MALE AND FEMALE TOTAL KNEE ARTHROPLASTY CANDIDATES AND HEALTHY CONTROLS DIFFER IN ANTHROPOMETRY, FUNCTIONAL CAPACITY AND BIOCHEMISTRY (INSULIN LIKE GROWTH FACTOR - I AND CYTOKINES)

BY

SONIA M. C. PAGURA

A THESIS SUBMITTED IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

GRADUATE DEPARTMENT OF REHABILITATION SCIENCE

UNIVERSITY OF TORONTO

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ABSTRACT

Male and Female Total Knee Arthroplasty Candidates and Healthy Controls Differ in Anthropometry, Functional Capacity and Biochemistry (Insulin Like Growth Factor - I and Cytokines)

Master of Science 1998, Sonia M.C. Pagura, Graduate Department of Rehabilitation Science, University of Toronto

PURPOSE: Osteoarthritis is associated with obesity, decreased functional capacity and dissatisfaction with function. Low serum IGF-I levels are associated with obesity, reduced function and possibly joint destruction. Cytokines have also been implicated in articular cartilage destruction. Thus the purpose of this study was to examine whether IGF-I and cytokine levels were altered in patients with osteoarthritis of the knee.

METHODS: Men and women aged 55-75 years (n=138) participated in this study. All subjects completed evaluation of: body composition, physical function, perceived function and serum IGF-I levels. A sub-population (n=38) underwent analysis of cytokines and IGF-I in synovial fluid. Differences were deemed significant at p <0.05.

CONCLUSIONS: Women versus men with OA had higher adiposity, altered cytokines, and lower IGF-I and functional capacity. The gender differences suggest a different etiology of osteoarthritic disease. These findings suggest that in women other factors (hormones) may influence IGF-I levels and ultimately osteoarthritis development.
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DEDICATION

This thesis is dedicated to my mother Silvana and father Remigio Pagura who without their love, support, and encouragement this thesis would not have evolved. They sacrificed much in their lives so that I may have mine and reap the rewards. Thank you mamma and papa for everything.
Chapter I

1.0 Introduction

Osteoarthritis (OA) has been around for centuries and generally tends to be a disease of the old. As the population continues to age, the prevalence of OA will rise. Many forms of OA are existent however, OA which affects the lower extremity tends to be more debilitating as it affects mobility and consequently results in secondary problems such as obesity, cardiovascular de-conditioning, decreased joint movement and muscular wasting surrounding the affected joint. The resultant effect of OA and the secondary consequences ultimately impair quality of life, which makes this area worthwhile exploring.

Despite the importance of studying OA due to its high rates and debilitating effects, the research effort to date has not yielded much insight into conclusive influencing factors on OA pathophysiology. Specifically the patho-physiology of knee OA is still murky today as it was 20 years ago with much research focus placed on biomechanical or anthropometric causes for OA, and its effect on function. Unfortunately, few researchers to date have delved into other causative factors, such as the role of systemic or local biochemistry. Preliminary studies have revealed that OA joints have altered levels of cytokines and growth factors when compared to apparently healthy individuals, and potentially are the implicating factors for joint degeneration. With exciting new evidence identifying biochemical causes for OA, a link between biochemistry and functional ability should be explored as convincing reproducible results have yet to be provided for anthropometric and biomechanical explanations. Thus the role which hormones or proteins play locally or systemically (biochemistry) in OA pathophysiology requires greater light shed upon its potential implications in this disease process and how it may effect function. It is with different, non-conventional thinking and approaches that potential causative or influencing factors may be discovered in this debilitating disease process.
In many studies evaluating contributing factors to OA, many have not explored potential gender differences that may exist in anthropometric, functional, or biochemical measures. It is quite odd that despite epidemiological evidence that suggests that peak prevalence occurs in different age categories between males and females, that gender differences quite often are not explored. The lack of this data, unfortunately in many situations, results in misleading results or half truths about the population in question. In fact in many other disease processes it is not uncommon to identify concrete gender differences, thus lack of exploration on functional, anthropometric and biochemical effects on OA is unfortunate. Only recently have gender roles been explored in the OA population revealing invariably that gender differences do exist. Hence with this new information on potential gender differences in the OA population, it is imperative that gender roles be explored.

This thesis will attempt to shed light on differences which may exist between apparently healthy individuals and Total Knee Arthroplasty Candidates (TKAC) in hopes of linking biochemistry with end stage knee OA. Additionally, this thesis will identify any differences in function and perceived function, as deficits in one's function most often is the impetus to seek medical intervention. Relationships between biochemistry and function will also be explored in hopes of establishing a link between these two areas as limited information has been reported to date attempting to bridge this gap. Finally, gender issues will be explored to identify if in fact males and females score similarly to identical measures performed.
2.0. Osteoarthritis Prevalence

Decided agreement exists amongst health care professionals that osteoarthritis (OA) is highly prevalent in the elderly population (Badley & Rothman, 1996; Felson, ; Felson et al., 1987; Felson & Radin, 1994; Greene & Hochberg, 1993; Hochberg, Kasper, Williamson, Skinner & Fried, 1995). Unfortunately, the effects which this disease exerts on men and women is not well established, thus certain cohorts may in fact have a higher prevalence of OA. Regardless of gender, OA remains an ubiquitous disease which ultimately results in reduced activity in the elderly, with morbidity becoming increasingly manifest (Hamerman, 1995).

In Ontario alone 18.5 % of the population are afflicted with some form of arthritis (Badley, 1995; Badley & Rothman, 1996). Of the total number affected, 75 % have been specifically diagnosed with OA, making OA the most common form of arthritis (Badley, 1995; Badley & Rothman, 1996). Studies conducted in other countries have also reported similar OA prevalence rates to those seen in Canada (Badley, 1995; Felson, 1988; Hamerman, 1995; Spector & Hart, 1992). Presently in Canada the ratio of OA to RA has been documented to be 7:1, and in other countries reported as high as 22:1 reinforcing that OA is a widespread form of arthritis (Badley, 1995; Badley & Rothman, 1996). It has been speculated by many health care professionals that the prevalence of OA will continue to rise as the life expectancy of the population advances. Presently the fastest growing segment of the population are those over the age of 85 (Hamerman, 1995). Past epidemiological studies have reported that the prevalence of OA undergoes a steep rise with age jumping to 51.2 % as individuals exceed 75 years of age and bottoming out at 6.3 %, in those aged 24 and under (Badley & Rothman, 1996). Thus as the population continues to age this debilitating condition will affect more and more individuals and levy a greater social and medical cost to the communities these individuals reside in (Felson, 1988; Hamerman, 1995; Lethbridge-Cejku et al., 1994).

Regardless of age, gender biasing is present in OA pathology. Women are more frequently plagued with OA when compared to men in all joints with the exception of hip where the ratio is roughly equal (Acheson, 1994; Badley, 1995; Badley & Rothman, 1996; Spector &
Hart, 1992; Van Saase, Van Romunde, Cats, Vandenbroucke & Walkenburg, 1989). At the knee joint it has been documented that the ratio (after age 45) of female to male prevalence can range anywhere from 1.5-5.0 : 1 (Felson, 1988). In addition to having overall consistently higher prevalence rates, women also peak considerably earlier. Women appear to have the highest prevalence of OA at an average age of 45 just as menopause sets in, whereas men peak at age 65 (Badley & Rothman, 1996; Hamerman, 1995). It has been speculated that at age 45, men are more often plagued with cardiovascular disease (CV) processes and as age 65 approaches, the incidence of CV declines.

As more epidemiological studies of the prevalence of OA are conducted, more information will be revealed about the definitive contributors to the predilection of OA in various cohorts. It is with these studies that researchers can then conclusively determine whether race, socio-economic status or occupations may alter prevalence rates of OA (Anderson & Felson, 1988; Felson, 1988; Felson, 1996; Greene & Hochberg, 1993; Van Saase et al., 1989). Furthermore, as longitudinal studies are conducted, more information can be ascertained about OA's natural history since at present not much information exists (Calin, 1993; Spector & Hart, 1992).

2.1.0. OSTEOARTHROISIS AND FUNCTION

It is not uncommon for humans to lose some of their physical ability as they age due to many typical aging processes which affect the neural, hormonal and musculo-skeletal system. However, outside of the normal aging process, there are certain pathologies which may affect the way in which the body functions. In osteoarthritis pathology there is not just one limiting factor present, rather a complex interaction between primary disease effects such as pain and limited range of motion, associated with secondary impairment (muscle, cardiovascular system) due to increased sedentariness, and all the consequences associated with obesity. In fact there is ample documentation in the literature to suggest that a trend exists in individuals, particularly women, who have OA as it is not uncommon that they have a history of other health problems (angina, myocardial infarction, hypertension, diabetes, and lung disease) which impede their daily lives (Hochberg et al., 1995). These aforementioned individuals are also more likely to
report, as well as be in, fair to poor health when compared to counterparts without OA (Hochberg et al., 1995). It is these complex interactions and secondary health problems which limit individuals with OA severely in all facets of their lives. Yet the limitations most often reported are not those physical in nature but usually those which affect the quality of life (Badley, 1995). Ironically, most individuals with OA, especially women, usually do not seek treatment until many of the secondary health risks come about because they feel that arthritis is part of the normal aging process (Badley & Rothman, 1996). Outside of this laymen's perception of OA, many health care professionals also have a "fatalistic" approach to OA and only intervene at the end stage without recognizing that the arthritic joint is only one of the problems once all the secondary health related issues are now visible (Spector & Hart, 1992). Osteoarthritis ultimately is a multi-factorial disease process which increases mortality due to the many systems it affects (Spector & Hart, 1992). It is quite surprising that although this pathology has a high prevalence and disability rate, it remains largely under funded with respect to research (Spector & Hart, 1992).

Current studies show that in Canada OA is the primary cause of long term disability affecting 2.3 % of the population and is ranked 2nd amongst all pathologies for restricting activities (Badley, 1995; Badley & Rothman, 1996). Disability associated with OA presently affects more women than men (Badley, 1995). Knee OA is by far the most common site of OA which leads to the greatest amount of loss of function, and has a greater social cost than any other site of OA (Felson, 1988; Felson, Anderson, Naimark, Walker & Meenan, 1988). Of those surveyed with OA, 12% - 90% reported experiencing some form of disability in some aspect of their lives (Badley, 1995; Badley & Rothman, 1996; Hochberg et al., 1995). The most common complaint cited which affects greater than 90 % of the people was difficulty encountered with any activity which required them to weight bear, such as walking or stair climbing (Badley, 1995; Badley & Rothman, 1996). In 25 % of the cases, the OA was sufficiently disabling that individuals were unable to leave their home or participate in hobbies or social activities unless they had an attendant present (Badley, 1995; Badley & Rothman, 1996). Yet these statistics are not solely exclusive to Canada, rather the rate at which OA affects the population is quite similar across the world. In fact OA is ranked as one of the leading worldwide causes of disability. In United States, the National Health Survey showed that of all individuals radiographically diagnosed with OA, less than 43 % of the women and 29 % of the men were
able to walk 400 meters. (Spector & Hart, 1992) In Wales, more than 56% will have some associated disability due to their knee OA which affects their daily lives (Spector & Hart, 1992). Furthermore, disability resulting from knee OA is not restricted to mobility alone, rather, quite often it will also limit their social and emotional well being (Badley, 1995; Hamerman, 1995; Spector & Hart, 1992). Thus OA is a disease process which affects an individual's ability to function in all aspects of daily life and is a real cause of concern as people age due to the high prevalence in the population.

2.2.0 DEFINING OSTEOARThRITIS

At present, there is discordance in the literature as to what or how OA should be defined or diagnosed (Puhl, 1994). No satisfactory definition appears present and no general consensus exists on any suitable diagnostic criteria for OA (Dieppe & Kirwan, 1994). Unfortunately, there are no distinct disease markers in blood or synovial fluid (SF) which allow for the accurate classification of abnormal findings into some meaningful diagnosis which can be used by all health care professionals (Badley & Rothman, 1996; Dieppe, 1995). OA has been described by the National Institute of Health as being “the most common human joint disease with diverse etiology and obscure pathogenesis ... it affects mainly weight bearing joints and is involved in a complex degenerative and reparative patho-mechanism which results in a final common pathway of joint damage “(Puhl, 1994). The ARA has stated that OA is “a heterogeneous group of conditions that lead to joint symptoms and signs which are associated with the defective integrity of cartilage, in addition to the related changes in underlying bone and joint margin”. (McAlindon & Dieppe, 1989). Yet despite the description of signs and symptoms associated with OA, no clear cut definitive criteria form the basis of a comprehensible definition.

Presently a myriad of OA definitions are floating in the literature whose use often corresponds to the tools and approach used by the clinician. Some clinicians will describe and diagnose OA from a purely radiographic perspective, others combine radiographic, function and symptomatic complaints and still others may define OA from a purely pathologic or histomorphologic viewpoint. Commonly the key denominator in all definitions is focal loss of
cartilage. However to describe OA as purely a focal loss of cartilage without describing the peri-articular deficits and pathologies (which include ligaments, synovium, muscle etc...) is to characterize OA incorrectly as it has been already established that radiographic change alone does not translate into loss of function (Brandt, Fife, Braunstein & Katz, 1991; Fife et al., 1991). Yet using loss of function as the sole indicator of OA pathology in the absence of AC loss is also incorrect as arthritis is a disease of the boney tissue which has irreparably changed. These varying and vague descriptions often create confusion and inconsistency when trying to identify the etiology of OA.

Despite the perplexity, the two most traditionally reported definitions of OA (in most research designs and clinically) are radiographic changes using the Kellegran and Lawrence Scale or the American Rheumatology Association definition which combines both clinical findings and radiographs (Calin, 1993; Dieppe, 1996). Both methods of diagnosis have imperfections and have been criticized. Some of the criticisms include the lack of correlation between x-ray findings and symptoms or lack of reproducibility of the symptoms (Brandt et al., 1991; Fife et al., 1991). Despite these aforementioned problems, many still continue to use radiographic and symptomatic findings as a true method of defining and diagnosing OA. It is sufficient to say that OA can be described as a collection of signs and symptoms which include radiographic findings, symptomatology (stiffness, pain, reduced range of motion of the joint, effusion etc...), abnormal cytology in serum or local joint fluid and most importantly a loss of overall function impeding individuals from fulfilling their roles in society. It is important to state that there is no real “gold standard” for defining OA at this point, rather a collection of symptoms and concrete findings which help describe one or more processes which unfortunately leave individuals with joint destruction, considerable pain and dysfunction. It is difficult to diagnose early stage OA and more certainty develops when the disease progresses to the end stages.

2.3.0 Osteoarthritis Defined Radiographically

As previously mentioned OA is a condition which is loosely defined. Most of its definition relies on the interpretation of radiographs of the joint in question. OA may manifest itself in the form of one or more of the following; focal cartilage loss, osteophyte formation,
joint space narrowing, chondral sclerosis, mechanical mal-alignment of the knee joint, and peri-articular soft tissue alterations. Finding a scale which is sensitive, reliable and able to monitor change is a difficult task when applied to this pathology.

The medical and scientific community have attempted several times to find and establish a diagnostic gold standard to grade or monitor OA. To date there are no such tools or scales that are sensitive yet specific enough to monitor this diffuse pathology. Radiographic diagnosis of OA is dependent on the expertise of the clinician reading the film, the scale used, the preciseness of the technician administering the film, the view used when radiographing the specific joint, standardized positioning of the patient radiographed and finally the assumption that radiographs can pick up minor changes in disease pathology. Unfortunately, if any one of the aforementioned factors is altered the preciseness of the diagnosis can be reduced by as much as 20% (Ravaud et al., 1996).

Formulating and standardizing a scale which all clinicians can use in practice appears simple on paper but rather difficult in practice. This is especially difficult when trying to devise a diagnostic scale geared towards OA which is sufficiently sensitive to pick up minor changes, yet specific enough to minimize the amount of false positives diagnosed. The first scale which was standardized and put into full use for all Rheumatologists was one by Kellegran and Lawrence (KL) (see table 2.3.1).

Kellegran and Lawrence Scale (KLS) used by many clinicians to classify levels of OA

<table>
<thead>
<tr>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No evidence of OA</td>
<td>Doubtful OA</td>
<td>Minimal: definite small osteophytes with minimal narrowing</td>
<td>Moderate: moderately sized osteophytes or definite small osteophytes with moderate narrowing</td>
<td>Severe: large osteophytes or small or moderate osteophytes with severe narrowing.</td>
</tr>
</tbody>
</table>

Table 2.3.1. The original KLS categorized OA in 5 stages and placed most emphasis osteophyte formation and very little focus on joint space narrowing (JSN).
This scale was based on a 5 point scale whose primary determining factor of OA was osteophytes. This became problematic as time went on because scientists found that the formation of osteophytes was a normal process of aging and did not necessarily confirm that the individual had OA changes on the AC (Fife et al., 1991; McAlindon, Cooper, Kirwan & Dieppe, 1993). Longitudinal studies done by Danielsson et al 1970, Hernbor and Nilsson 1973 and Masssardo 1989 found that after 10 years groups who were followed and initially found to have osteophytes, did not go on to develop joint space narrowing (JSN), decreased range of motion (ROM), crepitus or any of the symptoms associated with knee OA (Danielsson & Hernborg, 1970; Dougados et al., 1992; Herborg & Nilsson, 1977; Massardo, Watt, Cushingham & Dieppe, 1989). Yet despite this information as early as 1970, clinicians still chose to use the KLS to grade and diagnose OA.

Other problems associated with the KLS was that the initial development was based on radiographs taken while supine not in a weight bearing (WB) position. To accurately assess JSN, radiographs must be taken while weight bearing (Fife et al., 1991). Since the development of the KLS years ago, many other scales have since been developed to try and quantify OA more correctly and hopefully develop a better diagnostic tool.

In addition to the technical aspect which may lead to false interpretations, another problem which remains in radiographic diagnosis is that many individuals have radiographic changes yet remain asymptomatic. Current literature reveals that greater than 50 % of all individuals who have radiographic changes continue to function without any pain, disability or change in their lifestyle (see table 2.3.2) (Adams & Wallace, 1991; Davis, Ettinger, Neuhaus, Barclay & Segal, 1992; Felson et al., 1987). In fact were it not for incidental radiographs taken usually when another health issue is being investigated, individuals would continue on in life not experiencing any ill effects associated with their radiographic changes.
REPORTED PAIN AND KLS GRADING ACROSS COHORTS

<table>
<thead>
<tr>
<th>Radiographic Grade</th>
<th>45-54 years of age</th>
<th>55-64 years of age</th>
<th>65-74 years of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 1593</td>
<td>n = 1144</td>
<td>n= 941</td>
</tr>
<tr>
<td>Mean percent with pain</td>
<td>9.3 %</td>
<td>8.9 %</td>
<td>14.5 %</td>
</tr>
<tr>
<td>0</td>
<td>8.2</td>
<td>7.1</td>
<td>8.8</td>
</tr>
<tr>
<td>1</td>
<td>11.6</td>
<td>15.6</td>
<td>20.4</td>
</tr>
<tr>
<td>2</td>
<td>40.1</td>
<td>30.2</td>
<td>36.9</td>
</tr>
<tr>
<td>3 or 4</td>
<td>66.4</td>
<td>47.2</td>
<td>60.4</td>
</tr>
</tbody>
</table>

Table 2.3.2. KLS has historically been used to quantify/grade the OA throughout the joints in the body. This table represents the percentage of individuals currently experiencing symptoms and their respective KLS score. As can be seen in the oldest age group only the mean percentage which report pain are solely 14.5 %.

It is well established in the literature that the correspondence between severity of the joint pain and disability and the severity of pathologic changes in the articular cartilage (AC) found during arthroscopy or total knee replacement is poor (see table 2.3.2) (Davis et al., 1992; Felson et al., 1987). This information suggests it is inappropriate to accept radiographs as the "gold standard". Evidence reveals that the correspondence between radiographic damage and symptoms generally is weaker in women than men.

REPORTED SYMPTOMATOLOGY, KLS GRADING OF OA, AND THE DIFFERENCES BETWEEN GENDERS

<table>
<thead>
<tr>
<th>OA Grade</th>
<th>% with symptoms</th>
<th>% of men (symptoms)</th>
<th>% of women (symptoms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.6 %</td>
<td>4.4 %</td>
<td>9.6 %</td>
</tr>
<tr>
<td>1</td>
<td>10.8 %</td>
<td>8.5 %</td>
<td>13.0 %</td>
</tr>
<tr>
<td>2</td>
<td>19.2 %</td>
<td>9.1 %</td>
<td>24.8 %</td>
</tr>
<tr>
<td>3 or 4</td>
<td>40.0 %</td>
<td>34.8 %</td>
<td>43.4 %</td>
</tr>
</tbody>
</table>

Table 2.3.3. Gender differences which are revealed when comparing the reported symptomatology of OA and the actual OA grade using the KLS.
Methods in discerning whether a scale is appropriate or not is to compare it to a gold standard. In the realm of OA, scoring the articular surface from arthroscopic images is the "gold standard" and other methods such as X-ray, macro-radiographs, MRI etc. can be compared to this method to see how well they correlate to the person's actual AC destruction. Many studies to date have found quite poor correlation between OA scoring radiographically and articular damage found arthroscopically (Brandt et al., 1991; Hart & Spector, 1993; Lysholm, Hamberg & Gillquist, 1987). Brandt et al actually found that in 27 % of the cases where OA was graded as 3 or 4 on the KLS, when reviewed arthroscopically, were found to be mildly eroded and given a grade of 0 or 1 (Brandt et al., 1991). In fact 80 % of individual's who were graded with a 2+ KL score, 72 % were found to have grossly normal AC when arthroscopically reviewed (Brandt et al., 1991; Lysholm et al., 1987). Only in severe cases where individuals were graded a 4+ using the KLS were correlations found to be moderately good (Brandt et al., 1991; Lysholm et al., 1987). Unfortunately, this cannot be considered acceptable as a scale should be able to monitor changes over time not just confirm the results of an end pathological process.

To date what has been found to diagnostically be sensitive yet specific is magnetic resonance imaging technology. Studies which compared MRI findings to arthroscopic findings revealed that only 1 % of the time were false positives or negatives reported and that AC degradation graded by MRI correlated quite well with arthroscopic findings across varying levels of severity (LaPrade, Burnett II, Veenstra & Hodgmena, 1994). However, MRI are still too costly to be performed on a regular basis and presently in Ontario only 9 MRI exist, thus making them virtually impossible to access for routine scans.

Consequently when diagnosing OA, one must keep in mind that presently radiographs and their scaling systems are not sensitive or specific enough to accurately grade OA. At the present radiographs are unable to pick up micro changes occurring in chondrocytes, nor are they able to identify peri-articular structures which in fact may be the root of the individual's pain. Remembering that OA is a slow process which begins with microscopic damage and usually involves other structures which initially are responsible for pain encountered, caution should be used when deciding how to diagnose and grade OA. At present time most clinicians will diagnose based on reported symptoms, functional impairment, manual joint assessment
and confirmation with radiographs of their clinical picture, which in essence follows the ARA guidelines.

2.4.0. OSTEOARTHRITIS ETIOLOGY

Osteoarthritis is not a single disease entity, rather it encompasses a variety of metabolic processes which affects various joints and their surrounding tissues, and should be regarded and treated as a complex disease process (Dieppe, Brandt, Lohmander & Felson, 1995; Kuettner & Goldberg, 1995; Pelletier, Roughley & Martel-Pelletier, 1993). There are usually common underlying risk factors and general outcomes, yet each joint also has specific risk factors which need to be addressed. Thus when attempting to establish causation of a disease process, especially with multi-faceted processes such as OA, each joint or area needs to be reviewed separately.

Osteoarthritis can be generally classified into two groups, primary OA where the onset is insidious in nature and the cause idiopathic, and secondary OA where a traumatic event or disease earlier on in life will predispose individuals to procuring OA (Bleasel & Moskowitz, 1996). Currently, the consensus is that the etiology of OA (regardless of group classification) is a rather convoluted process due to various genetic and environmental factors which interact in unknown processes (Dieppe et al., 1995). These interactions make identification of a specific causal factor of OA almost impossible. However, there are proven influences demonstrated in the literature which affect how OA progresses. Well known factors for knee OA include obesity, hormonal imbalances, mechanical joint problems, genetic predisposition, gender, and race.

As previously discussed, it has been shown that certain races and occupations can predispose individuals to knee OA (Anderson & Felson, 1988; Felson, 1988; Felson, 1996; Greene & Hochberg, 1993; Van Saase et al., 1989). Additionally, obesity and stress loaded upon the knee due to excess weight are associated with poor outcomes in knee OA (Felson, ; Felson et al., 1988; Felson et al., 1987; Hart & Spector, 1993; Hochberg et al., 1995; Spector, Hart & Doyle, 1994). However, whether obesity occurs prior to the development of OA or is a result of some hormonal imbalance which then increases the risk of knee OA is unknown at this
point in time. Thus, the discordance does not lie with whether obesity is associated with increased risk of knee OA, rather controversy exists whether obesity is a causal factor or a secondary factor associated with an underlying cause such as hormonal imbalance.

Gender has also been explored as a contributor to OA. Studies have observed that females are at an increased risk of developing any type of OA, excluding hip OA where men and women have equal risks (Acheson, 1994; Badley, 1995; Badley & Rothman, 1996; Spector & Hart, 1992). Studies have shown that the onset of symptoms is 20 years sooner in women than men, and at any age the prevalence of OA exceeds that of men (Acheson, 1994; Badley, 1995; Badley & Rothman, 1996; Spector & Hart, 1992). Many reasons are suggested for the observed phenomenon of gender differences but none have been substantiated to date.

Familial patterns of OA prevalence suggest a genetic component (Williams & Jimenez, 1993). Preliminary studies suggest that certain types of OA are hereditary such as primary generalized OA which affects a multitude of joints (Williams & Jimenez, 1993). However the role which genetic make-up takes remains unproven and in its infancy with respect to research as no gene has been identified to date.
CHAPTER III

3.0 RELEVANT BACKGROUND (REVIEW OF THE LITERATURE)

3.1.0. BASIC JOINT ANATOMY

The knee is the largest joint in the human body. It is composed of incongruent femoral and tibial components which form a modified hinge joint which permits rolling, gliding and sliding for mobility to occur while the knee is flexed, and conversely, in full extension the knee provides complete stability (Kahle, Leonhardt & Platzer, 1986; Smith, 1993). These incongruent surfaces are lined with thick AC and lie on menisci which allow for smoother movement and greater stability (Arnoczky, 1992; Kahle et al., 1986). In fact in the absence of menisci it has been shown that anterior posterior displacement of the femur increases by 24 %, thus the menisci aid in passive stability of the knee (Smith, 1993). The patella is also part of the knee joint and functions as a lever to allow for greater mechanical advantage of the quadriceps group as well as decreasing joint compression forces (Smith, 1993).

Hyaline articular cartilage, more commonly known as articular cartilage (AC) covers subchondral bone of all joints. It has been shown that subchondral bone (SB) is stiffer than AC and more resilient than cortical bone such that SB aids in the dispersion of loads on the joint (Walker, 1996). AC functions to distribute and transmit loads like SB, yet it also protects against shear forces and protects underlying bone by providing a frictionless surface (Walker, 1996).

AC has been found to be primarily anerual, avascular, alymphatic and sparsely cellular (Bandara & Evans, 1992; Walker, 1996). As a result of these aforementioned attributes, AC relies considerably on synovial fluid (SF) to provide for its nutrition and for carrier growth factors in SF to aid in its homeostasis (Matsumoto, Gargosky, Iwasaki & Rosenfeld, 1996). Conversely, the SF is also the medium in which destructive enzymes and proteins circulate to aid in the destruction of the AC.
3.1.1. CHONDROCYTES

AC was initially thought of as a metabolically inert structure that once formed, remained static until death (Bandara & Evans, 1992; Pelletier, Roughley, DiBattista, McCollum & Martel-Pelletier, 1991). This ideology has since been disproven and scientists currently agree that AC is a metabolically active structure, and responsible for its own maintenance through mediators found locally and systemically. Maintenance of the AC is essential so that the AC provides pain and friction free joint movement, thus full pain free mobility.

AC is comprised of chondrocytes (cells) embedded in an abundance of extra-cellular matrix (ECM) which is comprised of proteoglycans (PG), and type II collagen fibers in a mesh like fashion (Bandara & Evans, 1992). Chondrocytes although few in number are highly active and are responsible for maintaining its matrix integrity via the equilibrium of various anabolic and catabolic cytokines (Pelletier et al., 1993; Westacott & Sharif, 1996). The biological function of chondrocytes is secreting PG's and collagen which in turn provide that consistency of the extra-cellular matrix (ECM) to transmit loads of force (Walker, 1996).

Chondrocytes secrete IGF-I which works in an autocrine fashion to stimulate further production of chondrocytes. This in turn increases the amount of PG and collagen released into the ECM to maintain its structure. IGF-I released from the chondrocytes also stimulates chondrocyte mitosis in younger individuals (Dore et al., 1995). When cartilage is damaged, it has been reported that greater amounts of IGF-I are secreted in hopes of repairing the damage and slowing down the articular cartilage loss (Schouten, Van Den Ouweland, Valkenburg & Lamberts, 1993). When chondrocytes are healthy they maintain their matrix diligently via regulation of biosynthesis of anabolic and catabolic products. In an "unhealthy" situation, the balance between anabolism and catabolism of the ECM may be skewed resulting in the chondrocytes increasing the amount of anabolic factors released in attempts to counter-act the deleterious effects of cytokines.

Ironically chondrocytes are also responsible for the production and secretion of catabolic proteins such as interleukin - 1 (IL-1), tumor necrosing factor alpha (TNF-α) and other catabolic enzymes which degrade the cartilage matrix (Pelletier et al., 1993). In comparison to
individuals with RA where the synovium is most responsible for joint destruction, in OA it appears the chondrocyte itself aids in its own demise (Pelletier et al., 1993). However, as long as the balance between catabolic cytokines and promoting growth factors are kept in check then the matrix and the AC remain unchanged and in perfect equilibrium (Pelletier et al., 1993; Westacott & Sharif, 1996).

3.1.2. SYNOVIAL LINING AND FLUID

Most text books will describe the covering of a joint as the "synovial membrane" however the usage of this term is rather inappropriate, as the "synovial membrane" found surrounding joints has no definite borders along the joint (Walker, 1996). Rather the synovial tissue lies on areolar, fibrous or adipose tissue which eventually extends and merges with the fibrous capsule of the joint and is supported by the dense fibrous joint capsule (Bandara & Evans, 1992; McCarty, 1989; Walker, 1996). The tissue which lines the joint space is called the "synovial intimal layer" and directly beneath that is the "sub-intimal layer" which merges with the joint capsule. The intimal layer is not continuous, rather it is a collection of cells which form a porous layer that is 1 - 3 layers thick, embedded in a ground substance without any basement membrane (McCarty, 1989; Walker, 1996). The porosity in this barrier is quite functional as it allows the synoviocytes to come into contact with the SF (Walker, 1996). The intimal layer appears to have a blood supply, where the actual vessels are fenestrated permitting the transfer of solutes rather quickly and easily (Walker, 1996). It is this fenestration which potentially allows for systemic factors to influence the synoviocytes in production of certain proteins or enzymes. The porosity of the ground substance is also important as synoviocytes are secretory vessels and are responsible for secreting certain cytokines into the SF which will act either in an anabolic or catabolic way towards the AC (McCarty, 1989; Walker, 1996).

There are Type A and Type B synoviocytes present in the lining. Type A synoviocytes most resemble a macrophage due to their ability to phagocytose. These cells have many vacuoles, vesicles, rough endoplasmic reticuli and Golgi complexes, they are thought to synthesize and secrete hyaluronic acid into the SF (Walker, 1996). In addition it is postulated that Type A cells secrete IL-1 and prostaglandin E₂ which again are responsible for ECM
degradation (Walker, 1996). Conversely, Type B cells have more nuclei and are better equipped for protein secretion. However, these cells are also quite capable of secreting enzymes and it is thought that these cells synthesize and secrete proteinases, cholinases and other enzymes designed to degrade cartilage matrix (Bandara & Evans, 1992; Walker, 1996). Conversely, synoviocytes are also capable of secreting anabolic cytokines {transforming growth factor beta (TGF-β)} which will aid in restoring AC and allow for joint homeostasis. Regardless of type, synoviocytes are stimulated by several factors which include; inflammation, cytokines and SF particles and once stimulated function in a paracrine and autocrine fashion (Bandara & Evans, 1992).

The amount of SF present in most normal joints is negligible. The knee which is the largest joint in the body only contains at a minimum a few drops of fluid to a maximum of 4 ml (McCarty, 1989). This makes “pure” SF extraction quite difficult in a disease free state where there is not excessive fluid production. In addition, in a “normal state” trying to quantify certain cytokines is rather difficult as assays cannot detect levels under a certain threshold and certain cytokines or anabolic factors are not produced unless there is an abundance of another stimulus which again is elevated so that it triggers a counter effect. Fluid production is dependent on several factors such as irritation of the synovial lining, the forces acting on the fluid and the permeability of tissue to water and solutes (Walker, 1996).

SF is a medium which harbors electrolytes, small molecules, proteins, and enzymes (depending on the joint status). The actual fluid itself in most cases is colourless or pale yellow, transparent with a high viscosity, good mucin clotting and no spontaneous clotting. Conversely an osteoarthritic knee often has greater amounts of fluid present in the knee which is xanthochromic in colour, transparent in clarity with a high viscosity and spontaneous clotting (McCarty, 1989; Walker, 1996). Since the lining is highly vascular and responsible for the fluid production, the actual turnover of SF is quite high in both disease free and pathological states.
3.2. PHYSIOLOGY OF GROWTH HORMONE

3.2.0. REGULATION OF GH AND IGF-I

The GH/IGF-I systemic regulatory system is believed to basically run on feedback loops from 4 primary sources, growth hormone releasing hormone (GHRH), somatostatin, circulating GH and IGF-I (See figure 3.2.0.1) (Corpas, Harman & Blackman, 1993; Cuttler, 1996). In vitro GHRH appears to simulate somatostatin release and inhibit its own release from the hypothalamus (Cuttler, 1996). Somatostatin in vitro also inhibits its own secretion and whilst stimulating GHRH from the pituitary (Cuttler, 1996). GH and IGF-I also work on a negative feedback but on several levels, directly on the pituitary and also at the level of the hypothalamus where GHRH and somatostatin are either stimulated or inhibited depending on circulating levels (Cuttler, 1996). In addition to these four primary feedback sources, other factors which may affect IGF-I levels positively or negatively are stress, nutrition and the immune system (Blum, ; Blum, 1996).
Figure 3.2.0.1. attempts to demonstrate the relationships that exist between GH production and release with other components in the human organism. Shown above are the known relationships, however others may exist which have yet been identified.

3.2.1. GROWTH HORMONE

Growth Hormone (GH) is a single chain poly peptide of 191 amino acids which is produced in the anterior pituitary gland by somatotropic cells. These somatotropic cells are responsible for the production, secretion and storage of GH (Cuttler, 1996). Healthy adults normally have 5 - 10 mg of GH stored in the somatotropes, ready for release into the circulation upon stimulation (Shetty & Duthie, 1995). GH itself is secreted in a pulsatile fashion from the
pituitary with periods of high activity followed by periods of quiescence. Most of the daily secretion of GH occurs at night within the first 4 hours of sleep, with increased peak amplitudes during slow wave sleep (Corpas et al., 1993; Cuttler, 1996; Hartman, Veldhuis & Thorner, 1993; Shetty & Duthie, 1995). It is thought that the actual pattern and frequency of GH release (circadian rhythm) depends on what stimuli directly affect the pituitary cells or how physiological factors (age, gender, exercise, sleep etc...) affect the hypothalamus and the release of hormones which are known to regulate GH release (Hartman, 1996; Hooghe-Peters & Hooghe, 1995).

GH production and release is regulated by two hypothalamic hormones, GHRH and somatostatin both of which are released into the portal venous system and exert their opposing effects on the pituitary gland (Corpas et al., 1993; Cuttler, 1996). GHRH is a 40 or 44 amino acid peptide which is released in a pulsatile fashion from the neurosecretory cells of the hypothalamic arcuate nuclei (Mayo, Godfrey, DeAlmeida & Miller, 1996). GHRH has a stimulatory effect on the pituitary somatotropes causing them to proliferate themselves, produce and secrete GH (Corpas et al., 1993; Cuttler, 1996; Hartman et al., 1993; Shetty & Duthie, 1995). It is thought that GHRH is responsible for approximately 60 - 70 % of total GH release from the pituitary (Cuttler, 1996). Conversely, somatostatin's (which exist in two forms) function is to inhibit the release of GH as well as inhibiting the release of other hormones (Corpas et al., 1993; Cuttler, 1996; Shetty & Duthie, 1995). It is thought that the release of somatostatin is actually responsible for modulating the amplitude of GH release (Hartman et al., 1993). It is the pulsatile and intermittent release of each hormone which either stimulates and/or inhibits the pituitary from secreting GH into the circulation (Corpas et al., 1993; Hartman et al., 1993; Shetty & Duthie, 1995).

GH once secreted, largely travels bound to GH-binding protein (GHBP) which protects GH from rapid degradation in the circulation (Borst, Millard & Lowenthal, 1994; Corpas et al., 1993; Cuttler, 1996). Complied GH has a half life of approximately 20 minutes, whereas an uncomplexed GH has a half life of approximately 5 minutes (Borst et al., 1994). There are at least two known forms of GHBP, one which is considered high affinity and another which is considered to be a low affinity form (Corpas et al., 1993). It appears that 80-85% of GH binds to the high affinity form (Corpas et al., 1993; Cuttler, 1996). The high affinity GHBP has also
been found to be identical to the extracellular portion of the GH receptor on target tissues, which then mediates the effects of GH on the target cell (Corpas et al., 1993; Cuttler, 1996). This finding suggests that it is the extracellular portion of the target cell which may be responsible for the generation of GHBP in circulation (Corpas et al., 1993; Cuttler, 1996).

GH has numerous metabolic actions outside of linear growth patterns seen in children. Primarily in adults, GH is responsible for regulating protein synthesis resulting in the production of lean muscle mass, maintaining adequate bone density, regulating fat metabolism (specifically aiding in lipolysis), and potentiating other hormones' effects (Borst et al., 1994; Corpas et al., 1993; Hartman, 1996; Rosen, Bosaeus, Toll, Lindstedt & Bengtsson, 1993; Scanes, 1995). Other functions outside of musculoskeletal maintenance include roles in cardiac function, osmoregulation, maintaining fluid and metabolic homeostasis, and immunology of the organism (Rosen et al., 1993). These aforementioned actions result from direct interaction between GH and cells or mediated via other organs and hormones. (Borst et al., 1994; Corpas et al., 1993; Hartman, 1996; Rosen et al., 1993; Scanes, 1995). Absence of GH results in abnormal body composition, alterations in metabolic function, chronic fatigue and deconditioning and compromised psychological well being (Daughaday & Harvey, 1995). Many of the effects that GH has on tissue and organ systems are mediated by insulin like growth factor I (IGF-I) which is produced primarily in the liver (Blum, 1996).

3.2.2. Insulin Like Growth Factor - I (IGF-I)

IGF-I, formerly known as somatomedin C, is a single chained non-glycosylated polypeptide with a molecular weight of 7649 Da which exhibits a high degree of sequence homology to proinsulin (Blum, ; Blum, 1996; D'Ercole, 1996). IGF-I appears to share 45% sequence homology with insulin chains A and B which suggest similar evolution from precursor forms (Hussain, Schmitz, Christiansen, Zapf & Froesch, 1996). The IGF-I gene is located on the long arm of chromosome 12 and is composed of 6 exons of which the last two result in the formation of different precursors forms of IGF-I (D'Ercole, 1996; Gilmour, 1994). It is thought that these different forms are expressed in various tissues and in fact may have very different biological roles. It is believed that the expression of IGF-I Ea mRNA has a role in non-hepatic
tissue thus functioning in a local autocrine/paracrine fashion and IGF-I Eb mRNA is expressed in hepatic tissue which is sensitive to GH levels and functions in an endocrine fashion (Corpas et al., 1993; D'Ercole, 1996; Gilmour, 1994). Regardless of form a or b, IGF-I plays an integral role in cell anabolism, maintenance, potentiator of other hormonal effects and a key player in an information network on the organism's homeostasis (Blum, ; Blum, 1996). Specifically IGF-I has been documented as being involved in many functions such as propagating cell cycle progression, cell proliferation, inhibition of cell apoptosis, cell differentiation, and cell maintenance (synthesis of glycosaminoglycans, synthesis of extracellular matrix in chondrocytes, amino acid uptake, glucose uptake, and regulating cell hormone secretion) to mention a few (Blum, ; Blum, 1996; Jones & Cleemons, 1995). However new evidence suggests that the role that IGF-I plays is no longer that of solely being a mediator of GH rather as a transmitter of information to other sources about the well being of the cell or organism as its production and secretion are directly affected by various conditions which will be mentioned shortly (Blum, ; Blum, 1996).

It is well established in the literature that the liver is the largest reservoir of IGF-I mRNA and secretor of IGF-I into the circulation (Blum, ; Blum, 1996; Corpas et al., 1993; Hussain et al., 1996; Malemud, 1993). As the liver itself has few or no IGF-I receptors it is not considered a target organ, rather it functions as an endocrine organ such that it synthesizes, and secretes IGF-I (GH mediator) for total body distribution with multiple distant sites of action (Blum, ; Blum, 1996; D'Ercole, 1996; Hooghe-Peters & Hooghe, 1995). Unlike other peptide hormones which are stored in secretory granules, IGF-I is not stored in the liver rather it is readily secreted soon after its production bound to their respective binding proteins which in essence act as a temporary reserve (Blum, ; Blum, 1996; D'Ercole, 1996). Hepatic production of IGF-I is strongly affected by the amplitude and rate of GH secretion from the pituitary, however it is also affected by other factors such as stress, variations in immune system function, and by the nutritional status of the individual (Blum, ; Blum, 1996; D'Ercole, 1996). Conversely, hepatic derived IGF-I serum levels also provide regulation to GH secretion directly and indirectly via a negative feedback loops to the pituitary and hypothalamus (See figure 3.2.2.1.) (Hartman, 1996; Hussain et al., 1996).
Yet to assume that IGF-I is solely produced by hepatic sources (via GH stimulation) would be incorrect as evidence supports that IGF-I is produced locally in many other tissues (Malemud, 1993). It has been hypothesized that local production of IGF-I results from GH sources secreted from the pituitary. It is thought that IGF-I mRNA transcription is activated directly by GH which subsequently results in local IGF-I production which then behaves in a paracrine/autocrine fashion (Corpas et al., 1993; Malemud, 1993). Recent studies also suggest that other factors in the tissue environment (catabolic cytokines) resulting in high cellular activity may also promote IGF-I synthesis by local tissue in attempts to maintain homeostasis (Dore et al., 1995). Thus hepatic produced IGF-I may not be the origin of all tissue IGF-I, moreover, serum levels of IGF-I may not reflect local tissue levels. At present there is still much to be revealed regarding local tissue production and regulation of IGF-I as most is speculative at this point in time. Regardless of origin, IGF-I is an extremely potent metabolic and mitogenic potentiator.

3.2.3. Binding Proteins

Biological effects of IGF-I are asserted through binding proteins which have an extremely high affinity to IGF-I and transport them through biological fluids (locally, and systemically) (Donnelly & Holly, 1996; Jones & Cleemons, 1995). Less than 1% of all secreted IGF-I is in free form (not bound) in circulation or tissue and 99% is bound to some protein complex (Blum, ; Blum, 1996; Corpas et al., 1993; Hussain et al., 1996). In the absence of these binding proteins (BP) the half life of IGF-I in serum would be 10 minutes, whereas the IGFBP complex increases its half life significantly (over 1000 fold) (Blum, ; Blum, 1996; Corpas et al., 1993; Jones & Cleemons, 1995). Presently six binding proteins have been identified for IGF-I, of which 1 and 3 are most commonly known and written about in the literature. IGFBP’s basically have 4 primary functions with respect to their biological activity. These BP’s act as transporters and temporary reservoirs of IGF-I, they prolong the half life of IGF-I, they regulate the metabolic clearance of IGF-I, and they directly modulate IGF-I’s bio-availability and interactiveness with its respective receptor (Blum, ; Blum, 1996; Hartman, 1996; Jones & Cleemons, 1995).
As previously mentioned IGFBP3 appears to be the most abundant BP in circulation as well as the BP which most prolongs IGF-I's half life (up to 18 hours) (Blum, ; Blum, 1996; Corpas et al., 1993; Fernihough, Billingham, Cwyfan-Hughes & Holly, 1996; Hussain et al., 1996). IGFBP-3 once coupled to IGF-I forms another association with a glycoprotein complex, an acid labile subunit (ALS), to form this new ternary complex of 150 kD (Blum, ; Blum, 1996; Corpas et al., 1993; Jones & Cleemons, 1995). It is the new complex which accounts for 75 - 95 % of all IGF-I present in circulation or in tissue (Blum, ; Blum, 1996; Corpas et al., 1993; Jones & Cleemons, 1995). It is thought that the ALS unit is responsible for increasing the longevity of IGF-I's half life as well as not allowing IGF-I to leave the vascular compartment easily thus preventing its early clearance from the body (Jones & Cleemons, 1995).

The regulation of IGFBP-3 appears to be controlled primarily by GH and controversy surrounds the question of whether IGF-I also regulates this binding protein (Blum, 1996; Corpas et al., 1993). Recent studies have shown that ALS glycoprotein is solely synthesized by hepatocytes and is strictly controlled by GH not IGF-I, thus this lends more credibility to GH control of IGFBP-3 production than IGF-I (Blum, 1996; Corpas et al., 1993; Hussain et al., 1996).

3.2.4. IGF-I RECEPTORS

Once IGF-I has been carried to its destination it binds to another high affinity receptor (surface cell receptors) which once again shares structural homology with insulin receptors (D'Ercole, 1996). The interaction of IGF-IBP complex with the receptor results in a conformational change of the receptor (tyrosine phosphorylation) which then leads to a cascade of events within the cell setting in motion a variety of cytosolic signaling networks and ultimately results in IGF-I mediated action (differentiation, cell proliferation, matrix maintenance etc..) (D'Ercole, 1996; Donnelly & Holly, 1996; Jones & Cleemons, 1995). Exact pathways are still under investigation, however it is known that the coupling and transformation of these receptors are what mediate IGF-I and GH action in the cell, and it is these mediated actions which play a critical role in local tissue homeostasis (D'Ercole, 1996; Gilmour, 1994).
3.2.5. **Growth Hormone and Osteoarthritis**

It has been speculated that GH plays a role in OA such that decreased levels of GH secretion result in decreased levels of IGF-I found in synovial fluid in the joints thus preventing chondrocyte maintenance. In addition it is thought that decreased GH results in increased adiposity and body mass which has also been shown to be correlated with knee OA (Felson, et al., 1988; Hart & Spector, 1993; Hochberg et al., 1995; Spector et al., 1994). Preliminary research in vitro has shown that when GH is released systemically and latches onto its GH receptor on immature chondrocytes it stimulates IGF-I expression and synthesis (Barnard, Kaynes, Werther & Waters, 1988; Isaksson, Lindahl, Nilsson & Isgaard, 1987). This would account for local tissue production of IGF-I. However in adults this actual mechanism has not been demonstrated and in fact is poorly defined. When Dore et al conducted their study on adult chondrocytes he found that there was no relationship between systemic GH and local IGF-I production (Dore et al., 1995). Dore concluded that GH receptor was not present in adult chondrocytes thus could not stimulate the expression of IGF-I locally.

Presently, studies conducted on the role of GH, IGF-I and OA appear to be diametrically opposed. Some studies communicate that serum levels of IGF-I are greatly reduced in individuals with OA (Moskowitz, Boja & Denko, 1991; Trippel, 1995), while others inform that there is no difference between age matched controls and individuals with OA (Hochberg, Lethbridgecejku & Scott, 1994; Matsumoto et al., 1996; McAlindon, Teale & Dieppe, 1993; Moskowitz et al., 1991; Trippel, 1995). Yet these same studies who reported subjects with normal serum IGF-I levels also reveal elevated synovial levels of IGF-I in SF content. These inconsistent findings would suggest that elevated IGF-I levels may indicate a catabolic state in a joint, signifying a lack of homeostasis and/or AC attempting to repair itself (Matsumoto et al., 1996). It also implies that there is some local factor(s) which may be the impetus to IGF-I levels responding to AC destruction, rather than central stimulus. Unfortunately this just reinforces that at present there is no indisputable evidence which supports the argument that GH stimulates IGF-I peripherally (specifically chondrocytes) thus reinforcing that more research is required to determine if in fact GH and IGF-I do play a role in knee OA.
3.2.6. GH AND IGF-I IN THE AGED:

Aging results in many tissues losing their ability to regenerate, maintain and continue to function optimally. Why these tissues "age" may be a question of degeneration of the tissue or a question of decreased or absent stimulative factors which previously maintained that tissue's homeostasis. Aging has been shown to reduce the amount of GH and IGF-I (which functions to maintain and promote tissue health) secreted in serum and locally in tissues. Levels of IGF-I systemically have been well documented by many individuals and all reports show a decline with age (See Table 3.2.7.1.) (Bellantoni et al., 1996; Benbassat, Maki & Unterman, 1997; Binnerts, Deurenberg, Swart, Wilson & Lamberts, 1992; Blum, Blum, 1996; Denko, Boja & Moskowitz, 1990; Femihough et al., 1996; Hochberg et al., 1994; Hoffman & Ho, 1996; Lloyd et al., 1996; McAlindon et al., 1993; Moskowitz et al., 1991; Rudman & Mattson, 1994; Schouten et al., 1993; Thompson et al., 1995; Vitiello et al., 1997).
**Documented Serum Levels of IGF-I (Age of 55+) in the Literature**

<table>
<thead>
<tr>
<th>INVESTIGATOR</th>
<th>AGE</th>
<th>COMBINED GENDER</th>
<th>MALE IGF-I VALUES</th>
<th>FEMALE IGF-I VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benbassat et al 1997</td>
<td>71.2</td>
<td>N/R</td>
<td>154 ± 8 (ng/mL)</td>
<td>N/R</td>
</tr>
<tr>
<td>(n=38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bellantoni et al 1996</td>
<td>61.6</td>
<td>N/R</td>
<td>N/R</td>
<td>161 ± 24 (µg/L)</td>
</tr>
<tr>
<td>(n=16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blum, Werner (review)</td>
<td>50-70 yrs</td>
<td>98.0 - 169.0 (µg/L)</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Denko et al 1990</td>
<td>63 yrs M</td>
<td></td>
<td>HC: 21.0 ± 5 (nM/L)</td>
<td>OA: 14.2 ± 4 (nM/L)</td>
</tr>
<tr>
<td>(n=71)</td>
<td>67 yrs F</td>
<td></td>
<td></td>
<td>OA: 10.2 ± 3 (nM/L)</td>
</tr>
<tr>
<td>Fernihough et al 1996</td>
<td>69</td>
<td>HC: 167 ± 72 (µg/L)</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>(n=16)</td>
<td></td>
<td>OA: 146 ± 72 (µg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoffman et al 1994</td>
<td>47.3 HC</td>
<td>HC: 150 ± 11 (µg/L)</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>(n=58)</td>
<td>45.0 GHD</td>
<td>GHD: 85 ± 10 (µg/L)</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Llyod et al 1996</td>
<td>54.2</td>
<td>N/R</td>
<td></td>
<td>23.0 ± 7.0 (nM/L)</td>
</tr>
<tr>
<td>(n=761)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McAlindon et al 1993</td>
<td>71.8 yrs</td>
<td>HC: 16.4 ± 5.4 (nM/L)</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>(n=177)</td>
<td></td>
<td>OA: 15.9 ± 4.7 (nM/L)</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Rudman et al 1994</td>
<td>70.0 **</td>
<td>N/R</td>
<td>219.6 ± 50.4 (µg/L)</td>
<td>N/R</td>
</tr>
<tr>
<td>(n=120)</td>
<td>65.4 †</td>
<td></td>
<td>168.3 ± 57.1 (µg/L)</td>
<td></td>
</tr>
<tr>
<td>Schouten, et al 1993</td>
<td>57.4 yrs</td>
<td>19.6 ± 8.0 (nM/L)</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>(n=141)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thompson et al 1995</td>
<td>71.9 yrs</td>
<td>N/R</td>
<td>N/R</td>
<td>95.4 ± 16.8 (µg/L)</td>
</tr>
<tr>
<td>(n=16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitiello, et al 1997</td>
<td>66.9 yrs M</td>
<td>122.4 ± 8.0 (µg/L)</td>
<td>134.7 ± 8.7 (µg/L)</td>
<td>110.7 ± 9.1 (µg/L)</td>
</tr>
<tr>
<td>(n=52)</td>
<td>67.1 yrs F</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2.6.1. Serum IGF-I values that are currently reported in the literature in those over the age of 55 and across various populations. IGF-I levels reported in the table are reported by various units (ie/ nM/ml, ng/L µg/L, and nM/L), however current literature reports IGF-I in µg/L. Short forms used in the table are as follows; M=male, F=female, N/R=not reported, HC=healthy control, and GHD=growth hormone deficient, ** indicates elderly sedentary subjects, and † indicates active elderly.

Values in young males and females aged 20 - 30, typically will range from 158 - 471 µg/L, where 158 µg/L represents the 0.1 percentile and 471 µg/L represents the 95th percentile (Blum, ).
As individuals get older, smaller amounts of GH are secreted which results in increased obesity, decreased muscle mass and bone mass, reduced rates of protein synthesis and impaired healing (Borst et al., 1994; Corpas et al., 1993; Cuttler, 1996; O'Connor, Stevens & Blackman, 1996; Shetty & Duthie, 1995). It has been estimated that lean body mass is reduced by 27% in men and 15% in women and conversely adiposity increased 18% in men and 12% in women by age 70 (Corpas et al., 1993). It has been speculated that the increase in adiposity reduces GH secretion through a feedback mechanism resulting in further adiposity (Corpas et al., 1993; Cuttler, 1996; Shetty & Duthie, 1995). There also appear to be selective patterns in adiposity whereby the elderly selectively deposit fat in the abdominal area which has also been associated with GH hyposecretion (Corpas et al., 1993).

GH secretion is reduced anywhere from 15-70% (compared to IGF-I levels of 20 year old people) in individuals aged 40-65 years of age (Borst et al., 1994; Corpas et al., 1993; Cuttler, 1996; Fernyhough et al., 1996; O'Connor et al., 1996; Shetty & Duthie, 1995; Zadik, Shalew, McCarter, meistas & kowarski, 1985). Some studies suggest that there is a 14% decrease of GH secretion with each decade of life and the GH half life also falls by 6% (Corpas et al., 1993; Iranmanesh, Lizarralde & Veldhuis, 1991). Furthermore studies show that past age 70 greater than 40% of all adults have serum IGF-I levels in the range found in GHD children (Shetty & Duthie, 1995).

Aging results in changes in the anterior pituitary resulting in a moderate decrease in size, along with histologic changes which include fibrosis, necrosis and cyst formation (Shetty & Duthie, 1995). Along with these histologic changes, it appears that the anterior pituitary also has a reduction in GHRH receptors and a decrease in the sensitivity of the remaining receptors available (Shetty & Duthie, 1995). All of these changes reduce release from somatotrophs in the pituitary. Yet there is no primary explanation as to why GH levels are reduced as humans age. It is speculated that outside of changes to the pituitary that there is a reduction in GHRH secreted from the hypothalamus as well as an increase in somatostatin tone which ultimately reduces GH release from the pituitary (Borst et al., 1994; Corpas et al., 1993; Cuttler, 1996; Shetty & Duthie, 1995). In addition to reduction of GH promoting hormones and increases in inhibitory GH hormones, there is also evidence that the number of GHBP are reduced as aging occurs and may play a factor in reduced serum GH levels (Corpas et al.,
However the decline in GHBP has been suggested to be rather variable in individuals as well as gender selective, occurring exclusively in males (Corpas et al., 1993; O' Connor et al., 1996). In addition the reduction in GHBP may be a reflection of lost peripheral GH receptors which are the precursors to GHBP production (Corpas et al., 1993). Whether there is a reduction in the production and secretion of GH by somatotrophs, reduced production of GHRH , reduced GHRH receptors, reduced GHBP or increased somatostatin tone, all these factors ultimately translate into impaired tissue function. This impairment may be exacerbated further with physiological factors mentioned earlier which would affect the release rate and amplitude of GH release such that the tissue rate of loss is accelerated.

Age also appears to affect serum IGF-I levels such that by the seventh decade there is a 30-50 % drop in circulating hormone (Corpas et al., 1993; O'Connor et al., 1996; Shetty & Duthie, 1995). It appears that a bimodal drop in IGF-I levels occurs with a rather steep decline occurring between 30-40 years of age and a second steep decline occurring from 80-90 years of age (O'Connor et al., 1996). There also appears to be a concomitant drop in IGFBP-3 with age which may fall in line with the reduction in circulating levels of GH (Corpas et al., 1993). Another change which occurs with aging is that IGF-I no longer correlates well with GH levels present in the body. As a result serum IGF-I levels appear less useful indicators of GH secretion in the elderly (O'Connor et al., 1996).

Even though the literature supports declines in serum IGF-I levels with age, and further declines in serum levels of those with OA, some studies focusing on local environments have revealed interesting results. Many studies have shown that there is in fact an increase in IGF-I levels in synovial fluid (SF) in individuals having OA of the knee (Dore et al., 1995; Matsumoto et al., 1996). It is also suggested that IGF-I is directly stimulated by and correlated to the levels of catabolic proteins IL-1, IL-6 and TNF- α (which degrade the AC of the knee) present in the SF. This finding may suggest that IGF-I has two different control mechanisms, one which is governed by GH systemically, and the other which is governed by conditions present in local tissue. As this area of control is not well established, one must consider that both systems may play a role in OA progression.
3.2.7. **Measuring GH and IGF-I Systemically**

Review of the literature reveals that the most important regulator of serum IGF-I and IGFBP-3 is GH (Blum, ; Blum, 1996; Corpas et al., 1993; Hoffman & Ho, 1996). Due to this relationship, the status of GH secretion appears to be directly reflected in serum and tissue levels of IGF-I and IGFBP-3 (Blum, ; Blum, 1996). Clinically though it is difficult to measure GH directly due to its diurnal variations and pulsatile secretion, therefore an initial and useful clinical measure (for screening potential GH deficiency) is serum IGF-I value. Despite elements which affect IGF-I production (GH, stress, nutrition, immune system), actual serum levels of IGF-I appear to be relatively stable with little or no diurnal variation thus making IGF-I a more convenient index of GH secretion than measuring GH itself (Blum, 1996; Lloyd et al., 1996). As previously mentioned due to GH being released in a pulsatile fashion, measurement for clinical diagnosis is rather difficult without frequent samples over 24 hours. Thus using IGF-I which has little variation may be a better preliminary indicator of serum GH levels (Blum, ; Blum, 1996; Borst et al., 1994). The changes in IGF-I levels over a lifetime mimic those of GH, in that pubescence has the highest levels of GH and IGF-I and there is a gradual decline in both with age (Hoffman & Ho, 1996). Hence it is this similarity in ontogeny that makes serum IGF-I a good approximation of GH levels and permits some assurance that the values reflected were similar to the available amounts of GH (Hoffman & Ho, 1996).

Recent research has also revealed that there is an exponential relationship between IGF-I and IGFBP-3 levels in biological tissues which suggests that both variables can be considered as good measures of GH secretion in an organism (Blum, ; Blum, 1996).

3.3. **Physiology of Cytokines:**

"Disease morbidity and mortality is frequently caused by an over expression of cytokines" (Tracey & Cerami, 1993). Cytokines are a group of family of pleiotropic proteins or glycoproteins which are produced in picomolar or nanomolar responses to infection, invasion, injury, inflammation or other stimuli and function to biochemically change and/or regulate host function (Hamblin, 1993; Tracey & Cerami, 1993; Westacott & Sharif, 1996). However the term
cytokine is quite generic and in today's environment inappropriate as cytokine literally means "release from cells" which ignores its potentially deleterious or positive effects (Westacott & Sharif, 1996). Early investigations presumed that cytokines acted in the "best interest" of the host, however recent studies show that they are more prone to cause death rather than maintenance (Trippel, 1995; Westacott & Sharif, 1996).

Cytokines as mentioned previously are released due to stimuli. They function in a local environment via autocrine (cells that produce them) or paracrine (adjacent cells) methods as their half-life is relatively short (Westacott & Sharif, 1996). With respect to the association held between cytokines and OA, that remains largely unknown at present (Pelletier et al., 1993; Westacott & Sharif, 1996). The concept of cytokine mediated destruction with respect to OA came into focus years ago with the work of Fell and Jubb which demonstrated via organ cultures that synovial tissue could induce articular destruction (Fell & Jubb, 1977; Pelletier et al., 1993). Since then many studies have followed in animal and human models trying to establish mechanisms of cartilage destruction in hopes of finding a therapy to block the destruction.

Much research has been done on cartilage metabolism and its successive degradation in both animal models and humans. Research to date shows that there is a complex feedback system locally and systemically of cytokines and hormones which affect turnover rates of many tissues such as the ECM of AC. The exact system (final common pathway) in which these catabolic and anabolic proteins interact is largely unknown as the mechanisms have not been subjected to intense scrutiny (See figure 3.3.1.) (Pelletier et al., 1993; Westacott & Sharif, 1996). What is known is that pro-degradation cytokines affect the cell by attaching themselves to specific receptors on the surface membrane, which then signals intra-cellularly to the nucleus and alters the DNA. This signaling then causes the cell to produce the proteolytic enzymes (metalloproteases, collagenase, gelatinase, stromelysin, prostaglandin E2) and stimulates the release of other cytokines and potentiators (tissue plasminogen activator, bFGF and PDGF) to help destroy the AC (Hollander, Dieppe, Atkins & Elson, 1993). Conversely, anabolic cytokines inhibit degradation of tissue with competitive inhibition of the catabolic binding sites on cells thus preventing the transcription of DNA and ultimately preventing the production of products for cell destruction. The exact levels required to promote either
anabolism or degradation of the AC are largely unknown but several investigators have attempted to map out what levels are existent in specific populations (OA population) and healthy controls (See Table 3.3.1.) (Bertazzolo et al., 1994; Cameron, Fu, Paessler, Schneider & Evans, 1994; Fernihough et al., 1996; Fong, Boey, Koh & Feng, 1994; Kahle et al., 1986; Matsumoto et al., 1996; Schneiderman et al., 1995; Walker, 1996).
### Synovial Levels of Cytokines and Growth Factors in Various Populations Cited in the Literature

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Age</th>
<th>Fluid</th>
<th>Group</th>
<th>IL-1 α (pg/ml)</th>
<th>IL-1 β (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>IGF-I (µg/L)</th>
<th>IRAP (pg/ml)</th>
<th>TGF-β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bertalozzo et al 1994</td>
<td>NR</td>
<td>SF</td>
<td>OA patients</td>
<td>ND</td>
<td>ND</td>
<td>89.4 ± 120.5</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>Cameron et al 1994</td>
<td>NR</td>
<td>SF</td>
<td>NR</td>
<td>ND</td>
<td>11.56</td>
<td>51.30 ± 13.4</td>
<td>9.17</td>
<td>NM</td>
<td>NM</td>
<td>5.12 ± 1.50</td>
</tr>
<tr>
<td>Fernihough et al 1996</td>
<td>69</td>
<td>SF</td>
<td>M and F OA patients</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>78 ± 26</td>
<td>146 ± 60</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>SF</td>
<td>M and F HC</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>26 ± 6</td>
<td>167 ± 72</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td>Fong et al 1994</td>
<td>49</td>
<td>SF</td>
<td>IL-1 type not specified</td>
<td>267 ± 58</td>
<td>NM</td>
<td>19 ± 0.5</td>
<td>35 ± 4.9</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>M and F OA patients</td>
<td>140 ± 11</td>
<td>NM</td>
<td>80.0 ± 30</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td></td>
</tr>
<tr>
<td>Kahle et al 1992</td>
<td>58</td>
<td>SF</td>
<td>M and F OA patients</td>
<td>NM</td>
<td>21 ± 13</td>
<td>88 ± 25</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td></td>
</tr>
<tr>
<td>Matsumoto et al 1996</td>
<td>69</td>
<td>SF</td>
<td>M and F</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>88 ± 25</td>
<td>NM</td>
<td>NM</td>
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<tr>
<td>Schneiderman et al 1995</td>
<td>73</td>
<td>SF</td>
<td>M and F HC</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>31.0 ± 12.5</td>
<td>150.3 ± 66.2</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>SF</td>
<td>M and F OA patients</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>73.6 ± 28.9</td>
<td>152.3 ± 28.9</td>
<td>NM</td>
<td>NM</td>
</tr>
</tbody>
</table>

Table 3.3.1 represents values reported in the literature on cytokines and IGF-I found in synovial and serum fluid. Short forms used are as follows; SF = synovial fluid, S = serum, HC = healthy controls, OA = osteoarthritic, M = male, F = female, NR = not reported, NM = not measured and ND = not detectable.
3.3.0. Catabolic Cytokines

3.3.1. Interleukin-1 (IL-1)

IL-1 was first identified in the late 1970's as an inflammatory mediator which was capable of tissue damage as well as being a potentiator of other catalytic mediators which evoked cellular damage (Westacott & Sharif, 1996). IL-1 is a part of the endogenous pyrogen family and was initially thought of as a mediator of fever (Dinarello, 1989). It was not until later
that the multiple roles as well as the multiple sites of production were identified (Dinarello, 1989).

IL-1 has been identified in several lines of human tissue, including connective tissue (synoviocytes, chondrocytes, osteoblasts, and monocytes) (Lotz et al., 1995; Pelletier et al., 1991). IL-1 is somewhat similar to IGF-I in that it has a role systemically as well as locally which are quite opposite. Systemically IL-1 levels are usually quite low and any large increases will result in hypotension, shock and in animals have been found to be lethal (Dinarello, 1994). Locally, IL-1 functions more as a mediator to other proteins in the local destruction of tissue. Some researchers believe it has no role to play in the normal joint homeostasis whereas others suggest that it is part of the cascade of events required to maintain a balance in the joint environment (Dinarello, 1994). As a result of its catabolic activity, it has come under much scrutiny especially with respect to its local effects and how it contributes to the pathophysiology of OA.

IL-1 is primarily produced by both macrophages and monocytes. When produced by these aforementioned cells IL-1 functions chiefly as an inflammatory agent whereas IL-1 produced by connective tissue appears to have a role in normal tissue turnover and in the maintenance of joint homeostasis (Pelletier et al., 1991). There are two forms of IL-1, one is an alpha type (IL-1α) which is the acidic form and the other is the beta form (IL-β) which is neutral. Each form of IL-1 is derived from a different amino acid sequence, yet they are related in that they both adhere to the same cell receptor and have similar biological activities (Dinarello & Wolff, 1993). Both forms also have a high affinity for surface receptors of chondrocyte cells, and both bind similarly (Bandara & Evans, 1992; Pelletier et al., 1991).

In joints IL-1 is produced by both chondrocytes and synoviocytes. It appears though that there is a preferential secretion of IL-1 β over IL-1α in synoviocytes for unknown reasons (Bandara & Evans, 1992). In pathology free individuals, IL-1 levels in serum and synovial fluid are quite small, less than 20 pg/ml and are usually not detectable by sandwich ELISA's (enzyme linked immuno-sorbent assays). Usually the limit of detection is 40 pg/ml in most assays, thus reinforcing the difficulties with trying to establish a profile of normal IL-1 levels in both serum and synovial fluid. Auxiliary problems would still be present even if assays were
very sensitive as IL-1 α remains in a pre-cursor form in the cytosol of cells or in synovial fluid and only functions in an autocrine or biologically active paracrine messenger to abutting cells (Dinarello & Wolff, 1993). Conversely, IL-1 β is also in an inactive form in the actual cell and it becomes active only with a portion of its protein being cleaved off by IL-1 β converting enzyme. Once the cytokine is activated it is then released either by exocytosis, active transport or by the death of the cell. Several studies show that in OA pathology there is an abundance of IL-1 α found in the extracellular spaces or in circulation as a result of being released by synoviocytes (Bandara & Evans, 1992; Dinarello & Wolff, 1993). With both proteins initially being in an inactive state, any bioassay technique is almost futile as it is not able to detect inactive forms. Additional problems which occur with detection are that IL-1 α is released at different times than IL-1 β, thus to see their interactions is quite arduous (Bandara & Evans, 1992). Despite the difficulty of just detecting inactive forms there are other factors to contend with such as many inhibitors of IL-1 (α and β) in synovial fluid and in serum which again, make it difficult to detect and produce an IL-1 profile (Bandara & Evans, 1992).

IL-1 may aid in joint homeostasis by potentiating or activating other catabolic cytokines. It is well known that IL-1 produced by synoviocytes activates IL-6 which is also a catabolin and destroys AC. Both IL-1 and IL-6 (where IL-1 is the stronger of the two) activate chondrocytes to produce additional catabolic cytokines such as; IL-1, PGE₂, metalloproteases/enzymes (collagenase, gelatinase, stromelysin), chondrocyte activating factors, plasminogen activators potentiators (bFGF, PDGF) (Bandara & Evans, 1992; Cameron et al., 1994; Dinarello & Wolff, 1993; Lotz et al., 1995; Pelletier et al., 1991). IL-1's strongest ability is to affect the macromolecular structure of the ECM by potentiating the synthesis and release of other matrix degraders (metalloproteases, stromelysin) which when released in unison have the ability to destroy the whole macro-molecular structure of the ECM of the chondrocyte (Pelletier et al., 1993). Ultimately, IL-1 is a strong catabolic mediator which activates other factors to initiate chondrocyte death.

Conversely, IL-1 also activates inhibitors or antagonists which would prevent its function. These inhibitors are Interleukin I Receptor Antagonist Protein (IRAP) and soluble IL-1 receptor. Both function similarly in that they block the binding sites of IL-1 on the chondrocyte thus limiting the amount of activation of other catabolic cytokines as well as limiting the amount
of overall destruction. As one can see (Figure 3.3.1.) IL-1 plays a critical role in joint homeostasis in that if it is not kept in check, IL-1 can activate many other factors which ultimately will destroy the AC of any joint and potentially result in OA.

3.3.2. TUMOR NECROSING FACTOR - α (TNF-α), INTERLEUKIN-6 (IL-6), AND OTHER RELEVANT CYTOKINES

Much study has been focused on IL-1 and its actions on tissues as it is a very potent mediator of destruction. As a result to date not much information has been reported on other catabolic cytokines as contributors to OA such as IL-6 and TNF-α. Of the information that exists it is known that both are contributors to AC damage and chondrocyte death.

TNF-α is produced by type A synoviocytes, macrophages, monocytes, B cells, T cells, NK cells, glial cells, and adipocytes which aids in the destruction of chondrocytes (Cameron et al., 1994; Tracey & Cerami, 1993; Westacott & Sharif, 1996). TNF-α closely resembles IL-1 in that they share similar effects on chondrocytes, however it is a weaker agonist than IL-1 (Tracey & Cerami, 1993; Westacott & Sharif, 1996). TNF-α has been reported to have one tenth of the potency that IL-1 has in terms of destruction (Tracey & Cerami, 1993; Walker, 1996). However when coupled with IL-1 this results in a "synergistic toxicity" which has disastrous consequences on living tissue (Tracey & Cerami, 1993). Specifically in the knee joint, TNF-α functions to reduce PG synthesis as well as degrade PG in the ECM, decrease the amount of type II and IX collagen in the chondrocyte thus affecting the ECM, and stimulate PGE2, IL-6, plasminogen activator and nucleometalloproteases (NMP’s) (Bandara & Evans, 1992; Lotz et al., 1995; Pelletier et al., 1991). These latter activations destroy ECM and ultimately cause chondrocyte death and aid in OA pathology. Akin to IL-1, TNF-α is also inhibited by TNF-α receptor which binds to the chondrocyte surface receptors and blocks the binding thus the activation of PG destruction.

Once again the quantity of TNF-α present in synovial fluid is usually small. It appears that the levels are found to be higher in patients with OA versus those with RA, however once again detection is difficult due to small quantities present normally in tissue (Westacott & Sharif,
Conflicting evidence also exists in the literature with respect to OA pathology in that some researchers have found elevated TNF-α levels (di Giovine et al. 1988 and Brennan et al. 1992) where as others found no elevation in TNF-α (Martel-Pelletier et al. 1990) as reported by Westcott et al (Westacott & Sharif, 1996).

One must discuss IL-6, metalloproteases and plasminogen activators in unison. All of these aforementioned factors need some sort of stimulus from another other catabolin in order to be synthesized or activated. What is surprising is that all these catabolins, with the exception of IL-6 which is synthesized by synoviocytes and induced by IL-1, are made by chondrocytes, the tissue that is actually being destroyed (Middleton, Manthey & Tyler, 1996). However just as IL-6 is able to activate chondrocyte death, it also activates inhibitors to keep its destructive capabilities in check. Again this is the case with all the NMP's, whereby their activation of ECM lysis also activates TIMP (tissue inhibitors of metalloproteases) which inhibits their function by binding to receptors such that their destructive effects cannot manifest themselves intracellularly (Westacott & Sharif, 1996).

IL-6 though is somewhat unique as it is a multi-functional cytokine in that it acts in some situations as an anti-inflammatory and in others as a mediator of joint destruction (Middleton et al., 1996; Westacott & Sharif, 1996). Since IL-6 functions in a dual capacity, it is becoming more apparent that one of its role is a regulator or limiting factor in absolute AC damage via feedback mechanisms (Middleton et al., 1996; Westacott & Sharif, 1996). However at present little can be concluded about IL-6's role in OA as the literature contains controversy.

3.3.3. ANABOLIC CYTOKINES

Chondrocytes are pleiotropic in nature such that they are able to produce pernicious substances, and also quite capable of self-preservation by producing anabolic substances to maintain their cell environment and ultimately allow the joint to function in a manner in which it was designed to do. Many of these anabolic factors are produced in a check and balance way just as the catabolins, and an augmentation in the levels of "growth factors" will stimulate the chondrocyte to produce factors which will limit growth (Malemud, 1993; Westacott & Sharif,
There are also other factors which are not part of joint homeostasis per say, rather they are inhibitors which react to an upsurge of catabolins and attempt to limit the amount of destruction. These factors may also be listed under the category of anabolic systems as they allow limited destruction and allow certain growth factors to function better.

3.3.4. Transforming Growth Factor Beta (TGF-β)

TGF-β belongs to a family of growth factors which have many isoforms that act on AC in various stages of development to allow for cell division or cell maintenance (Jahng, Lee, Han, Kim & Yoo, 1997; Malemud, 1993; Westacott & Sharif, 1996). TGF-β is produced by many cells in the body including chondrocytes (Malemud, 1993; Westacott & Sharif, 1996). TGF-β has been reported to be an influential factor on chondrocyte metabolism and potentiator of other anabolic factors (Jahng et al., 1997).

TGF-β has been studied profusely yet still remains rather enigmatic as diametrically opposed effects on tissue have been reported (Lafeber, Vander Kraan, Huber-Bruning, Vanden Berg & Bijlsma, 1993; Posever, Phillips & Pottengr, 1995; van den Berg, 1995). Through in vitro studies it has been shown that TGF-β has an inhibitory effect on PG and type II collagen synthesis on the explant culture of chondrocyte cells initially. However, following prolonged exposure, the addition of fibronectin or other agents, or depending on the stage of development in which the cell was in, TGF-β stimulated chondrocytes to produce PG and collagen (Malemud, 1993; Trippel, 1995; van den Berg, 1995). To elucidate how specific TGF-β is on tissue, some studies have revealed that if a cell is in the S phase of mitosis, there was enhanced proliferation of chondrocytes, however, if it was in the G phase TGF-β inhibited proliferation. But overwhelming evidence does show that TGF-β is more of an anabolin than a catabolin. In fact TGF-β is believed to have a more potent growth and mitogenic influence on cells than IGF-I (Guerne, Blanco, Kaelin, Desgeorges & Lotz, 1995; Guerne, Sublet & Lotz, 1994; Lotz et al., 1995; van den Berg, 1995). Yet seeing that it has some sort of catabolic effect via inhibition, it is uncertain as to how TGF-β will respond in an in vivo environment.
TGF-β is produced by synoviocytes in local tissue of a knee joint. TGF-β in local tissue predominantly behaves as an anabolic modulator by counter-acting IL-1 by down regulating its receptors on the cells, increasing interleukin 1 receptor antagonist protein (IRAP) expression, increasing enzyme inhibitors and up regulating PG synthesis in the chondrocyte (Hardingham, Bayliss, Rayan & Noble, 1992; Lotz et al., 1995; Malemud, 1993; van den Berg, 1995; Westacott & Sharif, 1996). Finally TGF-β also promotes the calcification of ECM thus leading to osteophyte formation along the joint line of knee joints (Hardingham et al., 1992; Lotz et al., 1995; Malemud, 1993; Trippel, 1995; van den Berg, 1995). It is hypothesized that TGF-β plays a very critical role in homeostasis of the knee joint as it reduces the effects of IL-1 which is a modulator of many other catabolins, as well TGF-β also acts as a stimulator of TIMP and other receptor antagonists which limit the amount of degeneration.

3.3.5. INTERLEUKIN 1 RECEPTOR ANTAGONIST PROTEIN (IRAP)

IRAP is the receptor antagonist which is a new addition to the IL-1 family and has only been identified in the past few years. IRAP has similar weight and is structurally related to IL-1 (Dinarello & Wolff, 1993). Unlike IL-1 or other catabolic cytokines, IRAP functions solely as an agonist such that it is a competitive inhibitor with IL-1 for the surface cell receptor on all chondrocytes (Dinarello & Wolff, 1993; Hung et al., 1994). IRAP also limits the stimulatory effect that IL-1 has on other catabolic cytokines (Dinarello & Wolff, 1993). However, IRAP is not considered to be a strong inhibitor of IL-1 as it only occupies few receptor sites thus limiting its ability to reduce AC death (Hung et al., 1994). Yet in studies it appears to function well in preventing OA degradation. Pelletier et al observed that treatment with IRAP subsequent to experimentally inducing OA in an animal model, limited the disease progression (Pelletier et al., 1997). Thus IRAP’s full potential as protector/inhibitor of AC has not been fully explored.

It appears that IRAP is not involved with day to day tissue turnover and joint homeostasis, rather it becomes activated solely when there is an imbalance in the level of IL-1 present in the synovial fluid (Dinarello & Wolff, 1993; Dinarello, 1994). Once activated, IRAP’s production is further enhanced by other growth factors such as TGF-β which act as a goad to IRAP production and result in further increased levels (Dinarello & Wolff, 1993). Preliminary
research suggests that in disease free individuals, IRAP will not be found in plasma or synovial fluid, only in pathological or in a state of imbalance will the levels of IRAP increase so that they become detected. It appears that IRAP is more of a counter-mechanism to destruction of tissue not protein which is involved with the maintenance of proliferation of AC.

3.3.6. Cytokine Analysis

Cytokine analysis has been an exciting area in the scientific community as levels of certain cytokines have been associated with identifying the presence of certain disorders or quantifying their severity (Heney & Whicher, 1995; Mire-Sluis, Gaines-Das & Thorpe, 1995; Roux-Lombard & Steiner, 1992). As cytokines are found in biological fluids in pico-molar amounts, they are usually undetectable by many methods unless pathology arises and there is a significant increase in their production (Heney & Whicher, 1995; Mire-Sluis et al., 1995). Thus quantifying normal concentrations of cytokines in SF are generally not possible (Westacott & Sharif, 1996). Presently the most common method used to analyzed cytokines in serum or synovial fluid are Enzyme Linked Immuno-sorbent Assay (ELISA) kits. (Heney & Whicher, 1995; Herzyk, Berger, Allen & Wewers, 1992; Mire-Sluis et al., 1995; Ravindranath, Ravindranath, Morton & Graves, 1994; Roux-Lombard & Steiner, 1992; Wicher & Ingham, 1990). These kits are used extensively due to their reproducibility, quantitative results and the requirement of little antigen or antibody (sera) whereas other immunoassays require greater amounts (Herzyk et al., 1992; Ravindranath et al., 1994). However errors and problems have been associated with the use of ELISA kits and identification of cytokines in biological fluids due to storage and preparation of the biological fluid, selection of microtitre plates, coating of wells with glycolipid, use of blocking procedure and detergent, use of varying test tubes, ‘in vitro’ release of cytokines, alteration of standard kits into in-house assays, interference from antibodies and choice of anti-coagulent (Heney & Whicher, 1995; Herzyk et al., 1992; Mire-Sluis et al., 1995; Ravindranath et al., 1994; Riches, Gooding, Millar & Rowbottom, 1992; Roux-Lombard & Steiner, 1992; Wicher & Ingham, 1990). It has been reported that variations may be as high as 20-25 % (Heney & Whicher, 1995; Jeffcoate & Das, 1977; Mire-Sluis et al., 1995; Wicher & Ingham, 1990). In addition not all kits are alike where identical biological solutions will result in different results depending on the kits used immunoassays (Roux-Lombard & Steiner,
As a result of these aforementioned problems the use of ELISA kits commercially is limited but as improvements continue to be made in the detection of cytokines, the kits may be more readily available (Heney & Whicher, 1995; Mire-Sluis et al., 1995).

Yet, despite the problems associated with ELISA or bioassays, these tools are generally quite sensitive to levels of cytokines in biological and are often more sensitive than immunoassays (Heney & Whicher, 1995; Mire-Sluis et al., 1995; Wicher & Ingham, 1990). In a comparison done by Whicher et al it was found that bioassays were more sensitive to cytokine levels than immunoassays however their precision was poor (Heney & Whicher, 1995; Mire-Sluis et al., 1995; Wicher & Ingham, 1990). Conversely, immunoassays were found to be quite susceptible to background noise, interfering anti-bodies and they require high signal levels in order to get results (Wicher & Ingham, 1990). In a study conducted by Roux-Lombard et al it was revealed that bioassays were able to detect synovial levels of TNF-α, IL-6, and IL-1β better than immunoassays, and in both assays tested (biological or immunoassay) IL-1α was almost always un-detectable (Roux-Lombard & Steiner, 1992). In fact synovial levels of TNF-α were undetectable using immunoassays (Roux-Lombard & Steiner, 1992).

ELISA techniques for detecting synovial fluid levels of cytokines appears to be the best measuring technique available at present (Mire-Sluis et al., 1995; Riches et al., 1992; Wicher & Ingham, 1990). Generally, it is suggested by most investigators attempting to show reliability, validity, sensitivity and specificity of any commercial or laboratory kit that variability can be minimized when the identical kits or in-house concoctions are used to sample each fluid, the preparation includes ethanol washing of plates, irradiation of plates, use of identical matrix, running duplicate and triplicate samples, measuring solely the active form of IL-1α, running standard curves, not coating the wells and using blocking agents to decrease non-specific binding (Heney & Whicher, 1995; Herzyk et al., 1992; Mire-Sluis et al., 1995; Ravindranath et al., 1994; Riches et al., 1992; Roux-Lombard & Steiner, 1992; Wicher & Ingham, 1990).

In this study the individual who analyzed the cytokine samples was well experienced in synovial fluid analysis, and followed commercial kit instructions exactly. Error was reduced by following the procedure discussed above. In addition standard curves were run and plotted
(see appendix) which identified that all antibodies used were being identified correctly as per the kits’ instructions, as well as duplicate samples were run to ensure consistency.

3.4.0. Pathophysiology of Osteoarthritis

3.4.1. Knee Joint Homeostasis in OA

In humans most systems rely on a harmonious series of checks and balances to allow for optimal functioning, which result in a total homeostatic condition throughout the body. Should any bodily mechanisms become in a state of unbalance, a cascade of deleterious or over compensatory events may occur which could result in dysfunction, limitations, chronic conditions and ultimately death if not counterbalanced. However most states of imbalance are usually transient until the body can accommodate or adjust to the new status.

As we age, normal changes occur in our musculoskeletal system. We see declines in type II fibers of muscle, reduced GH production, reduced cartilage thickness, cerebral atrophy etc... . However our body continues to function as optimally as possible adjusting and compensating for these naturally occurring changes. Certain imbalances which are associated with aging, or some other factor (ie/ prolonged obesity, trauma etc.. ) that the body can no longer accommodate results in impairment, disability and ultimately handicap. One specific example can be seen in the knee joint, whereby shifting to a state of unbalance may result in a debilitating condition, primarily OA.

Normally, the knee joint itself undergoes changes with age. There is reduced thickness of the AC, greater amounts of empty lacunae, reduced cellularity of the chondrocytes and finally the inability of chondrocytes to divide and thus maintain the ECM (Guerne et al., 1995). Studies have actually revealed a decreased cellularity density by up to 50 % in specimens from autopsy’s done on elderly donors (Quintero et al., 1984). This aforementioned reduction in cells results in a diminution in the amount of PG produced thus altering the matrix itself and affecting the ability of the AC to bear loads. Once there is focal pressure or the inability of the tissue to disperse loads adequately, degradation of the tissue may ensue. Yet what is boggling is most
aging individuals (estimated at 60% of the population) do not suffer symptoms of OA yet have AC degradation. Mechanisms to explain this are not available in the literature. However for those who are symptomatic, if AC loss due to aging is coupled with other factors which are known to expedite AC loss (obesity, GH deficiency, elevated levels of IL-1, IL-6 or TNF-α) the result can be grave. Ultimately imbalances which are not corrected may result in individuals requiring some sort of surgical intervention to allow for a better quality of life, or coping with reduced function.

3.4.2. CYTOKINES, IGF-I AND OSTEOARTHRITIS. JOINT HOMEOSTASIS?

Unfortunately not much focus has in the past been given to the role that biochemistry and the hormonal imbalances may play in OA. It has just been in the past few years where scientists have identified and quantified the deleterious effects that cytokines have systemically and locally. Furthermore with the effects of GH deficiency brought to the forefront of research and the effects of IGF-I locally and systemically presently under study, scientists are now attempting to determine how IGF-I, GH deficiency and cytokine homeostasis in the joint may relate to the initiation or progression of OA.

Alteration of joint homeostasis in the knee appears to be the most plausible reason explaining why OA develops. Researcher Scott Dye eloquently stated that “the knee is an asymmetrical moving parts whose sole function is to accept, transfer, and ultimately dissipate high loads generated at the ends of the long mechanical lever arms of the femur and tibia” and it is the imbalance of any component which will alter the function of the knee and ultimately shift the direction towards anabolism or catabolism (Dye, 1996). This imbalance in homeostasis of the knee occurs when anabolic factors in the knee are constantly trying to compensate for ongoing disturbances. This is detrimental as any increase load or focal pressure which may have normally been dealt with may result in a further negative balance in the knee and greater degeneration of the AC.

At present little is known about the progression of OA and the levels of IGF-I and cytokines locally or systemically. Some studies have examined SF content in acute and chronic
anterior cruciate injuries in a younger population (under 45 years of age) noting that acute response usually results in initially inhibitory effect on chondrocyte repair with a subsequent stimulatory effect in attempts to repair the AC (Cameron et al., 1994; Rodrigo, Steadman, Syftestad, Benton & Silliman, 1995). However these studies have attempted to establish SF profile via the removal of human synovial fluid (from chronic and acutely injured individuals) and administration on chick chondrocytes in vitro to observe the effects of certain levels of cytokines and growth factors (Rodrigo et al., 1995). Unfortunately, this information may not be completely useful due to species differences in cytokine effects. All in all there is an abyss in the literature with respect to cytokine and growth factor profiles locally in knee OA. This is primarily a result of the area's infancy, specifically in relation to knee OA. Hence in hopes of characterizing the biochemistry of the disease process in OA further longitudinal or multiple age group sampling is required. Given the uncertainty of OA definition it is prudent to start this work by examining well developed, endstage OA.

3.5.0. Obesity and Osteoarthritis

Agreement exists amongst researchers that obesity is associated with increased risk of an individual acquiring knee OA and potential hand OA (Davis, 1994; Felson, ; Felson et al., 1988; Hart & Spector, 1993; Hochberg et al., 1995; Spector et al., 1994). However, where the controversy and the dichotomy exists amongst these researchers is answering whether OA is a result of being obese or if OA is a result of some other process which also causes obesity. Many problems which exist in defining obesity's role in OA stem from the lack of longitudinal studies and inappropriate scaling systems to grade OA. Unfortunately, most studies conducted to date are cross-sectional which only give representation at the specific time measured without accounting for the possibility that subjects could have developed OA due to other factors which have lead to a sedentary lifestyle and ultimately obesity (Felson et al., 1988). Of the few longitudinal studies found in the literature there is the underlying assumption that all individuals tested were radiographically free of OA at their inception into the study. Thus conclusions on the role of obesity are made from initial assumption years later, as radiographic changes may have been originally present, thus negating that obesity resulted in boney changes (Felson et al., 1988). Other problems are the varied results in the literature where associations between
obesity and OA have not been observed consistently in all weight or non-weight bearing joints (Davis, 1994). Hence, to firmly establish and conclude that obesity causes mechanical stress resulting in OA without considering the other concomitant factors associated with obesity (hyperuricemia, hypercholesterolemia, diabetes, GH deficiency) which themselves may directly influence articular cartilage is incorrect (Davis, 1994).

Yet many studies which have attempted to establish obesity as a primary predisposing factor of OA have revealed that “obesity” in itself poses a risk solely to the knee and not hip, hand, ankle or foot (Davis, 1994; Kuettner & Goldberg, 1995). Moreover, there appears to be a gender bias, where women who are obese will have greater risks of developing radiographic OA in all joints, except in the hip where it appears there is an equal risk between men and women (Badley & Rothman, 1996; Kuettner & Goldberg, 1995). Cross sectional and longitudinal studies report a strong correlation between high BMI scores and radiographic OA development in hands, knees, and lumbar spine in both men and women (Felson et al., 1988; Hart & Spector, 1993; Hochberg et al., 1995; Spector et al., 1994). One study which enrolled patients with unilateral knee OA assessed the effects of BMI on the incidence of OA development in the asymptomatic contra-lateral limb 2 years post inception into the study. The results showed that 34% with OA developed radiographic OA in the contra-lateral limb. Additionally, the relative risk of OA increased in every individual with each 5 kilogram augmentation in weight (Hart & Spector, 1993; Spector et al., 1994). What these studies also concluded was despite obesity being a strong risk factor in increasing the prevalence of radiographic OA, less than half experienced symptoms (Felson et al., 1988; Hart & Spector, 1993). Therefore, most were able to show that obesity does play a role in OA, but no study was able to clearly conclude that obesity preceded OA and was the direct causal mechanism.

3.5.1. Adiposity and Growth Hormone Deficiency

As people age there is an increase in adiposity due to a variety of factors. One potential factor is the reduction of GH secretion which directly affects lipolysis (Rosen et al., 1993). Aging results in a 14% decrease per decade (Holloway, Butterfield, Hintz, Gesundheit &
Marcus, 1994; Iranmanesh et al., 1991; Jorgensen et al., 1996). Also demonstrated is the reduction of GH with every 6% per unit increase of BMI (Iranmanesh et al., 1991). Obesity reduces the secretion of GH thus resulting in reduced lipolysis, and increased lipogenesis resulting in greater obesity (De Boer, Blok, Voerman, De Vries & van der Veen, 1992; Gertner, 1993; Jorgensen et al., 1996). In fact several studies have shown inverse relationships with GH secretion and GHRH response with BMI (Baum et al., 1996; Weltman et al., 1994).

The senescent reduction of GH and IGF-I levels systemically are considered normal occurrences in the aging process and are sometimes referred to as somatopause (Gertner, 1993; Jorgensen et al., 1996). But the amount of reduction GH or IGF-I varies widely as does the amount of LBM, body fat, bone mass and the ability to function that an elderly individual has. It has been suggested that greater than half of all elderly individuals have some form of GH insufficiency (Borst et al., 1994; Sharma et al., 1997).

GH deficient adults are subject to the preferential deposition of fat in the truncal area thus increasing their risk of cardiac pathology and potentially limiting function. This pattern of truncal deposition has also been shown in normals to reduce GH release, once again impairing fat metabolism (Jorgensen et al., 1996). Fat cells normally have GH receptors attached to the surface membrane which respond to GH or IGF-I and commence lipolysis (Gertner, 1993). In GH deficiency, the lipolytic effect is lost and there is a sharp rise in obesity or fat mass (Gertner, 1993). In addition to this increased adiposity there is a reduction in LBM which ultimately limits those individuals ability to function and lead productive lives.

When viewing individuals with OA, one can see that there are often similar patterns of adipose deposition and overall reduced functional capacity. Increased body weight (due to GH deficiency) would increase the stressors across the knee joint thus increasing the risk of OA. Furthermore, decreases in IGF-I or GH levels may also affect the local joint environment by altering the levels of catabolic and anabolic cytokines which would then expedite the rate of AC destruction. However the question still remains whether OA may be attributed to the lower levels of IGF-I circulating systemically which then result in obesity and greater prevalence of OA or whether the pre-existing obesity drives the hormonal system and reduces circulating IGF-I levels leading to a cascade of events which additionally contribute to the OA process.
Yet the underlying factor remains that all these individuals are functionally impaired and the task at hand is to discern whether the impairment is related to reduced circulating IGF-I levels, increased body fatness or some multi-factorial process.

3.5.2. **Measurement of Adiposity**

The measurement of adiposity and the improvement of present methods has been consistently investigated in the medical and scientific community. It is also well known that the total amount of fat present in humans plays important physiological roles which include either promoting or hinder the effectiveness of drugs and their respective dissipation rates, a strong influence on morbidity and mortality, has functional purposes such as withstanding starvation and protection from the cold and finally provides shock absorption from daily activities (Broekhoff et al., 1992; Durmin & Womersley, 1974; Maughan, 1993). Unfortunately, direct and precise measures of total body fat are not possible in a living being, rather are feasible solely after death (Maughan, 1993). Thus all methods available to clinicians at present are indirect measures (BIA, hydrostatic weighing, skinfold estimation) which are all based on equations and basic assumptions on body tissue consistency (Baumgartner, Chumlea & Roche, 1990; Bosaeus et al., 1996; Broekhoff et al., 1992; Deurenberg, 1996; Deurenberg, Weststrate & van der Koor, 1989; Durmin & Womersley, 1974; Houtkooper, Lohman, Going & Howell, 1996; Maughan, 1993; NIH, 1996; Oldham, 1996).

The literature has reported time and time again that the gold standard in estimating body fatness or adiposity indirectly is hydrostatic weighing (Deurenberg, 1996; Kushner, 1992; Maughan, 1993). However to employ this method or any other proven valid, reliable and accurate method may be disadvantageous to many investigators. Many techniques which are considered desirable with respect to accuracy such as Dual Energy X-ray absorptiometry (DEXA), isotope dilution methods and hydrostatic weighing are expensive, require much skill on the technician's part, are not readily available in all settings and may not be applicable to certain populations due to cognitive problems or fear (Baumgartner et al., 1990; Bosaeus et al., 1996; Kushner, 1992). Thus many other methods are used for their convenience, cost
effectiveness and availability to most research and hospital settings such as BIA or skinfold measurement.

3.5.2.1. BIOELECTRICAL IMPEDENCE

Since the development of BIA 38 years ago much research has been invested in this method to hone and improve its measuring capacity such that its use would be wide in the medical and scientific community (Baumgartner et al., 1990). BIA is a safe, non-invasive technique which is based on the theoretical relationship between the volume of a conductor (human body) and the electrical impedance of the conductor (water content) (Deurenberg, 1996; Houtkooper et al., 1996; NIH, 1996). It is a misnomer that BIA measures fat, rather BIA provides an estimate of the total body water (TBW) content from which fat-free mass and body fat can be estimated through various equations (Deurenberg, 1996; Houtkooper et al., 1996; NIH, 1996). Equations vary according to manufacturers of the BIA equipment as well as investigators and their belief as to which equation best represents the population studied. A summary of some equations reviewed in the literature can be found in Table 3.5.3.1.
PREDICTIVE EQUATIONS TO CALCULATE TOTAL FAT FREE MASS IN MEN AND WOMEN

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Prediction Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lukaski et al 1986 n=114</td>
<td>18-50</td>
<td>F = 67</td>
<td>FFM = 0.821 Ht²/R + 4.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M = 47</td>
<td>FFM = 0.827 Ht²/R + 5.21</td>
</tr>
<tr>
<td>Segal et al 1988 n=1567</td>
<td>17-62</td>
<td>F = 498</td>
<td>FFM = 0.00108 Ht² - 0.02090 R + 0.23199 Wt + 0.06777 Age + 14.59453</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M = 1069</td>
<td>FFM = 0.00132 Ht² - 0.04394 R + 0.23520 Wt - 0.16760 Age + 22.66827</td>
</tr>
<tr>
<td>Deurenberg et al 1991 n=827</td>
<td>7 - 83</td>
<td>M &amp; F</td>
<td>FFM = 0.406 x 10¹ Ht² (m) / R + 0.360 Wt + 5.580 Ht + 0.56 Sex - 6.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 166 ≤ 15</td>
<td>FFM = 0.340 x 10¹ Ht² (m) / R + 15.34 Ht + 0.273 Wt - 0.127 Age + 4.56 Sex - 12.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n= 661 ≥ 16</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.5.3.1. Various equations which were employed by investigators to estimate FFM using BIA equipment.

BIA has been effectively tested across various populations more recently and compared to various other methods. In a study conducted by Broekhoff et al, BIA was compared to hydrostatic weighing, and anthropometry in healthy elderly women. The results indicated that the fat free mass (FFM) predicted from BIA in an elderly population was lower than that measured from hydrostatic weighing and that FFM prediction from summation of skinfold overestimated the percentage of FFM (Broekhoff et al., 1992). Segal et al found that prediction error was reduced by manipulating the equations such that different equations were used for various populations studied (Segal, Fitzgerald, Hodgdon & Van Itallie, 1988). McNeill et al and van der Kooy et al both demonstrated that in obese individuals FM was underestimated by impedance for reasons that most fat was carried in the truncal area and the contribution to total resistance is solely 10-20%, whereas the limbs contribute to 80-90% of resistance despite a smaller volume and less fat deposited. This small contribution from the trunk results in an underestimation of truncal and total body fat. (McNeill, Fowler & Maughan, 1991; van der Kooy et al., 1992). Reasons why the elderly, and obese individuals generally are over predicted or
underestimated stem from the equations used to predict FFM as well as height which is lost in the aging process due to kyphosis or just decreased disc height in the spine (Broekhoff et al., 1992). Thus BIA values are quite dependent on the physical characteristics of the population at hand.

Another population where BIA has been cross-validated to ensure proper estimation of FFM is in the GHD population. As previously mentioned GHD results in an imbalance of TBW content with a decrease in ECW and an absolute decrease in TBW (Binnerts et al., 1992; Bosaeus et al., 1996; Janssen, Deurenberg & Roelfsema, 1997). Thus with GHD population the prediction equations which assume that all individuals have a constant 73.2 % TBW content may not hold true. Studies conducted by Bosaeus et al., Binnerts et al and Janssen et al all concluded that body fat content was underestimated using BIA in this particular population (Binnerts et al., 1992; Bosaeus et al., 1996; Janssen et al., 1997). Specifically when Bosdaeus compared the DEXA method versus single frequency BIA there was an underestimation of 3.5 ± .71 kg of fat yet the correlation between the DEXA and BIA remained good (r = 0.88) (Bosaeus et al., 1996). Hence in a GHD population the use of BIA, single or multi-frequency models, may not be adequate as fluid balances are disturbed in this population, therefore FFM tends to be overestimated.

Initially many of the studies conducted using BIA in the estimation of body fat were executed on a healthy young population of which standard equations and normative data were established and used on every population. However research has shown that various populations such as the elderly or GHD have changes in structural composition (ratio of intra/extra cellular water, connective tissue plasticity, fat distribution), thus any normative data which exists cannot be applied to specific populations as the assumptions have not been shown to hold true (Bosaeus et al., 1996; Broekhoff et al., 1992; Deurenberg et al., 1989).

Variances (even in a normal healthy young population) anywhere up to 10 % depending on the type of machine or the protocol used are observed with BIA (NIH, 1996). Briefly it has been shown that body position (supine/prone/standing or abduction, crossing of legs), hydration status, consumption of food and beverages immediately before testing, skin temperature, physical activity, electrode placement, ambient air, and the examining table used will vary FM
predictions significantly (Deurenberg, 1996; Houtkooper et al., 1996; Kushner, 1992; Kushner, Gudivaka & Schoeller, 1996; NIH, 1996; Oldham, 1996).

BIA equipment varies depending on the manufacturer. BIA equipment can come in a single frequency or multi-frequency abilities. The difference between the machines appears to be that single frequencies (usually 50 kHz) do not fully penetrate cell membranes thus not accounting for the intracellular water (ICW) (that which is usually attached to glycogen) (Deurenberg, 1996; NIH, 1996; Oldham, 1996). The single frequencies appear to be conducted solely in the ECW space whereas the multi-frequency models appear to be able to penetrate cell membranes thus providing better measure of TBW in special populations where the distribution between the ratio of ICW and ECW vary (Deurenberg, 1996; NIH, 1996; Oldham, 1996).

Evidence exists through various studies that measurements of impedence taken immediately after being positioned supine and another measurement taken 10 minutes later results in a rise of impedence values and a continued rise up to 4 hours later due to the re-distribution of body fluid (Kushner et al., 1996; NIH, 1996). In standing fluid is basically sequestered in the extra-cellular space in the lower extremities whereas in a supine position the fluid is re-distributed to the central pool. As most of the resistance registered in the body is in the lower extremities, this shift would result in a rise in resistance (Kushner et al., 1996; NIH, 1996). Therefore, we standardized our measurement time.

Hydration status directly reflects BIA measurements by altering the amount of TBW and ECW volumes (Kushner et al., 1996; NIH, 1996). Measures made 3-4 hours post-prandially may be affected as absorption has occurred, and excess fluid is in the bloodstream (Houtkooper et al., 1996; Kushner et al., 1996; NIH, 1996). Conversely, immediate measures after food ingestion appear not to affect impedence values (Kushner et al., 1996; NIH, 1996). Yet, even if measures are taken immediately after the BIA measure may be altered as body weight is increased, which is one parameter used in the estimation equation, thus altering values again. It is recommended that an overnight fast occur with bowel movement and bladder emptying prior to BIA testing (Houtkooper et al., 1996; Kushner et al., 1996; NIH, 1996).
Body position also results in variances in impedance measures. The BIA model in the human body suggests that all segments are connected in a series (Kushner et al., 1996; NIH, 1996). Contact from the extremities to the body has been shown to short circuit the model and result in variations in Z values (resistance). It has been documented that leg crossing will result in a 18 % error and arm crossing or contact with the waist can result in a 43 % error in measurement (Kushner et al., 1996).

Skin temperature will have an effect on vascular perfusion, which in turn is reflected in BIA measures (Kushner et al., 1996; NIH, 1996). Studies have revealed that an increase or decrease in vascular perfusion to the skin resulting in temperature changes will alter resistance values. It appears that cooler conditions (cool skin) results in higher resistance values recorded (up to 4 %) and conversely warmer conditions result in lower resistance values reported to be as high as 8 % (Kushner et al., 1996; NIH, 1996). Hence on test-retest conditions ambient air and subject skin temperature should be kept in mind to minimize error.

Physical activity also appears to play a role in altering impedance values as it shares the same principle as skin temperature and fluid imbalance. Exercise results in a hemodynamic response shifting greater amounts of blood and fluid to the limbs (which account for 80-90 % of total body resistance) which reduces total body impedance because of greater fluid in the area (Kushner et al., 1996; NIH, 1996). In addition to fluid shifting exercise also increases skin temperature which again reduces resistance values (Kushner et al., 1996; NIH, 1996). The only adverse effect of exercise is the loss of fluid which would ultimately increase resistance values if not adequately replaced (Kushner et al., 1996). Thus it is recommended that heavy exercise not be done several hours prior to BIA testing (Kushner et al., 1996).

Electrode placement will also alter the readings obtained from BIA equipment. Positioning electrodes on the non-dominant arm results in a decrease of 7 - 18 Ω of resistance (Houtkooper et al., 1996). Actual electrode placement will also alter resistance values significantly where a 1 cm displacement of the electrode can result in a 2 % change in resistance either up or down (Houtkooper et al., 1996; NIH, 1996). Thus standardization is important.
3.5.5.2. Skinfold Measurement

Skinfold measurement has been in existence and used widely in many clinical settings for quite some time. It is a quick, inexpensive method of estimating total body fatness without requiring excessive training or potentially hazardous to be followed. More importantly the skinfold technique is a non-threatening test which does not deter most individuals from participating in and does not require any physical discomfort. Most equations which are used for fat approximation using skinfold technique come from the study conducted by Dumin and Womersley which found a relationship between skinfold thickness and hydrostatic weighing (Durnin & Womersley, 1974). Using Siri’s assumption (in hydrostatic weighing) that the body was mainly comprised of two compartments, fat mass and fat free mass, it was assumed that all FFM weighed 1.1000 kg/l and all FM weighed 0.90000 kg/l (Deurenberg et al., 1989). Based on these assumptions, FM equations were developed (Deurenberg et al., 1989). However what was found through various studies was body density does not remain constant as people age and in fact changes due to the changes in connective tissue, loss of compact bone and variances in gender (Deurenberg et al., 1989). As a result of this new information new densities were formulated to account for age and gender related losses making FM estimation better than the original equations used (Deurenberg et al., 1989).

Studies conducted comparing skinfold technique, BIA and hydrostatic weighing found the skinfold technique better estimated FM than BIA when compared to hydrostatic weighing (Maughan, 1993). Conversely Broekhoff et al found that BIA was a better indicator of FM than skinfold techniques when compared to hydrostatic weighing (Broekhoff et al., 1992). Despite these discrepancies, these measures appear to correlate well with each other (r > 0.70) (Broekhoff et al., 1992).

Despite their limitations, both methods were employed in this study to discern if they correlated well with each other and if in fact the values obtained appeared to be reasonable when comparing them to other physical measures. Unfortunately no criterion technique (as it was not the focus of this study) such as the DEXA or hydrostatic weighing was employed to see which method in fact gave a better estimation.
As the population continues to age the issue of mobility and the capability to live independently becomes a forefront issue in the realm of rehabilitation and medical care. Functional ability declines with every passing decade making it more difficult for the elderly to cope. Studies have shown that the ability and speed of walking declines with advancing age (Himann, Cunningham, Rechnitzer & Paterson, 1988). Documentation exists showing that the speed of walking declines in a curvi-linear fashion such that approximately 4.5 % reduction in walking speed per decade can be expected up until age 62 where the rate of decline then increases to 12 % per decade there after (Himann et al., 1988). It is this decrease in function which holds a great impact on the individual's capacity to function independently in society. It has also been documented that the "level of independence or "quality of life" of elderly persons is dependent upon their ability to do various daily physical activities" (Cunningham, Paterson, Himann & Rechnitzer, 1993). In many hospital settings evaluation forms which attempt to quantify function do not take into account the individuals' surrounding environment and their ability to negotiate in their surroundings. To be independent in society an individual must be able to negotiate stairs, ambulate appropriate distances at an adequate speed and be able to rise from a seated position and sit from a standing position with adequate control (Robinett & Vondran, 1988). Without these basic skills, and without accurate methods in assessing them individuals with medical problems (specifically lower extremity disorders) may be released into society without the ability to cope in their environment.

Presently measures which are most often used and found to be reliable in aiding to predict an individual's functional ability with lower extremity disorders are the Self Paced Walk (SPW), the Stairs Test and the Timed Get Up and Go test (TUG). In addition, the SPW has normative data from various populations which allow comparisons to see if individuals of interest are within the norm (See Table 3.5.3.2.)
**Walking Speeds Documented in the Literature for Various Age Groups and Genders**

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Walking Speed (m/s)</th>
<th>Standard Deviation of Walking Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bassey et al 1988</td>
<td>female</td>
<td>72</td>
<td>1.17 m/s</td>
<td>.17</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>71</td>
<td>1.33 m/s</td>
<td>.17</td>
</tr>
<tr>
<td>Cunningham et al 1982</td>
<td>male</td>
<td>55 - 66</td>
<td>1.33 m/s</td>
<td>.15</td>
</tr>
<tr>
<td>Cunningham et al 1986</td>
<td>male</td>
<td>60 - 65</td>
<td>1.30 m/s</td>
<td>.16</td>
</tr>
<tr>
<td>Fransen et al 1997</td>
<td>female and male</td>
<td>68</td>
<td>0.88 m/s</td>
<td>.22</td>
</tr>
<tr>
<td>Himann et al 1988</td>
<td>female</td>
<td>&gt; 63</td>
<td>0.89 m/s</td>
<td>.23</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>&gt; 63</td>
<td>1.21 m/s</td>
<td>.25</td>
</tr>
<tr>
<td>Keneko et al 1991</td>
<td>female</td>
<td>55 - 64</td>
<td>1.38 m/s</td>
<td>.19</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>65 - 74</td>
<td>1.01 m/s</td>
<td>.17</td>
</tr>
<tr>
<td>Kovar et al 1992</td>
<td>female and male</td>
<td>70</td>
<td>1.05 m/s</td>
<td>.31</td>
</tr>
<tr>
<td></td>
<td><strong>(fast pace only)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kroll et al 1989</td>
<td>female and male</td>
<td>68</td>
<td>0.82 m/s</td>
<td>N/R</td>
</tr>
<tr>
<td>Marks, 1995</td>
<td>female and male</td>
<td>53 - 73</td>
<td>0.82 m/s</td>
<td>N/R</td>
</tr>
<tr>
<td>Mattsson et al 1990</td>
<td>female and male</td>
<td>68</td>
<td>1.09 m/s</td>
<td>.43</td>
</tr>
<tr>
<td>Murray et al 1969</td>
<td>male</td>
<td>50 - 55</td>
<td>1.57 m/s</td>
<td>N/R</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 - 65</td>
<td>1.45 m/s</td>
<td>N/R</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67 - 73</td>
<td>1.18 m/s</td>
<td>N/R</td>
</tr>
<tr>
<td>Oberg et al 1993</td>
<td>female</td>
<td>50 - 59</td>
<td>1.10 m/s</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 - 69</td>
<td>1.16 m/s</td>
<td>.17</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>50 - 59</td>
<td>1.25 m/s</td>
<td>.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 - 69</td>
<td>1.28 m/s</td>
<td>.12</td>
</tr>
</tbody>
</table>

Table 3.5.3.2. The speeds reported above in m/s are representative of an individuals (healthy control or TKAC) self perceived normal walking speed across various age groups following various protocols.

Symbols (**†**) depicted in the table represent walking speed of TKA candidates prior to surgery, **‡** indicates individuals with OA symptom duration average of 4 years.
3.5.3.1. **Self Paced Walk (SPW)**

The SPW is an easy, cost efficient test which allows for a measure of gross motor function, mobility and cardio-respiratory status in the elderly population. The SPW was initially developed by Bassey et al. in attempts to make available a measure which would test cardiorespiratory ability for an elderly population where direct or indirect testing of aerobic function would not be possible nor advisable (Bassey, Fentem, MacDonald & Scriven, 1976). The task of walking itself was selected over cycle ergometry due to the familiarity of most individuals to walking and the cost efficiency of the task (Bassey et al., 1976). When Bassey et al. conducted their preliminary tests of reliability it was found that all subjects were quite willing and able to walk at the various speeds, however only 70% of the subjects were able to complete the cycle ergometry portion of the study (Bassey et al., 1976). Bassey concluded that the SPW was a relevant and realistic method of ascertaining an individual's daily demands in life (Bassey et al., 1976).

The distance walked for the SPW has been frequently modified. Rationale for various selections of distances include the testing of functional mobility over basic cardio-respiratory status (Connelly, Stevenson & Vandervoort, 1996). However parameters of interest which appear to be kept constant in all tests are the time required to complete the distance, heart rate and stride length (Bassey et al., 1976; Cunningham et al., 1993; Cunningham, Rechnitzer & Donner, 1986; Cunningham, Rechnitzer, Pearce & Donner, 1982; Himann et al., 1988; Unknown, 1988).

Reliability and validity of the SPW has been established by various studies. Studies have shown that the SPW is a reliable and reproducible measure with very good correlation coefficients \( r = 0.81 \) for speed and stride length \( r = 0.77 \) and no significant difference with repeated testing (Connelly et al., 1996; Cunningham et al., 1986; Cunningham et al., 1982; Unknown, 1988). Marks (1994) found excellent reliability (ICC= .88 - .93) for a short distance (13 m) version of the SPW when testing patients with knee OA (Marks, 1994).

The internal consistency of the SPW has been established by Bassey et al. where variations in speed did not exceed 3 - 6% (Bassey et al., 1976). In test-retest reliability the
variations again were low in that they did not exceed 11% for walking speed and 8.2% in heart rate measures in the elderly (Bassey et al., 1976).

The SPW has also been established as having good correlation to cardio-respiratory (VO₂ max) fitness in men under 60 as demonstrated in a study done by Cunnigham et al. which compared individuals across different age groups and tested the relationship between aerobic capacity and walking at 3 different paces (Cunningham et al., 1982). In this preliminary study it was found that the SPW was correlated to aerobic capacity (r = -0.25) better than with age (r = -0.13) (Cunningham et al., 1982).

The SPW is responsive to changing cardio-respiratory status in an elderly population. Cunningham et al. (1986) embarked a group of elderly individuals on a physical activity program for one year to establish whether the association between SPW and cardio-respiratory fitness would be responsive to cardio-vascular change and if the association between aerobic capacity and SPW speeds were still maintained (Cunningham et al., 1986). Maximum O₂ uptake and SPW speed (at all 3 speeds slow, normal and fast paces) were correlated and that HR was not correlated to SPW speed either prior to training or post training, thus showing that SPW can be used as a measure of fitness in the elderly (Cunningham et al., 1986; Cunningham et al., 1982).

Previous studies have confirmed that the SPW is a reliable, valid and reproducible test in the elderly in establishing cardio-respiratory fitness, mobility and gross motor function. Thus in the osteoarthritic population the SPW is an excellent measure of ability to cope in their respective environments, establishing cardio-respiratory fitness and assessing general mobility levels.

### 3.5.3.2. Stair Test

A good measure of mobility and potential indicator of independence which many physiotherapists employ in the decision making process at time of hospital discharge is the ability of the client to negotiate stairs. Unfortunately there is no standard measure which tests
a client’s ability to negotiate stairs which has been proven reliable, valid, reproducible, and correlated well with other functional tests (Marks, 1995). Many studies measure stair ability as a indicator of function yet until recently no specific group has conducted studies to confirm if the test is reliable and valid (Andriacchi, Galante & Fermier, 1982; Lundgren-Lindquist, Aniansson & Rundgren, 1983; Marks, 1995). To date preliminary studies have reported inter-session reliability \( r = 0.82 \) on pilot stair studies (Marks, 1995). Marks conducted a pilot study specifically on a knee OA population and their ability to negotiate stairs and walk 13 m in a corridor in a controlled setting on repeated sessions which encompassed a 6 week period. Marks found that the reliability for the walking was greater \( r = 0.86 \) than the reliability of the stairs \( r = 0.82 \) over repeated testing (Marks, 1995). The conclusion that Marks arrived at was stair negotiation was more painful than ambulation and the variability of symptoms on a day to day basis may account for some of the variability seen in test-retest values (Marks, 1995).

Other studies which tested both stair and walking ability found that those who had difficulty negotiating stairs also had difficulty ambulating at fast velocities, however no correlation showing the strength of the relationship was done (Lundgren-Lindquist et al., 1983).

Recently a group of researchers from the Centre of Studies for Physical Function at the Orthopaedic and Arthritic Hospital have conducted preliminary analyses on stair negotiation ability or better known as a "stair performance measure". The group studied was a heterogeneous population with lower extremity dysfunction specifically due to hip or knee OA. The test consisted of walking up 10 standard steps, turning around and descending the 10 steps at a comfortable pace using any assistive device which was needed. Upon completion of the test all individuals were asked to report their perceived pain using a horizontal 10 mm Visual Analogue Scale (VAS) and report their perceived exertion using the Modified Borg scale. Interrater reliability for this group across these measures was \( r = 0.95 \) for stair time, \( r = 0.59 \) for pain reported, and \( r = 0.89 \) for Rating of Perceived Exertion (RPE) (Finch, Kennedy, Walsh & Woodhouse, 1997).

These preliminary studies strongly suggest that in a OA population that the stair measure is a good indicator of function, and that repeated testing may in fact show responsiveness to treatment effects.
3.5.3.3. Timed Get Up and Go (TUG)

The TUG was first developed by Mathias et al in 1986 as a measure of balance to predict the risk of falls in the elderly (Mathias, Nayak & Isaacs, 1986). The initial testing revealed that the TUG related well with gait speed \((r = -0.75)\) such that the more impaired (taking longer to complete the test) individuals were also more impaired in their speed (Mathias et al., 1986). Initial criterion used on the TUG was based on a 5 point categorical scale. The categories used were rather obscure and initial attempts to clinically use the scale resulted in the inability to classify an individual at risk, especially between the scores of 2 to 4 (Podsiadlo & Richardson, 1991).

Podsiadlo et al modified the TUG a few years later such that time was now the parameter measured and that higher scores would indicate greater impairment than lower scores. This group of researchers tested the reliability and validity of the TUG as well as the test-retest reliability to ensure that the measure was able to endure the test of time. The TUG was also compared to other well known measures such as the SPW, and the Berg Balance Scale and the Barthel Index of Activities of Daily Living to establish that the TUG indeed was a good measure of mobility, balance, and functional capacity (Podsiadlo & Richardson, 1991). Results of the study showed that the modified TUG using time as method of determining function related well to gait speed, the Berg Balance test, and the Barthel Index \((r = -0.55, r = -0.72, \text{ and } r = -0.51)\) (Podsiadlo & Richardson, 1991). In addition to the good correlation with other tests, the intra and inter rater reliability of measuring the TUG over time was exceptionally high (Podsiadlo & Richardson, 1991). Podsiadlo concluded that those whose times were less than 20 seconds required to complete the TUG with a gait speed of 0.5 m/s were deemed to be independent and at less risk of falling (Podsiadlo & Richardson, 1991). Hence the TUG can be deemed a good basic test of mobility as it encompasses the most basic requirements and maneuvers for movement yet is quick, practical and economical.
3.5.3.4. **Rating of Perceived Exertion (RPE)**

The category scale more commonly known as the "Borg Scale" is used for quantifying the level of exertion which an individual perceives during an activity (Carton & Rhodes, 1985; Noble, Borg, Jacobs, Ceci & Kaiser, 1983). The scale was initially developed such that perceptual ratings of exertion increased linearly with workloads and heart rates of the individual while exercising on a cycle ergometer (Carton & Rhodes, 1985; Noble et al., 1983). The original 21 point scale was found to have good correlation with heart rate ($0.80 = r = 0.90$) in a light to moderate workload (Carton & Rhodes, 1985). The original scale was evaluated for reliability and validity of perceptual responses whilst walking on the treadmill, cycling on a cycle ergometer or stool stepping.

A modified scale was created with a 10 point scale which allowed ratio properties between the categories (Noble et al., 1983). The scale was shown to increase exponentially with physical work thus making it a better indicator of perceived exertion (Carton & Rhodes, 1985). Reliability and validity testing of this new scale (see appendix) was conducted and found to be excellent ($r = 0.98$) when compared across other modalities (Carton & Rhodes, 1985; Noble et al., 1983). Thus the RPE scale has been shown to be reliable, valid and reproducible in various populations to monitor perceived effort while doing various tasks (aerobic or anaerobic) (Carton & Rhodes, 1985).

3.5.3.5. **Visual Analogue Scale (VAS)**

The VAS has been utilized by many health professions for the quantification and monitoring of pain. The tool itself is a 10 mm vertical or horizontal line with qualifiers at one end of the line no pain whatsoever and at the other end maximal pain perceived. The horizontal line is marked by an individual quantifying the pain they are feeling at that particular moment.

The VAS for pain has undergone much testing and has been shown to be reproducible, reliable and valid for the subjective assessment of pain (de Nies & Fidler, 1997). Many studies have shown good correlation between VAS and other tools (de Nies & Fidler, 1997). In a study
conducted by de Nies et al. the VAS correlated exceptionally well ($r = 0.84$) with the Harris Hip Score and in the study conducted by Finch et al. the intra-class correlation ($r = 0.59$) was also reported to be good in the VAS for pain (de Nies & Fidler, 1997; Finch et al., 1997). Hence, the VAS measuring tool for pain is a good indicator of perceived pain in an osteoarthritic population.

### 3.6.0. Roles Questionnaires Play in Osteoarthritis Research

The collection of information on health and social issues using formalized scales took place as far back as 300 years ago (Ware, 1992). Health care has conventionally measured the health status of all individuals by morbidity, mortality, and life expectancy. Recently though, there has been a shift from this concern of "mortality and survival" to address issues more related to the quality of life. It appears that many health care professionals are now veering away from traditional indicators of poor health (i.e., symptoms of disease) and shifting their focus of research and documentation more towards how disease impacts an individual's social, mental, and personal life (McDowell & C, 1987; Streiner & Norman, 1989; Ware, 1992). Individuals in today's society have challenged health care to respond to their quality of life concerns rather than the biochemistry of their disease process. To address these concerns, researchers have attempted to formulate adequate scales which are standardized, reliable, yet address the public's concerns.

Since the early 1980's many researchers have attempted to devise a questionnaire which could quantify the amount of disability which is associated with osteoarthritis, yet, be responsive enough so that it could be used in clinical intervention trials to determine the efficacy of the treatment used. Many of the questionnaires attempted to address areas which were identified in the WHO model under the description of what defined health and combine them specifically with the disease process of OA. However, the WHO definition of health has always been a difficult term or outcome to measure as it is abstract and may mean many things to many individuals (McDowell & C, 1987). Furthermore, past scales which have attempted to encompass the term “health” have been not been sensitive to specific disease processes (Ware, 1992). A review of the literature by Bellamy et al. (Bellamy & Buchanan, 1984) revealed that most questionnaires lacked standardization and many outcomes chosen had excessive
variability when measured. The literature review also revealed that many existing scales used for research purposes were not even proven to be reliable, valid or responsive to changes in the disease process. As a result of these preliminary findings the task was undertaken by Bellamy and others to develop a questionnaire specifically designed to address OA pathology, yet be sensitive enough to identify any changes which resulted from an intervention.

3.6.1. **Western Ontario McMaster (WOMAC) Osteoarthritis Index**

The WOMAC was developed in the later part of the 1980's as a joint effort between the University of Western Ontario and McMaster University. Bellamy et al conducted several studies determining what information was relevant to patients and to clinicians with regard to the disease process of OA. A scale was created which captured all the preliminary variables of interest (Bellamy, Buchanan, Goldsmith & Campbell, 1988). Initially the scale contained categories which reflected the emotional and social capacities of individuals. However, upon formal testing they were found not to be quite as responsive as the pain, stiffness and difficulty categories, thus were excluded from the formal questionnaire (Bellamy et al., 1988). The scale originally utilized both the Likert and Visual Analogue Scale to rate each question and both were found to be reliable and sensitive to change in the disease process (Bellamy et al., 1988).

Once the scale was formalized, the same researchers conducted several studies which established the WOMAC scale's validity, reliability, responsiveness and sensitivity to pick up changes when specific interventions were applied (Bellamy, 1989; Bellamy et al., 1988; Bellamy, Kean, Buchanan, Gerecz-Simon & Campbell, 1992). Studies were first conducted using a drug versus placebo model to establish the scale's most basic sensitivity. Later on the WOMAC's sensitivity to differences between drugs with similar effects was evaluated in a multi-drug trial (Bellamy, 1989; Bellamy et al., 1992). The WOMAC proved able to pick up changes between multiple drug interventions better than the other scales (SF-36, HRQL, Doyle Articular Cartilage and Lequesne Knee Index) available while many of the others were more efficient at assessing general health status (Bellamy, 1989; Bellamy et al., 1992; Hawker, Melfi, Paul, Green & Bombardier, 1995). This indicated that the scale was more specific towards individuals who had OA of the lower extremity and that it was capable of detecting small changes in perceived pain and function better than other scales available. Further testing was
then undertaken to ascertain if a signal method (whereby one question was sufficient enough to capture changes as the aggregate scores) was just as efficient as the aggregate method. Studies revealed that both methods identified change well, however the aggregate method allowed for preferable profiling of the individual's problematic areas (Barr et al., 1994; Bellamy, Buchana, Goldsmith, Campbell & Duku, 1990). Since its formulation, the scale has been used extensively in the OA population as a reliable outcome measure to identify changes which occur with any treatment interventions.

3.6.2. LOWER EXTREMITY ACTIVITY PROFILE (LEAP)

The LEAP was developed as a result of the apparent scarcity of reliable, responsive and valid scales which addressed the social, emotional and physical aspects of disability experienced by those who have OA of the lower extremity (Finch & Kennedy, 1995). Preliminary construction of the LEAP began in the late 1980's, where the original categories developed were based on the MACTAR Patient Preference Disability Questionnaire (Finch & Kennedy, 1995). During the early stages of development of the LEAP, the investigators included 51 items in their scale which spanned several areas, from ability to put socks on to the individual's emotional well being (Finch & Kennedy, 1995). The Visual Analogue Scale using a 100mm horizontal line was chosen for the rating scale for each category (Huskisson, 1982). This initial scale was administered to a group of individuals with OA to see how well the scale captured their disease process. Pilot testing of the LEAP revealed that the original categories overlapped each other considerably, was quite exhausting for individuals to complete and was rather costly to execute as a physical therapist administered the questionnaire to each participant (Finch & Kennedy, 1995). As a result of these preliminary findings, Finch and Kennedy shortened the questionnaire to 23 categories instead of the original 51 and found that self-administration was just as accurate as interviewer format administration and appreciably less costly (Finch & Kennedy, 1995). Thus the final LEAP format contained 23 categories which addressed the difficulty, satisfaction, severity, frequency and effects of OA on an individual. Testing of this latest questionnaire on individuals with end-stage OA and 6 months post-operatively revealed that the questionnaire was sensitive enough to pick up changes across all categories. The scale was also able to reveal gender differences in the scoring, which
previously was not shown with other scales such as the WOMAC (Finch & Kennedy, 1995). The internal consistency of the scale was found to be moderate overall and could be improved upon with the removal of 2 categories, however it was felt by the researchers that the elimination of the categories would be of detriment to the overall scale (Finch & Kennedy, 1995).

3.7. Summary of the Literature Review

Osteoarthritis in itself is a complex disease process which is not fully understood. In past much focus was placed upon the biomechanics of the knee joint as well as the role that obesity played in OA. For years the literature has stressed that obesity is strongly related to OA, specifically knee OA, and that a reduction in body mass would either ameliorate or halt the degradation. In addition little focus was placed upon the role which hormonal deficiency plays, either locally or systemically, to contribute to OA directly, or contribute to its pathology indirectly by altering the organism's metabolism such that obesity is the outcome.

However, recent new areas have been explored and investigated in hopes of establishing other relationships to OA. Medical research has embarked upon the effects of disrupted local joint homeostasis with respect to cytokine and growth factor interactions and how the balance will result in either a maintenance of the joint or a destruction of the joint. Additionally, a new interest has been sparked on the role in which IGF-I, a mediator of GH, contributes to knee OA directly (maintenance of chondrocytes) or indirectly (increased obesity resulting in greater forces transmitted through the knee). Regardless of which viewpoint is taken, science is stepping forward and challenging old theories and attempting to explain other possible factors to an extremely prevalent disease in the elderly.

Currently what is of utmost importance to individuals accessing the health care system and health care professionals is the way OA impairs function and the ability to live independently in the community. All too often measures which are utilized look at pathology issues (enzyme levels, protein factors, etc.) and disregard factors which are important to the client, primarily function and independence. Thus addressing levels of disability via functional
tests and specific questionnaires are equally important as establishing exact disease pathology. Specifically in OA establishing functional levels provides critical information when attempting to delineate how OA affects an individual's quality of life and level of independence.

Yet to focus solely on function or solely on pathology appears to be the case all too often in health care professions. Negligible amounts of information presently exist in the literature on potential relationships between hormonal, or cellular levels and function. It appears though that more investigators are attempting to tease out factors which affect function on the cellular level as opposed to the macro level (such as obesity). Thus to investigate potential relationships of IGF-I and cytokines in OA appears warranted seeing the role that IGF-I may play in obesity and the role that cytokines play on joint homeostasis. Relevance to establishing such a relationship will allow future investigators as well as medical health care professionals to potentially use non-invasive measures to quantify levels of imbalance in the body.
CHAPTER IV

4.0 METHODOLOGICAL CONCERNS

It is relatively accepted that most studies conducted have some methodological concerns with respect to the methods employed. Many studies immediately will shed light on the shortfalls of the experiment thus warning the reader in advance that conclusions derived from the study must be held in caution as problems existed with protocol or measures used. This experiment is not an exception in that problems were identified early on and every attempt was endeavored to correct or ameliorate any procedure to limit the amount of uncertainty or error involved in the measures. Two areas which were identified as potentially being a problem were cytokine analysis and BIA.
CHAPTER V

5.0. FOCUS AND RATIONALE OF THESIS

Many individuals with OA are obese, sedentary people whose cardiovascular and musculoskeletal systems are compromised due to inactivity. Whether obesity causes OA or is a result of OA is a controversial topic of discussion which has plagued researchers for years. However new theories are emerging in the literature suggesting that a systemic hormonal deficiency may be the impetus to both OA and obesity. One avenue which researchers have embarked upon recently is determining the role of IGF-I in osteoarthritis and obesity. It is thought by some that OA is a reflection of reduced levels of IGF-I systematically and locally in tissues. It is widely known that reduced levels of systemic IGF-I (less than 85 μg/L) result in obesity, reduced lean body mass, altered fluid balances, and other systemic effects. Recent literature also reveals that in the local environments, IGF-I exerts its actions on many tissues resulting in proliferation and maintenance of articular cartilage (AC). In fact its role in the maintenance of articular cartilage is so vital that reduced levels result in ECM destruction and reduced PG synthesis resulting in chondrocyte death. Furthermore any imbalance in the synovial fluid (SF) environment may result in a rapid decline in chondrocytes and expedite AC destruction. As we age it is natural for systemic levels of IGF-I to decrease, possibly accounting for reductions in lean body mass and increased adiposity. However it is speculated that the amount of reduction in IGF-I may be contributing towards the development of OA. However much of the preliminary research of the role of IGF-I in OA development has been done on animal models or cadaveric explants, and to date no active AC change has been quantified in the knee joint of osteoarthritic patients. In addition, few attempts have been made to map out changes in IGF-I levels over time, resultant adiposity and their relationship to OA. As a result little information exists on the progression of the relationship between OA and declining IGF-I levels. This work targets specifically endstage OA in hopes of furthering or advancing our knowledge of OA pathology.

Yet to assume that reductions in IGF-I alone are responsible for chondrocyte death is quite wrong, rather a series or cascade of events which occur in the local environment are equally responsible for AC death and ultimately knee OA. Recent studies have revealed that
alterations in the levels of anabolic and catabolic cytokines will shift the homeostatic environment (Westacott & Sharif, 1996) resulting in AC death via the destruction of the ECM by PG synthesis inhibition in the chondrocyte. It appears that these cytokines are all interrelated, yet the exact mechanism or cascade of events which ensue prior to the shift of balance is unknown at the present time. However what is certain is that if there is an increase in catabolic cytokines in the SF, primarily IL-1 α and β, IL-6 and TNF-α, this will result in AC degradation. What is also certain is that IRAP and TGF-β and IGF-I constantly attempt to counteract the effects of these cytokines in hopes of neutralizing their effects as well as attempting to stimulate chondrocyte proliferation and maintenance. In OA of the knee it has been hypothesized that the catalytic cytokines appear to be produced and released in the SF in larger numbers than the anabolic cytokines which result in the overpowering of the effects of the anabolic cytokines and result in chondrocyte death. It was decided to measure the most mapped out cytokines in hopes of establishing a pattern or relationship in an OA knee. What is not well established to date is whether these imbalances of anabolism and catabolism are a result of direct trauma which alters the local environment, obesity or some systemic influence (reduced IGF-I levels) which causes a shift in the SF and chondrocyte environment.

Again similar to IGF-1, little information is known about cytokine homeostasis in joints over a lifetime and their contribution to OA. Due to the difficulty in detecting small quantities, difficulty in extracting SF in order to quantify levels, and the constant variable nature of the cytokine balance within a joint (potential skewed appearances of joint status) providing profiles on cytokines in SF is presently unfeasible. Yet despite these limitations, cytokine profiling in specific populations is important. Seeing that this area is in its infancy, despite the abundance of difficulty, even a little information such as quantifying cytokines in endstage OA remains just as important as discovering the actual mechanism of release as the final result is a piece of the puzzle in joint homeostasis.

We chose to recruit candidates for total knee arthroplasty as our osteoarthritic population for this study. Clinically this cohort represents an extreme degree of knee OA and in fact would represent end stage OA which requires surgery for amelioration of symptoms and function. This approach avoids the uncertainty of OA diagnosis that may occur in early stages.
of the disease. We also recruited apparently healthy volunteers with no lower extremity dysfunction (this included pain or lack of mobility) to represent our control.

Hence our purpose in this study was to contrast those with OA and those without OA such that we could discern if in fact there were differences in hormonal and protein levels systemically and locally which might relate to impaired function and obesity in individuals with OA.
5.1.0. Research Questions

1) Do serum IGF-I levels differ in TKA candidates when compared to their healthy age and gender matched controls?

2) Do serum IGF-I levels differ when comparing men and women in either the TKA group or the healthy age matched controls group?

3) Are serum IGF-I levels related to functional measures such as Self Paced Walk, Stairs, Timed Get Up and Go?

4) Are serum IGF-I levels related to fat and lean body mass in men and women with and without OA?

5) What are the relationships among catabolic (IL-1α, IL-1β, IL-6 and TNF-α), anabolic (TGF-β, and IRAP) cytokines, and IGF-I levels in synovial fluid of individuals undergoing TKA?

6) What are the relationships among synovial IGF-I levels and serum IGF-I levels in individuals undergoing TKA?

7) What is the relationship between synovial cytokines, synovial IGF-I levels and function (SPW, Stairs, and TUG)?
CHAPTER VI

6.0. MATERIALS AND METHODS

6.1. EXPERIMENTAL DESIGN

A cross-sectional design was employed to contrast patients with severe OA (end stage) to similar volunteers without diagnosed OA. Differences between study groups in serum IGF-I levels, function and perceived function and adiposity were examined. The relation of adiposity, IGF-I, and cytokines to function was examined. All testing was done at the Orthopaedic and Arthritic Hospital. TKA candidates were tested on their scheduled Patient Orientation Program (POP) visit and age matched controls were tested at their earliest convenience.

A sub-study was also conducted to evaluate differences in serum IGF-I, synovial IGF-I, synovial catabolic and anabolic cytokines and the potential relationships of the aforementioned with function. All measures were identical as the initial groups, with the three additional measures; completing the WOMAC questionnaire, and the removal of 15 ml of blood on the surgical date (from TKAC) and synovial fluid extraction from the TKAC on their surgical date.

6.2. SUBJECT RECRUITMENT AND TESTING PROCESS

One hundred and forty three individuals between the ages of 55 and 75 agreed to participate in this study. These individuals were subdivided into two categories, those scheduled to undergo TKA surgery (TKAC) and healthy individuals recruited from the Ontario population (Control group). In total 57 participants were recruited for the TKA group and 84 for the control group. In total 17 from the TKA group and 21 from the control group agreed to participate in the sub study and perform the additional measures.

Eligibility was based on the following set of inclusion/exclusion criterion: 1) women and men between the ages of 55 and 75 who were considered to have end stage osteoarthritis of the knee (determined by their surgeon) requiring a TKA or healthy individuals who have not
been diagnosed with osteoarthritis in the lower extremities; 2) participants could not be on NSAID'S for a period of 3 days prior to testing; 3) participants could not be receiving exogenous GH or GH hormone secretagogues; 4) participants could not be insulin dependent diabetics; 5) participants could not be taking L-dopa or clonidine; 6) participants should not be involved in any treatment requiring the intake of investigational drugs; and 7) participants should not have a history of alcohol abuse.

TKAC were recruited on the pre-operative patient orientation program (POP) visit at the Orthopaedic and Arthritic Hospital, a specialty adult hospital dedicated solely to the care of orthopaedic problems. Controls were recruited via newspaper advertisement, community flyers, word of mouth and radio. In total 159 individuals responded to these advertisement methods, of which 84 met the inclusion and exclusion criterion (see table 6.2.1). Every attempt was made to age and gender match all subjects based on the ratio of gender specific surgeries (1.63 : 1.00 female to male) done at the hospital in 1996. Participants were tested on a one time basis either on their scheduled pre-admission date or by appointment if they were in the control group.

**CHARACTERISTICS OF THE CONTROL GROUP**

<table>
<thead>
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<th>NUMBER OF INDIVIDUALS WHO RESPONDED TO ADVERTISEMENT</th>
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<table>
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<td>N=59</td>
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<tr>
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<td>Female</td>
</tr>
<tr>
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<table>
<thead>
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<th>RA</th>
<th>Age</th>
<th>Other</th>
</tr>
</thead>
<tbody>
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<td>n = 1</td>
<td>n = 1</td>
<td>n = 6</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.2.1. represents the total number of healthy participants who called to participate in the study. Reasons for ineligibility are also mentioned.
Upon completion of the consent form, each participant filled out a basic Health Questionnaire to gather general information, and the Lower Extremity Activity Profile (LEAP). Upon completion of the questionnaires anthropometric data were collected using various techniques (tape measure, skinfold calipers and bioelectrical impedance), followed by functional measures (fast self paced walk [FSPW], slow self paced walk [SSPW], the stairs measure, and the timed get up and go [TUG]). Those in the sub-population were required to fill out one additional questionnaire (WOMAC) and the TKAC were also required to undergo venous puncture on their surgical date with the removal of 15 ml of blood and extraction of 20 cc of synovial fluid prior to the commencement of their surgery. All participants were given the opportunity to withdraw from the testing at any point in time if they felt they were no longer able to continue or felt that the pain was too great to bear.

6.3.0. Testing Protocols

6.3.1 Informed Consent

All individuals prior to consenting to participate in the study were contacted by telephone and verbally given an explanation as to what the purpose of the study was and what their involvement required in terms of time and physical capacity. Upon agreeing to participate verbally, candidates were given an in depth information sheet which they could take home (if any further questions arose) and the consent form to read (Appendix 1 and 2). Both forms explained all details of the study in depth. Any questions were answered by the primary contact (Sonia Pagura) and if there was any concern or indecisiveness, then potential subjects were dissuaded from participating.

6.3.2. Questionnaire Administration

All participants were required to fill out a general Health Status Questionnaire, and the LEAP questionnaire. A sub-population of individuals were also required to complete the WOMAC questionnaire. All participants completed the questionnaire independently without
any help from the primary contact (PC). Any measure which had the VAS scaling system was explained in depth by the examiner to ensure that each individual understood how to utilize the scale so that they could indicate their perceived functional level. Throughout the questionnaire administration the PC was not present so that answers could not reflect the PC's interpretation or opinion.

6.3.3. Anthropometric Measurements

Anthropometric measurements were taken from all participants to attain an adequate characterization of each individual's body composition. The actual anthropometric measurements collected were height, weight, girth measurements of the waist and hips, and 5 skinfold measurements. Bioelectrical impedance analysis (BIA) was used to estimate total body water (TBW) (ultimately percentages of body fat and lean muscle mass via equations) content from the values obtained from the body's reactance and resistance to the microcurrent given.

Height was measured to the nearest quarter centimeter (cm) and weight was measured to the nearest one hundredth of a kilogram (kg) on the Health-o-meter standard beam scale system. Body Mass Index (BMI) (weight/height²) was calculated to allow for the analysis of each individual's body mass when compared to height to discern whether they were in a health risk zone when compared to others in their age category (Canadian Society For Exercise Physiology : Health Canada, 1996). Girth measurements (mm) of the waist and hips were taken according to the Canadian Physical Activity and Fitness Lifestyle Appraisal (CPAFLA) guidelines (Canadian Society For Exercise Physiology : Health Canada, 1996). A flexible measuring tape was used (which had a tensiometer on one end to standardize the tension) to ensure standardization each time a measurement was made.
6.3.4. Skin Fold Measurement

Skin fold measurements were taken on the right side of the body using Harpenden Calipers. The criterion for measuring and the five sites selected (biceps, triceps, subscapular, suprailiac and the calf) were chosen according to the CPAFLA manual (Canadian Society For Exercise Physiology: Health Canada, 1996). All averaged final values were added together to get the Sum of Skinfolds (SOS) and 2 pre-selected sites (suprailiac and subscapular site) were also added together to get the Sum of Trunk Skinfolds (SOTS) to allow for the determination of total body fatness and distribution of body fat.

All SOS values were then converted to percentage body fat using the Durnin and Womersley formulae which accounts for age and gender differences using the sum of 4 skinfolds (biceps, triceps, subscapular and suprailiac) (Durnin & Womersley, 1974). These values were later compared to BIA values.

6.3.5. Bioelectrical Impedance (BIA)

BIA technology allows for the measurement of electrical impedance of body tissues and the estimation of total body water [Health, 1994 #165]. Upon attaining total body water values, fat-free mass and total body fat can then be estimated through various algorithms or regression equations (Houtkooper et al., 1996; Kushner et al., 1996; NIH, 1996; Oldham, 1996). BIA actually measures the opposition of body tissues to the flow of mild alternating current which is less than 1 milliamp (NIH, 1996). The impedance measure obtained from any device is a function of two components, that of resistance of the tissues themselves to the current (called resistance) and an additional opposition due to capacitance of membranes, tissue interfaces and nonionic tissues (called reactance). This technique is based on the principle that impedance to electrical flow of a known current is related to the volume of a conductor and the square of the conductor's length which in this case is the human body (Deurenberg, 1996; Gray, Bray, Gemayel & Kaplan, 1989; Kushner, 1992; NIH, 1996; Oldham, 1996; Segal et al., 1988). The BIA device used was the BIA 101 Body Composition Analyzer from RJL Systems.
with the accompanied computer software package which reported percentage of body fat, water and lean tissue, as well as the fluid portion which identified extracellular water (ECW) and intracellular water (ICW) contents. The RJL impedance analyzer distributes an inappreciable constant 800 µA of alternating current at a fixed 50 KHz frequency via the distal electrode and the proximal electrode functions to sense the drop in voltage (Deurenberg, 1996; Kushner, 1992; NIH, 1996; Oldham, 1996). The participant was required to lie supine on a non-conducting surface with arms and legs abducted and resting comfortably. Surface electrodes were placed on the right side of the body on freshly cleaned skin (using alcohol to reduce surface impedance) and secured onto the dorsal surfaces of the hands and feet. Exact placement for the hand electrodes was the source electrode (that giving the voltage) being placed between the distal prominences of the radius and ulna and at the detector electrode (that sensing the drop in voltage) was placed at the level of the second metacarpal-phalangeal joint. Foot electrode placements were similar in that the source electrode was secured at the midpoint between the medial and lateral malleoli and the detector electrode was secured on the second metatarsal phalangeal joint. Values were recorded when both reactance and impedance values stabilized. Both values were inputted into a computer software program which used weight, height, reactance, resistance, age and gender to estimate TBW, percentage of fat and lean body mass percentage.

6.3.6. Biological Fluid Collection and Analysis

Blood samples were obtained by venous puncture from all candidates on their scheduled visit to the hospital. All candidates had 15 ml of blood removed from their arm of choice by a certified lab technician. Blood samples were temporarily stored in a red top tube which contained no preserving agents as such agents have been known to reduce the ability of radio immuno assays to detect IGF-I levels. Blood samples were immediately (within 5 minutes) centrifuged at 2500 rpm using the Western Scientific H103N series centrifuge for approximately 20 minutes. Upon completion, the supernatant (being plasma) was separated from the packed cells (comprised mostly of hematocrit), stored in a sterile, clearly identified (subject number) container and frozen at -40 °C until it was analyzed at a later date. All
samples were batch analyzed to reduce the possibility of lab error (documented as high as 5%) which may result in analyzing individual samples.

All blood samples were analyzed at the Wellesley Hospital by an experienced technician who was very familiar with using Nichols Institute Radio Immuno Assay (RIA) kits for IGF-I quantification. IGF-I can be dissociated from its binding proteins by various methods such as physical separation (size exclusion chromatography, solid-phase extraction), functional separation and via acid-ethanol extraction (Blum, ). The most widely used technique at present is the acid-ethanol extraction procedure as it has minimal steps and allows large batches to be analyzed at once (Blum, ). The disadvantages associated with this method are that IGFBP-1 and IGFBP-2 remain in solution in large quantities which may interfere with the assay, however in this particular study IGFBP-3 was analyzed thus making that concern a mute point (Blum, ). Thus the method of acid-ethanol extraction was deemed appropriate as a large number of samples were to be analyzed with good recovery allowable (Blum, ).

A sub-population of 16 TKAC had an additional 15 ml of blood removed on the morning of their surgery. All candidates were in a fasted and drug-free condition (which may not have been ideally controlled for at their POP visit) at the time of their blood collection. All samples collected were obtained and stored in the same aforementioned manner. Collection of surgical day blood samples was done to identify whether there were any variations in IGF-I or cytokine values which may be affected due to increased levels of stress (due to surgery), washout of medication or fasting status.

In addition to the blood collection the same sub-population had 20 cc of SF removed from their surgical knee prior to commencing the surgical procedure. A co-investigator in the study, Dr. Peter Welsh, injected 20 ml of saline fluid into the knee joint, then proceeded to flex and extend the knee ten times to ensure adequate mixing of the saline and existing SF, followed by removing 20 ml of combined fluid from the knee for analysis. This injecting and mixing technique was employed as there are very limited amounts of synovial fluid, even in a symptomatic knee. In addition, a fixed volume was injected in essentially a closed system, hence a relative and equal comparison would be made across individuals in the study allowing for equal conditions.
All samples once removed were brought immediately to the laboratory in the hospital to be centrifuged using the Jouan C 422 centrifuge. All samples were spun at 4000 rpm for approximately 20 minutes. Upon completion the supernatant was removed and the remaining cells, and proteins discarded. The supernatant was then stored at -20 °C in the hospital’s freezer until analysis.

All samples (serum and synovial fluid) were analyzed using specific R & D cytokine Enzyme Linked Immunosorbent Sandwich Assays (ELISA) Kits for IL-1α, IL-1β, IL-6, TNF-α, IRAP, and TGF-β. These kits are immunometric enzyme assays where the analyte of interest is captured by an antibody coated and placed on the microtitre plate. A second antibody is then conjugated to an enzyme and is then sandwiched such that the analyte becomes captured and subsequently immobilized with the enzyme on the microtitre well (R & D systems, 1997). Finally a substrate is added and the enzyme generates color which is proportional to the analyte and values are obtained (R & D systems, 1997).

Samples were transported on dry ice and analyzed at John Hopkins University Hospital by an orthopaedic research fellow who was experienced with the analysis technique. IGF-I levels in synovial fluid samples were analyzed in Toronto at the Wellesley Hospital by the same technician to keep consistency and once again were analyzed using RIA.

6.3.7. FUNCTIONAL PERFORMANCE

Functional performances were assessed using three measures, self paced walk (SPW) timed get up and go (TUG) and stair negotiation ability. All three measures were considered as ideal for this population as they would represent tasks encountered in daily living.
6.3.7.1. **Self Paced Walk**

SPW testing was done in a hallway which had a 20 m stretch (without any obstacles) that was delineated quite clearly. Dedicated photosensitive lights hooked to an IBM computer (computerized timing device) were placed at the beginning and end of the 20 m stretch in order to time the individuals’ walking speed to the ten thousandth second. All participants were required to walk at two different speeds; a normal pace and a fast pace. Participants were allowed to use any assistive device they normally required for walking. Participants were requested to walk the hallway distance eight times totaling the amount walked being 160 m for each speed. Standardized instructions were given to each participant prior to commencing each test. The instructions to each participant for the normal speed were “I want you to walk at a normal pace neither fast nor slow” and for the fast speed the instructions were “I want you to walk rather quickly but without over-exerting yourself” (Bassey et al., 1976; Cunningham et al., 1993; Cunningham et al., 1986; Cunningham et al., 1982).

The amount of time required to complete the 160 m, and Rating of Perceived Exertion (RPE) were recorded for each individual. RPE was recorded using the modified Borg Scale and walking speed was collected by the system previously mentioned. All participants walked at the fast speed first and were then given a rest until their HR returned to their resting value. Walking speed and mean heart rate were calculated for the middle 80 meters of the walk test.

6.3.7.2. **Stair Measure**

All participants were required to negotiate 10 steps (ascending and descending) of standard height (20 cm height and 28 cm depth) in a stairwell. Each participant was given the instructions “I want you to walk up the stairs in a manner in which you are accustomed to on a daily basis”. Again participants were allowed to use any assistive device and use the hand rails if need be throughout the testing phase. All participants were timed by the PC to the closest one hundredth second using a Timex stop watch from their first step to the last step. At the end of the test each participant was asked to rate their perceived exertion and perceived knee
pain using the modified Borg scale and a horizontal 10 mm visual analogue scale (VAS). All scores were recorded on a standard data collection sheet.

6.3.7.3. TImed GET UP AND Go (TUG)

All participants were required to sit in a standard height chair (height 44 cm) as this was the starting position for the test. On instruction all participants were required to stand from the chair, walk 3 meters to a clearly demarcated line on the floor, turn around, and sit back down in the chair. The instructions given to each participant were “I want you to get up out of the chair and walk to the line at a comfortable pace, upon reaching the line I want you to turn around and walk back to the chair and sit down”. Once again assistive devices could be used by any participant throughout the test. All participants were timed to the closest one hundredth second by the PC using a Timex stop watch. At the end of the test each participant was also asked to rate their perceived exertion and perceived knee pain using the modified Borg scale and a horizontal 10 mm visual analogue scale (VAS). All scores were recorded on a standard data collection sheet.
CHAPTER VII

7.0 STATISTICAL ANALYSIS

The required sample size for the primary study (IGF-I detection) was 15 in each group when using a power of $\beta$ of 0.85 at a probability of $\alpha = 0.05$ and a standard deviation originally drawn from the literature present at the time was 16 units (Rudman & Mattson, 1994). This sample size calculation basically allowed a 10 % or 20 unit detection between controls and TKA candidates.

All raw data collected were entered into Dbase IV (Ashton-Tate Corporation) and analyzed by SAS 6.10 software (SAS Institute Inc. Cary, North Carolina). All analyses which contained ratio or continuous data used the general linear model (proc glm) as our study model was a two way unbalanced design. This procedure accounts for any analytical difference resulting from unequal cell sizes (Cody & Smith, 1991).

Box plots were performed on several key variables to descriptively represent the data, and ensure that outliers were identified which may have unduly influenced the results.

Analytical statistics were executed to outline any differences between groups and genders in ages, height, weight, BMI, SOS, BIA values. A 2 way Analysis of Variance (ANOVA) was then performed to see if there were any significant differences by gender or group. All differences were deemed significant if $p \leq 0.05$.

All the other variables analyzed were continuous measures. A 2 way (ANOVA) coding for group and gender was employed to detect any differences in serum and synovial IGF-I levels, serum and synovial cytokines levels, functional measures (FSPW, NSPW, stairs, and TUG) and finally the mobility categories of the LEAP and WOMAC questionnaires.

The recruitment and design of the study resulted in subject groups presenting quite differently when tested, thus it was imperative to analyze each group and gender separately to account for these differences.
Simple Pearson Product Moment Correlations were performed on various measures to establish whether potential relationships existed between the biochemistry, functional measures and perceived function (questionnaires). All correlations were once again coded for group and gender as it was speculated that there may be a gender effect. Specifically with regard to the biochemistry, IGF-I values (both serum and synovial fluid), were correlated to cytokine values (both serum and synovial fluid) to reveal whether systemic and local levels were related. In addition both IGF-I and cytokines relationships were then tested against function, again to see if any relationships existed, such that biochemistry could indicate functional impairment. Function was then tested against perceived impairment to establish whether physical findings and perceived findings were similar. Finally IGF-I was correlated to perceived function to discern whether biochemistry was reflected in the severity of perceived disability.

Correlations were considered strong or significant if \( r \geq 0.70 \), moderate if \( 0.40 \geq r \leq 0.69 \) and poor if \( r \leq 0.39 \). Once again anything \( p \leq 0.05 \) was deemed significant. Scatterplots were used to visually check for non-linear relationships.

A prediction model was identified using multiple regression utilizing only specific "predictor variables" which were felt to have a strong influence on the independent "response variable" in question. Response variables used were functional measures (FSPW, NSPW, Stairs and TUG) and predictor variable chosen included IGF-I, BIA fat, BIA LBM, Age and waist measurements. All ideal models were selected from a set of criterion which included the Mallow’s Cp, \( r^2 \), mean square error and whether the model made clinical sense. The regression was carried out by group and gender as it was felt that this subdivision would identify any gender or group differences present. IGF-I was the sole biochemistry variable chosen as a predictor variable as cytokines or growth factors present in synovial fluid are quite variable and in many situations found in extremely small quantities, often eluding detection. Thus it was decided that a consistent measurable variable be used as a predictor variable. Furthermore, it is well established that IGF-I is related to body composition, which in turn affects function. Whereas cytokines would only affect the AC and relate to pain which was not tested in this study.
CHAPTER VIII

8.0. RESULTS

The results section will be displayed in three sections, one section dealing solely with potential differences encountered in the groups, section two exhibiting Pearson Product Correlations (PPC), and section three identifying predictor models.

All differences tested between healthy controls and TKAC were done by 2 way ANOVA's which reveal group, gender and group x gender interactions. However if no group or group x gender interactions were present, however the differences between means were large and potentially clinically significant, simple one way ANOVA's were carried out to establish if differences were masked by the 2 way omnibus ANOVA. Upon revealing any differences in either groups or genders, relationships were tested using PPC in hopes of linking biochemistry with function, adiposity and synovial fluid products. Finally, a predictor model was explored in hopes of establishing variables which would aid in predicting functional outcome.

8.1. SUBJECT DESCRIPTION

Attempts were made to age and gender match for both the TKAC and control groups. There were no problems in matching for age between both groups, however we were unable to recruit the same gender proportions with the final ratio of women to men in the TKAC group being 1.36:1.00 and in the control group being 2.43:1.00 (See table 8.1.1). The attempted gender match for participation was based on the ratio between women and men having undergone TKA at the Orthopaedic and Arthritic Hospital (O & A). The ratio of genders for TKA operations between April 01, 1996 to January 31, 1997 (9 month period) was 1.69 : 1.00 (female to male).
RATIOS OF MALE AND FEMALE PARTICIPANTS COMPARED BY GROUP AND GENDER

TKAC and controls physical characteristics were examined to reveal any differences in age, height and weight. The results indicated that no differences existed \((p > 0.05)\) in age or height between genders or across groups. What was identified was significant total body weight differences within and between gender \((p = 0.0001)\) and group differences \((p = 0.0014)\). Although no significant group X gender effect was observed with weight \((p = 0.2040)\). When a comparison was made between healthy controls and TKAC to establish the magnitude of difference with respect to weight, male TKAC were found to be 18.5 % heavier (kg's) than male healthy controls and TKAC females were found to be 26.4 % heavier than female healthy controls. Thus despite lack of age and height differences, male and female TKAC were considerably heavier than their healthy controls.
DESCRIPTION OF SUBJECT POPULATION BY GROUP AND GENDER

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>TKAC</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males  n = 25</td>
<td>Males  n = 24</td>
</tr>
<tr>
<td>Total N=138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) (mean ± SEM)</td>
<td>64 ± 1.39</td>
<td>66 ± 1.22</td>
</tr>
<tr>
<td>Height (cm) (mean ± SEM)</td>
<td>171.17 ± 1.16</td>
<td>159.65 ± 1.63</td>
</tr>
<tr>
<td>Weight (kg) (mean ± SEM)</td>
<td>91.0 ± 5.75</td>
<td>85.5 ± 3.69</td>
</tr>
</tbody>
</table>

Table 8.1.2. summarizes the characteristics of each group which participated in the study. As seen above no differences existed except in weight. The symbol † depicts a p < 0.0001 difference between the groups controls versus TKA, and ‡ signifies a difference existed p < 0.0014 between genders. No group and gender interaction was observed.

8.2. ANTHROPOMETRIC MEASURES

Differences were examined in all anthropometric measurements (BMI, waist girth, skinfolds and BIA) within and across both groups (Table 8.2.1.). The results indicated that in each variable examined, except for BMI, there were significant group, and gender differences (p < 0.05) (Table 8.2.1.). When BMI was examined between groups, it was revealed that no significant gender difference existed yet a prominent group difference was evident (p = 0.0001). Group x gender effects were present in some variables (waist, and BIA % of fat) however no group x gender interactions were seen in BMI, BIA LBM and skinfold measures.
### SUBJECT ANTHROPOMETRIC CHARACTERISTICS

<table>
<thead>
<tr>
<th>Variable tested</th>
<th>TKAC</th>
<th>Controls</th>
<th>Group p value</th>
<th>Gender p value</th>
<th>Group x Gender p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n = 25)</td>
<td>Females (n = 33)</td>
<td>Males (n = 24)</td>
<td>Females (n = 56)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²) (mean ± SEM)</td>
<td>31.3 ± 1.4</td>
<td>33.9 ± 1.4</td>
<td>25.6 ± 0.6</td>
<td>24.7 ± 0.6</td>
<td>0.0001 ♠</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.4309*</td>
</tr>
<tr>
<td>Waist (cm) (mean ± SEM)</td>
<td>97.6 ± 4.6</td>
<td>97.3 ± 3.5</td>
<td>95.0 ± 4.8</td>
<td>76.0 ± 1.3</td>
<td>0.0003 ♠</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0035 ♠</td>
</tr>
<tr>
<td>SOS (% fat) (mean ± SEM)</td>
<td>29.2 ± 1.4</td>
<td>40.5 ± 1.1</td>
<td>27.3 ± 1.2</td>
<td>35.6 ± 0.6</td>
<td>0.0001 ♠</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0001 ♠</td>
</tr>
<tr>
<td>BIA % fat (mean ± SEM)</td>
<td>22.7 ± 1.9</td>
<td>35.9 ± 2.4</td>
<td>16.8 ± 1.1</td>
<td>19.2 ± 1.0</td>
<td>0.0001 ♠</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0001 ♠</td>
</tr>
<tr>
<td>BIA % LBM (mean ± SEM)</td>
<td>71.0 ± 2.4</td>
<td>52.6 ± 1.8</td>
<td>58.9 ± 1.5</td>
<td>44.0 ± 0.9</td>
<td>0.0001 ♠</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0001 ♠</td>
</tr>
</tbody>
</table>

Table 8.2.1 provides all anthropometric data with their means, standard error means (SEM), and level of significance. All differences are indicated by the symbol ♠ and were deemed significant if p < 0.05. Group and gender interactions were only visible in waist and percentage BIA measures.

Simple one way ANOVA's were carried out on all variables whose results are identified by the symbol *. These ANOVA's were carried out to establish whether the omnibus ANOVA masked any effects. The ANOVA (BMI variable) was first coded for gender resulting in group differences existing in both males and females (females TKAC vs Controls p = 0.0001, males TKAC vs Controls p = 0.0007). However when coding for group, no gender differences existed in BMI in either TKAC (p = 0.3647) or Healthy Controls (p = 0.1909).

Simple one way ANOVA's were also carried out for SOS and BIA LBM. Once again overall group differences did exist in both variables (SOS % Fat: TKAC p = 0.228, Controls p = 0.0001; BIA LBM: TKAC and Controls p = 0.0001) and gender differences existed in both variables p = 0.0001 except when coding for gender for SOS % fat where males did not differ significantly p = 0.0930.
Male TKAC were calculated as having BMI values 18.2% higher than male controls, 2.7% larger waists, 25.9% fatter as determined by BIA, and 24.7% more LBM as predicted by BIA than healthy male controls. Conversely, female TKAC had BMI values in excess of 27.1%, were found to have larger waists by 21.9%, were 46.5% fatter as determined by BIA and had 16.3% more LBM when compared to their female healthy controls. Male controls when compared to the Canadian population (Canada, 1986) were ranked in the 80th percentile, female controls were ranked in the 65th percentile, male TKAC were found to be ranked in the 10th percentile and female TKAC were found to be ranked in the 5th percentile.

All in all it was apparent that when compared to healthy controls, TKAC women appeared to show a greater disparity as they were found to be heavier in overall mass, fatter (% body fat) and larger girth measurements than their male TKAC counterparts. Additionally, male and female TKAC were found to have higher BMI values than their healthy controls and were also found to be in the lowest percentiles when compared to people of similar age in the Canadian population.

8.3.0. Biological Fluid Analysis

8.3.1. Serum and Synovial IGF-I Values

Serum IGF-I values were compared across and within groups to identify if any differences existed. Results revealed that male TKAC and male controls were found to have similar levels of serum IGF-I (Table 8.3.1.). Women were consistently found to have significantly (p = 0.0002) less serum IGF-I regardless of group origin when compared to males. Additionally, TKAC women also revealed lower serum IGF-I levels than their female control group (Table 8.3.1.). The 2 way ANOVA revealed no group or group x gender interaction despite differences in the means. Despite group and group x gender differences not being deemed significant when using the omnibus ANOVA, IGF-I values were further analyzed with a planned contrast ANOVA. These planned contrasts were coded first for group then gender to identify if the lack of difference in IGF-I between male TKAC and controls masked the apparent difference between female TKAC and female controls. It was felt that despite gender
interaction being absent, that a female difference was in fact present and simple one way ANOVA should be conducted to explore this avenue. When coding for gender, what was revealed was no differences (p = 0.9132) between male TKAC and controls existed, and this was to be expected. However this planned comparison revealed a significant difference (p = 0.0345) between female TKAC and their age matched controls. ANOVA coding for group also revealed differences between gender within the TKAC group (p = 0.0010) with men having higher values of IGF-I than women TKAC and in the control group it approached significance (p = 0.0689) where once again men demonstrated higher values than women (See table 8.3.1.). Overall, serum IGF-I values were found to be significantly different across genders however there were no group differences nor was group x gender interactions significant, and planned one way comparisons revealed that gender and group differences existed (Figure 8.3.1.0.).

To ensure that a difference truly did exist in serum IGF-I between genders and was not a result of another variable's influence, a omnibus 2 way ANOVA was conducted adjusting for LBM (similar to an ANCOVA), which is known to affect serum IGF-I levels. The results of the statistical procedure revealed that an overall significant difference (p = 0.0023) still existed, however when breaking down gender, group and group x gender effects, values were slightly altered (8.3.1.1). Gender values approached significance ( p = 0.07) and no group or group x gender interactions were noted. Again upon review of the means, it appeared that males may have masked any gender effect, so a planned comparison was performed, once again adjusting for LBM on both groups. The statistics revealed that a significant gender difference existed ( p = 0.0364) with no difference in LBM between genders, as well as a significant group difference existed (p = 0.0201). However, group differences also revealed significantly different (p = 0.0006) LBM values thus suggesting that the difference noted most likely was due to LBM differences. These findings suggest that the lack of male difference may have influenced the gender difference in the omnibus 2 way ANOVA.
SERUM IGF-I CONCENTRATIONS BY GROUP, GENDER AND GROUP X GENDER

**ONE WAY ANOVA CODING FOR GROUP**

<table>
<thead>
<tr>
<th></th>
<th>TKA (IGF-I (μg/L) ± SEM)</th>
<th>Controls (IGF-I (μg/L) ± SEM)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male n=25</td>
<td>167.0 ± 10.9</td>
<td>165.0 ± 14.6</td>
<td>0.0010</td>
</tr>
<tr>
<td>Female n=33</td>
<td>120.2 ± 5.7</td>
<td>141.1 ± 5.6</td>
<td></td>
</tr>
</tbody>
</table>

**ONE WAY ANOVA CODING FOR GENDER**

<table>
<thead>
<tr>
<th></th>
<th>Males (IGF-I (μg/L) ± SEM)</th>
<th>Females (IGF-I (μg/L) ± SEM)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls n=24</td>
<td>165.0 ± 14.6</td>
<td>141.1 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>TKAC n=25</td>
<td>167.0 ± 10.9</td>
<td>120.2 ± 5.7</td>
<td>0.0345</td>
</tr>
</tbody>
</table>

**TWO WAY ANOVA CODING FOR GROUP AND GENDER**

<table>
<thead>
<tr>
<th>Overall p value</th>
<th>Gender p value</th>
<th>Group p value</th>
<th>Group x Gender p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0018</td>
<td>0.0002</td>
<td>0.3154</td>
<td>0.2240</td>
</tr>
</tbody>
</table>

Table 8.3.1. reveals serum IGF-I differences when coding for group and gender alone. A significant gender difference (p=0.0010) exists within the TKAC group, and within the control group the difference was approaching significance (p=0.0689). A significant between group differences existed for women (p=0.0345), but no difference existed between groups for men.

Serum IGFBP-3 was also analyzed between groups and found not to differ significantly by group (TKAC 5.46 ng/ml ± 0.22 SEM, Controls 5.57 ng/ml ± 0.16 ), gender (male 5.59 ng/ml ± 0.24, female 5.51 ng/ml ± 0.16) or group x gender (male TKAC 5.49 ± 0.3, female TKAC 5.43 ± 0.3, male controls 5.70 ± 0.7, female controls 5.55 ± 0.7).
**SERUM IGF-I CONCENTRATIONS ADJUSTING FOR THE VARIABLE LBM**

### ONE WAY ANOVA CODING FOR GROUP

<table>
<thead>
<tr>
<th></th>
<th>TKA (IGF-I (μg/L) ± SEM)</th>
<th>Controls (IGF-I (μg/L) ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 58</td>
<td>p value</td>
</tr>
<tr>
<td></td>
<td>167.0 ± 2.24</td>
<td>0.0201</td>
</tr>
</tbody>
</table>

### ONE WAY ANOVA CODING FOR GENDER

<table>
<thead>
<tr>
<th></th>
<th>Males (IGF-I (μg/L) ± SEM)</th>
<th>Females (IGF-I (μg/L) ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=49</td>
<td>p value</td>
</tr>
<tr>
<td></td>
<td>162.0 ± 3.31</td>
<td>0.0364</td>
</tr>
</tbody>
</table>

### TWO WAY ANOVA CODING FOR GROUP AND GENDER

<table>
<thead>
<tr>
<th>Overall p value</th>
<th>Gender p value</th>
<th>Group p value</th>
<th>Group x Gender p value</th>
<th>LBM p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0023</td>
<td>0.0744</td>
<td>0.1395</td>
<td>0.2083</td>
<td>0.2201</td>
</tr>
</tbody>
</table>

Table 8.3.1.1. reveals serum IGF-I differences when coding for group and gender alone, yet adjusting for LBM. Significant gender differences (p=0.0364) group differences (p = 0.0201) were observed, with group differences occurring due to significant differences in LBM which is to be expected. The omnibus 2 way ANOVA revealed an overall difference, with gender approaching significance.

When represented as percentages, TKAC males had IGF-I values which were 102 % of IGF-I values of control group males, and TKAC women had 85 % of IGF-I values for healthy women (which was found significantly different p = 0.0345). Overall women had 81 % of IGF-I levels of men when collapsing both groups, suggesting an overall male/female difference.
Figure 8.3.1.0. Box-plots depicting serum IGF-I levels in both groups and genders. A gender difference existed between male and females, however there were no overall group differences or interactions.

The top and bottom of the plots represent the 25th and 75th percentiles of the variable measured, the median is represented by the solid line within the shaded area, and the dashed line represents the samples' mean. Vertical lines which extend above and below each shaded box represents data which extends to a maximum of 1.5 times the interquartile range. Finally symbols represent all data which is outside the 1.5 range but below 3.0 interquartile ranges and are considered quite often outliers and not typical of the data.
Synovial levels of IGF-I were also compared between genders in the TKAC sub-population (males = 8, females = 7). Results revealed that synovial IGF-I values were significantly different between genders with male TKAC exhibiting significantly higher synovial levels (74.88 μg/L ± 5.04 SEM, p = 0.0001) than their female TKAC counterparts (62.0 μg/L ± 1.18 SEM). Group and group x gender differences were not explored as the control group did not have synovial fluid removed and analyzed (See figure 8.3.1.1.).

Comparisons revealed that when compared to serum levels, synovial levels of males were 40 % of serum levels (n=8, serum IGF-I 182.6 ± 22) and in women synovial levels were 44 % of serum levels (n=7, 137.6 μg/L ± 19.6) (Figure 8.3.1.2).
Figure 8.3.1.1. Box-plots depicting synovial IGF-I levels in a subsample of the TKAC group (males n=8, females n=7). A gender difference existed between male and females (p = 0.0001).
Comparison of Pre Surgery Serum and Synovial IGF-I Levels in TKA Candidates

Figure 8.3.1.2. compares synovial levels of IGF-I to pre-surgical serum levels of IGF-I in the TKAC group. Gender differences existed between men and women. Women were consistently lower in IGF-I values in both biological fluids. However what should be noted is this sub-populations higher mean serum IGF-I values when compared to the overall TKAC IGF-I means.
Synovial levels of IGFBP3 were also analyzed between genders in the TKAC group and in most instances were undetectable in all synovial fluid samples. In both males and females the IGFBP3 were unable to meet the detection limit. Samples were re-run several times (3 in total) as the results appeared somewhat odd, and each subsequent testing the results were identical to the first. Detection limits for the assay was 0.9 mg/ml.

Surgical and POP visit serum IGF-I samples were compared in the sub-population (TKAC n = 18), via repeated analysis, to identify if any differences existed in serum between test days due to outside factors such as stress, nutritional status, medication and sleep (Table 8.3.2). Our results revealed that no overall differences (p = 0.9695) were present when comparing POP serum samples to surgical day serum samples.

IGFBP-3 was also analyzed from serum samples obtained from the TKAC sub-sample at their respective POP visits and scheduled surgical day. Once again repeated analysis revealed no differences between both time points in either males or females (Table 8.3.2).

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>TKAC</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>POP VISIT SAMPLE</td>
<td>TKAC</td>
<td>PRE-SURGICAL SAMPLE</td>
</tr>
<tr>
<td>MALE n = 8</td>
<td>FEMALE n = 7</td>
<td>MALE n = 8</td>
</tr>
<tr>
<td>IGF-I (MEAN ± SEM)</td>
<td>182.63 ± 18.35</td>
<td>137.57 ± 23.74</td>
</tr>
<tr>
<td>IGFBP-3 (MEAN ± SEM)</td>
<td>4.59 ± 0.29</td>
<td>4.71 ± 0.59</td>
</tr>
</tbody>
</table>

Table 8.3.2. represents the lack of difference found between surgical and POP visit IGF-I levels indicating a stable IGF-I values over a mean 4 week period. Of note is the IGF-I means in both males and females in this sub-sample when compared to the whole TKAC group were found to be overall higher. IGFBP-3 was also noted not to be significantly different at POP and surgical day collections, also indicating stable values across a 4 week interval.
8.3.2. Serum and Synovial Levels of Cytokines

Anabolic and catabolic cytokines were analyzed from serum and synovial fluid samples in the TKAC sub-group (n=17) and serum cytokines in the control sub-group (n=21). Our results revealed that all catalytic cytokines (IL-1 α, IL-1 β, and TNF- α) in serum and SF, with the exception of IL-6, were undetectable across both groups and genders. Statistical analysis of IL-6 revealed no gender or group difference in either serum or SF.

Our results further revealed that in all anabolic growth factors analyzed (TGF- β and IRAP) in serum and synovial fluid, no significant differences were found between any of the groups, genders or group x genders (Table 8.3.2.1).

It appeared that gender differences in serum and synovial levels of IRAP, synovial levels of IL-6 and synovial levels of TGF-β could have possibly reached significance had the sample size been larger thus reducing the SEM (Figure 8.3.2.2 and Figure 8.3.2.3.), however without further testing of a larger sample size this cannot be ascertained.
Figure 8.3.2.2 Serum IRAP values between both TKAC and controls reveal large SEM's. Small sample sizes (TKAC: n=7 females, and n=8 males, Controls: n=11 females, and n=10 males) may have contributed to masking possible differences in biochemistry.
Figure 8.3.2.3. compares various biochemical factors in synovial fluid as well as revealing large standard deviations within each group. However, focus should be placed upon the differences which exist even with a small sample size. Although none are significant, one should consider the effect sample size may have contributed to masking any difference.
### Overview of Cytokine Values Using 2 Way ANOVA (Coding for Group and Gender)

<table>
<thead>
<tr>
<th>Variable tested</th>
<th>TKAC</th>
<th>Controls</th>
<th>Group p value</th>
<th>Gender p value</th>
<th>Group x Gender p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n = 9)</td>
<td>Females (n=8)</td>
<td>Males (n = 10)</td>
<td>Females (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Serum IL-1β (mean ± SEM)</td>
<td>0.57 pg/ml ± 0.6</td>
<td>1.06 pg/ml ± 1.1</td>
<td>0.00 pg/ml ± 0</td>
<td>0.00 pg/ml ± 0</td>
<td>0.6381</td>
</tr>
<tr>
<td>Serum IL-6 (mean ± SEM)</td>
<td>0.00 pg/ml ± 0</td>
<td>0.65 pg/ml ± 0.7</td>
<td>0.52 pg/ml ± 0.5</td>
<td>0.60 pg/ml ± 0.6</td>
<td>0.4912</td>
</tr>
<tr>
<td>Serum IRAP (mean ± SEM)</td>
<td>398.4 pg/ml ± 55.1</td>
<td>566.7 pg/ml ± 112.4</td>
<td>415.3 pg/ml ± 58.0</td>
<td>319.1 pg/ml ± 41.1</td>
<td>0.5921</td>
</tr>
<tr>
<td>Serum TGF-β (mean ± SEM)</td>
<td>28.5 pg/ml ± 7.3</td>
<td>23.1 pg/ml ± 6.1</td>
<td>20.4 pg/ml ± 4.7</td>
<td>30.5 pg/ml ± 5.6</td>
<td>0.7022</td>
</tr>
</tbody>
</table>

### Synovial Fluid Samples from TKAC Group Only

<table>
<thead>
<tr>
<th>Variable tested</th>
<th>Males (n = 8)</th>
<th>Females (n = 8)</th>
<th>Overall p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovial IL-6 (mean ± SEM)</td>
<td>212.1 pg/ml ± 85.31</td>
<td>95.0 pg/ml ± 55.9</td>
<td>0.2704</td>
</tr>
<tr>
<td>Synovial TNF-α (mean ± SEM)</td>
<td>0.13 pg/ml ± 0.1</td>
<td>0.00 pg/ml ± 0.0</td>
<td>0.3343</td>
</tr>
<tr>
<td>Synovial IRAP (mean ± SEM)</td>
<td>485.90 pg/ml ± 257.9</td>
<td>332.68 pg/ml ± 106.1</td>
<td>0.5914</td>
</tr>
<tr>
<td>Synovial TGF-β (mean ± SEM)</td>
<td>40.43 pg/ml ± 8.83</td>
<td>31.75 pg/ml ± 36.6</td>
<td>0.5886</td>
</tr>
</tbody>
</table>

Table 8.3.2.1. presents all cytokine and growth factor values found in serum and synovial fluid. As seen above, synovial values are only available for TKAC as harvesting of SF was not possible in the control group. In addition what should be noted are the small sample sizes and large SEM. As mentioned previously, no significant differences were found in group, gender or group x gender when analyzing the variables.
8.3.3. Functional Measures

Comparisons were conducted on all functional measures (FSPW, NSPW, Stairs, and TUG) to reveal if any differences existed between groups or gender. Our results revealed consistent findings with each statistical test conducted on each functional measure. TKAC's were significantly \( p = 0.0001 \) more disabled than controls, and TKAC females were consistently the most disabled across all groups and genders revealing the lowest values (Table 8.3.3.1). However in some measures (FSPW RPE, NSPW RPE, TUG VAS and Stairs VAS), gender differences were not seen despite large differences noted in means. Thus planned one way ANOVA's coding either by group or gender were carried out to reveal if any differences did exist between gender in FSPW RPE, NSPW RPE, TUG VAS and Stairs VAS (Table 8.3.3.1.). Our analyses revealed that significant \( p < 0.05 \) group and gender differences were found in NSPW RPE, and significant \( p < 0.05 \) group differences were identified in TUG and Stair perceived pain scores using VAS (Table 8.3.3.1.).

Overall male TKAC were more functionally able than their female counterparts, yet were still found to be significantly more impaired than their healthy male and female controls. No gender differences though were found between healthy controls, in fact women were slightly faster in the stair measure and extremely close in all the other measures (Table 8.3.3.1). Significant \( p < 0.05 \) group x gender interactions existed in all measures with the exception of FSPW RPE, TUG VAS and Stair measure VAS.

All perceived ratings of exertion and pain were found to be significantly different between TKAC and control groups. Female TKAC in all measures reported more exertion or reported more pain, however none of the differences between male and female TKAC were found to be statistically significant with the sole exception NSPW RPE \( p = 0.0486 \). No gender differences were found between men and women in the healthy control group with respect to perceived exertion or pain on each variable measured (Table 8.3.3.1).
Table 8.3.3.1 exhibits all functional differences between gender, group and group by gender. All values below \( p < 0.05 \) were deemed significant and depicted by the symbol \( * \). Values with the symbol \( + \) were found not to be significant using the omnibus ANOVA, however, when analyzing the variables using a one way ANOVA coding for either gender or group several values were then deemed significant. Gender differences always existed between TKAC females and healthy females in all variables \((0.0001 > p < 0.0006)\), where male TKAC only differed between their male counterparts in the VAS scores of the TUG and Stair measure \( (p = 0.0001) \). No differences within each group existed on any variable except for TKAC where NSPW RPE was marginally significantly different \((p = 0.0486)\), however no other variable revealed any within group differences between gender.
All functional data revealed greater disability amongst TKAC than healthy controls, however the stair function and FSPW were apparently the most difficult task for both genders of the TKAC group. It was these specific tasks (stairs and FSPW) which revealed the largest differences when comparing TKAC to their healthy controls (Figure 8.3.3.2 and 8.3.3.3.).
Figure 8.3.3.2 demonstrates the various speeds each group is able to achieve when requested to "walk rather fast without overexerting yourself". The larger the score indicates greater functional ability. Overall female TKAC are most impaired across and within groups.
Figure 8.3.3.3 represents functional ability on the stairs denoted by time. In this measure the lower the score, the greater functional ability. Female TKAC were again the most disabled across and within all groups. Both male and female healthy controls exhibited no significant differences in function thus showing that in a healthy population gender is not an issue with respect to function (FSPW, NSPW, Stairs and TUG).

In addition the stairs also appeared to produce greatest amounts of pain than any other physical measure, and the FSPW resulted in the greatest amount of exertion for the TKAC group (Figure 8.3.3.4.). In all functional variables measured, both genders of the healthy control group reported perceiving smaller amounts of effort and/or pain upon completion of any task.
Figure 8.3.3.4. demonstrates that reported RPE during the FSPW was significantly different in TKAC females than any other group or gender. In addition, the male TKAC and both male and female healthy controls reported similar RPE while doing the task. The stair measure though displays that both male and female TKAC reported greater amounts of pain while doing the stair measure which was significantly different that reported pain from healthy controls.

Female and male TKAC's did not differ in their reported levels of pain, however they did differ significantly on their perception of exertion during the FSPW (p = 0.049).
Overall with respect to function, female TKAC were able to walk at 60% of their healthy counterparts speed at a quick pace, negotiate stairs at 32% of the pace healthy counterparts achieved, and get in and out of a chair at 56% of healthy controls' capacity. When reviewing perceived exertion and pain, female TKAC perceived more exertion on tasks (3 fold) and rated greater amounts of pain (approximately 9 fold) upon doing the task than their healthy counterparts. TKAC males overall were able to walk at 77% of their healthy counterparts' speed, negotiate stairs at 58% of the pace, and rise and descend into a chair at 72% of healthy male capacity. Male TKAC also perceived more exertion on tasks (2 fold) and perceived greater amounts of pain (9 fold) than healthy controls. Functionally it was quite apparent that in general the TKAC group was most impaired when compared to healthy controls, however, overall it was the TKAC women who demonstrated the most functional disability.

8.3.4. WOMAC AND LEAP QUESTIONNAIRES (PERCEIVED PAIN & DISABILITY)

The pain and mobility components of both questionnaires were selected for analysis as they were considered most likely to be related to function and possibly any underlying biochemistry (cytokines and IGF-I levels). In both questionnaires, zero represented minimal pain and 10 represented maximal perceived pain. Conversely in the mobility questions, zeros represented most difficulty with mobility, whereas as 10 represented no difficulty with mobility (See Appendix). Scores on the LEAP mobility question were transformed to match the WOMAC's scale for easier comparison. LEAP mobility scores were transformed from their original scale to a scale (identical to the WOMAC's) where zero represented no difficulty and a score of 10 represented maximal difficulty.

Questionnaires were analyzed for differences. Our results revealed similar findings in 3 of the 4 questions (LEAP pain, WOMAC mobility and WOMAC pain). All 3 questions identified significant group differences between TKAC and healthy controls (p = 0.0001), however no gender differences or group x gender interactions were present in any of the categories analyzed (Table 8.3.4.1). Aggregate scores for both the LEAP and WOMAC are also
represented in Table 8.3.4.2 which demonstrates overall differences in perceived disability between groups. The LEAP mobility question was the sole question which revealed significant group (p=0.0001), gender (p=0.0141) and group x gender differences (p = 0.0093). It appeared once again that female TKAC were most impaired. That no gender effect was noted appeared odd upon examining some of the means, thus a planned one way ANOVA was employed to reveal if gender or group effect was present but just masked due to close similarities in the control group. These planned one way comparisons revealed that significant (p < 0.05) gender differences between groups existed (Table 8.3.4.1.) What was of interest is that even though women were functionally more impaired, on the whole they perceived levels of disability and pain equally to male counterparts (LEAP pain, WOMAC mobility and WOMAC pain) (Figure 8.3.4.2. and 8.3.4.3.).

When analyzing the LEAP and WOMAC mobility and pain questions, what was revealed was TKAC women reported greater disability across all 4 questions analyzed, although in many instances they were found to be not significantly different than male TKAC. Perceived disability or pain was reported to be anywhere from 2 to 9 fold greater than healthy controls. Not surprisingly in the LEAP question of mobility, females reported significantly more difficulty with (see Table 8.3.4.1) activity than TKAC males.
Figure 8.3.4.2 represents significant differences between groups (p = 0.0001) with perception of mobility in both the WOMAC and LEAP questionnaires. All analysis revealed no gender differences within groups, solely overall group differences. Both the LEAP and WOMAC showed similar responses in both groups with respect to a specific mobility question.
Comparison of Healthy Controls and TKAC with their respective perceptions of pain experienced as quantified by both the LEAP and WOMAC

Figure 8.3.4.3 represents perceived pain experienced in the past week (LEAP) and past 72 hours (WOMAC). As depicted there is a group difference (p=0.0001), as well as a group x gender difference (p=0.0001) when investigated by a one way ANOVA coding for gender, however no gender differences were exhibited within groups. Once again as was the case with perceived mobility, male and female TKAC have no significant difference between them despite women being more disabled in all functional tasks.
CATEGORICAL SCORES (MOBILITY AND PAIN) ON PERCEIVED DISABILITY BETWEEN GROUPS USING THE LEAP AND WOMAC QUESTIONNAIRES

<table>
<thead>
<tr>
<th>Variable</th>
<th>TKAC</th>
<th>Group p value</th>
<th>Gender p value</th>
<th>Group x Gender p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (VAS 100 mm)</td>
<td>Female (VAS 100 mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEAP mobility</td>
<td>5.02 ± 0.55</td>
<td>3.37 ± 0.37</td>
<td>0.0001 *</td>
<td>0.0083 *</td>
</tr>
<tr>
<td>LEAP pain</td>
<td>6.86 ± 0.46</td>
<td>6.51 ± 0.45</td>
<td>0.0001 *</td>
<td>0.6719 *</td>
</tr>
<tr>
<td>WOMAC mobility</td>
<td>4.14 ± 0.89</td>
<td>5.60 ± 1.03</td>
<td>0.0001 *</td>
<td>0.1477 *</td>
</tr>
<tr>
<td>WOMAC pain</td>
<td>4.77 ± 1.10</td>
<td>4.25 ± 1.15</td>
<td>0.0001 *</td>
<td>0.7226 *</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Controls</th>
<th>Group p value</th>
<th>Gender p value</th>
<th>Group x Gender p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (VAS 100 mm)</td>
<td>Female (VAS 100 mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEAP mobility</td>
<td>9.61 ± 0.06</td>
<td>9.59 ± 0.13</td>
<td>0.0001</td>
<td>n.s</td>
</tr>
<tr>
<td>LEAP pain</td>
<td>0.86 ± 0.44</td>
<td>0.87 ± 0.29</td>
<td>0.0001</td>
<td>n.s</td>
</tr>
<tr>
<td>WOMAC mobility</td>
<td>0.59 ± 0.51</td>
<td>0.54 ± 0.45</td>
<td>0.0001</td>
<td>n.s</td>
</tr>
<tr>
<td>WOMAC pain</td>
<td>0.19 ± 0.06</td>
<td>0.39 ± 0.20</td>
<td>0.0001</td>
<td>n.s</td>
</tr>
</tbody>
</table>

Table 8.3.4.1 represents the actual VAS scores (using the horizontal 100 mm scale) between healthy controls and TKAC. The higher the score in the mobility LEAP question represented greater mobility, whereas the other LEAP question and WOMAC questions the higher the score represented greater disability.

All significant differences are denoted with the symbol *. All values denoted with the symbol * were not initially found significant using the omnibus ANOVA, however when conducting a one way ANOVA coding for gender differences were found between the groups with p ranging from 0.0001 > p < 0.0026. When coding for group, no differences were found in healthy controls, and only in the LEAP mobility were differences noted (p = 0.0083) with females reported greater difficulty. Values which had n.s. represented no significant values regardless of analysis used, as no differences were found between gender within the healthy control group.
AGGREGATE SCORING OF PERCEIVED DISABILITY BETWEEN GROUPS USING THE LEAP AND WOMAC QUESTIONNAIRES

<table>
<thead>
<tr>
<th>Variable</th>
<th>TKAC</th>
<th>Healthy Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (VAS 100 mm)</td>
<td>Female (VAS 100 mm)</td>
</tr>
<tr>
<td>Total LEAP Pain (mean ± SEM)</td>
<td>14.58 ± 0.92 *</td>
<td>13.51 ± 1.03 *</td>
</tr>
<tr>
<td>Total LEAP Difficulty (mean ± SEM)</td>
<td>21.15 ± 1.98 *</td>
<td>25.45 ± 1.80 *</td>
</tr>
<tr>
<td>Total LEAP Satisfaction (mean ± SEM)</td>
<td>40.19 ± 3.35 *</td>
<td>44.65 ± 3.18 *</td>
</tr>
<tr>
<td>Total WOMAC Pain (mean ± SEM)</td>
<td>22.38 ± 3.78 *</td>
<td>26.22 ± 2.45 *</td>
</tr>
<tr>
<td>Total WOMAC Difficulty (mean ± SEM)</td>
<td>10.62 ± 1.67 *</td>
<td>12.40 ± 1.86 *</td>
</tr>
<tr>
<td>Total WOMAC Stiffness (mean ± SEM)</td>
<td>82.15 ± 15.32 *</td>
<td>84.04 ± 2.45 *</td>
</tr>
<tr>
<td>TOTAL LEAP (mean ± SEM)</td>
<td>85.83 ± 6.41 *</td>
<td>93.78 ± 6.29 *</td>
</tr>
<tr>
<td>TOTAL WOMAC (mean ± SEM)</td>
<td>115.16 ± 20.36 *</td>
<td>122.70 ± 23.80 *</td>
</tr>
</tbody>
</table>

Table 8.3.4.2 reveals the aggregate scores of both the LEAP and WOMAC in varying sub-categories (difficulty, satisfaction, pain and mobility) as well as total scores. Significant differences p = 0.0001, was revealed between groups (when comparing total scores in each category) and depicted by the symbol *. No group or group x gender interactions were identified.
8.3.5 SUMMARY OF GENERAL DIFFERENCES

Overall significant group and gender differences were found in anthropometry with healthy controls having significantly less body fat than TKAC. Additionally, upon closer examination of the data, it was apparent that female TKAC overall, had the greatest amount of body fat when compared to healthy females and male TKAC.

Once more gender differences prevailed when comparing serum and synovial IGF-I levels. Significant differences were seen both systemically and locally. TKAC women were found to have the lowest values overall, and were also found to have substantially lower values than their healthy counterparts. In synovial fluid IGF-I levels again women were found to have lower values than their male counterparts. However when examining this sub population closer, both male and female TKAC demonstrated higher serum IGF-I values than the main TKAC group. Furthermore in the male TKAC sub-population the values exceeded those of healthy controls considerably. In the female TKAC group the sub-population approached healthy control values. When adjusting for LBM in all 4 groups, no drastic differences were seen in serum IGF-I values which indicated that LBM was not a co-variate of IGF-I and that gender differences truly did exist.

When comparing POP visit serum samples and surgical day serum samples, no differences were found in both male and female TKAC in either IGF-I values or IGFBP-3 values. This lack of significance ($p > 0.05$) between these repeated samples of serum IGF-I and IGFBP-3 aided in establishing the stability of the variable measured.

No statistically significant group differences existed when examining cytokines and growth factors either systemically or synovially. However upon examining the data closer it appeared that serum IRAP and synovial levels of IL-6 and IRAP differed amongst genders with males exhibiting higher values than females. However due to small sample size and large standard deviations none were found to be statistically significant.
Overall differences were found in all aspects of function with group, gender and group x gender effects occurring. Healthy controls were evenly matched with no overall gender differences, however both male and female controls were functionally superior to TKAC. Specifically, female TKAC were once again found to exhibit greater disability across all functional measures.

Group differences were found in perceived mobility and perceived pain with TKAC reporting greater disability and pain. Perceived mobility was more impaired in female than male TKAC but no gender differences in reported pain were observed. Overall group differences were also found in RPE's and reported pain after completing a task where healthy controls were least affected. Within groups no gender differences existed in either the healthy control group nor the TKAC group with the exception of RPE while performing the NSPW, where female TKAC reported more exertion than their male TKAC counterparts.
8.4.0 Pearson Product Correlations (PPC)

8.4.1. Relationships between Anthropometry Techniques

An important aspect of the study was to tease out gender and group effects of variables tested. Initial PPC were run on the variables as a whole without considering gender or group influence. Upon re-examining the data it was discovered that group and gender did play an important role on the strength of the relationship between variables selected. Furthermore consultation was done with a statistician to ensure that analyzing correlations by group and gender would in fact be the correct course of action taken without over manipulating the variables. Thus all PPC's reported in the results section are described individually by group and gender.

PPC were carried out on both methods of determining body fatness (BIA and Skinfold technique) to identify the amount of agreement in assessing total body fatness. Across gender and groups, the relationship between these two techniques appeared to be moderately strong (male controls $r = 0.75$, female controls $r = 0.73$, male TKAC $r = 0.88$ and female TKAC $r = 0.72$), with no apparent gender or group differences in skewing $r$ values (Figure 8.4.1). All $r$ values were found to be significant ($p = 0.0001$).
Figure 8.4.1 reveals the moderately strong relationship between both techniques in quantifying total body fatness in both healthy controls and TKAC.
8.4.2. RELATIONSHIPS BETWEEN IGF-I AND BODY COMPOSITION, CYTOKINES, FUNCTION AND PERCEIVED DISABILITY

Correlations were performed on serum levels of IGF-I and age in all groups. Serum levels of IGF-I overall correlated poorly with age in all groups, especially in the women (both TKAC and healthy controls) where the correlations were negligible r < -0.08. The strongest relationship (r = -0.38), which also approached significance (p = 0.0591), was evident in TKAC males. All other correlations were found to be not significant.

Correlations performed on synovial levels of IGF-I and age in the TKAC group revealed different results with the PPC improving considerably and revealing low-moderate strength correlations (male TKAC r = -0.55, and female TKAC r = 0.48). Despite the increase in strength of the PPC, they were found to be not significant. What was interesting was that males and female IGF-I and age relationships were in the opposite in direction to each other.

8.4.2.1. PEARSON PRODUCT CORRELATION BETWEEN IGF-I AND ANTHROPOMETRY

PPC's were performed on IGF-I and percentage of body fat (BIA estimation, and Durnin & Womersley estimation), and percentage of lean body mass (using BIA estimation) to establish whether relationships existed between IGF-I and these variables. Correlation coefficients across all three methods of tissue estimation and IGF-I were uniformly low with r values consistently below 0.17 and not significant in all groups and genders (Figure 8.4.2.1.2.).
Comparing TKAC and Healthy Controls
Relationship between IGF-I and Body Fat
Using BIA Method

Figure 8.4.2.1.2 shows the relationship between IGF-I and body fatness by gender and group. As seen above the relationships in all are poor, with females overall having the poorest relationship.
Serum IGF-I and LBM shared a positive relationship in all cases except with female TKAC where the relationship was negative ($r = -0.02$) (Figure 8.4.2.1.4).

Figure 8.4.2.1.4. reveals relationships between IGF-I and LBM in both groups and genders. Once again no relationships existed in either female group with $r < 0.09$. 
Synovial levels of IGF-I and percentage body fat (BIA technique) were also analyzed, and surprisingly moderate correlations were found in female TKAC ($r = -0.58$), while poor correlations were found in TKAC males ($r = -0.26$) (Figure 8.4.2.1.3). For both genders PPC were once again found to be not significant.

Figure 8.4.2.1.3 demonstrates the relationship between body fatness and synovial levels of IGF-I.
8.4.2.2. RELATIONSHIPS BETWEEN SERUM AND SYNOVIAL IGF-I AND SERUM AND SYNOVIAL CYTOKINES

All cytokines and growth factors were analyzed using the PPC in hopes of identifying any relationship between variables as well as within variables in serum and SF. Our results disclosed that in most situations no significant relationships existed. Negligible to moderate relationships did exist in serum IGF-I when compared to serum TGF-β and IRAP, however none of these relationships were found to be significant, with one exception where serum levels of IGF-I and IRAP in TKAC males approached significance with a moderately strong negative relationship (Table 8.4.2.2.1). Serum IGF-I was not correlated with IL-1 (both forms), and TNF-α as no detectable levels were identified.

<table>
<thead>
<tr>
<th>CORRELATIONS BETWEEN SERUM IGF-I AND CYTOKINES</th>
</tr>
</thead>
<tbody>
<tr>
<td>VARIABLE</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>IGF-I vs. IRAP</td>
</tr>
<tr>
<td>IGF-I vs. TGF-β</td>
</tr>
</tbody>
</table>

Table 8.4.2.2.1. reveals moderate relationships between IGF-I and IRAP and TGF-β in males (both TKAC and healthy controls) however neither were found to be significant, thus indicating that the relationships may have been due to chance. TKAC and healthy women had poor relationships in both categories.

Synovial fluid IGF-I values were also compared to serum IGF-I values and all SF cytokines. Overall values were found to correlate low-moderately in TKAC males and correlated poorly in TKAC females (Table 8.4.2.2.2.). No relationships were identified with IGF-I serum or SF levels when correlated with IL-1 α or IL-1 β. This was to be expected as the amounts of both cytokines in most instances were reported as zero which was below the sensitivity of the assay.
CORRELATIONS BETWEEN SYNOVIAL LEVELS OF IGF-I AND SYNOVIAL CYTOKINES

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>Male (n=8)</th>
<th>Female (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovial IGF-I vs. Serum IGF-I</td>
<td>r = 0.23</td>
<td>r = 0.01</td>
</tr>
<tr>
<td></td>
<td>p = 0.5799</td>
<td>p = 0.9822</td>
</tr>
<tr>
<td>Synovial IGF-I vs. Synovial TGF-β</td>
<td>r = -0.57</td>
<td>r = -0.18</td>
</tr>
<tr>
<td></td>
<td>p = 0.1419</td>
<td>p = 0.6635</td>
</tr>
<tr>
<td>Synovial IGF-I vs. Synovial IRAP</td>
<td>r = 0.55</td>
<td>r = -0.29</td>
</tr>
<tr>
<td></td>
<td>p = 0.1595</td>
<td>p = 0.4859</td>
</tr>
<tr>
<td>Synovial IGF-I vs. Synovial TNF-α</td>
<td>r = -0.42</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>p = 0.2986</td>
<td></td>
</tr>
</tbody>
</table>

Table 8.4.2.2.2. reveals poor-moderate PPC in male TKAC with synovial IGF-I and cytokines, and female TKAC shows very poor relationships with cytokines. Overall no significant difference was found in any of the PPC. NC indicates no TNF-α was detected in synovial fluid in females thus no correlation was possible.

When comparing relationships between cytokines and growth factors in SF, interestingly IL-6 and TGF-α exhibited a moderate and significant relationship in males (r = .75, p = 0.0325), and not relationship in females (Figure 8.4.2.2.1). Females conversely revealed a strong and significant relationship between TGF-α and IRAP (r = 0.81, p = 0.0157) whereas males did not (Figure 8.4.2.2). All other relationships between men and women were found to be poor and not significant (r < .34, 0.14 > p < .76).
Figure 8.4.2.2.1. represents the relationship between synovial levels of IL-6 and TGF-α in men and women TKAC. Of interest is the positive significant relationship between the known catabolin IL-6 and the growth factor TGF-β in males ($r = 0.75, p = 0.0325$).
Figure 8.4.2.2.2 represents synovial levels of IRAP and TGF-β in males and females undergoing TKA. Of note is the strong and significant relationship between these variables in females ($r = 0.81$, $p = 0.0157$) and the lack of relationship in males.
8.4.3 Serum and Synovial Levels of IGF-I Correlated to Function

The association of serum levels of IGF-I and function (specifically FSPW, Stair measure and TUG) was tested in both groups. Solely one group revealed a significant correlation between serum IGF-I and function. This significant relationship was identified in male controls for FSPW and serum IGF-I ($r=0.51$, $p = 0.0120$). All other functional measures analyzed revealed non significant poor-moderate relationships between IGF-I and function (Figure 8.4.3.2 and 8.4.3.3).
Figure 8.4.3.2 represents the relationship between IGF-I and Stair function in TKAC and healthy controls. Relationships range from poor to moderate.
Figure 8.4.3.3. represents serum IGF-I levels and FSPW in both groups. Again PPC are poor to moderate depending on gender and group. Only male controls were found to have a significant correlation ($r=0.51$, $p = 0.0120$).
Synovial levels of IGF-I were also analyzed for correlations and revealed interesting results. Male TKAC revealed correlations values as $r = 0.4268$ (IGF-I vs. FSPW), $r = -0.2668$ (IGF-I vs. TUG) and $r = -0.45$ (IGF-I vs. Stairs Figure 8.4.3.4.), with no relationship approaching significance. One must consider that a sample size of eight may have influenced the results and accounted for the lack of significance. Females surprisingly revealed moderately strong correlation for FSPW and IGF-I ($r = 0.73$) with poor-moderate on the remaining two functional measures $r = -0.29$ (IGF-I vs. Stairs), and $r = -0.3773$ (IGF-I vs. TUG). Once again, similar to the male TKAC, there were no relationships found to be significant.
Figure 8.4.3.4. Represents the correlation between synovial IGF-I and Stair function. As seen in the graph relationship are poor-moderate and were found to be not significant indicating that the relationship exhibited may be due to chance.
8.4.4. Serum and Synovial Levels of IGF-I Correlated to Perceived Disability and Pain

Relationships between serum IGF-I and perceived disability (specifically mobility) and pain were tested across both groups. Our results revealed consistent findings, in that all relationships proved to be poor with PPC values ranging from \(-0.0378 > r < -0.3781\) across both male and females and across both groups. In addition none of the relationships were found to be significant in any group or gender.

Conversely, when synovial levels of IGF-I were tested against the same variables mentioned above, initially it appeared that similar results would be elicited (All PPC’s were below \(r = -0.20\)), however one exception did occur, that of synovial IGF-1 vs. the mobility question tested in the WOMAC. This pairing resulted in a correlation of \(r = -0.9076\) which was also found significant \(p = 0.0047\) in TKAC (Figure 8.4.4.1.).
Figure 8.4.4.1. exhibits the strong and significant relationship ($r = -0.9076, p = 0.0047$) between mobility as assessed by the WOMAC and synovial IGF-I levels. The relationship between females was not as strong ($r = 0.2830$).
8.4.5. Relationships Within Functional Measures

PPC were performed on the FSPW, TUG and Stair Measure to see if each was equally able to quantify functional impairment. Our results revealed once again consistent findings across each variable tested. All measures revealed strong significant correlations with one another (Table 8.4.5.1). These strong correlations held true across all groups and genders (Figure 8.4.5.2). Overall it appeared that the TKAC group demonstrated stronger relationships between variables than their healthy controls.

Relationships between Functional Measures

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>TKAC</th>
<th>Healthy Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n=25)</td>
<td>Female (n=32)</td>
</tr>
<tr>
<td>Stairs vs. FSPW</td>
<td>( r = -0.82 )</td>
<td>( r = -0.72 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.0001 )</td>
<td>( p = 0.0001 )</td>
</tr>
<tr>
<td>Stairs vs. TUG</td>
<td>( r = 0.78 )</td>
<td>( r = 0.78 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.0001 )</td>
<td>( p = 0.0001 )</td>
</tr>
<tr>
<td>FSPW vs. TUG</td>
<td>( r = -0.82 )</td>
<td>( r = -0.84 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.0001 )</td>
<td>( p = 0.0001 )</td>
</tr>
</tbody>
</table>

Table 8.4.5.1. reveals strong correlations between functional measures which are all significant \((p< 0.05)\).
Figure 8.4.5.2 reveals the strong significant correlations between FSPW and Stair Function across all groups.
8.4.6. **Summary of Pearson Product Correlations**

PPC were done on a variety of variables to reveal if in fact a relationship existed between biochemical and functional, perceived disability and anthropometric measures. All in all, analysis using PPC revealed poor-moderate relationships between biochemistry and functional, anthropometric parameters with few of the relationships being significant. The general trend revealed that any relationship found between serum and synovial levels of IGF-I and any variable tested (age, anthropometry, function and perceived disability and pain) was always stronger in the males (regardless of group), and females (regardless of group) consistently revealed extremely poor correlations. The general trend also revealed that few relationships were found significant, suggesting that relationships identified most likely were due to chance rather than the variables being related.

PPC carried out between serum or synovial levels of IGF-I and cytokines revealed a few moderate relationships (in male TKAC) however once again none were found to be significant. Additionally cytokines (catabolic or growth factor) tested against one another revealed that no significant relationships existed in either serum or synovial fluid. Some of the cytokines analyzed in the TKAC population revealed low-moderate strength, but in many cases were found to be not significant which suggests that the relationship attained may have been due to chance.

PPC carried out between various techniques of anthropometry and functional measures revealed moderate-strong relationships which were all found to be significant across all groups and genders. It appeared that the relationships were consistently found to be stronger in the TKAC group than healthy controls across all functional and anthropometric measures.
8.5. Prediction Models

Prediction models using stepwise regression were employed to establish the best subset of predictor variables for a particular response variable which were clearly identified in Table 7.1. As many of our previous analyses revealed, group and gender differences clearly affected results. As a result, the stepwise regression was carried out by coding for group and gender. In total five variables were chosen as potential predictors, and a 3 model maximum was employed. Best predictor models were selected by considering various parameters; Mallow CP (C(p)) statistic, mean sum of error (MSE) , and whether the model made any clinical sense with respect to the population selected. In many instances predictor variables were found to be not significant, however this was not of large importance. As discussed with a statistician, in many instances predictor models may not be significant, and variables found to be significantly explaining variance may not be the best predictors, thus one must decide if variance explained is the issue at hand or if prediction is the goal of the study (Corey, 1998). It was decided that predictive ability would be followed thus significance of each predictor variable was not conducted.

Our results revealed consistent findings for all the variables analyzed. Each response variable (FSPW, NSPW, TUG and stair function) exhibited low total $r^2$ (none exceeding $r^2 = 0.43$) values when using a 3 predictor model regardless of group or gender (Table 8.5.0.1.). There appeared to be no trend among males when collapsed into one group, however each group of males did identify consistent trends in predictive variables across all response variables (Table 8.5.0.1). Overall it appeared that walking ability was best predicted by the variables, with stairs and tug measures having lower predictive ability. Conversely solely female controls exhibit similar consistency across all predictive models, where as female TKAC demonstrated a variety of predictive models with walking functions using the same variables and stair and tug functions also using the same variables. Across all groups age was definitely a predictor of function, and in healthy men and women serum IGF-I was also used as predictor. In the TKAC group no real consistency existed between males and females.
### BEST REGRESSION PREDICTOR MODELS FOR FUNCTIONAL RESPONSE VARIABLES

<table>
<thead>
<tr>
<th>Measures</th>
<th>Gender</th>
<th>( r^2 )</th>
<th>Mallow Cp</th>
<th>Model Variables</th>
<th>Variables</th>
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<td></td>
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<td></td>
<td>TKAC</td>
</tr>
<tr>
<td>FSPW</td>
<td>Male</td>
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<td>2.51</td>
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<td>Age, LBM, Waist</td>
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<td>2.96</td>
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<td>Age, Fat, IGF-I</td>
</tr>
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<td>2.33</td>
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<td>Age, LBM, Waist</td>
</tr>
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<td>Age, Fat, IGF-I</td>
</tr>
<tr>
<td>Stairs</td>
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<td>3.66</td>
<td>3</td>
<td>Age LBM, Waist</td>
</tr>
<tr>
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<td>5.50</td>
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<td>Age, Fat, LBM</td>
</tr>
<tr>
<td>TUG</td>
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<td>2.01</td>
<td>3</td>
<td>Age, LBM, Waist</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.24</td>
<td>2.50</td>
<td>3</td>
<td>Age, Fat, LBM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HEALTHY CONTROLS</td>
</tr>
<tr>
<td>FSPW</td>
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<td>2.09</td>
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<td>3.40</td>
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</tr>
<tr>
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<td>0.16</td>
<td>4.12</td>
<td>3</td>
<td>Age, IGF-I, LBM</td>
</tr>
</tbody>
</table>

Table 8.5.0.1 reveals the best predictor models for the response variables chosen, without grossly exceeding the Mallow Cp statistic. Of note it appears that Age, and IGF-I are consistently chosen as predictor variables in the models in helping to explain the response variable. Also, in a few cases a two variable model was chosen as the 3 variable models did not increase the amount of variance explained.

To summarize the regressions it appears that overall the total variance explained was rather low using a combination model. There appeared to be a general lack of consistency amongst the predictor variables across genders and groups for each response variable tested, with the exception of age which was consistently chosen first as a predictor of function. However within the control group there appeared to be some consistency in the variables best selected to predict function. In healthy controls age and IGF-I were uniformly chosen across all functional measures and across all groups, the only variable which varied was LBM versus waist as the predictor. In the TKAC group uniformity was not repeatedly found, with women demonstrating different predictive models with each functional measure.
CHAPTER IX

9.0 DISCUSSION

Several hypotheses on end stage OA were formulated for this study. Of primary importance was to establish if in fact serum IGF-I in TKAC was lower than their healthy controls and conversely if synovial levels of IGF-I were elevated in TKAC when compared to healthy controls. Both hypotheses were formulated in hopes of distinguishing whether the OA process was more systemically driven or locally driven. Another hypothesis formulated was the speculation that in end stage OA there would be an elevation of catabolic cytokines in comparison to anabolic cytokines in the synovial fluid in the TKAC group; once again potentially supporting locally driven mechanisms in this disease process. Finally, it was speculated that function (FSPW, NSPW, Stair function, and TUG) and perceived function (WOMAC and LEAP) would differ in these two populations.

Overall our results indicated that significant IGF-I gender differences existed in both serum and synovial levels of IGF-I whereby women (TKAC and healthy controls) had consistently lower levels than their male (TKAC and healthy controls) counterparts. Additionally a significant difference in serum IGF-I levels existed between healthy females and female TKAC, with female TKAC having lower amounts of IGF-I. No difference was identified in serum IGF-I when comparing male controls and TKAC. In fact male TKAC had higher serum IGF-I levels (although not significant). LBM was found not to influence gender differences when analyzed, which reinforced that despite males having larger amounts of LBM than females, a true serum IGF-I difference did exist.

In synovial fluid there were no trends identified with respect to elevated catabolic cytokines, rather anabolic cytokines (growth factors) appeared to be consistently elevated. Both of these previous findings suggested different mechanisms of OA pathology in males and females. Our results also fully supported the hypothesis that function was impaired in the TKAC group significantly more than the healthy control group with slower walking speeds, stair negotiation and rising from and sitting into a chair requiring more time. Additionally, the TKAC group reported significantly more disability with respect to mobility and experienced more pain.
Overall it appeared that TKAC women were more disabled functionally as well as exhibiting lower IGF-I scores.

Other hypotheses speculated that IGF-I (serum or synovial) would correlate to cytokines (serum or synovial), function, and perceived function. Our results on the whole did not support these hypotheses, however a few exceptions did exist. For the most part no correlations of moderate strength which were also significant were identified across any measure.

What this study revealed overall was significant differences do exist in serum IGF-I in females, specifically TKAC females which potentially plays a role in their limited function. Unfortunately the role which IGF-I plays in end stage OA was not clarified in the study rather strong speculations existed. What the study revealed is more variables need to be explored in hopes of establishing a link between OA, and lowered IGF-I levels and impaired function.

9.1. Anthropometry

It was imperative to recruit individuals with similar ages in each group, as it has been shown that GH and IGF-I are related to age with a 14 % decline each decade in GH and subsequent declines in IGF-I (Corpas et al., 1993; Iranmanesh et al., 1991; O'Connor et al., 1996; Shetty & Duthie, 1995) . In our study both males and females were matched to age, height and gender. Overall significant differences which were revealed, were identified in variables such as weight, BMI, waist girth measurements, and percentage of body fat.

All in all TKAC were found to be heavier despite similar ages and heights. When compared to other studies it appeared that our healthy controls weighed less (approximately 2.0 - 9.2 %) than subjects used in other studies (Baumgartner, Stauber, McHugh, Koehler & Garry, 1995; Walsh, 1995). When comparing the BMI of our TKAC group to other studies conducted on individuals with OA, it appeared that our subjects overall had a higher BMI which ranged from 10.5 % for males up to 23.8 % for females (Hochberg et al., 1995; Spector et al., 1994). What was surprising in the two studies previously mentioned was an identified
increased risk of OA and a higher prevalence of OA in those with BMI's greater than 28.2 for males and 25.8 for females. Comparing these values to our TKAC, they were certainly well within the risk or zone for developing OA, however our controls were fast approaching these levels of risk yet were deemed healthy. Furthermore that our subjects exhibited much higher BMI scores, it was easily surmisable that they were at end stage OA for a longer period of time thus increasing the amount of inactivity and increasing total body fatness. In the studies previously cited no mention was made of duration at the particular stage of OA, thus it was not unreasonable for our subjects to be considerably heavier than those reported in the literature.

It was of importance to establish total body fatness in this population as correlations with IGF-I were to be carried out between the two variables. It has been well documented in the literature that IGF-I deficiency has been linked to adiposity and increased truncal fatness, and increases in adiposity have been linked to further reduction in GH and IGF-I secretion (De Boer et al., 1992; Gertner, 1993; Jorgensen et al., 1996; Rosen et al., 1993). Using both techniques, significantly (p=0.0001) increased overall fatness was noted in both male and female TKAC when compared to healthy controls. Regardless of technique used, women (healthy controls and TKAC) generally had greater percentages of adiposity than males, and overall female TKAC were identified as having the greatest body fatness. It was not abnormal that females generally have more adipose tissue than males, rather what was surprising was that female TKAC were 47% fatter (using BIA technique) than female controls, while male TKAC were 36% fatter than their controls.

Skinfold technique using the Dumin and Womersley method appeared to give considerably higher percentage fat values across all groups (Table 9.1.1.) when compared to the BIA technique. The largest discrepancies between the two techniques appeared to manifest themselves in the controls, whereas the values remained more constant in the TKAC group.
Table 9.1.1 reveals differences in body fat estimation techniques. Of note are the consistently higher estimation of body fat using the Durnin and Womersley method. Additionally, this discrepancy appears more well noted in the control group than the TKAC. The symbol * indicates significant differences (p = 0.001) between groups and genders regardless of technique used.

In the TKAC group the differences between each measure varied from 11 - 20 %, however in the control group the differences from each technique varied from 38 - 46 %. The literature acknowledges that BIA may underestimate total body fat, especially in specific populations such as the elderly or GHD, however skinfold estimation also varies depending on the population used as densities of tissues no longer remain constant with age related changes (Broekhoff et al., 1992; Deurenberg, van der Kooy, Leenen, Weststrate & Seidell, 1991; Deurenberg et al., 1989; Durnin & Womersley, 1974; McNeill et al., 1991; Segal et al., 1988; van der Kooy et al., 1992). Specifically with skinfold techniques the loss of height can result in underestimation of fat free mass (overestimation of body fat) (Broekhoff et al., 1992). It appears that in this study the BIA in all likelihood underestimated body fatness in the controls and possibly underestimated the TKAC group. Correlations conducted between these two techniques appeared to be quite good (0.73 > r < 0.88, p=0.0001) and proved significant across all groups and genders (Figure 8.4.1.). However despite the good correlation, caution must be taken when choosing the technique to determine body fatness as under or overestimation may ensue.

Total body fatness (both techniques) and LBM were not significantly correlated to IGF-I in TKAC controls. Our negative findings are clearly supported by Benbasset et al 1997 who observed that no relationship existed between IGF-I, IGFBP3 and anthropometric measurements (Benbassat et al., 1997). The lack of relationship suggests that IGF-I levels may
not be the primary determinant of adiposity in the TKAC or healthy control population. Physical activity level and dietary habits may be other possible determinants.

9.2.0. BIOCHEMISTRY

9.2.1. SERUM AND SYNOVIAL LEVELS OF IGF-I

Adequate documentation exists in the literature regarding serum values for older individuals who are apparently healthy, however it is quite difficult to establish normative values of serum IGF-I for individuals with OA (Table 3.2.6.1 and Table 3.3.1.). What is even more difficult to establish are age matched normative or OA values of IGF-I in synovial fluid of the knee. As identified earlier, few investigators have actually profiled knee synovial IGF-I levels in healthy normals and even fewer profiles on individuals with OA (Table 3.2.6.1 and Table 3.3.1.). Of all healthy profiles of SF, those reported in the literature most have been conducted on individuals under the age of 45, thus comparisons of our synovial fluid results are rather difficult. Additionally, of those who have documented synovial fluid in OA patients, no gender differentiation was incorporated into the analyses thus not allowing for potential gender effects to be revealed. Yet, despite these difficulties, results obtained proved to be quite interesting as they revealed differences in healthy individuals and OA patients.

Our results revealed an overall significant (p = 0.0002) gender difference with females consistently having lower levels of serum IGF-I than males. When comparing serum values between genders, healthy women had 75.2 % serum IGF-I of healthy males, and TKAC women had 66.2 % serum IGF-I levels of TKAC males. This gender difference was clearly supported by the literature with men consistently having higher IGF-I levels than females (Denko et al., 1990; Moskowitz et al., 1991; O'Connor et al., 1996). Our results further revealed that TKAC females had significantly (p=0.0345) lower serum IGF-I values than their female healthy controls. These findings (in women) were generally supported in the literature with OA subjects consistently having lower IGF-I values than controls. The IGF-I levels in our healthy controls are low relative to some sources and rather high in other instances (Bellantoni et al., 1996; Denko
et al., 1990; Moskowitz et al., 1991; Thompson et al., 1995; van Beuningen, Amtz & van den Berg, 1993; Vitiello et al., 1997).

In males the IGF-I levels were marginally higher in TKAC group (although not significant) than healthy controls which was surprising as this was unanimously not supported by any findings in the literature. Of all the literature reporting serum IGF-I levels on individuals with OA, consistently those with OA were identified as having reduced IGF-I levels regardless of gender (Denko et al., 1990; Fernihough et al., 1996; Hoffman & Ho, 1996). Serum IGF-I values for our male TKAC group were on average 15 % higher than previously reported (Fernihough et al., 1996; Vitiello, 1997 #363; McAlindon et al., 1993; Moskowitz et al., 1991). Conversely, our healthy males values were, when compared to the literature, found to be variable both exceeding and being inferior to the reported values. When compared to studies conducted by Denko et al 1990 and Rudman et al 1994, our healthy males values were low, ranging from 21.0 - 23.0 % lower than the average reported (Denko et al., 1990; Rudman & Mattson, 1994). When compared to studies conducted by Benbasset et al 1997 and Vitiello et al 1997, our males exceeded the reported results ranging 7 - 18 % (Benbassat et al., 1997; Vitiello et al., 1997). It appears in the former studies that large age ranges were used which may potentially account for various discrepancies in serum IGF-I.

Two explanations were considered for the elevated IGF-I levels in our TKAC group when compared to Denko et al 1990; firstly that TKAC individuals were not truly at end stage OA, and secondly their mechanism of development of OA may be different than that of women. To ensure that the improperly staged OA was not the reason for this elevation in serum IGF-I, a sub-sample of individual's x-rays were reviewed to ensure that all were classified as end stage OA. On two separate occasions, graded by two orthopaedic specialists whose readings correlated quite strongly (0.84> r < 1.00 ) using the ARA recommended KLS (Appendix 4), greater than 65 % were graded as severe (4 ⋆ ) on the KLS and the remaining 35 % were graded as moderately severe (3 ⋆ ) in at least one compartment of the knee. These OA gradings were identical to the gradings used in all studies reviewed. In fact many of the studies included any individual who had a 2 ⋆ grading according to the ARA, thus wrongful staging of OA most likely was not the reason for elevated IGF-I levels (Denko et al., 1990; Moskowitz et al., 1991; Vitiello et al., 1997).
The other possibility for elevated IGF-I was the mechanism of OA (traumatic versus idiopathic). The whole TKAC group was reviewed and what was revealed was that 63% of males recalled having some sort of injury to the affected knee earlier on in life, where as only 15% of women recalled traumatic injury to the knee. In fact when viewing serum IGF-I levels in our sub-population (where reported percentage of trauma was comparable 5/8), the mean serum value was higher than that of the overall TKAC male population tested (182.6 μg/L ± 18.35). Suggestions have often been made that following trauma to the knee, many individuals (approximately 44 - 60%) subsequently develop knee OA (Cameron et al., 1994). Davis et al 1989 reported that “history of injury was strongly related to the presence of ipsilateral OA” which suggested that injury was a major risk factor in the development of OA (Davis, Ettinger, Neuhaus, Cho & Hauck, 1989). Thus one may speculate that in males who develop OA the mechanism may be a disruption to the local joint homeostasis, whereas in female TKAC the OA process may be more systemic. Specifically, with respect to our results it appears that local trauma may truly account and explain for higher levels of IGF-I associated with our male TKAC. However to date few longitudinal studies exist to conclusively support this hypothesis.

Synovial samples of the TKAC group indicated that relative to the females, males once again had significantly (p = 0.0001) elevated IGF-I levels. The values obtained from our male subjects were similar to those reported in the literature with elevated synovial IGF-I levels in OA patients when compared to healthy controls (Fernihough et al., 1996; Matsumoto et al., 1996; Schneiderman et al., 1995). However the values cited in these aforementioned studies had combined values from both men and women with no gender distinction made. In our study it was rather evident that females had reduced synovial IGF-I levels (in many instances below the RIA detection limit). An explanation for elevated IGF-I levels in synovial fluid may include continued AC destruction resulting in joint attempts to repair or diminish further damage. However exact mechanisms (local versus central regulation) and rationale for elevated levels of IGF-I in synovial fluid is largely unknown at present.

When comparing serum and synovial levels of IGF-I, synovial levels were significantly lower (p = 0.0246) than serum levels overall. Male TKAC synovial IGF-I levels were 40% of
serum values and female TKAC reported synovial IGF-I levels 44% of serum values. These figures appeared to correspond to values reported by Femihough et al, whereby he reported synovial levels of IGF-I in OA patients to be 53% of serum levels (Femihough et al., 1996). Schneiderman et al observed in his OA population similar values found in Femihough's study with synovial fluid representing 48% of serum IGF-I values (Schneiderman et al., 1995). However values reported in this study combined males and females thus not accounting for gender differences. Yet the general trend in an OA population appeared to be a reduction of synovial levels of IGF-I when compared to serum levels of IGF-I. Authors speculate that the serum to synovial differences occur because synovial IGF-I is regulated locally by mechanisms such as cytokines, chondrocyte homeostasis and synoviocytes, while serum IGF-I is primarily regulated by GH secretion (Dore et al., 1995; Malemud, 1993). Dore et al. 1995 conducted a study on human chondrocytes to see if GH directly stimulated IGF-I release and found that local synovial levels of IGF-I appeared to be GH/GHR independent and local IGF-I was stimulated by other factors such as TGF-β, or catalytic cytokines (Dore et al., 1995). However, to date there are considerable inadequacies in the literature explaining local levels of IGF-I. Thus the question remains whether central signals drive local environments, or do local environments stimulate and regulate themselves. With respect to IGF-I, strong speculation continues to exist (in the absence of concrete findings) that local IGF-I levels are locally controlled and are not affected by central GH levels.

Serum and synovial levels of IGF-I were poorly correlated (0.01 > r < 0.23), statistically insignificant, and clinically irrelevant. Our results were supported by the literature which also found no correlation between serum and synovial levels of IGF-I which suggests separate mechanisms of control (Femihough et al., 1996; Matsumoto et al., 1996). However in both studies conducted by Femihough and Matsumoto, the sample sizes were small (10 subjects) which may have influenced their findings (Femihough et al., 1996; Matsumoto et al., 1996). Similarly, our study sample size was also quite small (males = 9, females = 8) possibly masking true relationships which may be revealed with a larger samples. Yet if one were to agree with the hypothesis that both serum and synovial levels are regulated by different mechanisms, it would not be of surprise that no correlation existed between the two variables. It appears that further investigation with larger sample sizes are required to put this question to rest.
Serum levels of IGFBP3 were found not to vary across both groups and genders indicating that any difference in serum IGFBP3 was insignificant. Our values were found to be somewhat high to reported IGFBP3 levels in the literature with our values exceeding other authors reported normative values by 12 - 40 % (Benbassat et al., 1997; Blum, ; Hoffman & Ho, 1996). Reasons for this elevation in both groups are uncertain.

Synovial levels of IGFBP3 was not detectable as levels were below the detection limit of the assay. Implications of this were potential elevated levels of free synovial IGF-I, however in many of the females (5/8) IGF-I levels were below the detection limit of 60 μg/L, thus true IGF-I levels were also not available. Yet in the TKAC males levels were mostly above 60 μg/L yet synovial IGFBP3 were still not detectable. A potential explanation for this phenomenon was provided by Olney et al who demonstrated that articular cartilage chondrocytes produce IGFBP3 in humans, and this production is elevated when exposed to IL-1α and TNF-α (Olney, Wilson, Mohtai, Fielder & Smith, 1995). This finding may be important as in end stage OA, the number of chondrocytes are reduced due to destruction, as well as with the normal decline of chondrocytes which occurs with aging. Hence with the reduction in chondrocytes, the amount of binding proteins produced and secreted may also be reduced thus accounting for non-detectable levels. The reductions in IGFBP3 in males potentially may result in higher levels of free SF IGF-I which may also react with surrounding tissue and exert anabolic effects. Unfortunately at the present time free IGF-I levels are not measurable in any biological fluid to prove this previous statement.

9.2.2. Catabolic Cytokines and Growth factors

Although OA is considered to be a non inflammatory disease process, recent studies have indicated elevated levels of cytokines (both anabolic and catabolic) and growth factors in synovial fluid of individuals with OA (Table 3.3.1.) (Bertazzolo et al., 1994; Fong et al., 1994; Holt, Cooper, Denton, Meager & Hopkins, 1992 ; Sipe, 1995). At present a complete profile of normative data does not exist for either an OA population or a healthy control population on synovial levels of cytokines and growth factors. Due to this lack of information, comparing results becomes exceedingly difficult and interpretation virtually impossible.
Synovial levels of cytokines in many cases were undetectable in our TKAC population. In fact our catalytic cytokines, IL-1 α, IL-1 β, TNF-α were quantified as near zero or zero as they were undetectable by the assay kit whose sensitivity's were <0.1 pg/ml for IL-1 α and β and 5.0 pg/ml for TNF- α. These findings were not considered abnormal as most of the literature reviewed revealed that chronic inflammatory responses or OA pathology cited these specific catalytic cytokines to be usually low or undetected (Bertazzolo et al., 1994; Cameron et al., 1994; Kahle et al., 1992). Only one study, that of Kahle et al 1992, reported elevated levels of IL-1 β and TNF-α in synovial fluid of OA patients (Kahle et al., 1992). Kahle measured these aforementioned SF cytokines in various populations, those with OA (ages ranged from 17-59), trauma, other arthritides and RA. The study reported that those with RA were identified with the largest values (IL-1 ~ 193 pg/ml and TNF-α ~ 170 pg/ml ) whereas those with OA reported lesser values (IL-1β ~ 21.0 pg/ml and TNF-α ~ 80.0 pg/ml) (Kahle et al., 1992). The lack of consistency with other author's may be explained possibly by the large range of age (15 - 79 yrs), as well as potential lack of uniformity in disease staging (no mention of end stage OA or severe OA).

When viewing the lack of SF catalytic proteins in our sub sample it was hypothesized that due to the destructive nature of OA, our end stage OA sample would have minimal amount of AC or chondrocytes remaining. Potentially the catalytic proteins had in essence destroyed as much as possible and subsequently “burnt out” or down-regulated themselves. Recalling that chondrocytes are responsible for the production of IL-1, IL-6 and TNF-α, upon reduction in number (due to destruction or aging) or activity, subsequent production is also reduced, potentially resulting in low SF values. It has also been suggested by Holt et al 1992 that IL-1 β which is secreted into synovial fluid may be biologically inactive (in precursor form), only functioning in close proximity of tissue (Holt et al., 1992). Alternatively, reduced IL-1 levels and TNF-α may possibly reflect the elevation of counter acting factors such as IRAP, IGF-I and TGF-β which may potentially suppress activity. Unfortunately either explanation for the lack of IL-1 and TNF-α is presently not substantiated in the literature as cytokine interactions and exact SF cytokine levels in various disease processes are rather complex and not well understood at present.
Synovial levels of IL-6 were not found statistically different between genders despite the 117 pg/ml difference between males and females. In all likelihood the small sample sizes and large standard error means resulted in this lack of statistical significance. Our synovial values appeared to be rather high when compared to the works of Bertazzolo et al 1994 where mean values were reported as 89.45 ± 120 pg/ml (Bertazzolo et al., 1994). Our males reported values 2.37 times greater and our females reported values 1.06 times greater than Bertazzolo’s results. Once again no normative values were available in the literature to properly compare our subjects. What appeared inexplicable in our subjects was the elevation of IL-6 despite negligible amounts of IL-1 (either α or β). As discussed earlier, it is well documented that IL-1 induces or promotes IL-6 production. However, controversy presently exists on the true role or function of IL-6 in the knee joint. Presently some researchers are hypothesizing that IL-6 may function in a dual capacity, one as joint catabolin, and the other as an anti-inflammatory agent or protector (Middleton et al., 1996; Westacott & Sharif, 1996). Its exact role has yet to be discovered, however with our elevated levels of IL-6 and undetectable levels of IL-1 (either form), one may possibly surmise that in end stage OA, IL-6 may function as a joint protector. This theory was partially demonstrated with our data. Correlations performed on cytokines (catabolic and anabolic) revealed a positive, moderately strong, significant relationship (r = 0.75, p = 0.0325) between IL-6 and IRAP in synovial fluid (Figure 8.4.2.2.1.) in male TKAC (not demonstrated in female TKAC). This surprising finding suggests two possibilities: firstly that IL-6 may in fact function as a joint protector independent of IL-1’s influence in the knee joint in male TKAC. As previously mentioned, if IL-1 were a strong producer of IL-6, reduction in IL-1 would indirectly affect IL-6 production which is clearly not seen in this situation. Secondly IL-6 may very well be a catabolin solely responding to the elevation of IRAP which in essence blocks IL-1’s activity on the AC. Thus elevated IL-6 levels are in fact heightened attempts to continue AC destruction. Both possibilities are presently not substantiated in the literature as establishing exact cytokine interactions are quite difficult in vitro and exceedingly difficult in vivo. However it was somewhat unexpected to find such a relationship in a small sample.

Synovial levels of anabolic cytokines appeared to be elevated once again possibly suggesting final attempts of the AC at repairing or preventing further damage. Female TKAC had values 6.2 times and TKAC males had 7.90 times greater levels of TGF-β than those found in the chronic anterior cruciate ligament (ACL) deficient patients in Cameron et al’s (1994)
study. No significant gender differences were found, however, consideration must be given that potential differences may have been masked as a result of small sample sizes and that future investigations account for this. Finding normative data again proved to be difficult with few studies profiling serum or synovial levels of TGF-β in an OA population or normal population. Due to this apparent void in available information on normative data, comparisons become terribly difficult. However when comparing our results to Cameron et al 1994, one may suggest that due to the chronicity of injury in our population one may expect our subjects’ elevated levels of TGF-β to be a futile attempt to retard further damage to the AC (Cameron et al., 1994).

IRAP was also found to be elevated in synovial fluid, yet again no significant differences were found between males and females. Normative values were not available in the literature reviewed for individuals with OA, or healthy controls. Referring once more to Cameron et al 1994, acute ACL injuries revealed elevated IRAP levels (1892.7 pg/ml) whereas chronically deficient ACL’s (greater than 3 months - 14 years) revealed no detectable levels of IRAP in SF (Cameron et al., 1994). Our results did not concur values reported by Cameron et al 1994 as our values were elevated (300 - 400 pg/ml) when compared to her chronic ACL patients which reported no detectable IRAP levels. Elevated IRAP levels may suggest continued attempts of the joint in preventing further degradation of the AC by competing for receptor sites of IL-1. However due to IL-1 not being detected in either form, IRAP may also represent a normal delayed response in activation with chronic elevation to aid in preventing further destruction.

When comparing the relationships between anabolic cytokines and growth factors a strong, significant (r = .81, p =0.0157) relationship was identified in female TKAC (not males) between TGF-β and IRAP levels (Figure 8.4.2.2.2). It was believed that this relationship potentially indicated that despite undetectable levels of catalytic cytokines in SF in end stage OA patients, the AC appeared to still be attempting to protect itself against further damage. No literature was found to substantiate our results with respect to the relationships found between cytokines (anabolic or catabolic). Although all relationships between anabolic cytokines were found to be not significant, one must again consider the small sample size and its potential influence on the noise/signal ratio of the results.
Overall it appeared that synovial growth factors were still in essence attempting to recover or further prevent destruction despite negligible levels of strong catalytic cytokines present in the synovial fluid. Normally, both growth and catalytic factors are in tight regulation of one another. However if one exceeds the other, the opposite group (eg. growth factors) may remain elevated in hopes of deterring or repairing damage. Additionally there appeared to be gender differences with males secreting larger amounts of all cytokines (Figure 9.2.2.1) when compared to females which potentially suggests either greater damage overall or larger local stimuli potentiating the response. At present our results do not substantiate this conclusively, nonetheless one should not completely refute the results as sample size was rather low, suggesting further investigation is required.

To bring closure to the roles and stimuli which growth factors and catalytic cytokines play is almost impossible to do. As reviewed earlier on in the thesis, the interactions between anabolic growth factors and catalytic cytokines are quite complex (Figure 3.3.1.). The exact system (final common pathway) of these interactions at present is largely unknown due to the infancy in this area of interest, as well as the lack of controlled in vitro studies (Pelletier et al., 1993; Westacott & Sharif, 1996). Evidence provided by preliminary studies suggests that chondrocytes and synoviocytes are the prime driving influences on the regulation of these synovial agents, and that systemic input is minimal (Bandara & Evans, 1992; Bandara, Georgescu, Lin & Evans, 1991; Pelletier et al., 1993; Walker, 1996). Evidence underlying minimal systemic input is not readily available in the literature, thus to speculate at this point in time appears premature. However whatever the impetus is for the cascade of events in the joint, it remains rather interesting and ironic that the chondrocyte, that which maintains the AC, is also responsible for its own death (via IL-1, TNF-α and other catabolin secretion). Thus the question remains as to whether there is some systemic input that drives the chondrocyte to produce local agents, or is there one event in the local environment which is the catalyst to these cascade of events which alters the homeostasis in the joint. At present, scientific studies have not been able to shed light on controlling mechanisms.
Comparison of Synovial Fluid Cytokines and Growth Factors in TKAC Males and Females

Figure 9.2.2.1 reveals cytokine profiles in male and female TKAC of their synovial fluid prior to surgery. As can be seen, males overall have a larger amount of anabolic cytokines, and a larger amount of IL-6 (catabolic ?, anabolic ?). Also of interest is that none of these differences were deemed significant due to large SEM and small sample sizes.
No relationship existed between serum and synovial levels of cytokines (anabolic or catabolic) in either gender. No literature was unearthed identifying studies linking local and systemic cytokines in end stage OA. Although in literature which studied individuals with rheumatoid arthritis, a well known systemic disease, some cytokines were found to correlate moderately well when examining cytokine levels in serum and synovial fluid (Holt et al., 1992). However OA and RA are quite different disease processes, thus comparisons are not advisable.

9.3. Functional Measures

Our results revealed that healthy men and women were significantly ($p = 0.0001$) superior in performance, reported significantly ($p = 0.0001$) less pain and reported significantly ($0.0001 > p < 0.0026$) less exertion in all functional measures (FSPW, NSPW, Stairs and TUG). Overall TKAC males and females were quite disabled and functioned at a fraction of their healthy counterparts ability (Table 9.3.1.). No gender differences existed in our healthy population as males and females performed equally, however gender differences did exist in our TKAC population with males functioning significantly better ($p < 0.01$) in all tasks than female TKAC.

### Performance of Functional Measures of the TKAC Group (indicated as Percentages)

<table>
<thead>
<tr>
<th>VARIABLE MEASURED</th>
<th>TKAC (% PERFORMANCE WHEN COMPARED TO HEALTHY CONTROLS)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>FSPW</td>
<td>83 %</td>
</tr>
<tr>
<td>NSPW</td>
<td>86 %</td>
</tr>
<tr>
<td>Stair Function</td>
<td>58 %</td>
</tr>
<tr>
<td>TUG</td>
<td>74 %</td>
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</tbody>
</table>

Table 9.3.1 represents a comparison of function between Healthy Controls (male and female) and TKAC (male and female). The actual values indicates the calculated percentage of performance when comparing each gender with each task. In this scale normals were considered 100 %, thus anything less was functioning at less of a capacity.
Our results of superior functioning by our healthy controls in all the functional measures were clearly substantiated in the literature when comparing reported walking speeds of healthy controls to individuals with OA (Bassey et al., 1976; Cunningham et al., 1986; Cunningham et al., 1982; Fransen, Crosbie & Edmonds, 1997; Himann et al., 1988; Kaneko, Morimoto, Kimura, Fuchimoto & Fuchimoto, 1991; Kovar et al., 1992; Kroll et al., 1987; Marks, 1995; Mattsson & Brostrom, 1990b; Murray, Duthie, Gambert, Sepic & Mollinger, 1985; Oberg, Oberg & Oberg, 1994). Overall our healthy controls performed the NSPW and FSPW somewhat better (ranging from 7 - 30%) than those reported in the literature, but were quite similar to controls used in another study of OA conducted by Walsh (1995). The TKAC candidates' performance when compared to results in the literature were mixed, as they exceeded performance levels in some instances and lagged in performance in others (Table 3.4.4.1). What must be kept in mind though are the subjects recruited for these studies and the combining of males and females thus not revealing potential gender differences. Our stair function results were substantiated by those found in Walsh et al 1995, with TKAC being more impaired than healthy controls overall (Walsh, 1995). Surprisingly though, our pre and Walsh's post TKA stair functional values were not much different, where male TKAC pre-surgery performed 17 % faster than male TKAC post surgery, and our females performed at 96 % of female TKAC post surgery (Walsh, 1995).

Overall it appeared once again that TKAC women were found by far more functionally disabled when compared to their male surgical counterparts. TKAC women performed consistently below the male TKAC ability (ranging from 59 % - 74 %) in the speed of all functional tasks. Reasons for reduction in ability are potentially many, however it was speculated that adiposity possibly may have played a significant role in the great functional disability seen in TKAC females but not males.

Correlations performed on adiposity and function revealed moderate correlations in female TKAC between adiposity performed by BIA and all functional measures (Table 9.3.2.). Surprisingly, this relationship was solely seen in TKAC females and not TKAC males nor in the control group. Speculation around this finding suggests that TKAC females may once again have a systemic process which potentially affects their functioning, whereas in males a more local process may affect functioning.
Correlations were also examined between serum IGF-I and function which revealed no relationships existed except in healthy males (r = 0.51, p = 0.0120). Preliminary studies were conducted by Rudman et al 1994, attempting to establish a relationship with serum IGF-I levels and overall amounts of physical activity in two different populations. What Rudman et al 1994 reported was no relationship existed between activity and serum IGF-I levels, furthermore that sedentary older men actually reported significantly (p<0.05) higher values of serum IGF-I than physically active older men (Rudman & Mattson, 1994). Another study conducted by Papadakis et al 1995, compared some strength measures, physical performance test and cognitive function and found that age, not IGF-1 was the most important variable in predicting functional decline (Papadakis et al., 1995). Unfortunately no other literature to date has been located comparing relationships between functional measures, or physical function and serum IGF-I. Ergo, we were unable to determine if our results were substantiated by others. It is quite important to distinguish that although each author previously mentioned looked at some aspect of strength, function was truly not assessed as it combines many variables such as strength, balance, endurance and vision.

Overall, what was rather interesting with our results though were female controls exhibiting equal or marginally better performances, yet having overall lower IGF-I levels than their male counterparts even when LBM was accounted for. This finding would in theory be similar to the results Rudman et al reported that physically active individuals had lower IGF-I levels. However exact reasons for this finding are not available.

Relationships between synovial levels of IGF-I and function (FSPW, NSPW, Stair Function and TUG) were found to possess no gender differences, and no clinical, or statistical

<table>
<thead>
<tr>
<th></th>
<th>FSPW</th>
<th>NSPW</th>
<th>Stairs</th>
<th>TUG</th>
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<tbody>
<tr>
<td>% Fat</td>
<td>r = -0.58</td>
<td>r = -0.57</td>
<td>r = 0.49</td>
<td>r = 0.46</td>
</tr>
<tr>
<td></td>
<td>p = 0.0004</td>
<td>p = 0.0006</td>
<td>p = 0.0072</td>
<td>p = 0.0102</td>
</tr>
</tbody>
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Table 9.3.2. represents significant, moderately correlated values between measured adiposity and function.
significance. Presently no literature exists comparing function and synovial IGF-I levels, thus our values can neither be substantiated nor refuted as being correct.

Alternate functional measures were highly correlated (Table 8.4.5.1 and Figure 8.4.5.2.) across all groups and genders. This indicated that any measure presently used was acceptable to quantify functional ability in either population. As function is increasingly important for independent living, our results indicating that any test chosen to quantify function would indirectly address other functional issues, was quite pleasing. This suggested that any clinician could adequately assess function with any measures and that results would indicate levels of function across other measures.

Multiple Regressions were performed on each functional measure to evaluate which predictor variable (independent variable) could explain most of the variance in the response (dependent) variable. What was revealed indicated that in most instances not much of the response variable variance was explained using multiple predictors. In fact the best predictor model of any response variable only explained 43% of the variance, which was viewed as rather poor. This indicated that the models chosen, or variables entered did not account for variance and other factors may be better suited. However, clinically the variables chosen were believed to have the most influence on a person's functional capacity overall.

Predictor variables age and fat were the primary variables which consistently explained the most variance in each response variable chosen and were also found to be significant (p < 0.05). However as previously mentioned the prediction model was used to identify which variable could best predict function regardless of significance. Overall it appeared that functional measures are not predicted well based upon anthropometric or biochemical factors.

9.4. PERCEIVED FUNCTIONAL DISABILITY

Specific questions of both the LEAP and WOMAC questionnaire were identified and analyzed between groups. Many studies to date have been conducted on the WOMAC in the OA population (Barr et al., 1994; Bellamy, 1989; Bellamy et al., 1990; Bellamy & Buchanan,
1984; Bellamy et al., 1988; Bellamy, Goldsmith, Buchanan, Campbell & Duku, 1991; Bellamy et al., 1992; Bellamy, Sothem & Campbell, 1990). Recent and current studies of the LEAP are demonstrating its usefulness as a reliable, valid, sensitive tool which is capable of identifying change in this population (Finch & Kennedy, 1995; Finch, Walsh, Thomas & Woodhouse, 1997). Both aggregate and signal measurements have recently been identified as picking up differences in a population due to intervention (Barr et al., 1994; Bellamy et al., 1990). As a result of these preliminary studies, it was decided upon to embark on similar conditions in this study to identify if signal measurements would identify patterns or trends in the populations at hand. Signal measurements were chosen as it was felt that future correlations with IGF-I would most likely manifest themselves with pain measurement and mobility measurement. Unfortunately, nothing is present in the literature to refute or substantiate this rationale.

Overall the control group reported significantly (p = 0.0001) less pain and less perceived difficulty with mobility when compared to the TKAC group. Our results were partially substantiated by a study conducted by Finch et al., 1997 which indicated that group differences in perceived mobility capabilities exist between healthy controls and individuals post TKA (Finch et al., 1997). What was surprising was the equivalent reports of pain between men and women despite greater functional disability and greater perceived functional disability overall among women. These findings of equal pain ratings are at a variance with Finch et al findings in their post TKA candidates (Finch et al., 1997). The variance in findings may be due to differences between pre and post TKA status.

Relationships among serum and synovial IGF-I and perceived pain and mobility were quite interesting. Correlations of serum IGF-I with perceived mobility and pain were disappointing in that no real trend or relationship was found in either group or gender. However, when reviewing relationships between synovial IGF-I and perceived function and pain, a strong, significant negative relationship (r = -0.91, p = 0.0047) was revealed in male TKAC between mobility using the WOMAC questionnaire and synovial levels of IGF-I. No other correlations were found clinically relevant or significant. Once again due to small sample sizes our findings still may be due to chance despite the strong significance as only one relationship (WOMAC) became visible and not the other.
9.5. **Clinical Significance of IGF-I, Cytokines, Function, and Perceived Function**

It is often quite difficult to bring together biochemical findings and relate them to a disease process and the individual's ability to function. Quite often perceived ability and biochemistry may not correlate or match due to a variety of reasons (confounding factors, limited detection ability of tests, improper design format, etc...). However to dismiss this bridge between these two areas may be calamitous to health care as underlying links may exist which if properly understood might lead to improved treatment methods.

We know individuals with reduced serum IGF-I levels will experience decreases in LBM, increases in adiposity and potential hormonal imbalances which in turn may result in limited functional ability. In vitro testing reveals that reductions in synovial IGF-I (in the presence of heightened catabolins) results in the reduction of chondrocyte proliferation and maintenance, thus less reparative effect and ultimately reductions in function due to pain, or inability to accept loads. In vivo results show that in OA patients, IGF-I levels rise which may aid in joint repair. Minimal reductions in quantitative function (walking, stair climbing etc.) are quite often well categorized by scales. We have attempted to pull the diverse areas of biochemistry, function and perception in the OA population and form a model gelling these areas together. An overall model (Figure 9.5.1) showed that TKAC women as the group which reported most difficulty with perceived function, were most disabled as classified by functional measures, revealed lowest IGF-I levels and had the greatest amount of total body adiposity. The relations depicted for the female TKAC population suggested a potential systemic component to their OA pathophysiology. The substantial and pervasive differences between female TKAC and controls suggests that females are truly affected in all areas which may be indicative of a systemic process.

Furthermore looking at the model one also sees that TKAC males are certainly functionally impaired and perceived greater amounts of impairment, yet their biochemistry (IGF-I) and adiposity are not very different from their healthy male counterparts. Looking at this model overall, one may speculate that in male TKAC the OA process may be affected by
something local, not systemic as their biochemistry and anthropometric measures are quite similar to their male counterparts.

Thus to firmly say in the aforementioned paragraphs that due to lack of significant relationships proved by statistical analysis that variables have no relationship may be premature as a number of factors may be responsible for these relationships not being revealed. It is quite apparent that despite firm relationships, overall TKAC females are certainly the most impaired, and disabled in this population tested. Furthermore that their disability most likely is multi-factorial and needs to be explored further.
Figure 9.5.1. reveals how females are quite different from their age matched controls, and overall present a picture of 4 systems (biochemistry, anthropometric, functional ability and perceived disability) being grossly affected by OA. Conversely male TKAC depict a overall normal anthropometric and biochemical profile and abnormal function and perceived function profile. Both these findings potentially suggest that the OA process works through two different systems in males (locally) and females (systemically).
9.6. Study Limitations

Several limitations existed in our study which in all likelihood limited our ability to establish true roles which IGF-I and cytokines play in end stage OA. Unfortunately we were unable to harvest synovial fluid samples from the knees of healthy controls which would have provided sufficient information to profile cytokine and growth factor levels such that comparisons could be made between groups to establish normality. Additionally due to the scarcity of literature on synovial levels of cytokines and growth factors for healthy individuals above the ages of 55, the synovial fluid data's interpretation became quite limited.

In addition to the lack of healthy controls for synovial fluid harvesting, few TKAC volunteered for the additional measures. In total solely 17 individuals (female = 8, males = 9) volunteered, with one sample of fluid being lost in storage, making the analysis quite difficult. This was especially noticeable in correlations performed between synovial IGF-I and cytokines, function and perceived function. Correlations appeared to be low-moderate in strength but were shown not to be significant, most likely due to a small sample size as they were analyzed by gender. It was felt that a larger sample size would have allowed for more conclusive speculations.

Another limitation which presented itself towards the end of the study was the potential role which estrogen could play on levels of IGF-I in serum and synovial fluid in women. Exact dosages of estrogen, route of administration (oral or transdermal) and the length of treatment were not documented originally. It was felt that had these factors been accounted for further information would have resulted in helps of explaining reduced levels of IGF-I in women. Previous studies have documented that postmenopausal women have less spontaneous GH secretion (pulse and amplitude of pulse) than pre-menopausal females (Bellantoni et al., 1996). Additionally that endogenous estrogen levels are positively correlated to GH and IGF-I levels in premenopausal females, and have been cited to amplify spontaneous GH secretion (Bellantoni et al., 1996, Corpas et al., 1993; Jorgensen et al., 1996). Despite the known relations between estrogens and IGF/GH, some controversy continues to exist with the mode of estrogen delivery and the subsequent effects to this population.
Exogenous estrogen treatment/supplementation for postmenopausal may be administered in two forms, orally or through transdermal patches. However until recent, the route of exogenous estrogen administration was not fully explored to identify if method of administration affected serum levels of IGF-I. Via animal and human models, it was revealed that route of administration (oral versus transdermal) appeared to directly affect GH and serum IGF-I levels in postmenopausal females. Studies have documented that oral estrogen supplementation reduces serum IGF-I levels via a reduction of IGF-I messenger RNA specifically in the liver with subsequent elevation in the spontaneous pulses and release of GH (Bellantoni et al., 1996; Hartman et al., 1993). Conversely, transdermal estrogen supplementation resulted in minor elevations or no change in serum IGF-I levels with no effect on spontaneous GH secretion (pulse frequency and amplitude) (Bellantoni et al., 1996; Corps et al., 1993; Hartman et al., 1993; Ho, O'Sullivan, Weissberger & Kelly, 1996). In addition to this identification of how mode of estrogen supplementation affects GH/IGF-I axis, other studies have also suggested that in fact the mode may be irrelevant, rather the dosage of estrogen is more important (Bellantoni et al., 1996). Bellantoni et al (1996) reported that higher dosage of transdermal estrogen produced similar effects as oral estrogen, increased spontaneous GH release and reduced serum IGF-I levels. Hence, recording dosage, method and duration of estrogen supplementation is vital, as its' effects on IGF-I levels systemically may potentially skew results. Unfortunately with this information absent in our study, our gender differences of IGF-I remain somewhat unexplained, and we are reliant on future studies to document dosage and route of estrogen administration.

The final limitation in the study was the basic design of the study. Overall this was a cross sectional design which in essence presents a snap shot view of the variables measured. Specifically, prevalence of OA is quite different between men and women as noted by Badley et al, (Badley & Rothman, 1996), that a gender difference does exist in this population as shown by our results, multiple cohorts should have been tested such that a clearer picture be identified. Multiple age categories would have enabled us to profile the progression of knee OA biochemically, and thus discern if in fact IGF-I was a strong predictor of knee OA.
Patients with OA and patients with low serum IGF-I share similar features including increased adiposity, reduced function, and reduced cardiovascular status. Do patients with end stage OA have low serum IGF-I? Our most important finding is that the answer depended on the patient's gender. Women with OA demonstrated low levels of serum IGF-I and this suggested the possibility of developing OA through a different mechanism.

The primary premise of this study was to identify IGF-I differences in both serum and synovial levels between groups and possibly between genders. It is well documented that injury often leads to knee OA. Additionally, suppositions have been made that local injury, not a systemic process, is what drives the OA process. The emphasis on local versus systemic factors was partially supported by our findings as males demonstrated equivalent serum levels of IGF-I when compared to healthy males. Furthermore, synovial levels of IGF-I were elevated when compared to reported values of normals and other OA populations. This last finding suggested that OA in TKAC males, may be resultant of a local effect, not systemic influence. Finally, as no correlation existed between serum and synovial fluid levels, it was felt that this lent further support to this premise. Hence one may suggest that males with OA most likely develop OA through a local process.

TKAC females though did not exhibit similar patterns to their male counterparts. TKAC females demonstrated overall lower levels of serum IGF-I (lower than healthy females) as well as lower synovial levels of IGF-I when compared to male TKAC. The reduced IGF-I levels, high adiposity and markedly impaired function when compared to healthy females, and TKAC males suggests that the OA pathophysiology may be different in women. Females did not exhibit elevated levels of synovial IGF-I as males did, in fact many subjects synovial IGF-I levels were undetectable and this finding lent credibility to a systemic etiology rather than local etiology of OA. Finally, that women appear to be affected more globally in all measures than males one may consider that OA development in female TKAC is most likely idiopathic, due to some systemic factor.
The underlying mechanism for different OA pathology in males and females is presently unknown. One can speculate that hormones, specifically estrogen, or lack of may potentially have played a role in overall lowered serum IGF-I levels in women. It has been well documented in the literature that endogenous and exogenous estrogen does affect IGF-I in healthy women. We did not systematically assess estrogen status or use in our patients and can therefore only speculate that this effect could have shed light on differences between men and women, as well as intra-group differences between females with respect to IGF-I levels. Additionally, since that mechanism of injury was not thoroughly explored, one can only speculate again that this information could have shed further light on OA etiology in males and females.

Across all variables measured, it was quite obvious that the common theme was the marked impairment, and disability in female TKAC’s when compared to TKAC males. Despite similar damage to the knee joint, and ultimately the same end result being that of TKA replacement, women generally were worse off across all categories measured. Although previous studies report similar findings (Finch et al., 1997; Finch et al., 1997; Walsh, 1995), no explanations are available as to why this trend is evident and pervasive. Potentially, one may speculate that since the prevalence of OA peaks at a younger age than males, females may live with the disease longer and ultimately wait longer before surgery. This outcome would render them more functionally disabled which in turn affects them systemically. Alternatively, changes in hormonal status during menopause may result in certain females experiencing extreme drops of IGF-I systemically which may affect their metabolism resulting in obesity, loss of function and ultimately as many studies suggests greater risk of developing OA. However neither theory has been conclusively tested in the literature.

The most difficult aspect of the study was to determine if our cytokine profiles in synovial fluid were representative of the end stage OA population. As little information on OA synovial profiles exist in the literature it was rather difficult to conclusively determine if results obtained in fact were comparable to others with the same disease process. Overall our profile obtained was somewhat confusing, but did suggest biochemical support for some reparative processes. All major catalytic cytokines were absent in the knee, yet reparative factors (TGF-β, IGF-I, and IRAP) still remained elevated. These conditions would support continued AC repair but the
tissue destruction being too advanced to allow for any significant repair. What was interesting was the elevation of IL-6 in the absence of detectable IL-1 levels. This elevation suggested that IL-6 in fact may be an anabolic cytokine rather than a catabolic one. Presently in the literature controversy exists, hence this elevation may substantiate the theory that IL-6 in synovial fluid may aid in repair rather than destruction.

Gender differences in synovial levels of growth factors and cytokines between male and female TKA candidates may exist as demonstrated by our findings. The larger amounts (although differences were not statistically significant) of growth factors and anabolic cytokines observed were in male TKAC. This finding once again pointed in the direction of gender specific pathophysiology where local process due to injury or trauma may drive specific pathways in males, whereas females potentially develop OA through various other methods.

What was quite evident, and comforting as a clinician was the strong correlations between various functional measures suggesting that any measure used (FSPW, NSPW, TUG and Stairs) would accurately quantify the amount of disability in either group. This relationship held across all groups and genders which suggests that regardless of pathology, function could be adequately assessed by any of the tests utilized.

Signal measurements have been proven in past to be quite sensitive to change. It appeared that both the WOMAC and LEAP questionnaires were able to pick up differences in perceived mobility and function quite well in both groups. When reviewing the relationship between perceived ability and pain to biochemical indicators, no clear evidence was exhibited that strong relationships existed. Unfortunately no previous literature has explored the relationships between signal measurements as indicators of biochemical processes occurring systemically or locally in any environment, thus no precedent is available. Although solely one variable was found to be correlated and significant (synovial IGF-I and WOMAC mobility) in this study, this may lead potentially to future studies identifying particular signal questions which may reflect biochemical processes. In the advent of better diagnostic technology, and more readily available biological fluid in all populations, this link between perceived ability and biochemistry may be established.
In conclusion, our results indicate that both male and female TKAC are more functionally disabled, report more disability, and generally reveal greater adiposity when compared to healthy controls. These findings indicate that OA certainly does affect individuals in multiple systems. Our findings also indicate that female TKAC appear to be more impaired and disabled across all variables measured when compared to healthy controls and male TKAC. Additionally, that male TKAC despite portraying a different physical, mental and anthropometric profile than healthy male controls, demonstrated equal serum IGF-I as male controls. Ultimately, what these findings suggest are gender differences in the pathophysiology of OA development which affect functional ability, perceived ability and body composition differently.
11.0 Future Directions

OA is a disease process which in all likelihood will remain ever present and ever disabling for many older individuals. Specifically in females, the gross disabilities displayed in this study were rather disconcerting as demographics suggest that females outnumber males when exceeding age 65 and the prevalence of OA is higher. It is important to determine if our speculations about gender differences in the etiology and progression of OA are correct since these differences suggest different therapies may be employed in the prevention or retardation of OA.

Suggestions for future directions in the area of dysfunction and biochemistry may include conducting studies on the role of estrogen and women in OA to establish whether in fact various amounts, and types (transdermal versus oral) of estrogen replacement prior to or after menopause influence in serum IGF-I levels and function. Additionally, future studies may want to address the role in which exercise plays on serum IGF-I levels, function and perceived function in an OA population and healthy controls to see if changes occur, and if they do are they symmetrical across and within groups.

For males, we may want to investigate the types of knee injuries which occurred and any surgical interventions performed previous to their TKA. Further to injury history, advances in arthroscopic surgery may also allow identification of individuals who are at risk of OA earlier on and synovial environment may be serially studied to profile cytokine levels. Finally, as Cameron et 1994 suggested, many ACL injuries go on to develop moderate to severe OA. It would be worth while to explore how therapy, return to work, and return to sport may affect the local knee environment.
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APPENDICES
INFORMATION ON THE
SERUM AND SYNOVIAL LEVELS of IGF-1 AND CYTOKINES
RESEARCH STUDY

Serum and Synovial Levels of Insulin Like Growth Factor (IGF-1) and
Cytokines in Total Knee Replacement (TKR) Candidates, Anterior Cruciate
Deficiency (ACL) Patients and Healthy People

BACKGROUND INFORMATION

Many individuals will develop arthritis in their knees and other joints throughout their lifetime. Although there are many reasons suggested why arthritis comes about, there is no scientific evidence to show a causal relationship of a certain event and arthritis. It is believed that decreased amounts of IGF-1 and increased amounts of cytokines affect the severity of osteoarthritis (OA).

Every individual produces growth hormone (GH) which stimulates the production of IGF-1. It is believed that IGF-1 can directly affect articular cartilage (a smooth covering at the end of long bones which help the bones move over each other with little friction), by maintaining it healthy and intact so that arthritis is delayed. Some researchers believe that IGF-1 is produced by the covering of your joints (the synovial lining) while others think levels of IGF-1 are present in the blood which then affect the articular cartilage. Therefore, our goal is to compare blood levels of IGF-1 and synovial levels of IGF-1 to see if the cartilage protecting compound is lacking at the blood level or synovial level.

Cytokines are compounds that are believed to be present in all tissues in the body (especially joint fluid and linings). There are many different types of cytokines, those which help protect the articular cartilage (IRAP and TGF-b), and those which help breakdown articular cartilage (IL-1, IL-6 and TNF-a). There is a very delicate balance of cytokines within the synovial fluid (a clear lubricating fluid in most joints of the body), and any disruption may lead to OA. Therefore our aim is to look at the levels of cytokines in the knee joints of those individuals who are undergoing knee surgery and those individuals who have no problems with their knees. This information may reveal what are the normal levels and balances of cytokines which individuals, such as yourself should have in a knee joint to keep it healthy.

The goals of this study are as follows:

1. To Compare blood IGF-1 levels and synovial fluid IGF-1 levels in healthy individuals and TKR candidates.
2. To compare the relationship between synovial IGF-1 and synovial cytokines levels in healthy individuals and TKR candidates.

3. Determine if IGF-1 or cytokine levels relate to functional capacity or strength in TKR and ACL candidates and healthy individuals.

PARTICIPANT DESCRIPTION

We are looking for men and women, between the ages of 55 and 75 and 20 and 35 who are undergoing TKR or ACL reconstruction in one of their knees. Your participation would require one attendance at the Orthopaedic and Arthritic Hospital lasting approximately 2.0 hours (maximum).

For the TKR and ACL patients this would be done along with your Patient Orientation Program (POP) visit. During this visit, the measures described below would be made. Two additional measurements would be made during your surgery, in the presence of your surgeon and other medical staff.

DESCRIPTION OF STUDY

An investigator will take a brief medical history (specifically a health and OA history) to determine if you have had previous knee problems or health problems which may exclude you from this study.

Your height, weight and body fat will be measured. Your percentage of body fat will be measured using two techniques; bio-electrical impedance (a machine which sends a small current through your body and measures how much resistance there is to this current) and skinfolds using a caliper (a device which measures in millimetres the amount of fat around certain areas of your body). For skinfold measurements, you will be required to expose your trunk to the investigator behind an enclosed area. Female participants may keep their brassieres on during these measurements.

You will do a timed Self Pace Walk (SPW), where you are asked to walk at two paces; 1) your normal walking speed and 2) rather fast, but without over exerting yourself. At each pace you will walk a total of 160 metres (8 times down a 20 metre corridor), you may use your assistive device if need be. Your heart rate will be measured and you will be asked how hard you are exerting yourself.

Your knee strength will be tested at various speeds on our Isokinetic machine (a machine which uses a computer to measure the strength of your muscles while doing certain movements at specific speeds). You will be tested in a sitting position with your lower limb strapped in the machine. After you familiarize yourself to the machine, you then straighten and bend your knee as quickly, and forcefully as you can. The machine measures the force you are able to generate. You will be tested at slow and moderate
speeds. You should wear comfortable clothing; shorts, T-shirts, track pants and runners when participating in the SPW and the Isokinetic measurements.

In addition we will remove 10-15 ml of blood from the vein in your arm by a certified technologist at your POP visit and the day of your surgery. One sample of blood will be taken regardless of your participation in the study since it is normal hospital procedure to take a sample of blood prior to surgery.

TKR and ACL patients will have 2-3 tablespoons of synovial fluid removed from their operative knee by a needle during surgery. This would be done while you are completely anaesthetized (asleep) by your surgeon. This procedure will only be done once.

If you choose to participate in our study, your identity will be kept confidential, along with all your medical records, findings and personal information.

**RISKS AND BENEFITS**

There are minor risks involved in this study. When participating in the SPW and strength measures you may experience muscle soreness or joint soreness for several days after the measurements. Infrequently, the soreness may be severe enough to limit your activities for several days, however, it will resolve over time.

A final risk associated with this study is the removal of blood. Although blood is removed by a certified technologist, there is a potential for bruising around the site of extraction which may lead to soreness and reduced ability to use your arm temporarily.

Although these risks are minor it is imperative that you understand them completely. If you do not understand them, ask a study investigator (Paul Marks, Scott Thomas, Sonia Pagura or Linda Woodhouse) who will then explain them to you.

The benefits from your participation in this study are that you will aid the scientific community in understanding what factors or deficiencies are involved with OA. Your participation will allow investigators to establish a data base on normal and abnormal levels of cytokines and IGF-1 in the synovial fluid and blood. This information might, in the future, help the scientific community develop new means via medications or therapies to help people with OA.
Appendix 2
INFORMATION ON THE
PLASMA LEVELS OF IGF-1 IN TOTAL KNEE ARTHROPLASTY
CANDIDATES RESEARCH STUDY

Are Plasma IGF-1 Levels in Total Knee Replacement (TKR) Candidates and
Healthy Aged Matched Controls the Same?

BACKGROUND INFORMATION:

Many individuals will develop arthritis in their knees and other joints throughout their lifetime. Although there are many reasons suggested why arthritis comes about, there is no scientific evidence to show a causal relationship of certain events and arthritis. As research continues, we are able to shed light on causal factors, which may in turn help to discover methods to control this crippling ailment. It is believed that decreased amounts of IGF-1 affect the severity of osteoarthritis (OA).

Every individual produces growth hormone (GH) which stimulates the production of IGF-1 (produced and released by the liver). Growth Hormone levels are difficult to measure so we look at IGF-1 levels as a marker of Growth Hormone. Growth Hormone is important for adults as well as children. In adults it helps to increase muscle and bone growth and helps decrease the amount of body fat. Its effect on muscle and bone may be important in the process of recovering from operations. Moreover, it is also believed that IGF-1 can directly effect articular cartilage (a smooth covering at the end of long bones which help the bones move over each other with little friction), by maintaining it healthy and intact so that arthritis is delayed. Some researchers believe that the low levels of IGF-1 in the blood may be a determining factor of OA. Therefore, our aim is to compare IGF-1 levels in the blood of those individuals undergoing knee surgery and those individuals who have no problems with their knees. This information may reveal what the normal levels of blood IGF-1 should be in individuals, such as yourself, so that the knee joint can remain healthy.

The goals of this study are as follows:

1. To compare blood IGF-1 levels in healthy individuals and TKR candidates

2. To reveal if there are any differences in functional capacity with TKR candidates and healthy individuals.
PARTICIPANT DESCRIPTION

We are looking for men and women, between the ages of 55 and 75 who are undergoing TKR in one of their knees. We are also looking for healthy individuals with no past injuries or problems with their knees, and have not been diagnosed with any form of arthritis. Your participation would require one attendance at the Orthopaedic and Arthritic Hospital lasting approximately 45 minutes (maximum). For individuals who are undergoing knee surgery, this would be done along with your Patient Orientation Program (POP) visit. This visit would include various measurements and questionnaires. If you choose to participate in our study, your identity will be kept confidential, along with all your medical records, findings and personal information.

DESCRIPTION OF STUDY

An investigator will take a brief medical history to determine if you have had previous knee problems or health problems that may change the levels of IGF-1 in the blood. Physical activity may also influence levels of GH and IGF-1 and so we will use a questionnaire to find out how physically active you are. Should you meet the acceptance criterion, your data will only be recognized as an number to ensure that all information is kept confidential.

In addition to these questionnaires, your height and weight will be measured. Your percentage of body fat will also be measured using two techniques; bioelectrical impedance (a machine which sends a small current through your body and measures how much resistance there is to the current) and skinfolds using a caliper (a device which measures in millimeters the amount of fat around certain areas of your body. For skinfold measurements, you will be required to expose your truncal area to the investigator behind an enclosed area. Female participants may keep their brassieres on during these measurements.

You will be required to do a timed Self Pace Walk (SPW) where you will be asked to walk at two paces: 1) your normal waking speed and 2) rather fast, but without over exerting yourself. At each pace you will walk a total of 160 meters (8 times down a 20 meter corridor), you may use your assistive device. This test will enable us to determine your functional capacity. Your heart rate will be measured and you will be asked how hard you are exerting yourself.

Finally, we will also require the removal of 10-15 ml (1 small tube) of blood from the vein in your arm by a certified technologist at your visit. For the TKR patients, one sample of blood will be taken regardless of your participation in the study.
since it is normal hospital procedure to take a sample of blood prior to surgery. Furthermore, TKA candidates will have an extra vial (small tube) of blood taken just before surgery.

**RISKS AND BENEFITS**

There are minor risks involved in this study. When participating in the SPW, you may experience muscle soreness or joint soreness for several days after the measurements.

There is a small risk with the removal of blood. Although blood is removed by a certified technologist, there is a potential for bruising around the site of extraction which may lead to soreness and reduced ability to use your arm temporarily.

Although these risks are minor, it is important that you understand them completely. If you do not understand them, ask any of the investigators (Scott Thomas, Sonia Pagura or Linda Woodhouse) who will explain them to you.

The benefits from your participation in this study are that you will aid the scientific community in understanding what factors are involved with OA. Your participation will allow investigators to establish a data base on normal and abnormal levels of IGF-1 in the blood. This information can then potentially lead to the discovery of how we, the scientific community, can develop new means via medications or therapies to prevent OA in all individuals. The final benefit is knowing that your participation may help yourself or others in the future from developing OA, a crippling ailment of the joints.
Appendix 3
CONSENT TO PARTICIPATE IN A RESEARCH STUDY
at the Orthopaedic and Arthritic Hospital

Serum and Synovial Levels of Insulin Like Growth Factor (IGF-1) and Cytokines in Total Knee Replacement (TKR) Candidates, Anterior Cruciate Deficiency (ACL) Patients and Healthy People

You are invited to participate in a research study at the Orthopaedic and Arthritic Hospital. We are studying how IGF-1 levels and cytokine levels differ in people with knee problems (TKR or ACL surgery) and healthy people of the same age. This consent form is provided to help you make a well informed decision about participating in this study. Along with this consent you will be given an information sheet to take home which explains our study in more depth.

Should you have any questions please do not hesitate to ask either Scott Thomas (the principle researcher), Linda Woodhouse, Sonia Pagura or Paul Marks at 967-8717.

BACKGROUND INFORMATION

Many individuals develop arthritis in their knees and other joints through their lifetime. As yet, there is no scientific evidence linking certain event and arthritis. Better understanding of how osteoarthritis develops may lead to methods to control this crippling ailment. It is believed that decreased amounts of IGF-1 and increased amounts of cytokines affect the severity of osteoarthritis (OA). In this study we will compare levels of these compounds among two patient groups (ACL, TKR) and healthy people.

DESCRIPTION OF STUDY

To participate, you would come once to the Orthopaedic and Arthritic for approximately 2 hours (maximum). This visit would include measurements and questionnaires as listed below. If you are undergoing surgery 2 more measures would be made during your surgery, in the presence of your surgeon and other medical staff.

As a healthy study participant you would come to the Orthopaedic and Arthritic Hospital for the measures listed below. As a patient the following measures would be made prior to your surgery at your POP visit. An investigator and hospital staff would take the following measurements:

a) A health and Osteoarthritis history to determine whether you are eligible for the study;
b) Percentage of body fat using electrical impedance (a machine sends a small current through your body and measures how much resistance there is) and skinfold calipers to measure the thickness of your fat at five places on your body (2 arm, 2 trunk and 1 leg measure);

c) Self paced walk in which your heart rate will be measured while you walk in a corridor;

d) Your strength and muscular endurance in bending and straightening at the knee;

d) Removal of 10-15 ml of blood from a vein in your arm by a certified technologist so that it can be analyzed for IGF-1 and cytokine levels.

For patients, on the day of your surgery, your surgeon will be taking two measurements so that IGF-1 and cytokine levels can be analyzed. Your surgeon will:

a) Ask a technologist to remove 10-15 ml of blood from a vein in your arm just before you enter your surgery;

b) Remove 2-3 tablespoons of synovial fluid from your knee joint using a needle during the surgery while you are anaesthetized (asleep).

**RISKS AND BENEFITS**

The risks involved in this study are as follows:

a) muscle soreness from the strength and Self Paced Walk measures;

b) bruising around the site where blood was removed;

The benefits to you directly are that you will know your percentage of body fat and strength in your lower extremities. The indirect benefits include knowing that your participation may help the scientific community in understanding osteoarthritis.

**VOLUNTARY PARTICIPATION**

I certify that I have read or have had read to me all the preceding information, including requirements of involvement, risks and benefits, and fully understand the information. Any questions I have or may have prior to participation will be addressed by the principle investigator to my satisfaction. My signature below confirms my understanding of what is required of me and my voluntary willingness to participate in this study.
CONFIDENTIALITY

I give full consent to the investigators to analyze all data collected and potentially publish it in a journal as long as my identity is not revealed. I also understand that all data collected; questionnaires, blood and synovial samples, height, weight, age, sex and articular cartilage photographs will be kept completely confidential, with the sole use of analyzing them for future publication.

NEW INFORMATION

Any new information during the study which comes to the attention of an investigator, which would directly relate to your willingness or ability to continue participation, will be told to you directly.

If you have any questions about the research before or after participation or you need to report any injuries as a result of participation, contact Paul Marks, Scott Thomas Linda Woodhouse or Sonia Pagura at (416) 967-8626.

I fully understand that my participation in this research is voluntary, and that my decision to participate will not adversely affect my standing or care at this hospital. If I elect to participate in the study, I have the right, at any time to withdraw from the study without affecting my care or benefits in which I was entitled in the hospital. I also fully understand that the investigators may also require that I withdraw from the study at any time.

I, __________________________ HAVE READ AND FULLY UNDERSTAND ALL THAT IS REQUIRED OF ME IN THIS RESEARCH STUDY.

I HAVE BEEN GIVEN THE OPPORTUNITY TO ASK QUESTIONS OF THE RESEARCHERS AND THEY ANSWERED TO MY SATISFACTION.

I AM SIGNING THIS FORM VOLUNTARILY, INDICATING MY WISH TO PARTICIPATE IN THE STUDY FULLY KNOWING ALL THE RISKS AND BENEFITS.

PARTICIPANT: WITNESS:

_________________________ __________________________
Print name Print name

_________________________ __________________________
Signature Signature

_________________________ __________________________
Date Date
Appendix 4
ACTIVITY PROFILE

(LEAP)

The questionnaire concerns the way any disability of your leg(s) affects your daily routine. There are questions about:

1. difficulty experienced over the past month in performing various activities.
2. your level of satisfaction with a particular ability.
3. how often in the past month you have been affected by a particular problem.

INSTRUCTIONS:

When marking the lines to indicate your rating on these questions, think about yourself and how you have felt in general in the past month. The words at the ends of the line are to assist you in making the rating. You may mark any point along the length of the line.

EXAMPLE:

Difficulty Line:

a lot of difficulty moderate difficulty a little difficulty
unable to do ______________________ no difficulty

Satisfaction Line:

partially dissatisfied neutral partially satisfied completely satisfied
completely dissatisfied ______________________

Frequency Line:

occasionally sometimes frequently
never ______________________ always

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LOWER EXTREMITY ACTIVITY PROFILE

Patient ID ______

SELF CARE:

1. In general, how much difficulty would you have with activities involved in taking care of your body? Think about such activities as dressing, bathing, going to the toilet, and taking care of your hair and nails. To answer, mark the appropriate place on the difficulty line below.

Difficulty Line:

unable to do __________________________ no difficulty

2. How satisfied or dissatisfied are you with your ability to do the activities involved in taking care of your body? To answer, mark the appropriate place on the satisfaction line below.

Satisfaction Line:

completely satisfied __________________________ completely dissatisfied

MOBILITY:

3. In general, how much difficulty would you have moving around your home and community? Think about such activities as bending toward the floor, getting up and down from a chair, stairs, outdoor walking, use of public transportation, and getting in and out of a car. To answer, mark the appropriate place on the difficulty line.

Difficulty Line:

unable to do __________________________ no difficulty

4. How satisfied or dissatisfied are you with your ability to move around? To answer the question mark the appropriate place on the satisfaction line.

Satisfaction Line:

completely satisfied __________________________ completely dissatisfied

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LOWER EXTREMITY ACTIVITY PROFILE

HOUSEHOLD ACTIVITIES:

5. In general, how much difficulty would you have in doing household activities such as meal preparation, housecleaning, grocery shopping, and outside chores? To answer, mark the appropriate place on the difficulty line below.

Difficulty Line:
unable to do ______________________________ no difficulty

6. How satisfied or dissatisfied are you with your ability to do household activities? To answer mark the appropriate place on the satisfaction line below.

Satisfaction Line:
completely ______________________________ completely satisfied
dissatisfied ______________________________

WORK:

7. Do you normally work for wages? (Yes or no) ____

If NO, proceed to question # 10. If YES, answer question # 8 and 9.

8a. What type of work do you do? ______________________________

b. In general, how much difficulty do you have in your work because of any disability of your leg(s)? To answer, mark the appropriate place on the difficulty line below.

Difficulty Line:
unable to do ______________________________ no difficulty

9. How satisfied or dissatisfied are you with your ability to work for wages? To answer, mark the appropriate place on the satisfaction line below.

Satisfaction Line:
completely ______________________________ completely satisfied
dissatisfied ______________________________
LEISURE ACTIVITIES:

10. What kind of activities do you do in your spare time? (Include activities you would do at any time of the year.)

11. In general, how much difficulty would you have doing these activities? To answer, mark the appropriate place on the difficulty line below.

Difficulty Line:

unable to do ____________________________ no difficulty

12. How satisfied or dissatisfied are you with your ability to do these spare time activities? To answer, mark the appropriate place on the satisfaction line below.

Satisfaction Line:

completely ____________________________ completely satisfied
dissatisfied ____________________________ satisfied

EMOTIONAL HEALTH:

13. Over the past week, how often were you frustrated because of any disability of your leg(s)? To answer, mark the appropriate place on the frequency line below.

Frequency Line:

never ____________________________ always

14. In general how often has your emotional health been affected by any disability of you leg(s)? To answer, mark the appropriate place on the frequency line below.

Frequency Line:

never ____________________________ always

15. How satisfied or dissatisfied are you with your present emotional health? To answer, mark the appropriate place on the satisfaction line below.

Satisfaction Line:

completely ____________________________ completely satisfied
dissatisfied ____________________________ satisfied
SLEEP AND REST:

16. How frequently do you take medication to help you sleep? To answer, mark the appropriate place on the frequency line below.

Frequency Line:
never __________________________ always

17. How frequently has your sleep been affected by any disability of your leg(s)? To answer, mark the appropriate place on the frequency line below.

Frequency Line:
never __________________________ always

18. How satisfied or dissatisfied are you with your ability to sleep? To answer, mark the appropriate place on the satisfaction line below.

Satisfaction Line:
completely __________________________ completely
dissatisfied __________________________ satisfied

SOCIAL ACTIVITIES:

19. In general, how much difficulty would you have participating in social activities? To answer, mark the appropriate place on the difficulty line below.

Difficulty Line:
unable to do __________________________ no difficulty

20. How satisfied or dissatisfied are you with your ability to participate in social activities? To answer, mark the appropriate place on the satisfaction line below.

Satisfaction Line:
completely __________________________ completely
dissatisfied __________________________ satisfied
21. Thinking about any changes in your appearance as a result of disability of your leg(s), how satisfied or dissatisfied are you with your appearance? To answer, mark the appropriate place on the satisfaction line below.

Satisfaction Line:
completely ----------------------------- completely satisfied
dissatisfied ---------------------------

22. In general, in the past week how much leg pain have you had? To answer mark the appropriate place on the pain line below.

Pain Line:
no pain ----------------------------- pain as bad as it can be

23. In the past week, how often have you had leg pain? To answer, mark the appropriate answer on the frequency line below.

Frequency Line:
ever ----------------------------- always

THANK YOU FOR ANSWERING THESE QUESTIONS.

LEAP - D. E. Finch, J. Kennedy, Centre for Studies of Physical Function
Appendix 5
In this table what is revealed are the correlation coefficients (r value) between both raters using the KLS scoring on both separate occasions, as well as the r value on individuals between both readings. As note the correlations were all exceptionally strong, and found to be significant (p < 0.05) in all cases.