NOTE TO USERS

Insulin-like growth factor-I, insulin-like growth factor binding protein-3 and the risk of cervical squamous intraepithelial lesions.

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ABSTRACT

Insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) have been associated with an increased risk of several cancers. This case-control study investigated the relationship between IGF-I and IGFBP-3 plasma levels and the risk of squamous intraepithelial lesions (SILs) of the cervix, as well as the risk of HPV infection in women. 366 cases and 366 controls were recruited from five Montreal area hospitals. There was a significantly decreased risk of LSIL for the highest quartile of IGFBP-3 relative to the lowest quartile (Odds Ratio (OR)=0.25, 95% confidence interval (CI) 0.08-0.77), adjusted for age, HPV status and IGF-I. Also, there was a significantly increased risk of being positive for HPV, specifically high-risk types, for the highest quartiles of IGFBP-3 relative to the lowest quartile in controls (OR=4.53, 95% CI 1.33-15.40), adjusted for age and IGF-I. IGF-I was not significantly associated with SILs or HPV infection.

RÉSUMÉ

Les IGFs (insulin-like growth factors) et les IGFBPs (insulin-like growth factor binding proteins), ont été associés avec un risque élevé de plusieurs cancers. On a étudié le rapport entre le niveau de IGF-I et de IGFBP-3 dans le plasma du sang, et le risque de développer les lésions squameuses intra-épithéliales (SILs) du col utérin, ainsi que le risque d'être positif pour le papillomavirus humain (HPV) dans une population Montréalais. Il y avait une diminution signifiante du risque de SILs de bas grade (LSIL) pour le quart supérieur de IGFBP-3 par rapport au quart inférieur (rapport de cotes (OR)=0.25, 95% intervalle de confidences (CI) 0.08-0.77), ajusté pour l'age, la présence d'une infection HPV et l'IGF-I. En plus, it y avait une augmentation de risque d'être positif pour HPV, particulièrement les variants haut risques, pour le quart supérieur de IGFBP-3 par rapport au quart inféfieur dans le groupe témoin (OR=4.53, 95% CI 1.33-15.40), ajusté pour l'age et l'IGF-I. IGF-I n'était pas associé avec les SILs ou l'infection HPV d'une façon signifiante.

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AGC	Atypical glandular cells
AIDS	Acquired immunodeficiency syndrome
ALS	Acid-labile unit
ASC-H	Atypical squamous cells, cannot exclude HSIL
ASCUS	Atypical squamous cells of undetermined significance
BCC	Benign cellular changes
BCCR	Biomarkers of cervical cancer risk
BMI	Body mass index
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
ELISA	Enzyme linked immunosorbent assay
FGF	Fibroblast growth factor
FIGO	International federation of gynecology and obstetrics
GH	Growth hormone
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPV	Human papillomavirus
HRT	Hormone replacement therapy
HSIL	High-grade squamous epithelial lesion
ICC	Invasive cervical cancer
IGF	Insulin-like growth factor
IGF-IR	Insulin-like growth factor-I receptor
IGFBP	Insulin-like growth factor binding protein
LSIL	Low-grade squamous epithelial lesion
OC	Oral contraceptives
OR	Odds ratio
Pap	Papanicolaou
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PSA	Prostate specific antigen
RR	Relative risk
SCC	Squamous cell carcinoma
SD	Standard deviation
SHBG	Sex hormone binding globulin
SIL	Squamous intraepithelial lesion
STD	Sexually transmitted disease
TNM	Tumour-Node-Metastasis
WHR	Waist hip ratio
WNL	Within normal limits

1 INTRODUCTION

Cervical cancer is unique as it is the only cancer for which a necessary cause has been identified. Human papillomavirus (HPV) infection has been accepted to be necessary to the development of cervical cancer, and can be identified in nearly all cases (1). Even so, only a small percentage of women who are infected with HPV go on to develop cervical cancer or its precursors (2). Current research strives to determine why certain HPV positive women develop cervical cancer and others do not.

The insulin-like growth factor (IGF) family consists of IGF-I, IGF-II and several IGF binding proteins (IGFBPs). Whereas IGF-II mainly plays a role as a key regulator in embryonic and fetal development (3), IGF-I continues to play a role throughout an individual's life, and exerts its actions through the IGF-I receptor (IGF-IR).

IGF-I is a broad spectrum growth factor whose primary source is the liver; it then travels in the bloodstream to its target tissues. It has a positive effect on cell proliferation and transformation, and a negative effect on apoptosis (4). Based on these characteristics, it was postulated that IGF-I was involved in the progression to carcinogenesis. Several experimental studies have supported this hypothesis, and *in vivo* and animal experiments have shown that IGF-I causes proliferation of human breast cancer cells (5), decreases apoptosis (6), and more significantly, has a positive effect on tumour development in mice (6).

To date, six different IGFBPs have been identified. The most abundant is IGFBP-3, and over 90% of IGF-I in the serum is bound to this binding protein (7). IGFBP-3 has both IGF-dependent and IGF-independent functions (8). By binding to IGF-I, IGFBP-3 increases its half-life by protecting it from degradation, facilitates its transportation to target tissues, and regulates its interaction with the IGF-IR (7). IGFBP-3 also has an inhibitory effect on cell growth, which it exerts via its own receptor independent of IGF-I (8). Because of the way it interacts with IGF-I, it is important to take IGFBP-3 into consideration when considering the role of IGF-I in cancer progression.

Several epidemiological studies have found a positive relationship between IGF-I and cancer risk; the majority have studied breast cancer, prostate cancer and colorectal cancer. Other cancers that have been investigated include lung cancer, endometrial cancer, ovarian cancer and bladder cancer. Many of the magnitudes of risk obtained have been higher than those seen for several currently accepted cancer risk factors. To date, only one epidemiological study has been done on the relationship between IGF-I, IGFBP-3 and cervical cancer precursors (9). It found a strong positive relationship between high levels of IGF-I and cervical cancer precursors.

Because of its apparent role in the development of many other cancers, we postulate that IGF-I may play a role in cervical cancer development. The results of the recently published study (9) on the relationship between cervical cancer precursors and IGF-I only reinforce this hypothesis and suggest that this is a promising area of research.

The main purpose of this case-control study was to investigate the relationship between IGF-I plasma levels as well as IGFBP-3 plasma levels and the risk of cervical cancer precursors. Because of the rarity of cervical cancer, and our knowledge of the natural history of cervical cancer, precursors were used as the main outcome; this also prevents the outcome from having any effect on the exposure in question, so that the measures reflect as accurately as possible pre-cancer levels of IGF-I and IGFBP-3. A secondary goal of this study was to investigate the relationship between IGF-I, IGFBP-3 and HPV infection in controls. This study was carried out in the Montreal area in five participating hospitals. Information was gathered on potential confounders and all laboratory testing was blinded. If a relationship is found, this may help to explain the natural history of cervical cancer, as well as to serve as a biomarker for cervical disease.

2 LITERATURE REVIEW

2.1 CERVICAL CANCER

2.1.1 Epidemiology

Despite the decreasing incidence of cervical cancer that has been observed in the Western industrialized world, it remains the second leading cause of cancer morbidity in women worldwide, second only to breast cancer (10). In 2000, there were an estimated 471 000 new cases in the world and nearly half as many deaths, with these numbers projected to triple by 2050 (10), further increasing the global burden of cervical cancer. In Canada, the rates have been decreasing mostly due to implementation of Papanicolaou (Pap) smear screening programs, which lead to early identification and treatment of potential cancer precursors. Even so, in Quebec this year there will be an estimated 280 new cases and 90 deaths from the disease (11).

The burden of cervical cancer lies disproportionately in specific populations, particularly those in Central and South America, sub-Saharan Africa and the Caribbean, with developing countries accounting for nearly 80% of all cases. In these regions, cervical cancer is the leading cause of cancer mortality in women (10). Even within Canada, Aboriginal women are excessively affected, with rates several times the national average (12).

Cancer is the leading cause of premature death in Canada (11). Even though the prognosis for cervical cancer patients is quite good in low-risk areas, the 5-year survival rate (66%) is still significantly less than that for breast cancer patients (84%) in the

United States (13). In addition, the incidence of cervical cancer usually peaks between 45-49 years of age, a relatively younger age than for most cancers (10), with 10 000 potential years of life lost in 1999 in Canada (11).

2.1.2 Biology

Precursor stages to the development of cervical cancer have been identified and extensively studied. Progression to cervical cancer can be divided into two phases: the preinvasive phase (also known as cervical intraepithelial neoplasia (CIN), or as dysplasia) and the invasive phase. In the preinvasive phase, the changes are limited to the cervical epithelium and are asymptomatic; they can only be discovered by the Papanicolaou technique (Pap test) and confirmed by colposcopic examination and biopsy, followed by a histological examination.

According to the more recent Bethesda classification system, CIN is recognized as squamous intraepithelial lesions (SILs) (14). SILs can be classified as either low-grade (LSIL) or high-grade (HSIL). A lesion is defined as high-grade if it extends to the full thickness of the cervical epithelium, which can happen if a low-grade lesion is left untreated.

In a significant proportion of women with untreated high-grade lesions, the disease will traverse the lining formed by the basement membrane that separates the epithelium from the underlying connective tissue. This is known as invasive carcinoma. The progression from HSIL to invasive cancer may take upwards of 10 yrs (15).

In the US, approximately 4% of Pap smears are abnormal smears that have changes consistent with precursor lesions (16). There are an even greater number of cases which can be defined as ASCUS, or "atypical squamous cells of undetermined significance," that is, those which have equivocal or borderline atypia. Overall, both ASCUS cases and SIL cases account for about 10% of all Pap smears processed in screening programs (16). These cases need to be closely monitored for persistence and progression and this places a substantial burden on the health care system.

2.1.3 Etiology

Cervical cancer is unique, as a necessary cause for the disease has been identified. Research has led to the implication of the sexually transmitted HPV infection to the development of cervical cancer and its precursors (17). With new and more sensitive assays, HPV can be identified in nearly 100% of cervical cancer cases (1). Many risk factors that have been identified to date for cervical cancer, such as a younger age at first sexual intercourse, increased number of sex partners and a history of STDs, in fact increase one's risk for HPV infection (18).

Although it is deemed to be a necessary cause of cervical cancer, HPV infection is far from being a sufficient cause. Latent HPV infection is the most common sexually transmitted viral infection today, with 5%-40% of sexually active women of reproductive age being infected (2) and only a small proportion developing cancer. It appears that those women with an infection that persists into later life are at a greater risk of developing the disease than those who clear the infection early on (19). Despite the many studies focusing on the role of HPV infection, there is less research on other etiological

factors, which may help define why only some HPV infected women eventually develop cervical neoplasia.

Cofactors for progression to cervical disease which have been identified to date include tobacco smoking, parity, oral contraceptive (OC) use, dietary factors, genetics and human immunodeficiency virus (HIV) infection (20). Smoking likely affects the development of cervical disease in either one or both of two ways: 1) it may have a direct carcinogenic effect on the cervix and (21) 2) it may negatively affect the immune response to HPV infection (22).

Secondly, epidemiologic studies have consistently found a positive association between the number of live births and the risk of cervical cancer (23). Multiple pregnancies may have an immunosuppressive effect or there may be a hormonal effect on the cervix. Long-term oral contraceptive use appears to increase one's risk of cervical cancer as well (24).

Diet seems to play a large role in the development of several cancers. Some dietary factors that have been shown to decrease the risk of cervical cancer include food containing carotenoids and vitamin C, and also vitamins A and E, perhaps because of their antioxidant activity. A poor diet can also affect have a negative effect on immunity (25).

The immune system plays a large role in the acquisition and clearance of HPV infection. It is postulated that certain human leukocyte antigen (HLA) alleles affect susceptibility to

HPV infection and cervical neoplasia by affecting the immune response (20). HIV infection suppresses an individual's immune system and makes it less able to fight off any infection, including HPV infection. Latent HPV infections are quite common in HIV-infected women.

2.2 INSULIN-LIKE GROWTH FACTOR (IGF) FAMILY

One recent focus of cancer research has been on growth factors as risk factors for cancer progression. Growth factors have been postulated to play a role in carcinogenesis, initially based on their biological actions and *in vitro* experiments (4). One family of growth factors receiving attention lately with respect to cancer etiology is the insulin-like growth factor family.

2.2.1 Biological Actions

Insulin-like growth factors (IGFs) were discovered as a result of the fact that they possessed insulin-like activity that could not be abrogated by adding anti-insulin antibodies. They are broad spectrum growth factors, which means that they promote cell proliferation of several different cell types. Similar growth factors include platelet derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). The IGF family consists of IGF-I, IGF-II, the trans-membrane receptors IGF-I receptor (IGF-IR) and IGF-II receptor (IGF-IIR), the IGF binding proteins (IGFBPs) 1-6, and IGFBP proteases which hydrolyze the IGFBPs.

2.2.1.1 Insulin-Like Growth Factors I and II

IGF-I and IGF-II are single chain polypeptides which have 62% homology to each other (7). The expression of the IGF-I gene is primarily regulated by growth hormone (GH), but the primary regulator of the IGF-II gene is not known (7). Expression of IGFs can also be influenced by several hormones including estrogens, adrenocorticotropic hormone, thyrotropin, luteinizing hormone, follicle-stimulating hormone and human chorionic gonadotropin, as well as other growth factors like PDGF, EGF and FGF (26).

In adult humans, there is much inter-individual variation in the concentration of IGF-I in the blood. The majority of circulating IGF-I is produced in the liver in response to GH, which can then travel to target tissues. IGFs can also be produced by cells in many tissues, where they can act in an autrocrine or paracrine fashion (27).

IGF-I has both immediate and long-term effects on cellular activity, and these effects are mediated mainly through the IGF-I receptor (IGF-IR). The acute effects include an increase in the cellular uptake of amino acids and glucose which has an effect on protein and carbohydrate metabolism, as well as the stimulation of glycogen and protein synthesis (7). In the long-term IGF-I affects cell proliferation, differentiation and apoptosis (7). IGF-I provokes cells to progress through the phases of the cell cycle by stimulating expression of cyclin D1 and increasing DNA synthesis (28). As well, IGF-I can stimulate the expression of Bcl proteins and suppress expression of Bax proteins, which alters the Bcl/Bax ratio, which in turn blocks initiation of the apoptotic pathway (29).

The concentration of IGF-II in the blood of adult humans is higher than IGF-I and are relatively stable after puberty, at about 400-600 ng/mL (26). Even though it has its own receptor, most of its biological actions are mediated through the IGF-IR. IGF-II does not play a large role in body development and growth in adults, but rather may play a key role only during embryonic and fetal growth (3). After birth the role of IGF-II is replaced by that of IGF-I (30).

2.2.1.2 Insulin-Like Growth Factor Receptor

The majority of the cellular effects of the IGFs are mediated through binding to the IGF-IR (7). There is 60% homology between the insulin receptor and IGF-IR and in fact, insulin and IGF-I can cross-bind to each other's receptor, though with weaker affinity than for their respective receptors (31).

The IGF-IR is a trans-membrane $\alpha 2\beta 2$ heterotetramer and possesses tyrosine kinase activity. Once bound by the ligand, a signal transduction pathway is activated which mediates the mitogenic and antiapoptotic actions of the IGFs (31).

Expression of IGF-IR is stimulated by steroid hormones such as estrogens and glucocorticoids, and growth factors (32). On the other hand, IGF-IR expression is inhibited by tumour suppressor gene products like p53 and Wilms' tumour protein (WT1) (31). Also, IGF-I itself can act as a negative feedback signal for IGF-IR; high levels of the polypeptide result in a decline of IGF-IR (31).

There also exists a IGF-II receptor (IGF-IIR), but little is known about its expression. Unlike IGF-I, it is monomeric and has no tyrosine kinase activity. It binds only to IGF-II and binding of the ligand results in its degradation, thus reducing its biologic activity (32).

2.2.1.3 Insulin-Like Growth Factor Binding Proteins

Unlike insulin, IGFs are bound to binding proteins in the circulation. There are six different insulin-like growth factor binding proteins (IGFBPs), which are mostly produced in the liver. Each binding protein has a specific binding affinity for the IGFs, which is determined via post-translational modification (33). The most important of the binding proteins is IGFBP-3, as it is the most abundant binding protein in the serum. More than 90% of IGF-I in circulation is bound to IGFBP-3 in a 150 kDa ternary complex which also includes an acid-labile subunit (ALS) (20). The rest of the circulating IGFs are bound to other IGFBPs and less than 1% circulates unassociated (34). IGFBP expression is regulated by several hormones and growth factors, and GH plays an important role in the expression of IGFBP-3 (26).

IGFBP-3 has two types of functions: IGF-dependent and IGF-independent (8). The IGF-dependent functions serve either to enhance or inhibit IGF action. Binding of IGFBP-3 to IGF-I plays 3 roles: 1) transportation of IGF-I, 2) protection of IGF-I from degradation and 3) regulation of the interaction between IGF-I and its receptor (7). Since IGF-1 has a higher affinity for IGFBP-3 than for its receptor, IGFBP-3 binding to IGF-I prevents the IGF-IR from binding its ligand which prevents IGF action; on the other hand, by binding

IGF-I, the binding protein protects it from degradation which in turn enhances its bioavailability (7). Independent of IGF-I, IGFBP-3 has an inhibitory effect of its own on cell growth, which is regulated by IGFBP-3 specific cell receptors (8).

Because of its apparent dual role with regards to IGF-I, it is difficult to predict in what way IGFBP-3 levels in the serum would predict risk of cancer. It has been postulated that perhaps a more relevant measure is the molar ratio between IGF-I and IGFBP-3, which more accurately represents the bioavailable fraction of IGF-I.

2.2.1.4 Insulin-Like Growth Factor Binding Protein Proteases

Proteases act in the target organs to cleave IGFBPs. An example of an IGFBP protease is prostate-specific antigen (PSA), which can cleave IGFBP-3 and IGFBP-5 (35). Proteolysis reduces the affinity of the binding protein for the IGFs, thus releasing them and potentiating their biological action (36). In the case of IGFBP-3, normally the ternary complex of IGFBP-3/IGF-I/ALS cannot cross the vascular endothelial barrier. When the protease cleaves the IGFBP, the IGF is then free to the cross the barrier to the target tissue (7).

Regulation of proteolysis is poorly understood, but it is known that it can be influenced by certain changes in physiologic conditions; for instance, serum from pregnant women has higher proteolytic activity than normal serum (37). IGFBP protease levels are also higher in individuals with certain illnesses or conditions such as AIDS and diabetes mellitus (38, 39). The degree of variability of protease activity in healthy individuals is not known.

2.3 DETERMINANTS OF IGF LEVELS

IGFs and their associated proteins are present in all humans and are in fact essential to life. There is much inter-individual variation in blood levels. Although the determinants of these differences are not all known, it is universally accepted that age influences IGF-I and IGFBP-3 levels, and is likely the most important one. IGF-I levels increase throughout adolescence, peaking at around 14.5 years for girls at 500 ng/mL (40).

In adults, IGF-I levels decrease slowly toward pre-pubertal levels, reaching around 100 μg/L by age 80 (40-42). Several studies on age and IGF-I have found a correlation between the 2 variables from –0.60 to –0.70 in both American and Swedish women (40-42). The relationship appears to be linear, with 2 studies predicting a decrease in IGF-I levels of about 3.7 ng/ml for every increasing year of life (42, 43).

IGF-I is also affected by long-term fasting and certain disease states, such as malnutrition, liver disease and acromegaly, the latter of which is characterized by increased levels of IGF-I (44-47). Interestingly, whereas undernutrition appears to be linked to reduced IGF-I concentrations (48), it has been more difficult to link states of caloric excess to an increase in IGF-I levels. Most studies have found no significant association between body mass index (BMI) and blood levels of IGFs and IGFBPs (48-53), but one study by Pfeilschifter *et al* (54) found a Pearson correlation coefficient of 0.16 between IGF-I and BMI in German postmenopausal women. Counterintuitively, some current data links obesity to lower plasma IGF-I concentrations. It is believed that perhaps obesity may impair GH secretion, although it is unclear whether obesity precedes GH deficiency or vice versa (48).

Two studies have shown a positive association between smoking and IGF-I and IGFBP-3 levels (43, 51), but findings on this predictor have been inconsistent. There has also been no consensus on the role of physical activity on blood levels of IGFs and IGFBPs. It has been suggested that exercise increases synthesis of both IGF-I and IGFBP-3; therefore, any increase in IGF-I will be counterbalanced by the increase of IGFBP-3 thereby little affecting the bioavailable fraction of IGF-I (50). Also, aside from alcoholics whose heavy alcohol consumption can lead to liver damage, studies on the effect of alcohol consumption and IGF/IGFBP levels have been inconsistent as well (43, 51, 55).

Serum estradiol has been correlated with IGF-I in men and in women. After menopause, which is characterized by a decrease in estradiol, the decrease in serum IGF-I in women is more closely related to time since menopause rather than chronological age (56). This is supported by a study by Poehlman *et al* (57) that found that the decline in IGF-I after menopause was greater than associated with normal ageing. However, different studies have found opposite effects of hormone replacement therapy (HRT) on IGF-I levels, with one study showing an increase of IGF-I (58) with HRT use and another finding a decrease (51). The results are unclear, most likely due to small sample sizes. No effect has been found between IGF-I/IGFBP-3 serum levels across the menstrual cycle (59, 60).

Finally, there appears to be no variation in serum IGF-I or IGFBP-3 after short-term fasting, several hours after a meal or immediately after a meal (44). There also appears to be no diurnal variation (44).

2.4 IGFs AND CANCER

2.4.1 Experimental Evidence

Observations of the effects of IGF-I in cell culture are what led to the hypothesis that this growth factor may be linked to the cancer process *in vivo*. The progression to malignancy is a multi-stage process and is reliant upon the uncontrolled division of cells, as well as the abrogation of the cell's natural defense against such unrestrained growth (61). IGF-I stimulates cell growth and proliferation, promotes cell transformation from a normal cell to a cancer cell, and suppresses apoptosis (programmed cell death), that is, prolongs the survival of cells that would normally be eliminated. Dunn *et al* (6) have quantified these effects: in their experiment IGF-I reduced apoptosis 10-fold and increased proliferation 6-fold.

An experimental study in mice has revealed a direct effect of IGF-I on tumour progression (6), by showing that by adding only IGF-I, the protective effect of dietary restriction on tumour development can be eliminated. A second experiment by Yang *et al* (5) demonstrated that IGF-I causes cell proliferation of human breast cancer cells transplanted in mice.

Experimental studies on cervical cancer are also encouraging. Two studies by Steller *et al* (62, 63) showed that IGF-I can induce proliferation of cervical cancer cells, while a study by Hembree *et al* (64) showed that IGF-I can promote proliferation of non-cancerous cervical epithelial cells infected with HPV. In fact, many types of cancer cells,

including cervical cancer cells, overexpress the cellular receptor for IGF-I, thus potentiating its effect (63, 65).

2.4.2 Epidemiological Evidence

The majority of epidemiological studies on IGF-I and cancer to date have focused on the four major cancers in the Western industrialized world: prostate cancer, breast cancer, colorectal cancer and lung cancer. Though the findings are not unanimous, most of the studies have found a positive relationship between cancer risk and IGF-I levels, with one prospective study finding the risk increased more than 7-fold for high levels of IGF-1 (66). Recent studies are summarized in Table 2.1.

Of nine recent studies on prostate cancer risk and IGF-I levels, seven showed an increased risk when comparing men with high levels of IGF-I to men with low levels, with measures of risk ranging from 1.43 to 4.32 (67-73). Five of these studies obtained significant results. Of the two that showed negative associations, both were non-significant and had small sample sizes; one (74) did not specify the adjustment factors, so one can only speculate on potential sources of bias, and the other was a small a pilot study (75).

The largest prospective study on IGF-I levels and prostate cancer risk was conducted by Chan *et al* (67), who conducted a case-control study nested within the Physicians' Health Study, a cohort of 14,916 men. Based on a recruitment of 152 cases and 152 controls, they found a Relative Risk (RR) of 4.32 for the highest quartile of IGF-I levels using the lowest quartile as reference.

A similar study by Stattin *et al* (72), which recruited 149 cases and 298 controls from the Northern Sweden Health and Disease Cohort Study (n = 54,560) in Sweden, found a non-significant Odds Ratio (OR) of 1.57 for IGF-I but when restricted to patients less than 59 years at study entry, a strong positive trend in risk was found for increasing tertiles of IGF-I; the OR for the highest vs the lowest tertile of IGF-I was 4.75.

The findings appear to be applicable to female cancers as well. Many studies on breast cancer have shown this to be true, with several case-controls studies (76-79) reflecting the results of larger cohort studies (66), with measures of effect ranging from 1.08 to 7.34. Interestingly, the positive association between high IGF-I levels and the risk of breast cancer seems to be restricted to premenopausal women, especially those under 50 years of age, which suggests that IGF-I concentration may be a marker of breast cancer risk among premenopausal women (66, 76, 79).

A large prospective case-control study by Kaaks *et al* (79) did not find a positive relationship between breast cancer in women less than 50 years of age, but the number of breast cancer cases in women less than 50 years of age at recruitment was small.

The most startling result was found by Hankinson *et al* (66). They conducted a prospective case-control study nested within the Nurses' Health Study (n = 32,826). The association between IGF-I concentration and breast cancer risk only appeared after restriction to women who were less than 50 years of age and premenopausal both at blood draw and the occurrence of cancer. The RR for the highest vs the lowest tertile of IGF-I

was 7.28, which is much greater than the RRs for many of the known breast cancer risk factors currently under study.

Studies of colorectal cancer have had similar results, both in men and in women (Table 2.1) (81-86). The limited research on lung cancer has not been as consistent (87-89), and there are not enough studies to draw any conclusions, although confounding by smoking may play a factor in the results to date. Interestingly, three case-control studies on endometrial cancer have found a negative association, with ORs ranging from 0.63 to 0.90, though none of these results were statistically significant (90-92). One prospective study on ovarian cancer and IGF-I (93) found a significant OR of 4.98 in women less than 55 years at diagnosis and a recent case-control study by Zhao *et al* (94) on bladder cancer risk found a significant OR of 3.88.

There has been one study recently published looking at the relationship between serum levels of IGF-I and the risk of cervical cancer precursor lesions (9). After adjusting for age, ethnicity, and smoking status, IGF-I levels were associated with an increased risk of SILs in a dose-dependent manner. The OR for the risk for the fourth quartile relative to the first quartile was 8.54 (95% CI 4.15-17.60). They also found a dose-dependent relationship between IGFBP-3 levels and an increased risk of SILs, but the OR was significant only before adjustment for IGF-I levels. Also, after adjustment for confounding factors the IGF:IGFBP-3 molar ratio was significantly higher for those in the fourth quartile vs the first quartile (OR = 5.29), with an obvious dose-dependent relationship (test for trend, $p<0.0001$).

Wu *et al* (9) also analyzed the relationship between mean IGF-I, IGFBP-3 and IGF-I:IGFBP-3 and SIL grade. Individuals with both high-grade SILs and low-grade SILs had higher mean IGF-I and IGFBP-3 levels and higher ratios than controls ($p<0.01$ for all).

Although most studies looking at IGF-I concentrations and cancer risk show a positive relationship, the studies that have looked at the effect of IGFBP-3 on cancer risk are very inconsistent. The measures of effect have been both below 1.0 and above 1.0, though it is difficult to compare as not all adjusted for IGF-I (which is highly correlated with IGFBP-3) and most values were non-significant

If IGF-I is found to be a risk factor for cancer, it may help to explain the relationship between many general risk factors and malignancy. Increased levels of IGF-I and decreased levels of IGFBP-3 may partly explain the mechanism of action of poorly understood risk factors such as diet, physical activity, calorie restriction and height. It may also be a contributing factor to the increased risk seen with smoking, alcohol consumption, BMI and aging.

Elucidation of the role of IGF-I in cancer will help us to better target screening programs to high-risk groups. There are already several anti-cancer drugs which act as antagonists of IGF-I. Tamoxifen, an inhibitor of estradiol, is also a potent inhibitor of IGF-I-induced growth and fenretinide, a synthetic analogue of retinoic acid, reduces IGF-I levels by more than 20% (95). Both drugs have been used to treat breast cancer.

It is also important to take into consideration that growth hormone (GH) and IGF-I are used to treat several disorders, including various neurological conditions, diabetes, osteoporosis, renal failure, congestive heart failure and the changes of body composition associated with aging (96). Most trials of growth factor therapy are unlikely to monitor long-term outcome. Therefore it is unknown whether this therapy will increase an individual's risk of cancer. Further studies will be needed to determine if the benefits of such therapy outweigh the possible risk of cancer. Consideration should be given to whether use of these therapies is prudent in individuals already at a high risk of cancer.

Table 2.1: A summary of epidemiological studies on IGF variables and cancer

Cancer Site	Study	Study Design and Population	RR or OR (95% CI)	Adjustment factors
CERVIX	Wu *et al*, 2003 (9)	Case-control study; 267 cases diagnosed with HSIL or LSIL and 238 controls matched on age.	Highest vs lowest quartile: OR = 8.54 (4.15-17.60)	Age, ethnicity, smoking status, IGFBP-3
PROSTATE	Chan *et al*, 1998 (67)	Prospective nested case-control; 152 cases and 152 controls from Physicians' Health Study (n=14,916) matched on smoking, duration of follow-up and age	Highest vs lowest quartile: RR = 4.32 (1.76–10.6)	PSA, height, weight, BMI, CAG polymorphisms of the androgen receptor, lycopene, estrogen, testosterone, dihydrotestosterone, sex hormone binding globulin (SHBG), prolactin, 3α-androstanediol glucuronide (3α-diol G), IGFBP-3
	Chokkalingam *et al*, 2001 (68)	Population-based case-control; 128 newly diagnosed cases and 306 population controls	Highest vs lowest quartile: OR = 3.92 (1.58–9.70)	Age, IGFBP-I, IGFBP-3, SHBG, 3α-diol G
	Harman *et al*, 2000 (69)	Prospective case-control; 72 cases and 127 controls from the Baltimore Longitudinal Study on Aging matched on age	Highest vs lowest tertile: OR = 3.11 (1.11–8.74)	Age, date, date squared, IGF-II, IGFBP-3, PSA
	Oliver *et al*, 2004 (71)	Population-based case-control study; 176 cases and 324 controls from the PROTECT matched on age, general practice and calendar date of recruitment.	Highest vs lowest quartile: OR = 3.00 (1.50–6.01)	Smoking, IGFBP-3.
	Li *et al*, 2003 (70)	Case-control; 408 cases and 437 matched sibling controls.	Clinically less aggressive disease: Highest vs lowest quartile: OR = 2.78 (1.06-6.80)	Age

	Stattin *et al*, 2000 (72)	Prospective nested case-control; 149 cases and 298 controls from Northern Sweden Health and Disease Cohort Study (n=54,560) matched on age, town/village of residency	Highest vs lowest quartile: OR = 1.72 (0.93–3.19)	Age, BMI, smoking, fasting time
	Wolk *et al*, 1998 (73)	Population-based case-control; 210 newly diagnosed cases and 224 controls matched for age	Highest vs 2 lowest quartiles: OR = 1.43 (0.88–2.33)	Age, height, BMI
	Schaefer *et al*, 1998 (74)	Prospective cohort; 45 cases and 179 controls from a cohort of 765 male health plan members matched on age	Highest vs lowest quartile: RR = 0.81 (0.36–1.80)	Not specified
	Lacey Jr. *et al*, 2001 (75)	Prospective cohort (pilot); 30 cases and 60 controls from the Washington County Serum Bank (n=20,305) matched on age, race, date of blood draw	Highest vs lowest quartile: OR = 0.6 (0.1–2.9)	Age at blood draw; IGFBP-3
BREAST	Bruning *et al*, 1995 (77)	Case-control; 150 cases and 441 controls matched on age	Highest vs lowest quintile of IGF-I/IGFBP-3 ratio: RR = 7.34 (1.67–32.16)	Age, menopausal status, family history, premenopausal BMI, height, waist-hip ratio, albumin, C-peptide, testosterone, C-reactive protein
	Hankinson *et al*, 1998 (66)	Prospective nested case-control; 397 cases and 620 controls from the Nurses' Health Study (n=32,826) matched on age, time and month of blood draw, fasting status, use of hormones at blood collection	Premenopausal and <50 yrs: Highest vs lowest tertile: RR = 7.28 (2.40–22.0)	IGFBP-3
	Li *et al*, 2001 (78)	Case-control; 40 newly diagnosed cases and 40 controls matched on age and race	>median vs <median of free IGF-I: OR = 6.31 (1.03–38.72)	Menopausal status, IGFBP-3

Bohlke *et al*, 1998 (76)	Case-control; 94 cases and 76 controls, all premenopausal and <50 yrs	Highest vs lowest tertile: OR = 3.7 (1.1–12.2)	IGFBP-3, age, age at first birth, age at menarche, height, BMI, log estradiol, ethnic group, parity, family history of breast cancer
Yu *et al*, 2002 (79)	Population-based case-control study; 300 incident cases and 300 controls matched on age and menopausal status.	Premenopausal: Highest vs lowest quartile: OR = 2.66 (1.40–5.05)	
Krajcik *et al*, 2002 (97)	Nested case-control; 63 cases and 63 controls from the Kaiser Permanente Medical Care Program matched on age, date of exam, menopausal status, duration of follow-up	Premenopausal: Highest vs lowest quartile: OR = 2.01 (0.33–12.4)	IGFBP-3, insulin, glucose, BMI
Yu *et al*, 2002 (98)	Population-based case-control in Shanghai; 300 incident cases and 300 controls	Premenopausal: Highest vs lowest tertile: OR = 1.92 (0.88–4.20)	BMI, age at menarche, age at first live birth, total energy intake, waist hip ratio, history of fibroadenoma, family history of breast cancer, IGFBP-3
Toniolo *et al*, 2000 (99)	Prospective nested case-control; 172 premenopausal cases and 486 premenopausal controls from the New York University Women's Health Study (n=14,275) matched on age, date of blood draw, day of menstrual cycle at blood draw	Premenopausal and <50 yrs: Highest vs lowest quartile: RR = 1.90 (0.82–4.42)	History of benign breast disease, family history of breast cancer, parity, IGFBP-3
Del Giudice *et al*, 1998 (100)	Case-control; 99 premenopausal cases and 99 premenopausal controls matched on age	Highest vs lowest quintile: OR = 1.47 (0.66–3.27)	Age
Kaaks *et al*, 2002 (80)	Nested case-control; 513 cases and 987 controls from Sweden matched on age, date of blood draw, menopausal status, and use of ERT/HRT	Highest vs lowest quartile: OR = 1.08 (0.76 – 1.53)	Smoking, BMI, age at menarche

	Schairer *et al*, 2004 (101)	Case-control; 185 cases and 159 controls with nonproliferative breast changes, all post-menopausal.	Highest vs lowest quartile: OR =0.9 (0.4–2.0)	Age at diagnosis, age at menopause, Quetelet index, nulliparity, year of diagnosis, hour of blood draw, study hospital, c-peptide:fructosamine, IGFBP-3
	Keinan-Boker *et al*, 2003 (102)	Prospective nested case-control; 149 cases and 333 controls matched on cohort, age, date of blood donation, place of residence.	Highest vs lowest quartiles: OR = 0.7 (0.3–1.5)	BMI, age at menarche, age at first full term delivery, IGFBP-3
COLORECTUM	Ma *et al*, 1999 (83)	Prospective nested case-control; 193 male cases and 318 male controls from the Physicians' Health Study (n=14,916) matched on age and smoking	Highest vs lowest quintile: RR = 2.51 (1.15–5.46)	Age, smoking, IGFBP-3, BMI, alcohol intake
	Giovannucci *et al*, 2000 (81)	Prospective nested case-control; 79 female cases and 158 female controls from the Nurses' Health Study (n=32,826) matched on age, fasting status, month of blood draw	Highest vs lowest tertile: RR = 2.18 (0.94–5.08)	Alcohol intake, BMI, IGFBP-3
	Kaaks *et al*, 2000 (82)	Prospective nested case-control; 102 female cases and 200 female controls from the New York University Women's Health Study (n=14,275) matched on menopausal status, age, date, number of blood donations, time of blood draw	Highest vs lowest quintile: OR = 1.88 (0.72–4.91)	Day of menstrual cycle, fasting status, smoking, age, menopausal status
	Nomura *et al*, 2003 (84)	Nested case-control; 282 male newly diagnosed cases and 282 male controls matched on age.	Highest vs lowest quartiles: OR = 1.5 (0.8–2.8)	Smoking history, BMI, alcohol intake, IGFBP-3
	Palmqvist *et al*, 2002 (85)	Prospective nested case-control; 168 incident cases and 336 controls from the Northern Sweden Health and Disease cohort study matched on age, sex, date of blood sampling, fasting status	Highest vs lowest quintile: OR = 1.27 (0.65–2.47)	Smoking

	Probst-Hench *et al*, 2001 (86)	Prospective cohort; 135 male cases and 661 male controls from the Shanghai cohort study matched on neighbourhood of residence, age at interview, date of blood draw	Highest vs lowest quintile: OR = 1.18 (0.55–2.53)	Cigarette smoking, alcohol intake, BMI
LUNG	Yu *et al*, 1999 (89)	Case-control; 204 cases and 218 controls matched on age, sex, race, smoking	Highest vs lowest quartile: OR = 2.75 (1.37–5.53)	Age, sex, race, smoking, BMI, family history of any cancer, IGFBP-3
	London *et al*, 2002 (87)	Cohort; 230 male cases and 740 male controls from Shanghai matched on neighbourhood of residence, age at interview, timing of sample collection	Highest vs lowest quartile: OR = 0.86 (0.47 – 1.57)	Smoking, IGFBP-3
	Lukanova *et al*, 2001 (88)	Prospective nested case-control; 93 female cases and 186 female controls from the New York University Women's Health Study (n=14,275) matched on age, date of blood draw, menopausal status, day of menstrual cycle, smoking	Highest vs lowest quartile: OR = 0.54 (0.14–2.07)	Age, date of recruitment, menopausal status, day of menstrual cycle, current smoking, fasting status, cotinine, BMI, IGFBP-3
ENDOMETRIUM	Lukanova *et al*, 2003 (91)	Nested case-control study; 166 cases and 315 controls from the New York University Women's Health Study, Northern Sweden Health and Disease Study and the Study of Hormones and Diet in the Etiology of Breast Cancer matched on study cohort, age, date at recruitment, menopausal status and day of menstrual cycle for premenopausal women.	Highest vs lowest quintile: OR = 0.90 (0.44–1.82)	BMI, parity, OC use, HRT use
	Weiderpass *et al*, 2003 (92)	Population-based case-control study; 288 cases and 392 controls, all over 50, matched on age	Highest vs lowest quartiles: OR = 0.86 (0.46–1.58)	Age, BMI, diabetes mellitus, physical activity, menopausal status, different types of HRT and OC
	Lacey Jr. *et al*, 2004 (90)	Case-control study; 174 cases and 136 population-based controls matched on age, race and location, all post-menopausal.	Highest vs lowest tertile: OR = 0.63 (0.30–1.32)	Age, study site, race, log-SHBG, BMI

OVARIES	Lukanova *et al*, 2001 (93)	Nested case-control; from the New York University Women's Health Study, Northern Sweden Health and Disease Study, and the Study of Hormones and Diet in the Etiology of Breast Cancer matched on cohort, menopausal status, age, date at recruitment, day of menstrual cycle at blood draw	<55 yrs: Highest vs lowest tertile: OR = 4.98 (1.21 – 20.6)	Age, study cohort, date at recruitment, menopausal status, day of menstrual cycle, full-term pregnancy, BMI, smoking, IGFBP-3
BLADDER	Zhao *et al*, 2003 (94)	Case control; 154 newly diagnosed cases and 154 controls in Houston matched on sex, age, ethnicity	Highest vs lowest quartile: OR = 3.88 (1.74 – 8.65)	Age, sex, ethnicity, cigarette smoking status

3 METHODOLOGY

3.1 OBJECTIVES

The main objective of this case-control study was to determine whether there is a relationship between plasma IGF-I and IGFBP-3 levels and cervical cancer precursors.

The primary hypothesis is that an elevated plasma level of IGF-I is associated with an increased risk of cervical cancer precursors and that an elevated plasma level of IGFBP-3 is associated with a decreased risk of cervical cancer precursors.

The secondary objective of this study was to determine whether plasma levels of IGF-I and IGFBP-3 are associated with HPV positivity in controls.

3.2 STUDY DESIGN

3.2.1 Overview

In order to answer the above questions the study design is a case-control. This study was conducted in affiliation with several Montreal area hospitals and clinics. Cases were identified as those women undergoing cervical biopsy at the colposcopy clinics of these hospitals. Controls were selected from women undergoing a routine Pap smears at the participating family medicine and gynecology clinics. All participants answered questions in a self-administered questionnaire inquiring about possible confounding factors and underwent a blood test; blood levels of IGF-I and IGFBP-3 were assessed by laboratory testing on the blood.

3.2.2 Setting

We identified the majority of cases in both the French and English populations in the Montreal area by selecting the subjects from five collaborating teaching hospitals and clinics: the Jewish General Hospital, Hôpital Notre-Dame, Royal Victoria Hospital, Montreal General Hospital and Hôpital St.Luc.

3.2.3 Subject Selection

3.2.3.1 Cases

Potential cases consisted of all women undergoing cervical biopsy for suspected high-grade lesions at the colposcopy clinics of the participating hospitals. If the physician performing the colposcopy determined that a biopsy was needed, he/she introduced the study to the patient via a study information brochure (Appendix 1). Once a verbal agreement was given, a cervical specimen was gathered prior to the biopsy and then the woman was referred to the study research nurse. The research nurse explained the study to the potential case and obtained written informed consent (Appendix 2). The potential case then filled out the questionnaire and had her blood drawn. The woman was free to refuse to participate at any time, and if she chose to do so later on, her cervical specimen was discarded.

The patient was enrolled as a case only if she was later newly diagnosed with one of the following histologically confirmed conditions:

- Low grade pre-invasive cervical intraepithelial (LSIL), classified using the CIN terminology as mild dysplasia (CIN-I), or
- High grade pre-invasive cervical intraepithelial neoplasia (HSIL), classified using the CIN terminology as moderate dysplasia (CIN-II), severe dysplasia or in situ cancer (CIN-III).
- Early invasive cancer classified as microinvasive (T1a) (according to the tumor-node-metastasis (TNM) staging) (103) or IA (according to FIGO stages) (104) or minimal stage cervical cancer (TNM stage T1b or FIGO stage IB).

To minimize the possibility of the disease state affecting the measures of plasma IGF-I and IGFBP-3, women with later stage cancers were not eligible.

3.2.3.2 Controls

Potential controls were women who presented for their annual routine Pap smear at the participating family medicine and gynaecology clinics and who had no history of cervical abnormalities. The potential control was introduced to the study by a brochure (Appendix 1), and if she was interested she was referred to the study research nurse by the physician, nurse or clinic assistant. The research nurse explained the study to the potential control and obtained written informed consent (Appendix 3) if she was interested in participation. The potential control completed the questionnaire and had her blood drawn, and her medical chart was flagged to indicate to the physician that a cervical specimen was to be collected at the time of her Pap test for the purposes of the study.

For a potential control to be enrolled as a control, her Pap test results had to be classified as "within normal limits" (WNL) or had to be consistent with "benign cellular changes" (BCC). Potential controls were excluded if their cytological diagnosis indicated abnormalities consistent with ASCUS, ASC-H, AGC, LSIL or HSIL or invasive disease, but were eligible to become cases if they attended one of the participating colposcopy clinics during the study period.

3.2.3.3 Eligibility

Potential cases and controls were excluded if they met any of the following criteria:

- They were pregnant. Changes to the cervix occur during pregnancy and these can interfere with cytological tests (105).
- They had previous treatment for cervical abnormalities.
- Their cervix had been removed as a result of a hysterectomy or conization surgery.
- They had a personal history of any cancer, as IGF-I and IGFBP-3 are postulated to be involved with the cancer process in general and is not specific to cervical cancer.

3.3 DATA COLLECTION

3.3.1 Questionnaire

A questionnaire was administered to gather information on important covariates which may have confounded the main analysis (Appendix 4). The questionnaire was available both in French and in English and collected detailed information on sociodemographic

variables, smoking history and alcohol consumption, reproductive and sexual history and medical history. The questionnaire was a subset of one previously used in a separate study in the Montreal area and had been validated.

3.3.2 IGF-I and IGFBP-3 Testing

A 10 ml blood sample was collected from consenting subjects by venipuncture in a heparinized Vacutainer tube. Blood samples were centrifuged at 1500 x G for 20 minutes. Plasma and buffy coat were aspirated and stored separately in individual Nunc vials which were stored at -70 C. Plasma levels of IGF-I and IGFBP-3 were assayed by Dr. Michael Pollak's laboratory. Specimens were identified by a unique identifying number, so that laboratory personnel who carried out IGF-I and IGFBP-3 were blinded as to the case-control status of each specimen.

Plasma levels of IGF-I were determined using assay kits based on enzyme-linked immunosorbent assay (ELISA) provided by Diagnostics Systems Laboratory (Webster, TX) preceded by IGFBP removal via acid-ethanol extraction. IGFBP-3 was also quantified using ELISA assays from DSL. Each sample was assayed twice, and the mean of the two determinants was used for data analysis. If the relative difference between the two assays exceeded 10%, the assay was repeated. For quality control, aliquots from a single pooled serum sample were randomly placed within each batch

3.3.3 Cervical Specimen Testing and DNA Extraction

Exfoliated cervical cell specimens were collected from both cases and controls using an Accelon biosampler (Medscand, Inc.), a cytobrush that samples a fixed area of the

endocervix and ectocervix in a standardized way. The Accelon biosampler which contained the exfoliated cells was then agitated in 2 ml of PreservCyt (Cytyc Corp., Boxborough, MA) (106), a methanol-based transport media that preserves the integrity of the cells. The resulting cell suspension was then stored at 4°C at hospital research laboratories until it was transferred to the laboratory of Dr. Coutlée for specimen processing and DNA extraction. Specimens were centrifuged at 13000 X G for 15 minutes at 22 C. The supernatant was then discarded and the cell pellet was left to dry (106). Pellets were later resuspended in 300 µl of 20 mM Tris buffer, pH 8.3, and DNA was purified using the Master pure procedure (107). A segment of the human β-globin gene was amplified to confirm the presence of human DNA and specimen integrity. The extracted DNA was later used for HPV testing and typing.

3.3.4 HPV Testing and Typing

Dr. Coutlée's laboratory performed HPV detection and typing. Specimens were identified by a unique identifying number, so that laboratory personnel who carried out HPV testing were blinded as to the case-control status of each specimen.

HPV DNA was amplified using PCR with novel L1 consensus primers that are more sensitive than previously used consensus primer systems (108). The primer pair designated PGMY09/11 was used to amplify the HPV DNA. HPV typing was performed using the reverse line blot assay which reliably detects the presence of 10 HPV DNA copies (109). An extended line blot strip was used which probed for 37 genital HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89 and IS39, a subtype of 82.

Samples were considered HPV negative if they were not positive for any of these types. As well, if a specimen was negative for both β-globin and HPV, this was an indication that there was insufficient DNA for PCR, and thus was labelled as inadequate.

3.3 STATISTICAL ANALYSIS

3.4.1 Definition of Variables

3.4.1.1 HPV Status

HPV types were classified as having either high-risk oncogenic potential or low-risk oncogenic potential (110). HPV types considered to be high-risk are: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82. HPV positive women were classified into three overlapping categories: those who tested positive for any HPV type, those who tested positive for at least one high-risk HPV type and those who tested positive for HPV 16. Positivity for HPV 16 is considered separately as it is the HPV type most commonly found in cervical cancers.

3.4.1.2 IGF-1 and IGFBP-3

IGF-I and IGFBP-3 levels are extremely variable among individuals. The distribution is not normal and tends to skew towards upper values. Therefore, for the purposes of this study, IGF-I and IGFBP-3 were divided into quartiles, based on the distribution in the controls. IGF-I and IGFBP-3 levels were also divided into tertiles in order to increase the power.

3.4.2 Analysis of the Relationship Between IGF-I, IGFBP-3 and SILs

The associations between IGF-I and both LSIL and HSIL, as well as between IGFBP-3 and both LSIL and HSIL, were tested using multiple logistic regression. Odds Ratios (OR) were calculated, as well as their 95% confidence intervals (CIs). Age and HPV infection were considered *a priori* to be confounders to these relationships. Other factors suspected to be confounders were empirical tested, using a backwards deletion procedure. Age and HPV status were forced into the model, and if the removal of any variable changed the OR for the relationship between IGF-I/IGFBP-3 by more than 10%, it was considered to be a confounder. Also, both IGF-I and IGFBP-3 were included in a single model.

3.4.2 Analysis of the Relationship Between IGF-I, IGFBP-3 and HPV Status

Similarly, the associations between IGF-I and HPV positivity and IGFBP-3 and HPV positivity were tested using a multiple logistic regression. This analysis was restricted to controls, to see if a relationship existed between IGF variables and HPV infection independent of disease status. Three types of analyses were performed, one where the outcome was positivity for any HPV type, one where the outcome was positivity for at least one high-risk HPV type, and one where the outcome was positivity for HPV 16. Once again, ORs and 95% CIs were calculated. Age was considered *a priori* to be a confounder to this relationship, and other factors suspected to be confounders were tested using a backwards deletion procedure. Age was forced into the model, and if the removal of any variable changed the OR for the relationship between IGF/IGFBP-3 and HPV

positivity by more than 10%, it was considered to be a confounder. Also, both IGF-I and IGFBP-3 were included in a single model.

4 RESULTS

4.1 RECRUITMENT AND ELIGIBILITY

Between February 2001 and September 2003, 504 potential cases and 572 potential controls were approached to participate in this study (Table 4.1). Eleven cases and 29 controls did not consent to participate in the study, for a participation rate of 97.8% and 94.9%, respectively. Although they provided cervical specimens and filled out questionnaires, 49 potential cases and 75 potential controls did not consent to have their blood drawn. There was a slight tendency for these women to be younger than those who provided blood samples.

True case-control status was confirmed in 83.8% of potential cases (n=372) and 90.0% of potential controls (n=412). Of the potential cases that were excluded, 7 had invasive cancer, 28 had normal cervical epithelia and no sign of SILs, 25 had other conditions such as cervicitis, squamous metaplasia, or parakeratosis, 6 had an equivocal biopsy result and did not return for a repeat biopsy, and 1 had condyloma. 321 cases were diagnosed with HSIL and 51 were diagnosed with LSIL. Of the potential controls that were excluded, 21 had abnormal results, 1 was unsatisfactory and 3 could not be contacted further.

For the purposes of this study, the blood samples from 366 cases and 366 controls were tested for IGF-I and IGFBP-3. This subsample did not differ with regards to those who had their blood drawn but were not tested in the subsample on sociodemographic characteristics such as age, ethnic group, financial situation or highest levels of schooling.

Table 4.1: Recruitment and eligibility

	Cases	Controls	Total
	n	n	n
Approached	504	572	1076
Agreed to participate	493	543	1036
Had blood drawn	444	458	902
Case/control status confirmed	372	412	784
Blood sample tested for IGF variables	366	366	732

4.2 DESCRIPTION OF STUDY POPULATION

4.2.1 Sociodemographic characteristics

Table 4.2 presents the sociodemographic characteristics of cases and controls. Cases tended to be younger than controls; the mean age for all cases was 32.6 years (SD=9.5) while for controls it was 36.0 years (SD=11.2). This is consistent with the fact that the prevalence of SILs peaks between 25 and 29 years (20). The mean age of women diagnosed with LSIL was 33.5 years and 32.5 years for women diagnosed with HSIL.

The majority of participants in the study were French Canadian, and this proportion was higher in cases who were diagnosed with HSIL than in controls or cases who were diagnosed with LSIL. Most cases and controls described their financial situation as being moderate to comfortable, though cases tended to describe their financial situation as

slightly worse than controls. The highest level of schooling was similar among cases and controls.

Table 4.2: Distribution of sociodemographic characteristics by outcome status

Variable	Controls	Cases	
		LSIL	HSIL
	n (%)	n (%)	n (%)
Age			
<25	68 (18.68)	9 (17.65)	70 (22.22)
25 – 29	57 (15.62)	14 (27.45)	65 (20.63)
30 – 34	54 (14.79)	11 (21.57)	58 (18.41)
35 – 39	51 (13.97)	4 (7.84)	61 (19.37)
40 – 44	48 (13.15)	5 (9.80)	24 (7.62)
45 – 50	43 (11.78)	5 (9.80)	21 (6.67)
50	44 (12.05)	3 (5.88)	16 (5.08)
Ethnic Group			
French Canadian	199 (54.67)	27 (52.94)	198 (63.26)
English Canadian	32 (8.79)	8 (15.69)	41 (13.10)
Other	133 (36.54)	16 (31.37)	74 (23.64)
Financial Situation			
Difficult	25 (7.02)	5 (9.80)	36 (11.54)
Moderate	151 (42.42)	21 (41.18)	155 (49.68)
Comfortable	139 (39.04)	19 (37.25)	99 (31.73)
Very Comfortable	44 (12.36)	6 (11.76)	22 (7.05)
Highest Level of Schooling			
Elementary	5 (1.37)	0 (0.00)	5 (1.60)
Secondary	76 (20.82)	14 (27.45)	84 (26.92)
CEGEP/Technical post-secondary	114 (31.23)	18 (35.29)	108 (34.62)
Undergraduate	118 (32.33)	13 (25.49)	84 (26.92)
Graduate	52 (14.25)	6 (11.76)	31 (9.94)

4.2.2 Cigarette Smoking and Alcohol

A significantly higher number of cases (63.7%) than controls (47.3%) had ever been regular smokers, and 41.7% of cases were current smokers as compared to 26.5% of controls. A higher proportion of women diagnosed with HSIL had ever drunk alcohol at

least once a month during their lives than did women diagnosed with LSIL and controls. When women were asked about their alcohol consumption in the last five years, 33.6% of cases did not drink on a weekly basis, as compared to 45.6% of controls (Table 4.3). Among those who did drink on a weekly basis, cases drank a mean of 5.4 drinks per week, compared to controls who drank a mean of 6.1 drinks per week.

Table 4.3: Distribution of cigarette smoking and alcohol consumption by outcome status

Variable	Controls	Cases	
		LSIL	HSIL
	n (%)	n (%)	n (%)
Cigarette Smoking			
Never	178 (50.28)	15 (29.41)	106 (34.30)
Former	82 (23.10)	16 (31.37)	73 (23.62)
Current	94 (26.48)	20 (39.22)	130 (42.07)
Alcohol Drinking			
Never	90 (25.00)	11 (22.00)	45 (14.42)
Ever	270 (75.00)	39 (78.00)	267 (85.58)
Number of alcoholic drinks per week in the past 5 yrs			
0	162 (45.63)	17 (33.33)	104 (33.66)
2/week	59 (16.62)	8 (15.69)	47 (15.21)
3-6/week	88 (24.79)	19 (37.25)	99 (32.04)
7/week	46 (12.96)	7 (13.73)	59 (19.09)

4.2.3 Reproductive health characteristics

Table 4.4 shows the distribution of reproductive health variables among cases and controls. The distribution of age at menarche and parity did not differ between cases and controls. More cases were current users of oral contraceptives, though the proportion who had ever used oral contraceptives was similar among cases (89.5%) and controls (86.9%). Slightly more controls reported using hormones to treat conditions such as

infertility and menopause, though overall the number of participants who reported such use was small (Table 4.4).

Table 4.4: Distribution of reproductive health variables by outcome status

Variable	Controls	Cases	
		LSIL	HSIL
	n (%)	n (%)	n (%)
Age at menarche			
<11 yrs	35 (9.56)	7 (13.73)	26 (8.25)
11 – 12	143 (39.07)	20 (39.22)	130 (41.27)
13 – 14	142 (38.80)	20 (39.22)	115 (36.51)
15 yrs	46 (12.57)	4 (7.84)	44 (13.97)
Number of pregnancies			
0	132 (36.26)	17 (34.00)	117 (37.50)
1	73 (20.05)	10 (20.00)	67 (21.47)
2	64 (17.58)	8 (16.00)	52 (16.67)
3	95 (26.10)	15 (30.00)	76 (24.36)
Ever OC use			
No	47 (12.84)	7 (13.73)	31 (9.84)
Yes	312 (85.25)	44 (86.27)	281 (89.21)
Current OC use			
No	249 (68.03)	30 (58.82)	187 (59.37)
Yes	117 (31.97)	21 (41.18)	128 (40.63)
Length of OC use			
Never	47 (13.17)	7 (13.73)	31 (10.13)
0 – 4.9 yrs	139 (38.94)	17 (33.33)	121 (39.54)
5 yrs	171 (47.90)	27 (52.94)	154 (50.33)
Hormone use			
No	309 (86.31)	46 (92.00)	284 (91.91)
Yes	49 (13.69)	4 (8.00)	25 (8.09)

4.2.4 Sexual behaviour characteristics

Table 4.5 presents the sexual behaviour characteristics of cases and controls. As expected, cases first had vaginal sexual intercourse at a younger age than controls. The proportion of cases who first had vaginal intercourse before age 16 (33.0%) was over twice the proportion of such controls (13.8%). Cases also had a significantly higher

number of lifetime male vaginal sexual partners. Cases had a mean of 9.8 male sexual partners, while controls had a mean of 7.1 male sexual partners. 22.9% of controls had only one male sexual partner over their lifetime as compared to 8.33% of cases diagnosed with LSIL and 8.39% of cases diagnosed with HSIL (Table 4.5).

Table 4.5: Distribution of sexual behaviour variables by outcome status

Variable	Controls	Cases	
		LSIL	HSIL
	n (%)	n (%)	n (%)
Age at first vaginal intercourse			
<16 yrs	48 (13.75)	15 (31.25)	100 (33.22)
16-17	109 (31.23)	19 (39.58)	88 (29.24)
18-19	99 (28.37)	7 (14.58)	66 (21.93)
>19	93 (26.65)	7 (14.58)	47 (15.61)
Lifetime number of male vaginal sexual partners			
1	76 (22.89)	4 (8.33)	24 (8.39)
2-4	89 (26.81)	5 (10.42)	51 (17.83)
5-8	80 (24.10)	19 (39.58)	91 (31.82)
>8	87 (26.20)	20 (41.67)	120 (41.96)

4.2.5 HPV status

Table 4.6 describes the HPV infection status of cases and controls as determined by HPV DNA testing by PCR. Five women were excluded from the HPV analyses because their cervical sample was not adequate for HPV testing; that is, the sample tested negative for β-globin and negative for HPV. 84.3% of LSIL cases and 96.1% of HSIL cases were positive for any HPV type. This is in contrast to only 24.9% of controls. Given the strong causal association between HPV positivity and the risk of developing SILs, this was not unexpected.

When HPV types were classified as either high-risk or low-risk, the prevalence of positivity for high-risk types was higher in cases than in controls, whereas positivity for low-risk types was higher in controls. Of HPV-positive cases diagnosed with LSIL, 95.3% were positive for at least one high-risk HPV type, as were 96.0% of HPV-positive cases diagnosed with HSIL, while only 54.9% of HPV-positive controls were positive for at least one high-risk HPV type.

HPV-16 was uncommon among controls, and only a fraction of controls who tested HPV-positive were infected with HPV-16 (9.9%). HPV-16 was much more prevalent among HSIL cases then LSIL cases; 30.2% of HPV-positive cases diagnosed with LSIL were positive for HPV-16. In contrast, 56.0% of HPV-positive cases diagnosed with HSIL were positive for HPV-16.

Table 4.6: Distribution of HPV positivity by outcome status

Variable	Controls	Cases	
		LSIL	HSIL
	n (%)	n (%)	n (%)
HPV negative	275 (75.14)	8 (15.69)	12 (3.87)
Positive for any HPV type	91 (24.86)	43 (84.31)	298 (96.13)
Positive for only a low risk HPV type	41 (11.20)	2 (3.92)	12 (3.87)
Positive for at least one high risk HPV type	50 (13.66)	41 (80.39)	286 (92.26)
Positive for any high risk HPV type except HPV 16	41 (8.93)	28 (54.90)	119 (38.91)
Positive for HPV 16	9 (2.46)	13 (25.49)	167 (53.35)

4.2.6 Distribution of IGF-I and IGFBP-3

The mean plasma level of IGF-I was 282.3 ng/ml in LSIL cases (median=276.9 ng/ml) and 292.3 ng/ml in HSIL cases (median=275.6 ng/ml). The mean plasma level of IGF-I

in controls was slightly higher than in cases, at 294.5 ng/ml (median=286.3 ng/ml). The mean plasma level of IGFBP-3 was 4961.9 ng/ml (median=4877.5 ng/ml) in LSIL cases and 5112.1 ng/ml (median=5054.7 ng/ml) in HSIL cases. The mean plasma level of IGFBP-3 in controls was higher, at 5146.2 ng/ml (median=5122.9 ng/ml). Table 4.7 shows the distribution of the quartiles an tertiles of IGF-I and IGFBP-3 by case-control status. The cutpoints were defined on the basis of the distribution in the control group.

Table 4.7: Distribution of IGF-1 and IGFBP-3 in entire sample by case/control status

Variable	Controls	Cases	
		LSIL	HSIL
	n (%)	n (%)	n (%)
IGF-I (quartiles)			
Q1 (<216.8 ng/ml)	92 (25.14)	13 (25.49)	63 (20.00)
Q2 (216.8 – 286.3 ng/ml)	91 (24.86)	17 (33.33)	115 (36.51)
Q3 (286.3 – 356.0 ng/ml)	91 (24.86)	10 (19.61)	69 (21.90)
Q4 (>356.0 ng/ml)	92 (25.14)	11 (21.57)	68 (21.59)
IGF-I (tertiles)			
T1 (<242.8 ng/ml)	122 (33.33)	18 (35.29)	109 (34.60)
T2 (242.8 – 334.3 ng/ml)	122 (33.33)	20 (39.22)	117 (37.14)
T3 (>334.3 ng/ml)	122 (33.33)	13 (25.49)	89 (28.25)
IGFBP-3 (quartiles)			
Q1 (<4634.7 ng/ml)	91 (24.86)	18 (35.29)	85 (26.98)
Q2 (4634.7 – 5122.9 ng/ml)	92 (25.14)	15 (29.41)	80 (25.40)
Q3 (5122.9 – 5714.5 ng/ml)	91 (24.86)	9 (17.65)	86 (27.30)
Q4 (>5714.5 ng/ml)	92 (25.14)	9 (17.65)	64 (20.32)
IGFBP-3 (tertiles)			
T1 (<4813.1 ng/ml)	122 (33.33)	24 (47.06)	117 (37.14)
T2 (4813.1 – 5462.8 ng/ml)	122 (33.33)	15 (29.41)	111 (35.24)
T3 (>5462.8 ng/ml)	122 (33.33)	12 (23.53)	87 (27.62)

When comparing the plasma levels of IGF-I and IGFBP-3 in HPV positive controls to HPV negative controls, the mean plasma levels of IGF-I was 326.5 ng/ml (median=309.9 ng/ml) in those who were HPV positive and 283.9 ng/ml (median=274.4 ng/ml) in those who were HPV negative. A more detailed analysis of the association between IGF markers and HPV outcomes is presented elsewhere in this section.

4.3 UNIVARIATE ANALYSIS

For all analyses, women with IGF-I and IGFBP-3 levels in the lowest category (tertile or quartile) were used as the reference group.

4.3.1 Crude association between IGF-I and IGFBP-3 and the risk of LSIL

Table 4.8 presents the OR for the crude association between the IGF variables and the risk of LSIL. Whereas women with IGF-I levels in the second quartile had an increased risk of LSIL (OR=1.32, 95% CI 0.61-2.88), women with IGF-I levels in the upper two quartiles had a decreased risk of LSIL. Women with IGFBP-3 levels in the second, third and fourth quartiles had a decreased risk of LSIL, with the OR for the highest quartile in reference to the lowest being 0.51 (95%CI 0.22-1.18). However, none of these fluctuations in risk levels could be deemed statistically significant, or indicative of a dose-response trend.

Table 4.8: Crude association between IGF variables and risk of LSIL

IGF marker	OR	95% CI
IGF-1 (quartiles)*		
Q1	1.00	Ref.
Q2	1.32	0.61 – 2.88
Q3	0.78	0.33 – 1.86
Q4	0.85	0.36 – 1.99
IGF-1 (tertiles)		
T1	1.00	Ref.
T2	1.11	0.56 – 2.20
T3	0.72	0.34 – 1.54
IGFBP-3 (quartiles)†		
Q1	1.00	Ref.
Q2	0.83	0.40 – 1.75
Q3	0.51	0.22 – 1.18
Q4	0.51	0.22 – 1.18
IGFBP-3 (tertiles)		
T1	1.00	Ref.
T2	0.63	0.31 – 1.25
T3	0.50	0.24 – 1.05

*p-value for trend = 0.4198
†p-value for trend = 0.1222

4.3.2 Crude association between IGF-I and IGFBP-3 and the risk of HSIL

Table 4.9 presents the Odds Ratios for the crude association between the IGF variables and the risk of HSIL. Women with plasma levels of IGF-I in the second, third and fourth quartiles had increased risks of HSIL, with the risk being highest for those with plasma levels in the second quartile (OR=1.85, 95% CI 1.21-2.82). Although there were only small changes in risk for women with plasma levels of IGFBP-3 in the second and third quartiles, women with IGFBP-3 levels in the fourth quartile had a decreased risk of HSIL (OR=0.76, 95% CI 0.49-1.18). In all, all results were inconsistent with a dose-response relationship.

Table 4.9: Crude association between IGF variables and risk of HSIL

IGF marker	OR	95% CI
IGF-1 (quartiles)*		
Q1	1.00	Ref.
Q2	1.85	1.21 – 2.82
Q3	1.11	0.71 – 1.73
Q4	1.08	0.69 – 1.69
IGF-1 (tertiles)		
T1	1.00	Ref.
T2	1.07	0.75 – 1.54
T3	0.82	0.56 – 1.19
IGFBP-3 (quartiles)†		
Q1	1.00	Ref.
Q2	0.94	0.62 – 1.43
Q3	1.02	0.67 – 1.55
Q4	0.76	0.49 – 1.18
IGFBP-3 (tertiles)		
T1	1.00	Ref.
T2	0.95	0.66 – 1.36
T3	0.74	0.51 – 1.08

*p-value for trend = 0.7749
†p-value for trend = 0.5672

4.3.3 Crude Association between IGF-I and IGFBP-3 and HPV status among controls

The association between the IGF variables and HPV status among controls was also examined, with the results presented in Table 4.10. There was a significant trend toward an increased risk of being positive for any HPV type in the higher quartiles of both IGF-I and IGFBP-3. The OR for the fourth quartile of the plasma level of IGF-I was 3.30 (95% CI 1.51-7.22), whereas the OR for the fourth quartile of the plasma level of IGFBP-3 was 2.91 (95% CI 1.36-6.24).

When restricting to only high-risk HPV types, there was a similar significant trend toward an increased risk of being positive for a high-risk HPV type in the higher quartiles of both

IGF-I and IGFBP-3, but the increase in risk was even greater than for overall positivity. The OR for the fourth quartile of the plasma level of IGF-I was 4.00 (95% CI 1.42-11.26), whereas the OR for the fourth quartile of the plasma level of IGFBP-3 was 7.67 (95% CI 2.20-26.74).

Lastly, the outcome was restricted to positivity for HPV-16 only. Increased risks were observed for women with plasma levels in the highest quartiles/tertiles, but due to small numbers of women with a positive outcome, the confidence intervals were very wide (Table 4.10). Except for HPV 16, all dose-response trends were significant at the 5% level.

Table 4.10: Crude association between IGF variables and HPV status among controls

IGF	Overall Positivity		High risk HPV types		HPV 16	
marker	OR	95% CI	OR	95% CI	OR	95% CI
IGF-1 (quartiles)*						
Q1	1.00	Ref.	1.00	Ref.	1.00	Ref.
Q2	2.23	0.98 – 5.06	2.23	0.74 – 6.76	1.02	0.06 – 16.55
Q3	2.60	1.17 – 5.80	2.80	0.96 – 8.20	4.00	0.44 – 36.68
Q4	3.30	1.51 – 7.22	4.00	1.42 – 11.26	3.00	0.30 – 29.54
IGF-1 (tertiles)						
T1	1.00	Ref.	1.00	Ref.	1.00	Ref.
T2	2.23	1.16 – 4.29	2.47	1.04 – 5.90	3.96	0.43 – 36.08
T3	2.41	1.26 – 4.62	2.72	1.15 – 6.43	3.96	0.43 – 36.08
IGFBP-3 (quartiles)†						
Q1	1.00	Ref.	1.00	Ref.	1.00	Ref.
Q2	2.03	0.92 – 4.49	4.53	1.25 – 16.48	1.94	0.17 – 21.93
Q3	2.24	1.02 – 4.91	3.29	0.87 – 12.46	0.99	0.06 – 16.08
Q4	2.91	1.36 – 6.24	7.67	2.20 – 26.74	5.00	0.57 – 43.93
IGFBP-3 (tertiles)						
T1	1.00	Ref.	1.00	Ref.	1.00	Ref.
T2	1.51	0.81 – 2.81	1.87	0.79 – 4.41	0.99	0.14 – 7.17
T3	1.79	0.98 – 3.28	2.64	1.16 – 5.98	2.47	0.47 – 13.07

*p-value for trend = 0.0008, 0.0005 and 0.2754 for Overall positivity, High risk HPV types and HPV 16 respectively

†p-value for trend = 0.0355, 0.0081 and 0.0815 for Overall positivity, High risk HPV types and HPV 16 respectively

4.4 MULTIVARIATE ANALYSIS

Age was considered to be a potential *a priori* confounder of the relationship between IGF variables and SILs *a priori*. The relationship between HPV status and the IGF variables is less clear, but because of its strong association with the risk of SILs, it was also included as a potential *a priori* confounder as well. This strategy also permitted the assessment of age and HPV as mediating factors of the putative IGF-SIL association.

4.4.1 IGF-I and IGFBP-3 and the risk of LSIL adjusted for age and HPV status

As evident in Table 4.11, there was a non significant increase in the risk of LSIL for those women whose IGF-I levels were in the second quartile when adjusted for age; when further adjustment was made for HPV status, there was a non-significant decrease in risk. Women with IGF-I levels in the third and fourth quartiles had a decreased risk of LSIL when adjusted for age, but non-significant. When also adjusted for HPV infection status, the protective effect was greater (Table 4.11). Women with IGFBP-3 levels in the highest quartile had a decreased risk of LSIL when adjusted for age, which became significant when also adjusted for HPV infection status (OR=0.24, 95% CI 0.09-0.66) (Table 4.11).

Table 4.11: Association between IGF variables and risk of LSIL adjusted for age and age plus HPV positivity

IGF marker	Age adjusted		Age and HPV status adjusted	
	OR	95% CI	OR	95% CI
IGF-1 (quartiles)*				
Q1	1.00	Ref.	1.00	Ref.
Q2	1.06	0.48 – 2.37	0.82	0.33 – 2.05
Q3	0.49	0.19 – 1.27	0.43	0.15 – 1.27
Q4	0.49	0.19 – 1.29	0.40	0.13 – 1.20
IGF-1 (tertiles)				
T1	1.00	Ref.	1.00	Ref.
T2	0.84	0.41 – 1.74	0.66	0.29 – 1.52
T3	0.46	0.20 – 1.08	0.42	0.16 – 1.20
IGFBP-3 (quartiles)†				
Q1	1.00	Ref.	1.00	Ref.
Q2	0.72	0.34 – 1.54	0.50	0.20 – 1.21
Q3	0.41	0.17 – 0.97	0.29	0.11 – 0.79
Q4	0.39	0.94 – 1.00	0.24	0.09 – 0.66
IGFBP-3 (tertiles)				
T1	1.00	Ref.	1.00	Ref.
T2	0.53	0.26 – 1.08	0.55	0.25 – 1.22
T3	0.40	0.18 – 0.86	0.35	0.15 – 0.82

*p-value for trend = 0.0644 and 0.0360 for Age adjusted and Age and HPV status adjusted respectively

†p-value for trend = 0.0396 and 0.0155 for Age adjusted and Age and HPV status adjusted repectively

4.4.2 IGF-I, IGFBP-3 and the risk of HSIL adjusted for age and HPV status

Similarly to the results observed with LSIL, a non significant increase in the risk of HSIL was observed in those women whose IGF-I levels were in the second quartile as compared to the first quartile when adjusted for both age and age and HPV status. In the third and fourth quartiles, women had a decreased risk of HSIL when adjusted for age, and these ORs changed little when further adjustment was made for HPV status (Table 4.12). Women with IGFBP-3 levels in the highest quartile had a decreased risk of HSIL.

When adjusted for age and HPV status, the OR for the fourth quartile was 0.43 (95% CI 0.22-0.83) (Table 4.12).

Table 4.12: Association between IGF variables and risk of HSIL adjusted for age and age plus HPV positivity

IGF marker	Age adjusted		Age and HPV status adjusted	
	OR	95% CI	OR	95% CI
IGF-1 (quartiles)*				
Q1	1.00	Ref.	1.00	Ref.
Q2	1.43	0.92 – 2.22	1.24	0.63 – 2.41
Q3	0.68	0.41 – 1.11	0.67	0.33 – 1.39
Q4	0.57	0.34 – 0.95	0.53	0.25 – 1.11
IGF-1 (tertiles)				
T1	1.00	Ref.	1.00	Ref.
T2	0.77	0.52 – 1.14	0.64	0.36 – 1.14
T3	0.45	0.29 – 0.70	0.42	0.22 – 0.79
IGFBP-3 (quartiles)†				
Q1	1.00	Ref.	1.00	Ref.
Q2	0.80	0.52 – 1.24	0.66	0.34 – 1.26
Q3	0.82	0.53 – 1.27	0.66	0.35 – 1.26
Q4	0.59	0.37 – 0.93	0.43	0.22 – 0.83
IGFBP-3 (tertiles)				
T1	1.00	Ref.	1.00	Ref.
T2	0.81	0.56 – 1.18	0.89	0.51 – 1.53
T3	0.59	0.40 – 0.87	0.54	0.31 – 0.94

*p-value for trend = 0.0044 and 0.0087 for Age adjusted and Age and HPV status adjusted respectively

†p-value for trend = 0.0883 and 0.0700 for Age adjusted and Age and HPV status adjusted respectively

4.4.3 Multivariate analysis of empirical confounders

Age and HPV status were determined to be confounders *a priori* for the relationship between the IGF variables and the risk of SILs, therefore these variables were forced into the model. Also, given that IGF-I and IGFBP-3 interact with each other in the physiologic environment, they were mutually adjusted for each other. In order to decide which other variables to include in the model, a backward deletion procedure was

performed with all variables suspected to be risk factors for the development of SILs initially included in the model. To facilitate the procedure, the outcome (IGF-I levels) was dichotomized to below the median and above the median, the median being based on IGF-I levels in controls.

4.4.3.1 Backward deletion procedure for LSIL

The results of the backward deletion procedure for LSIL can be seen in Table 4.13. There were no variables which, when removed, changed the OR of the IGF-LSIL relation by more than 10%. The highest change in OR occurred when the variable "parity" was removed from the model, which decreased the OR by 7.3%.

Table 4.13: Backward deletion procedure for LSIL

Variable to be removed from the model in sequence	OR for IGF-I		% change in OR
	Variable in model	Variable removed	
Length of OC use	0.615	0.634	+3.1
Ever use of hormones to treat menopause/infertility	0.634	0.616	-2.8
Lifetime number of vaginal sexual partners	0.616	0.621	+0.8
Age at menarche	0.621	0.615	-1.0
Age at first vaginal intercourse	0.615	0.596	-3.1
Cigarette smoking (Current, Former, Never)	0.596	0.577	-3.2
Highest level of schooling	0.577	0.590	+2.3
Parity	0.590	0.547	-7.3
Ethnic group (French Canadian vs Other)	0.547	0.552	+0.9
Ever use of alcohol on a monthly basis	0.552	0.570	+3.3
Financial situation	0.570	0.572	+0.4

4.4.3.2 Backward deletion procedure for HSIL

The results of the backward deletion procedure for HSIL are presented in Table 4.14. There were no variables which, when removed, changed the OR by more than 10%, the highest percent change being 1.9%.

Table 4.14: Backward deletion procedure for HSIL

Variable to be removed from the model in sequence	OR for IGF-I		% change in OR
	Variable in model	Variable removed	
Length of OC use	0.503	0.509	+1.2
Ever use of hormones to treat menopause/infertility	0.509	0.518	+1.7
Lifetime number of vaginal sexual partners	0.518	0.524	+1.6
Age at menarche	0.524	0.534	+1.9
Age at first vaginal intercourse	0.534	0.528	-1.1
Cigarette smoking (Current, Former, Never)	0.528	0.535	+1.3
Highest level of schooling	0.535	0.535	0.0
Parity	0.535	0.530	-0.9
Ethnic group (French Canadian vs Other)	0.530	0.530	0.0
Ever use of alcohol on a monthly basis	0.530	0.529	-0.2
Financial situation	0.529	0.536	+1.3

4.4.3.3 Backward deletion procedure for HPV status

The same procedure was performed using HPV status as the outcome among controls. Age was forced into the model, and IGF-I and IGFBP-3 were mutually adjusted for each other. Once again, none of the variables, when removed, changed the OR by more than 10% (Table 4.15).

Table 4.15: Backward deletion procedure for HPV status as outcome

Variable to be removed from the model in sequence	OR for IGF-I		% change in OR
	Variable in model	Variable removed	
Financial situation	1.178	1.161	-1.4
Length of OC use	1.161	1.189	+2.4
Ever use of hormones to treat menopause/infertility	1.189	1.158	-2.6
Ever use of alcohol on a monthly basis	1.158	1.163	+0.4
Highest level of schooling	1.163	1.180	+1.5
Cigarette smoking (Current, Former, Never)	1.180	1.129	-4.3
Parity	1.129	1.142	+1.2
Ethnic group (French Canadian vs Other)	1.142	1.143	+0.1
Age at first vaginal intercourse	1.143	1.093	-4.3
Age at menarche	1.093	1.056	-3.4
Lifetime number of vaginal sexual partners	1.056	1.106	+3.8

4.4.3.4 Test for interaction

Since IGF-I and IGFBP-3 interact with each other *in vivo*, a test for interaction between the two variables was performed. IGF-I and IGFBP-3 were dichotomized to above and below the median, the median being based on values in controls. The data were stratified by IGFBP-3 level (above or below the median) and the OR was calculated for the IGF-SIL relationship for both strata. The homogeneity of the two ORs was tested using the Breslow-Day test.

The p-value for the homogeneity of the two ORs for the relationship between IGF-I and LSIL was 0.6159. Similarly, the p-value for the homogeneity of the ORs for the relationship between IGF-I and HSIL was 0.6993.

Interaction between IGF-I and IGFBP-3 was also assessed in the relationship between IGF-I and HPV status. The p-value for homogeneity of the ORs was 0.6599. Based on

these results, the interaction term for IGF-I and IGFBP-3 was not included in any of the final models.

4.4.4 Multivariate Models

4.4.4.1 Multivariate model for LSIL

Table 4.16 shows the results of the model with LSIL as the outcome, adjusted for age, HPV status and mutual adjustment for IGF-I and IGFBP-3. A slight decreased risk of LSIL was observed for women with IGF-I levels in the second quartile, and was even greater for women with IGF-I levels in the third and fourth quartiles, though in each case the results were non-significant. The OR for women with IGF-I levels in the highest quartile was 0.69 (95% CI 0.20-2.41). No appreciable gains in precision were obtained when the IGF-I levels were divided into tertiles.

On the other hand, a significant result was obtained for the highest quartile of IGFBP-3. The OR for women with IGFBP-3 levels in the highest quartile was 0.25 (95% CI 0.08-0.77).

Table 4.16: Association between IGF variables*, and risk of LSIL adjusted for age and HPV status in two separate regression models

Model	IGF marker		OR	95% CI
1) Quartiles	**IGF-1†**			
		Q1	1.00	Ref.
		Q2	0.95	0.34 – 2.66
		Q3	0.60	0.18 – 1.98
		Q4	0.69	0.20 – 2.41
	IGFBP-3‡			
		Q1	1.00	Ref.
		Q2	0.60	0.22 – 1.58
		Q3	0.35	0.11 – 1.05
		Q4	0.25	0.08 – 0.77
2) Tertiles	**IGF-1**			
		T1	1.00	Ref.
		T2	0.70	0.28 – 1.75
		T3	0.50	0.18 – 1.44
	IGFBP-3			
		T1	1.00	Ref.
		T2	0.68	0.29 – 1.62
		T3	0.38	0.15 – 0.97

*IGF-I and IGFBP-3 are mutually adjusted for each other
†p-value for trend = 0.2986
‡p-value for trend = 0.0716

4.4.4.2 Multivariate model for HSIL

Table 4.17 shows the results of the model with HSIL as the outcome, adjusted for age, HPV status and mutual adjustment for IGF-I and IGFBP-3. A non-significant increase in risk was observed for women with IGF-I levels in the second quartile (OR=1.32, 95% CI 0.66-2.66). There was a decreased risk for women both in the third and fourth quartiles, although both were non-significant. A significant result was obtained when IGF-I levels were divided into tertiles. The OR for women with IGF-I levels in the third tertile was 0.47 (95% CI 0.24-0.93).

Women with IGFBP-3 levels in the fourth quartile had a non-significant OR of 0.51 (95% CI 0.25-1.06). Similar results, though also non-significant, were obtained when IGFBP-3 levels were divided into tertiles.

Table 4.17: Association between IGF variables* and risk of HSIL adjusted for age and HPV status in two separate regression models

Model	IGF marker		OR	95% CI
1) Quartiles	**IGF-1†**			
		Q1	1.00	Ref.
		Q2	1.32	0.66 – 2.66
		Q3	0.75	0.35 – 1.61
		Q4	0.64	0.29 – 1.44
	IGFBP-3‡			
		Q1	1.00	Ref.
		Q2	0.64	0.33 – 1.25
		Q3	0.69	0.35 – 1.36
		Q4	0.51	0.25 – 1.06
2) Tertiles	**IGF-1**			
		T1	1.00	Ref.
		T2	0.64	0.35 – 1.18
		T3	0.47	0.24 – 0.93
	IGFBP-3			
		T1	1.00	Ref.
		T2	0.95	0.54 – 1.67
		T3	0.66	0.37 – 1.20

*IGF-I and IGFBP-3 are mutually adjusted for each other
†p-value for trend = 0.0647
‡p-value for trend = 0.3172

4.4.4.3 Multivariate model for HPV status

Table 4.18 shows the results of the model with HPV positivity as the outcome among controls, adjusted for age with mutual adjustment for IGF-I and IGFBP-3. When overall positivity was used as the outcome, no significant results were obtained. An increased risk of positivity was observed for women with IGF-I levels in the highest quartile

(OR=1.34, 95% CI 0.55-3.26), as well as for women with IGFBP-3 levels in the highest quartile (OR=1.91, 95% CI 0.85-4.30).

When positivity for any high risk HPV type was used as the outcome, a non-significant increase in risk was observed for women with IGF-I levels in the highest quartile (OR=1.20, 95% CI 0.38-3.76), which was slightly less than for when overall positivity was used as the outcome.

On the other hand, there was a significant increase in risk of positivity for any high risk HPV type for women with IGFBP-3 levels in the highest quartile (OR=4.53, 95% CI 1.33-15.40), which was much greater than for when overall positivity was used as the outcome.

Lastly, positivity for HPV16 was used as the outcome, despite the small number of subjects who were positive for HPV16. No significant results were obtained.

Table 4.18: Association between IGF variables* and risk of HPV positivity among controls adjusted for age in two separate models

Model	IGF marker	Overall positivity		High risk HPV types		HPV 16	
		OR	95% CI	OR	95% CI	OR	95% CI
1) Quartiles	**IGF-1†**						
	Q1	1.00	Ref.	1.00	Ref.	1.00	Ref.
	Q2	1.46	0.65 – 3.28	1.45	0.47 – 4.49	1.32	0.07 – 23.95
	Q3	1.19	0.50 – 2.84	0.96	0.30 – 3.07	2.49	0.19 – 33.22
	Q4	1.34	0.55 – 3.26	1.20	0.38 – 3.76	1.44	0.09 – 22.28
	IGFBP-3‡						
	Q1	1.00	Ref.	1.00	Ref.	1.00	Ref.
	Q2	1.72	0.78 – 3.81	3.68	1.10 – 12.32	2.02	0.19 – 22.02
	Q3	1.46	0.64 – 3.30	1.69	0.46 – 6.24	0.60	0.03 – 10.99
	Q4	1.91	0.85 – 4.30	4.53	1.33 – 15.40	2.06	0.19 – 22.29
2) Tertiles	**IGF-I**						
	T1	1.00	Ref.	1.00	Ref.	1.00	Ref.
	T2	1.71	0.85 – 3.44	1.72	0.67 – 4.44	4.68	0.34 – 65.06
	T3	1.45	0.68 – 3.08	1.22	0.46 – 3.26	2.61	0.17 – 40.35
	IGFBP-3						
	T1	1.00	Ref.	1.00	Ref.	1.00	Ref.
	T2	0.78	0.40 – 1.53	0.87	0.35 – 2.18	0.49	0.06 – 3.89
	T3	1.13	0.59 – 2.18	1.64	0.69 – 3.91	1.20	0.20 – 7.21

*IGF-I and IGFBP-3 are mutually adjusted for each other

†p-values for trend = 0.3086, 0.4706, and 0.7158 for Overall positivity, High risk HPV types, and HPV 16, respectively

‡p-values for trend = 0.3755, 0.1328 and 0.2075 for Overall positivity, High risk HPV types, and HPV 16, respectively

5 DISCUSSION

As of this writing, this is the second study to investigate the relationship between IGF-I and IGFBP-3 serum levels and the risk of SILs, and the first study to investigate the relationship between IGF-I and IGFBP-3 and the risk of HPV infection. The results of this study are consistent with a decreased risk of LSIL and HSIL with increased levels of IGF-I and IGFBP-3. Most striking is the significantly decreased risk of LSIL observed in women with IGFBP-3 levels in the highest quartile (OR=0.25, 95% CI 0.08-0.77). None of the other results in the adjusted model were statistically significant.

Also, in this study there was an increased risk of being positive for any HPV type for women with increased levels of IGF-I and IGFBP-3, which persisted when the outcome was restricted to only high-risk HPV types. The OR for the risk of being positive for a high-risk HPV type for women with IGFBP-3 levels in the highest quartile was 4.53 (95% CI 1.33-15.40).

In vitro and animal studies have demonstrated that IGF-I has a positive effect on cell proliferation and a negative effect on apoptosis (5, 6, 28, 113), and that IGF-I is correlated with tumour development (6, 112). This supports the fact that IGF-I is potentially positively associated with cancer risk, as many epidemiologic studies have confirmed. Even though several large prospective studies have found an increased cancer risk in individuals with increased IGF-I levels (66, 67, 83), there is no consensus as the range of magnitude of risk varies widely.

This study differs from those previously carried out in several ways. The outcome was cancer precursors and not cancer itself, and thus the mean age of the study population was much younger than previous studies, with less than 10% being over 50 years of age. Often, as with the studies on female cancers, the women being studied were post-menopausal; even so, the studies on breast and ovarian cancer suggest that the positive relationship between IGF-I levels and cancer risk was strongest in pre-menopausal women under 50 years of age.

Also, cervical cancer is unique in that HPV infection is considered to be a necessary cause. Thus, the natural history of cervical cancer differs from other cancers, specifically those which have been the focus of studies on IGF variables and cancer risk, for which sex hormones play a large role, such as breast cancer and prostate cancer.

Our results are also in contrast to those of Wu *et al* (9), the only other epidemiologic study on the relationship between IGF-I, IGFBP-3 and the risk of SILs. Wu *et al* found a large, significant increase in risk of SILs for women with IGF-I serum levels in the highest quartile (OR=8.54, 95% CI 4.15-17.60), which persisted when the outcome was stratified by low- and high-grade. Similarly, women with IGFBP-3 serum levels in the highest quartile had an increased risk of SILs, though non-significant.

There are several differences between our study and the one described above, which may have contributed to the different results. The mean IGF-I and IGFBP-3 levels found by Wu *et al* were much lower than those in this study, even though the mean age of women in the Wu *et al* study was 10 years younger. The population of the Wu *et al* study was

mostly Hispanic and African American and from an economically disadvantaged population, whereas the women in this study were mostly Caucasian and of moderate to comfortable means. Both race and socio-economic status may influence IGF-I levels, the latter potentially influencing IGF-I levels through indirect ways such as diet and nutrition (55, 114, 115).

It is unclear why increased levels of IGF-I would have a protective effect on the risk of cervical cancer precursors; although, since the confidence intervals were non-significant, this may have been a statistical artifact. It is possible that given the causative role of HPV infection in cervical cancer, IGF-I does not play a large a role in cancer development as for other cancers. As well, IGF-I could play a role downstream of what we tested here, that is, in the progression of HSIL to cervical cancer. It also cannot be ruled out that the presence of SILs had an effect on IGF levels.

Past studies have found both a positive and negative relationship between IGFBP-3 and cancer risk. The role of IGFBP-3 in cancer development is less clear, as it plays several functions at the cellular level, which can affect cell proliferation both positively and negatively (116). The protective effect of IGFBP-3 may come from the fact that it binds IGF-I and prevents it from crossing circulation to the tissue, where IGF-I exerts its cellular effects.

No other studies have looked at the relationship between HPV positivity, IGF-I and IGFBP-3. The results of the association between IGF-I and HPV positivity were inconclusive as the confidence intervals encompassed the null value, but controls with

IGFBP-3 levels in the highest quartile were at a significantly increased risk of being positive for high risk HPV types. It is possible that IGFBP-3 somehow positively affects HPV viral load and persistence, persistent infections being more likely to be detected.

There are several limitations and sources of potential bias to this study. Ideally, IGF-I and IGFBP-3 would have been measured prior to the development of the outcome. Due to the retrospective nature of this study, the possibility of the outcome having an effect on IGF-I and IGFBP-3 levels cannot be discounted.

Although many of the factors suspected to be associated with IGF-I and IGFBP-3 levels were measured, including age, tobacco smoking and alcohol consumption, residual confounding may have occurred if these factors were not measured accurately enough. Other determinants postulated to be associated with IGF-I and/or IGFBP-3 were not measured. Some studies have found a relationship between IGF variables and body mass index (BMI) (42, 54, 116, 117), diet (55, 118) and physical activity (116). Any confounder associated with IGF variables that differed between cases and controls that was not measured could have led to bias. Variables related to energy balance and caloric intake are to be considered.

As well, any study that relies on voluntary participation may lead to self-selection, which may affect the generalizability of the results. The socio-demographic characteristics of women who provided a blood sample for this study and those who did not were compared, and did not differ significantly on any of the characteristics examined. No data is available on those who refused to both fill out a questionnaire and give a blood

sample, but given the small number of women who refused to participate, it is unlikely this was a major source of bias.

Misclassification could also have occurred at the level of the outcome, exposure, or confounders. Case status was confirmed by cervical biopsy, whereas controls were confirmed by cytology only. Given that cases were more rigorously examined, it is more likely that misclassification would occur among controls. There may have been several false negatives among controls, which may have led to a misrepresentation of the OR. To avoid misclassification with respect to IGF-I and IGFBP-3 levels, each sample was tested twice, and tested in as few batches as possible by the same laboratory technician. Lastly, since most confounders were self-reported, some misclassification is certain, but unlikely to have created much bias.

On the other hand, there were many strengths to this study. SILs are a known precursor to the development of cervical cancer, and cervical cancer is very rare is this population. By using SILs as the outcome, it lessens the possibility that the outcome would affect IGF levels, which would be more likely with cancer, and is a common problem with many case-control studies in this area. Also, by stratifying the outcome by SIL grade, it allowed us to examine whether the relationship between IGF variables and SILs varied by grade.

This study had a very high participation rate lessening the chance of selection bias. Controls were drawn from a similar population as the cases, and controls who later on were determined to have LSIL or HSIL were eligible to be cases. Much care was taken to

ensure the accuracy of the measurement of the exposure, and laboratory personnel were blinded to case-control status.

There is very little research on the relationship between IGF variables and cervical cancer and its precursors. Because of the contrasting results obtained in this study as compared to that by Wu *et al* (9), further studies are warranted to elucidate the relationship between IGF-I, IGFBP-3, HPV infection and SILs. The relationships between IGFBP-3 levels and LSIL and high-risk HPV infection observed in this study are much stronger than for many currently accepted risk factors for cancer, and should be explored. Not only could this information help to better understand the natural history of cervical cancer and why some HPV-positive women progress to disease and others do not, IGF variables could potentially serve as biomarkers of disease progression.

6 CONCLUSION

This study makes an important contribution to the field of IGF-I, IGFBP-3, and cervical cancer research. We found a statistically significant, negative relationship between having IGFBP-3 plasma levels in the highest quartile and the risk of LSIL. We also found a strong, statistically significant, positive relationship between having IGFBP-3 plasma levels in the highest quartile and the risk of being positive for a high-risk type of HPV. Whereas the other results were not statistically significant, overall, they were consistent with high levels of IGF-I and IGFBP-3 having a protective effect on both LSIL and HSIL risk. The results of this study contrast greatly with those obtained in the one previous study on IGF variables and SIL risk. Given the potential for IGF variables to serve as biomarkers for cervical cancer and its precursors, and to help explain the natural history of cervical cancer development, further research is needed in this area to help clarify what role, if any, IGF-I and IGFBP-3 play in HPV infection and cervical cancer.

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APPENDIX 1

Study Information Brochures

McGill
Department of Oncology

CHUM

IF YOU ARE BETWEEN 18 AND 35 YEARS OF AGE, YOU CAN HELP US.

INFORMATION SHEET

RESEARCH PROJECT ON CERVICAL CANCER

Our research team investigates the natural history of cervical cancer and its risk factors, particularly human papillomavirus infection. Our research goal is to understand what causes cervical cancer and to implement better screening and prevention practices.

Cervical cancer and human papillomavirus

Cervical infection by human papillomavirus (HPV) is the main causal factor of cervical cancer and its precursor lesions. However, most women with cervical HPV infection do not develop cancer.

The aim of our research

Our current study focuses on determining why some women with a cervical HPV infection develop cancer, while most women do not. Specifically, we are looking at biological factors that may affect a woman's susceptibility to HPV.

How can you help us?

To investigate this, we need the participation of patients with cervical lesions as well as patients who do not have any cervical disease. You can help us by allowing us to obtain a sample of your cervical cells. This sample will be obtained at the time of your colposcopic examination or your Pap smear test. Additionally, we require a small blood sample (10 ml or, equivalently, about 2 teaspoons) and a questionnaire to be filled out by you.

Risks and confidentiality

There are no potential risks to you as a result of participating in this study; however, the collection of blood may cause some discomfort. Strict confidentiality will be maintained throughout the study. Names will not be linked to any information provided by you.

Your collaboration is important because our results could be used to identify women who are at a higher risk of developing cervical cancer and would benefit from frequent screening.

If you would like to know more about this project, please ask to speak with our research nurse.

We thank you for your cooperation.

Eduardo Franco, PhD
Professor and Director
Principal Investigator, BCCR Study

McGill

Département d'oncologie

CHUM

SI VOUS AVEZ ENTRE 18 ET 35 ANS, VOUS POUVEZ NOUS AIDER.
FEUILLET D'INFORMATION

PROJET DE RECHERCHE SUR LE CANCER DU COL DE L'UTÉRUS

Notre équipe de recherche s'intéresse à l'histoire naturelle du cancer du col de l'utérus et à ses facteurs de risque, particulièrement à l'infection au virus du papillome humain. Le but de notre recherche est de comprendre ce qui cause le cancer du col de l'utérus afin de mettre en oeuvre des moyens de prévention et de dépistage plus efficaces.

Cancer du col utérin et virus du papillome humain

L'infection du col utérin par le virus du papillome humain (VPH) est le principal facteur causant le cancer du col de l'utérus et le développement des lésions précancéreuses. Toutefois, la plupart des femmes qui contractent une infection au VPH ne développeront pas de cancer du col utérin.

L'objet de notre recherche

L'étude actuellement en cours vise à déterminer pourquoi certaines femmes ayant une infection au VPH développent un cancer alors que la plupart n'en développeront pas. De façon plus spécifique, nous désirons observer les facteurs biologiques qui peuvent affecter la susceptibilité de la femme au VPH.

Comment pouvez-vous nous aider?

Pour cette étude, nous avons besoin de la participation de patientes présentant des lésions du col de l'utérus ainsi que de patientes qui n'en ont jamais eues. Vous pouvez donc nous aider en nous autorisant à effectuer un prélèvement de cellules de votre col utérin. Cet échantillon sera obtenu en même temps que votre visite prévue soit pour un test PAP ou un examen par colposcopie. De plus, nous vous demanderons de fournir un petit échantillon sanguin (10 ml ou 2 cuillerées à thé) et de bien vouloir répondre à notre questionnaire.

Risques et confidentialité

Vous ne courrez aucun risque en participant à cette étude; sauf peut-être l'inconfort relié à la prise de sang. La plus stricte confidentialité est assurée tout au long de cette étude. Les noms des participantes ne seront jamais associés à toute information provenant d'elles.

Votre collaboration est importante parce que nos résultats pourraient servir à cibler les femmes qui ont un risque élevé de développer un cancer du col de l'utérus et qui bénéficieraient de tests de dépistage plus fréquents.

Si vous désirez en connaître davantage sur cette étude, demandez à parler avec notre infirmière de recherche.

Nous vous remercions de votre coopération.

Eduardo Franco, PhD
Professeur et Directeur
Chercheur principal, Recherche sur les biomarqueurs du cancer du col utérin

APPENDIX 2

Consent Forms for Cases

MONTREAL STUDY OF WOMEN'S HEALTH
PRINCIPAL INVESTIGATOR: DR. EDUARDO FRANCO

INFORMED CONSENT FORM

Purpose:

We are conducting a study in the Montreal area to determine whether certain markers affect susceptibility to cervical cancer. To investigate this we need the participation of patients with lesions of the cervix which require examination and biopsy. You may be eligible to participate in this study. This investigation is being done by clinical and basic scientists at this hospital and at McGill University and Université de Montréal.

What is necessary?

Your doctor will perform a biopsy on your lesion that will be checked for tumour cells. If you consent to participate, we ask your permission to allow us to test the biopsy tissue for signs of infection by human papillomaviruses and for the presence of the genetic characteristics that are of interest in this study.

We will also ask you to donate a small blood sample (10 ml or, equivalently, about 2 teaspoons) that will be tested subsequently for circulating levels of certain proteins which may be a risk factor for cervical cancer development

You will also be asked to complete a questionnaire which will assess whether you have one or more of the risk factors for cancer of the cervix. This questionnaire should take 15-20 minutes.

Benefits:

By participating in this study, you will be contributing to our understanding of what causes cervical cancer. Our results could help us to design new laboratory tests to determine which women are susceptible to cervical disease and should be monitored more closely, allowing us to prevent high grade lesions and cervical cancer. This will help future patients at risk of developing cervical cancers.

Risks:

The are no potential risks to you as a consequence of your participation in this study. The collection of the blood sample, however, may cause some discomfort and you will have to spend about 15 to 20 minutes filling out the questionnaire. In the questionnaire you will be asked a number of questions about family life, sexual activity, and medical history, some of which are of a sensitive nature.

Date: _____

Confidentiality:

The results from the analyses of your blood sample, cervical cells, and of the biopsy, as well as the responses you gave to the questionnaire will be treated with strict confidentiality. No names or other information that could identify you as a patient will be released. All the data from this study will be analyzed in aggregate statistical form only, again with no names linked to any data.

The actual samples from your blood and cervical cells will not be made available to investigators that are not involved with this study, nor will they be sold for commercial use. They will only be used for the purposes outlined in this consent form. They will be securely stored for as long as they are needed for the verification of laboratory tests and for research audit purposes. Your name will not be linked to any specimen.

Your rights:

You may refuse to participate in the study now or later, without any negative consequences. Nothing will change in terms of the quality of health care that you are receiving in this hospital. You may also refuse to answer any questionnaire questions with which you do not feel comfortable.

There are no costs to you, direct or indirect. All the tests will be paid out of research funds that our scientific team received to investigate the causes of cervical cancer.

Additional information:

If you would like to obtain additional information about this study you may call our research nurse, Solange Piché, at (514) 229-8019 or our study coordinator, Ms. Anita Koushik, at (514) 398-4992.

Your consent:

I understand the general purpose of the study, what will be required of me, and my rights as a participant. I consent to participate in the study. My participation is voluntary and if I agree to participate I may withdraw my consent and discontinue my participation from the study at any time without prejudice or loss of benefits to which I am otherwise entitled. I understand that my participation may be terminated with or without my consent.

Patient's name:	Signature:	Date:
Nurse:	Signature:	Date:

ÉTUDE MONTRÉALAISE SUR LA SANTÉ DES FEMMES
CHERCHEUR PRINCIPAL : D[R] EDUARDO FRANCO

FORMULAIRE DE CONSENTEMENT

Objectif:

Nous conduisons une étude dans la région de Montréal afin de déterminer si certains marqueurs influencent la susceptibilité au cancer du col utérin. Afin d'étudier ces aspects, nous avons besoin de la participation de patientes ayant une lésion du col utérin requérant un examen colposcopique et une biopsie. Vous pourriez répondre aux critères d'éligibilité pour cette étude exécutée par des cliniciens et des chercheurs à cet hôpital, à l'Université McGill et à l'Université de Montréal.

Qu'est-ce qui est requis?

Votre médecin prélèvera une biopsie de votre lésion et la fera analyser afin de déterminer si des cellules tumorales sont présentes. Si vous acceptez de participer à cette étude, nous vous demandons l'autorisation d'utiliser le tissu prélevé lors de la biopsie afin de vérifier la présence ou l'absence d'infection par le virus du papillome humain ainsi que de caractéristiques génétiques spécifiques.

Nous vous demanderons également de donner un petit échantillon sanguin (10 ml, l'équivalent d'environ 2 cuillerées à thé) sur lequel des tests seront effectués afin de détecter les niveaux de certaines protéines circulant dans votre sang. Ces protéines pourraient être associées au développement du cancer du col de l'utérus.

Nous vous demanderons de compléter un questionnaire qui nous permettra de déterminer si vous possédez un ou plusieurs facteurs de risque pour le développement du cancer du col utérin. Ce questionnaire devrait demander 15 à 20 minutes de votre temps.

Avantages :

Par votre participation à cette étude, vous contribuerez à améliorer notre compréhension des causes du cancer du col de l'utérus. Les résultats de notre étude pourraient nous aider à concevoir des nouveaux tests de laboratoire permettant de déterminer qui sont les femmes plus susceptibles aux maladies du col de l'utérus et requérant un suivi médical plus étroit, permettant ainsi de prévenir des lésions de haut grade et des cancers du col utérin. Les futures patientes à risque de développer un cancer du col de l'utérus bénéficieront des résultats de cette étude.

Risques :

Vous n'encourez pas de risques en participant à cette étude. Par contre, la prise d'un échantillon sanguin peut causer de l'inconfort et vous devrez prendre de 15 à 20 minutes pour compléter le questionnaire. Les questions porteront sur votre vie familiale, votre activité sexuelle et votre histoire médicale. Certaines questions seront de nature délicate.

Date: _____

Confidentialité:

Les résultats des analyses de l'échantillon sanguin, des cellules du col de l'utérus, de la biopsie, ainsi que les réponses au questionnaire seront traités dans la plus stricte confidentialité. Aucun nom ou autre information permettant de vous identifier ne sera divulgué ou publié. Toutes les données de cette étude seront analysées en groupe, sans que les noms soient associés aux données.

Les échantillons de sang et de cellules du col de l'utérus que vous fournirez ne seront pas utilisées par des chercheurs ne faisant pas partie de cette étude. De plus, vos échantillons ne seront pas vendus pour des fins commerciales. Ils seront uniquement utilisés pour les raisons décrites dans ce formulaire de consentement. Ils seront conservés en un lieu sécuritaire aussi longtemps qu'il sera nécessaire pour permettre la vérification des tests de laboratoire ainsi que pour un audit de recherche. Votre nom ne sera associé à aucun spécimen.

Vos droits :

Vous pouvez refuser de participer à cette étude, maintenant ou plus tard, sans aucune conséquence. Votre refus de participer ne changerait pas la qualité des soins de santé que vous recevrez dans cet hôpital. Vous pouvez également refuser de répondre à certains items du questionnaires, s'ils vous rendent mal à l'aise.

Cette étude ne vous occasionnera aucun frais. Tous les tests seront payés grâce à des subventions que notre équipe de recherche a reçues dans le but d'étudier les causes du cancer du col de l'utérus.

Informations supplémentaires:

Si vous désirez des informations supplémentaires concernant cette étude, vous pouvez joindre notre infirmière, Solange Piché, au 229-8019 ou la coordonnatrice de cette étude, madame Anita Koushik, au 398-4992.

Votre consentement :

Je comprends le but général de cette étude, ce qui me sera demandé, ainsi que mes droits en tant que participante. Je consens à participer à cette étude. Ma participation est volontaire et je peux décider de retirer mon consentement et d'arrêter ma participation en tout temps sans préjudice ou perte d'avantages auxquels j'aurais autrement droit.

Nom du patient:	Signature:	Date:
Infirmière :	Signature:	Date:

APPENDIX 3

Consent Forms for Controls

MONTREAL STUDY OF WOMEN'S HEALTH
PRINCIPAL INVESTIGATOR: DR. EDUARDO FRANCO

INFORMED CONSENT FORM
CONTROL PARTICIPANTS

Purpose:

We are conducting a study in the Montreal area to determine whether certain markers affect susceptibility to cervical cancer. To investigate this we need the participation of patients with lesions of the cervix as well as of patients with any other condition except cancer, the latter to serve as a control group. You have been contacted by our resesarch nurse because you could be one of these control patients. This investigation is being done by clinical and basic scientists at this hospital and at McGill University and Université de Montréal.

What is necessary?

If you consent, we will need to collect an additional sample of cells from your cervix which will undergo a Pap test to ensure that you do not have high grade intraepithelial lesions. We also ask your permission to test the cell sample for signs of infection by human papillomavirus and for the presence of the genetic characteristics that are of interest in this study.

We will also ask you to donate a small blood sample (10 ml or, equivalently, about 2 teaspoons) that will be tested subsequently for circulating levels of certain proteins which may be a risk factor for cervical cancer development.

You will also be asked to complete a questionnaire which will assess whether you have one or more of the risk factors for cancer of the cervix. This questionnaire should take 15-20 minutes.

Benefits:

By participating in this study you will be contributing to our understanding of what causes cervical cancer. Our results could help us to design new laboratory tests to determine which women are susceptible to cervical disease, allowing us to prevent high grade lesions. This will help future patients at risk of developing cervical cancers. As well, if any lesions are detected during your Pap test, your doctor will notify you so that you can be treated if necessary.

Risks:

The risks in this study are minimal as the collection of cervical cells for the Pap test is a safe examination. As with any gynaecological examination, there is a possibility that a slight discomfort might be felt during the insertion of the cervical sampler to collect the cells. The collection of the blood sample may also cause some discomfort, and you may have to spend about 15 to 20 minutes altogether filling out the questionnaire. In the questionnaire you will be asked a number of questions about family life, sexual activity, and medical history, some of which are of a sensitive nature.

Date: _____

Confidentiality:

The results from the analyses of your blood sample and cervical cells, as well as the responses you gave to the questionnaire will be treated with strict confidentiality. No names or other information that could identify you as a patient will be released. All the data from this study will be analyzed in aggregate statistical form only, again with no names linked to any data.

The actual samples from your blood and cervical cells will not be made available to investigators that are not involved with this study, nor will they be sold for commercial use. They will only be used for the purposes outlined in this consent form. They will be securely stored for as long as they are needed for the verification of laboratory tests and for research audit purposes. Your name will not be linked to any specimen.

Your rights:

You may refuse to participate in the study now or later, without any negative consequences. Nothing will change in terms of the quality of health care that you are receiving in this hospital. You may also refuse to answer any questionnaire questions with which you do not feel comfortable.

There are no costs to you, direct or indirect. All the tests will be paid out of research funds that our scientific team received to investigate the causes of cervical cancer.

Additional information:

If you would like to obtain additional information about this study you may call our research nurse, Solange Pichet, at 229-8019 or our study coordinator, Ms. Anita Koushik, at 398-4992.

Your consent:

I understand the general purpose of the study, what will be required of me, and my rights as a participant. I consent to participate in the study. My participation is voluntary and if I agree to participate I may withdraw my consent and discontinue my participation from the study at any time without prejudice or loss of benefits to which I am otherwise entitled. I understand that my participation may be terminated with or without my consent.

Patient's name:	Signature:	Date:
Nurse:	Signature:	Date:

ÉTUDE MONTRÉALAISE SUR LA SANTÉ DES FEMMES
CHERCHEUR PRINCIPAL : D[R] EDUARDO FRANCO

FORMULAIRE DE CONSENTEMENT
GROUPE CONTRÔLE

Objectif:

Nous conduisons une étude dans la région de Montréal afin de déterminer si certains marqueurs influencent la susceptibilité au cancer du col utérin. Afin d'étudier ces aspects, nous avons besoin de la participation de patientes ayant une lésion du col utérin ainsi que de patientes avec d'autres diagnostics (sauf un cancer) afin de constituer un groupe contrôle. Vous pourriez faire partie du groupe contrôle. Cette étude est exécutée par des cliniciens et des chercheurs à cet hôpital, à l'Université McGill et à l'Université de Montréal.

Qu'est-ce qui est requis?

Si vous consentez à participer, le médecin prélèvera un échantillon de cellules de votre col utérin. Une cytologie gynécologique (test PAP) sera effectuée afin de vérifier que vous n'avez pas de lésion intra-épithéliale épidermoïde de haut grade du col utérin. Nous vous demandons également la permission d'utiliser cet échantillon de cellules afin de vérifier la présence ou l'absence d'infection par le virus du papillome humain ainsi que de caractéristiques génétiques spécifiques.

Nous vous demanderons également de donner un petit échantillon sanguin (10 ml, l'équivalent d'environ 2 cuillerées à thé) sur lequel des tests seront effectués afin de détecter les niveaux de certaines protéines circulant dans votre sang. Ces protéines pourraient être associées au développement du cancer du col de l'utérus.

Nous vous demanderons de compléter un questionnaire qui nous permettra de déterminer si vous possédez un ou plusieurs facteurs de risque pour le développement du cancer du col utérin. Ce questionnaire devrait demander 15 à 20 minutes de votre temps.

Avantages :

Par votre participation à cette étude, vous contribuerez à améliorer notre compréhension des causes du cancer du col de l'utérus. Les résultats de notre étude pourraient nous aider à concevoir des nouveaux tests de laboratoire permettant de déterminer qui sont les femmes plus susceptibles aux maladies du col de l'utérus dans le but de prévenir des lésions de haut grade et des cancers du col utérin. Les futures patientes à risque de développer un cancer du col de l'utérus bénéficieront des résultats de cette étude. De plus, si une lésion était détectée lors de votre test PAP, votre médecin vous aviserait de façon à ce que vous puissiez recevoir un traitement, si nécessaire.

Date: _____

Risques :

Les risques encourus en participant à cette étude sont minimes puisque le prélèvement de cellules du col de l'utérus est une procédure courante et sans danger. Comme pour tout examen gynécologique, un léger inconfort pourrait être ressenti lors du prélèvement de cellules. La prise d'un échantillon sanguin peut également causer de l'inconfort. Vous devrez prendre de 15 à 20 minutes pour compléter le questionnaire. Les questions porteront sur votre vie familiale, votre activité sexuelle et votre histoire médicale. Certaines questions seront de nature délicate.

Confidentialité:

Les résultats des analyses de l'échantillon sanguin, des cellules du col de l'utérus, de la biopsie, ainsi que les réponses au questionnaire seront traités dans la plus stricte confidentialité. Aucun nom ou autre information permettant de vous identifier ne sera divulgué ou publié. Toutes les données de cette étude seront analysées en groupe, sans que les noms soient associés aux données.

Les échantillons de sang et de cellules du col de l'utérus que vous fournirez ne seront pas utilisées par des chercheurs ne faisant pas partie de cette étude. De plus, vos échantillons ne seront pas vendus pour des fins commerciales. Ils seront uniquement utilisés pour les raisons décrites dans ce formulaire de consentement. Ils seront conservés en un lieu sécuritaire aussi longtemps qu'il sera nécessaire pour permettre la vérification des tests de laboratoire ainsi que pour un audit de recherche. Votre nom ne sera associé à aucun spécimen.

Vos droits :

Vous pouvez refuser de participer à cette étude, maintenant ou plus tard, sans aucune conséquence. Votre refus de participer ne changerait pas la qualité des soins de santé que vous recevez dans cet hôpital. Vous pouvez également refuser de répondre à certains items du questionnaires, s'ils vous rendent mal à l'aise.

Cette étude ne vous occasionnera aucun frais. Tous les tests seront payés grâce à des subventions que notre équipe de recherche a reçues dans le but d'étudier les causes du cancer du col de l'utérus.

Informations supplémentaires:

Si vous désirez des informations supplémentaires concernant cette étude, vous pouvez joindre notre infirmière, Solange Piché, au 229-8019 ou la coordonnatrice de cette étude, madame Anita Koushik, au 398-4992.

Date: _____

Votre consentement :

Je comprends le but général de cette étude, ce qui me sera demandé, ainsi que mes droits en tant que participante. Je consens à participer à cette étude. Ma participation est volontaire et je peux décider de retirer mon consentement et d'arrêter ma participation en tout temps sans préjudice ou perte d'avantages auxquels j'aurais autrement droit.

Nom du patient:	Signature:	Date:
Infirmière :	Signature:	Date:

APPENDIX 4

Questionnaires

STUDY NO: _____

MONTREAL WOMEN'S HEALTH STUDY QUESTIONNAIRE

McGill University & Université de Montréal

INSTRUCTIONS FOR THE QUESTIONNAIRE

This questionnaire is composed of the following sections:

General information
Smoking history and alcohol consumption
Reproductive and sexual history
Medical history

Most questions require that you simply check a box ❑ with an "X" to indicate your choice. Other questions require a specific answer, such as age, date, or another number. Depending on your answer for some questions, you will be told to skip the next question and go to a different part of the questionnaire. This is to save you time, so that you won't have to go over questions that do not apply to you.

There are no right or wrong answers to any question. Many questions require that you think back over your adult years, particularly over the past year, to recall specific information. Please take the time to reflect. You will be surprised that by being "forced" to recall specific information of one type, some of the answers for other questions may come more naturally to you later on. If you can't possibly remember the information, skip the question, but we would like to encourage you to try to answer all questions. A good guess is always better than no information at all. If you'd like to tell us more about any specific items please use the available space at the end of the questionnaire.

WE APPRECIATE YOUR COOPERATION WITH THE STUDY

STUDY NO: _____

GENERAL INFORMATION

This portion of the questionnaire concerns general information about you and where you live. These questions help us to understand your sociodemographic background.

1. What is your date of birth? _____/_____/_____ ***(very important)***
D M Y

2. In what country were you born? _____

If born in Canada: indicate province: _____

3. What is your **current** marital status?

❑ Married ❑ Single ❑ Unmarried, but living with a partner
❑ Divorced/separated ❑ Widowed

4. a) The Montreal area is made up of many ethnic groups. We would like to know in which group you would place **yourself**. Check the most appropriate category:

❑ French Canadian ❑ English Canadian ❑ Hispanic/Portuguese
❑ Greek ❑ Black Canadian ❑ Italian ❑ Native Indian
❑ Asian/Oriental ❑ Arab ❑ Jewish Other:_____

b) In which group would you place your **mother?** Check the most appropriate:

❑ French Canadian ❑ English Canadian ❑ Hispanic/Portuguese
❑ Greek ❑ Black Canadian ❑ Italian ❑ Native Indian
❑ Asian/Oriental ❑ Arab ❑ Jewish Other:_____

c) In which group would you place your **father?** Check the most appropriate:

❑ French Canadian ❑ English Canadian ❑ Hispanic/Portuguese
❑ Greek ❑ Black Canadian ❑ Italian ❑ Native Indian
❑ Asian/Oriental ❑ Arab ❑ Jewish Other:_____

5. a) What is your current occupation? _____

b) Would you say that your family's financial situation is:

❑ Difficult ❑ Moderate ❑ Comfortable ❑ Very comfortable

c) What was the highest level of schooling you attained?

❑ Elementary school ❑ Secondary ❑ CEGEP/Technical post-secondary
❑ University (undergraduate) ❑ University (Master's, doctoral, or equivalent studies)

SMOKING HISTORY AND ALCOHOL CONSUMPTION

The following questions are about your tobacco smoking and alcoholic beverage consumption habits. Please try to be as specific as possible in your answers.

6. Have you ever smoked cigarettes **regularly**, that is, **one** cigarette or **more** each **day** for a **year** or more?

❑ Yes ❑ No

7. Have you smoked a total of at least 100 cigarettes in your lifetime?

❑ Yes ❑ No

→ **If No**, go to question 11

8. At what age did you start to smoke? _____years

9. Do you still smoke? ❑ Yes ❑ No

↓

If No, at what age did you stop? _____years

10. On average, how many cigarettes do/did you smoke a day? _____cigarettes per day

11. Has there ever been a period in your life when you drank beer, wine or liquor AT LEAST ONCE A MONTH?

❑ Yes ❑ No

→ **If No**, go to question 13

12. Has there ever been a period in your life when you drank beer, wine or liquor AT LEAST ONCE A WEEK?

❑ Yes ❑ No

↓

If Yes,

indicate the average number of drinks per week that you consumed during the past 5 years *(consider a drink as being equivalent to a 12 oz. can of beer or to a 4 oz. glass of wine or to 1.5 ounces of hard liquor such as gin, vodka, whiskey, scotch, rum, tequilla, etc.).*

a) Beer: _____ cans per week

b) Wine: _____ glasses per week

c) Liquor: _____ drinks per week

REPRODUCTIVE & SEXUAL HISTORY

In this section of the questionnaire we would like to know about your reproductive health including all your pregnancies as well as miscarriages and abortions. We would also like to know about your sexual history. We realize this is a personal subject, but it is very important to the study. Please take the time to recall this information as accurately as possible. Note that some questions in this section refer to your entire life as an adult, whereas others refer only to your recent experience. We would like to remind you that all the information you give us will be kept entirely confidential.

13. At what age did you have your first menstrual period?_____years

14. To the best of your knowledge, are you currently pregnant?

❑ Yes ❑ No ❑ Don't know

15. Have you ever been pregnant before?

❑ Yes ❑ No

↓ → **If No**, go to question 18

If Yes,
how many times? _____ times

16. How many of your pregnancies resulted in:

a) livebirths: _____

b) stillbirth: _____

c) miscarriage _____

d) abortion: _____

17. How many of your full-term pregnancies resulted in:

a) vaginal deliveries: _____

b) cesarean sections: _____

18. Have you ever engaged in vaginal sexual intercourse?

❑ Yes ❑ No

↓

If Yes,

how old were you when you first had vaginal sexual intercourse? _____years

19. THROUGHOUT YOUR LIFE, what is the number of male partners with whom you have had vaginal sexual intercourse?

Number *(approximately)*_____

20. With how many of these male partners did you have a sexual relationship involving intercourse on a regular basis for **three months or longer?**

Number_____ ❑ None

21. For MOST OF YOUR SEXUALLY ACTIVE LIFE, how often on the **average**, did you have sexual intercourse? Please give your answer in number of times per week, month, or year, whichever is easiest:

Number of times per week_____
OR
Number of times per month_____
OR
Number of times per year _____
OR
Less than once a year ❑

22. During THE LAST YEAR ONLY, what is the number of male partners with whom you have had sexual intercourse?

_____ Number ❑ None in the past year

↳ How many of those partners were new?_____ Number

MEDICAL HISTORY

The next questions are about use of contraceptives, the frequency with which you have taken PAP smears and about some medical problems including sexually transmitted diseases. We realize that this is a sensitive subject but, again, it is very important to the research. We appreciate your honesty and want to remind you that all information you give us is kept private and confidential.

23. THROUGHOUT YOUR LIFE, did you use oral contraceptives (birth control pill)?

❑ Regularly ❑ Sometimes ❑ Never

24. If you have used oral contraceptives, please indicate how old you were when you first took them?

Age: _____ years ❑ Never used oral contraceptives

25. Considering only the times when you were taking the pill, for how long have you been relying on this method of birth control (add together all periods during which you took any oral contraceptives)?

_____months

OR

_____years

OR

❑ all periods combined were less than 3 months

26. THROUGHOUT YOUR LIFE, did you and your partner(s)/husband(s) use condoms?

❑ Regularly ❑ Sometimes ❑ Never

27. Do you currently use oral contraceptives?

❑ Regularly ❑ Sometimes ❑ Never

28. Do you and your partner(s)/husband(s) currently use condoms?

❑ Regularly ❑ Sometimes ❑ Never

29. Thinking back over your adult years, how often have you usually had a PAP smear? Choose one category below:

❑ this is my first PAP smear
❑ 2-3 times
❑ 4-5 times
❑ 6-10 times
❑ more than 10 times

30. What is the month and year of the last PAP smear you had? _____/_____
Month Year

31. Did a doctor ever tell you that you had one of the following conditions? Check all that apply, if you are in doubt check the "don't know" column.

a) Vaginal yeast infections:	❑ Yes	❑ No	❑ Don't know
b) Trichomonas vaginal infections:	❑ Yes	❑ No	❑ Don't know
c) Venereal warts, condylomas, or papilloma virus infections:	❑ Yes	❑ No	❑ Don't know
d) Chlamydia:	❑ Yes	❑ No	❑ Don't know
e) Genital herpes:	❑ Yes	❑ No	❑ Don't know
f) Syphilis:	❑ Yes	❑ No	❑ Don't know
g) Gonorrhea:	❑ Yes	❑ No	❑ Don't know
h) Ulcers or genital sores:	❑ Yes	❑ No	❑ Don't know

32. Sometimes women are given female hormones by their doctors because of a variety of reasons (regulate or eliminate painful periods, menopausal symptoms, reduce discomfort during intercourse due to vaginal dryness, prevent miscarriage, among others). To the best of your recollection, were you ever prescribed any female hormones by your doctor?

❑ Yes ❑ No

↓ ↳ **If No**, go to question 35

If Yes,
in what month and year did you start taking them and also, in what month and year did you last take them?

Start: _____/_____ End: _____/_____
month year month year

33. Between the above two dates, for how long (number of months) did you take the female hormone medication on a continual basis, altogether? _____months

34. If you were given hormone replacement therapy for menopausal symptoms, what age were you when you began taking them? _____years

35. Would you please indicate the date when you finished filling in the questionnaire?

_____/_____/_____
DAY MONTH YEAR

USE THE SPACE BELOW IF YOU HAVE ANY ADDITIONAL INFORMATION YOU FEEL WOULD BE IMPORTANT FOR US TO KNOW:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

This is the end of the questionnaire. We would like you to take a few seconds to review your answers in all sections of the questionnaire. Again, try to answer all questions; a good guess will be more useful to the study than leaving the question blank.

THANK YOU VERY MUCH FOR YOUR COOPERATION

NO ÉTUDE: _____

ÉTUDE MONTRÉALAISE SUR LA SANTÉ DES FEMMES

Université McGill et Université de Montréal

INSTRUCTIONS POUR LE QUESTIONNAIRE

Ce questionnaire comprend les sections suivantes:

Informations générales
Histoire de la consommation de tabac et d'alcool
Histoire reproductrice et sexuelle
Histoire médicale

Pour la plupart des questions, vous devrez simplement faire un "X" dans le carré ❑ afin d'indiquer votre choix. Pour d'autres questions, vous devrez fournir une réponse spécifique, par exemple : âge, date ou autre nombre. Selon vos réponses à certaines questions, vous pourrez passer des groupes de questions et aller compléter une autre partie du questionnaire. Vous épargnerez ainsi du temps en ne complétant que les parties du questionnaire pertinentes à votre situation.

Il n'y a pas de bonnes ou mauvaises réponses. Pour plusieurs questions vous devrez vous référer à votre vie adulte ou plus particulièrement à la dernière année, afin de vous souvenir d'informations spécifiques. Prenez le temps de réfléchir. Vous réaliserez qu'en devant réfléchir pour vous rappeler un certain type d'information, vous pourriez trouver plus facilement les réponses à d'autres questions. Vous pouvez laisser une question sans réponse si vous n'arrivez vraiment pas à vous souvenir de l'information. Nous vous encourageons par contre à essayer de répondre à toutes les questions. Il est plus utile pour nos recherches d'avoir une réponse approximative de votre part plutôt que pas d'information du tout. Si vous désirez nous communiquer plus d'informations pour certaines questions, veuillez utiliser l'espace prévu à cette fin à la dernière page du questionnaire.

NOUS VOUS REMERCIONS POUR VOTRE COLLABORATION À CETTE ÉTUDE.

NO ÉTUDE: _____

INFORMATIONS GÉNÉRALES

Cette section comporte des questions de nature générale sur vous-même et votre lieu de résidence. Ces questions nous aiderons à définir votre profil socio-démographique.

1. Quelle est votre date de naissance? _____/_____/_____ ***(très important)***
J M A

2. Dans quel pays êtes-vous née? _____

Si vous êtes née au Canada, indiquez la province: _____

3. **Présentement**, quel est votre état civil?

❑ Mariée ❑ Célibataire ❑ Pas mariée, mais vivant en union de fait
❑ Divorcée/Séparée ❑ Veuve

4. a) La population de la région de Montréal est composée de plusieurs groupes ethniques. À quel groupe ethnique **vous** identifiez-vous? Veuillez cocher la catégorie la plus appropriée :

❑ Canadien français ❑ Canadien anglais ❑ Hispanique/Portuguais
❑ Grec ❑ Noir canadien ❑ Italien ❑ Amérindien
❑ Asiatique/Oriental ❑ Arabe ❑ Juif Autre:_____

b) À quel groupe ethnique identifiez-vous votre **mère?** Veuillez cocher la catégorie la plus appropriée :

❑ Canadien français ❑ Canadien anglais ❑ Hispanique/Portuguais
❑ Grec ❑ Noir canadien ❑ Italien ❑ Amérindien
❑ Asiatique/Oriental ❑ Arabe ❑ Juif Autre:_____

b) À quel groupe ethnique identifiez-vous votre **père?** Veuillez cocher la catégorie la plus appropriée :

❑ Canadien français ❑ Canadien anglais ❑ Hispanique/Portuguais
❑ Grec ❑ Noir canadien ❑ Italien ❑ Amérindien
❑ Asiatique/Oriental ❑ Arabe ❑ Juif Autre:_____

5. a) **Présentement**, quelle est votre occupation?_____

b) Diriez-vous que votre situation financière familiale est :

❑ Difficile ❑ Dans la moyenne ❑ Confortable ❑ Très confortable

c) Quel est le plus haut niveau de formation que vous avez atteint?

❑ École primaire ❑ École secondaire ❑ CÉGEP/Diplôme d'études professionnelles
❑ Université (premier cycle) ❑ Université (Maîtrise, doctorat, études équivalentes)

HISTOIRE DE LA CONSOMMATION DE TABAC ET D'ALCOOL

Les questions suivantes portent sur vos habitudes de consommation de tabac et d'alcool. Veuillez tenter de répondre de façon aussi précise que possible

6. Avez-vous déjà fumé la cigarette **régulièrement**, c'est-à-dire **une** cigarette ou **plus**, chaque **jour,** durant un **an** ou plus?

❑ Oui ❑ Non

7. Avez-vous fumé un total d'au moins 100 cigarettes dans votre vie?

❑ Oui ❑ Non

↳ **Si non**, allez à la question 11

8. À quel âge avez-vous commencé à fumer? _____ans

9. Fumez-vous encore? ❑ Oui ❑ Non

↓

Si non, à quel âge avez-vous arrêté? _____ans

10. En moyenne, combien de cigarettes fumez/fumiez-vous par jour?
_____cigarettes par jour

11. Y-a-t-il une période de votre vie durant laquelle vous avez bu de la bière, du vin ou des spiritueux AU MOINS UNE FOIS PAR MOIS?

❑ Oui ❑ Non

↳ **Si non**, allez à la question 13

12. Y-a-t-il une période de votre vie durant laquelle vous avez bu de la bière, du vin ou des spiritueux AU MOINS UNE FOIS PAR SEMAINE?

❑ Oui ❑ Non

↓

Si oui,

indiquer en moyenne le nombre de verres consommés par semaine durant les 5 dernières années *(un verre est défini comme une bouteille ou canette de bière de 12 onces (375 ml) ou un verre de 4 onces (125 ml) de vin ou 1,5 onces (45 ml) de spiritueux tel que gin, vodka, whisky, scotch, rhum, tequila, etc.).*

a) Bière: _____ canettes ou bouteilles par semaine

b) Vin: _____ verres par semaine

c) Spiritueux: _____ verres par semaine

HISTOIRE REPRODUCTRICE ET SEXUELLE

Cette section portera sur votre santé reproductrice, incluant grossesses, fausses-couches et avortements. Nous vous poserons également des questions concernant vos pratiques sexuelles. Nous réalisons que c'est un sujet très personnel, mais cette information est de la plus haute importance pour notre étude. Veuillez prendre le temps de vous souvenir aussi précisément que possible. Certaines questions porterons sur toute votre vie d'adulte alors que d'autres couvriront seulement vos expériences plus récentes. Nous désirons vous rappeler que toute l'information dont vous nous faites part est entièrement confidentielle.

13. À quel âge avez-vous eu vos premières menstruations?_____ans

14. Au meilleur de votre connaissance, êtes-vous enceinte présentement?

❑ Oui ❑ Non ❑ Je ne sais pas

15. Avez-vous déjà été enceinte?

❑ Oui ❑ Non

↓ → **Si non**, allez à la question 18

Si oui,
combien de fois? _____ fois

16. Indiquez combien de vos grossesses ont eu le résultat suivant:

a) naissance: _____

b) enfant mort-né: _____

c) fausse-couche: _____

d) avortement: _____

17. Indiquez combien de vos grossesses à terme ont eu le résultat suivant:

a) accouchement par voie naturelle (vagin): _____

b) accouchement par césarienne: _____

18. Avez-vous déjà eu des relations sexuelles vaginales?

❑ Oui ❑ Non

↓

Si oui,

quel âge aviez-vous lors de votre première relation sexuelle vaginale? _____ans

19. DURANT VOTRE VIE ENTIÈRE, quel est le nombre de partenaires sexuels masculins avec lesquels vous avez eu des relations sexuelles vaginales?

Nombre *(approximativement)* _____

20. Avec combien de ces partenaires sexuels masculins avez-vous eu une relation durant laquelle vous aviez des relations sexuelles de façon régulière pour une période de **trois mois ou plus**?

Nombre_____ ❑ Aucun

21. Durant LA MAJEURE PARTIE DE VOTRE VIE SEXUELLE ACTIVE, combien de fois en **moyenne** avez-vous eu des relations sexuelles? Veuillez donner votre réponse en nombre de fois par semaine, mois ou année, selon ce qui est le plus facile pour vous:

Nombre de fois par semaine_____
OU
Nombre de fois par mois_____
OU
Nombre de fois par année_____
OU
Moins d'une fois par année ❑

22. Dans la DERNIÈRE ANNÉE SEULEMENT, quel est le nombre de partenaires masculins avec lesquels vous avez eu des relations sexuelles?

_____ Nombre ❑ Aucun dans la dernière année

↳ Combien de ces partenaires étaient de nouveaux partenaires?_____ Nombre

HISTOIRE MÉDICALE

Les questions suivantes portent sur l'utilisation de contraceptifs, la fréquence à laquelle vous avez consulté un médecin pour une cytologie gynécologique (test Pap) et certains problèmes médicaux incluant les maladies transmissibles sexuellement. Nous réalisons que ce sujet est délicat, mais une fois de plus, il est très important pour notre étude. Nous apprécions votre honnêteté et désirons vous rappeler que toute l'information que vous nous fournissez est strictement confidentielle.

24. DURANT VOTRE VIE ENTIÈRE, avez-vous utilisé des contraceptifs oraux (pilule anticonceptionnelle)?

❑ Régulièrement ❑ Quelquefois ❑ Jamais

24. Si vous avez utilisé des contraceptifs oraux, veuillez indiquer l'âge auquel vous en avez pris pour la première fois?

Âge: _____ ans ❑ Jamais utilisé de contraceptifs oraux

25. En tenant compte seulement des périodes où vous preniez la pilule, combien de temps avez-vous utilisé cette méthode de contrôle des naissances (additionnez toutes les périodes de temps durant lesquelles vous avez pris des contraceptifs oraux)?

_____mois

OU

_____ans

OU

❑ moins de trois mois au total

26. DURANT VOTRE VIE ENTIÈRE, est-ce que vous et votre(vos) partenaire(s)/conjoint(s) avez utilisé des condoms?

❑ Régulièrement ❑ Quelquefois ❑ Jamais

29. Présentement, utilisez-vous des contraceptifs oraux?

❑ Régulièrement ❑ Quelquefois ❑ Jamais

30. Présentement, est-ce que vous et votre(vos) partenaire(s)/conjoint(s) utilisez des condoms?

❑ Régulièrement ❑ Quelquefois ❑ Jamais

29. Durant votre vie adulte, combien de fois avez-vous eu une cytologie gynécologique (test PAP)? Choisissez une des catégories ci-dessous:

- ❑ ceci est mon premier test PAP
- ❑ 2-3 fois
- ❑ 4-5 fois
- ❑ 6-10 fois
- ❑ plus de 10 fois

30. À quand remonte votre dernière cytologie gynécologique (test PAP)? ____/____
Mois Année

31. Est-ce qu'un médecin vous a déjà dit que vous souffriez d'un des problèmes médicaux suivants? Répondez à chacune des questions. Si vous êtes incertaine, indiquez "je ne sais pas".

a) Infections vaginales à levures:	❑ Oui	❑ Non	❑ Je ne sais pas
b) Infections vaginales à Trichomonas:	❑ Oui	❑ Non	❑ Je ne sais pas
c) Verrues génitales, condylomes, ou infections par le virus du papillome humain:	❑ Oui	❑ Non	❑ Je ne sais pas
d) Chlamydia:	❑ Oui	❑ Non	❑ Je ne sais pas
e) Herpès génital:	❑ Oui	❑ Non	❑ Je ne sais pas
f) Syphilis:	❑ Oui	❑ Non	❑ Je ne sais pas
g) Gonorrhée:	❑ Oui	❑ Non	❑ Je ne sais pas
h) Ulcères ou plaies génitales:	❑ Oui	❑ Non	❑ Je ne sais pas

32. Des femmes se voient quelquefois prescrire des hormones féminines par leurs médecins pour différentes raisons (régulariser les menstruations, éliminer ou contrôler les douleurs menstruelles et les symptômes de la ménopause, diminuer l'inconfort causé par la sécheresse vaginale durant les relations sexuelles, prévenir les fausses-couches, etc.). Un médecin vous-a-t-il déjà prescrit des hormones féminines?

❑ Oui ❑ Non

↓ → **Si non**, allez à la question 35

Si oui,
quand avez-vous commencé à les prendre (mois et année) et quand les avez-vous prises pour la dernière fois (mois et année)?

Début: ____/____ Fin: ____/____
mois année mois année

33. Entre les deux dates indiquées ci-haut, pour combien de temps au total (nombre de mois) avez-vous pris des hormones féminines de façon régulière? _____mois

34. Si vous avez pris des hormones pour contrer les symptômes de la ménopause, quel âge aviez-vous lorsque vous avez commencé ce traitement? _____ans

35. Veuillez indiquer la date à laquelle vous avez complété ce questionnaire:

_____/_____/_____
JOUR MOIS ANNÉE

VEUILLEZ UTILISER L'ESPACE CI-DESSOUS SI VOUS DÉSIREZ NOUS COMMUNIQUER TOUTES AUTRES INFORMATIONS QUE VOUS CROYEZ PERTINENTES:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Le questionnaire est maintenant terminé. Nous aimerions que vous preniez quelques secondes afin de vérifier vos réponses pour toutes les sections du questionnaire. Il est important que vous essayiez de répondre à toutes les questions. Une bonne approximation est plus utile pour notre étude qu'une question laissée sans réponse.

NOUS VOUS REMERCIONS DE VOTRE COLLABORATION

APPENDIX 5

Certificates for Ethics Approval