baseline testing. A surprising result is the progression of asymmetry in the LFD ACL-X group. Previous data indicates lean animals are asymmetric immediately following ACL-X, but then tend to recover after several weeks [11,13,14,15]. The results in the present study suggest an agreement with this data however the trend between baseline and 16-week AI values for the LFD ACL-X group animals is not statistically significant. Our results demonstrated exacerbated AI for the DIO ACL-X 1-week post surgery compared to the LFD ACL-X group, as well

as both DIO groups compared to the lean controls at 8 and 16 weeks post surgery. Importantly, it is worth noting the similarities between the DIO ACL-X and the DIO SHAM group, demonstrating that increased asymmetry occurs regardless of surgical intervention in this cohort. Opening the joint capsule, as was done in the SHAM surgery, has been shown to alter synovial inflammation in the joint that may cause animals to offload [22]. This could explain why asymmetries were seen in the same direction for both DIO ACL-X group and DIO SHAM.

Obesity has been shown to be associated with muscle weakness [23]. Consequently, we speculate this muscle weakness, in conjunction with a heavier body mass, results in more joint instability. Future work will include evaluating intramuscular fat content in lean and DIO animals to determine if differences exist. Recovery of gait symmetry in LFD groups in the literature suggests that animals develop gait compensations to deal with the progression of OA in the affected limbs [11,13,14,15]. However the results of this study suggest that obesity has a larger affect on AI impulse, and a recovery of gait symmetry was not observed. Due to the elevated asymmetry in these DIO animals, it can be speculate that the intrinsic factors of DIO have a greater affect on gait than the mechanical driver of surgery alone. Obesity may affect the joint through factors such as inflammation and muscle weakness, resulting in asymmetries similar to ones observed in DIO ACL-X subjects.

The LFD SHAM group was not included in the analysis because the animals did not meet the minimum inclusion criteria, for each of the time points. The exclusion of the LFD SHAM group was unexpected because this group was expected to have the least deviation from normal values, whereas the DIO animals were expected to have the greatest. Due to a large expected variation in the DIO animals, it was speculated that more animals would be excluded from this group and so more subjects were included in this group to begin the study (n=18). Having no LFD SHAM group is a limitation of the study. Future work should consider including larger numbers to more comprehensively study gait in this group. It has been previously shown that, control rats had more symmetrical loading on the hind limbs, indicating a more symmetrical walk, than ACL-X experimental group animals [10]. Therefore, we speculate that the AI for the LFD SHAM group would have been similar or lower than that of the LFD ACL-X.

Rats inherently walk with a non-uniform motion, and tend to pause frequently while walking [24]. Video analysis was used to eliminate unsuccessful trials. Only vertical forces were obtained, so it was not possible to determine movements in the medial and lateral direction by video analysis, or force plate analysis. 3D analysis could be used to better characterize gait characteristics. Future work will include evaluating medial/lateral and anterior/posterior force vectors to provide insight into gait properties. Future gait studies will also employ real time speed feedback using timing gates.

Conclusion

Diet-Induced Obesity increases gait asymmetries in the presence and absence of an ACL-X. Obesity may have biological drivers as well as mechanical drivers that increase gait asymmetries. With the ACL-X as a valid model for OA onset and progression [11,13], and gait analysis as a means to assess functional deficits in OA subjects [10], it can be speculated that due to the observed increased asymmetries in the DIO groups, obesity may accelerate the onset and progression of OA. Further work is needed to determine the individual effects of obesity from high fat/high sucrose diet versus ACL-X alone, on gait characteristics. A larger cohort of non-surgical diet-induced obesity animals is currently under experimentation to examine the effect of diet on gait symmetry without a surgical intervention. Future work is needed to support the preliminary findings of this study and further investigation is needed to better understand the interaction between intrinsic factors of obesity and function, specifically in the medial/lateral and anterior/posterior directions.

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