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SERUM OMENTIN AS A POTENTIAL BIOMARKER FOR COMPLEX ATYPICAL HYPERPLASIA AND ENDOMETRIOID ENDOMETRIAL ADENOCARCINOMA

Laura L. Holman MD

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**SERUM OMENTIN AS A POTENTIAL BIOMARKER FOR COMPLEX ATYPICAL
HYPERPLASIA AND ENDOMETRIOID ENDOMETRIAL ADENOCARCINOMA**

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**SERUM OMENTIN AS A POTENTIAL BIOMARKER FOR COMPLEX ATYPICAL
HYPERPLASIA AND ENDOMETRIOID ENDOMETRIAL ADENOCARCINOMA**

A

THESIS

Presented to the Faculty of
The University of Texas
Health Science Center at Houston
and
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In Partial Fulfillment

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by

Laura L. Holman, M.D.
Houston, Texas

May, 2015

SERUM OMENTIN AS A POTENTIAL BIOMARKER FOR COMPLEX ATYPICAL HYPERPLASIA AND ENDOMETRIOID ENDOMETRIAL ADENOCARCINOMA

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Abstract

Obesity is a significant risk factor for endometrial cancer, the most common gynecologic malignancy in the United States, but much remains unclear about the relationship between obesity-related factors and the development of endometrial cancer. Omentin, a recently discovered adipokine, has been shown to be present in lower levels in patients who are obese and/or insulin resistant. A case-control study was conducted using the serum of 140 women with endometrial cancer and 75 women with endometrial hyperplasia who were matched 1:1 based on body mass index (BMI) and menopausal status to women with no history of endometrial cancer (controls). The concentration of omentin in the patients' serum was experimentally determined by conducting tests in triplicate on an enzyme-linked immunoadsorbent assay (ELISA) specific to human omentin. The mean serum omentin levels of women with endometrial hyperplasia and endometrial cancer were statistically significantly lower than controls, regardless of BMI. The mechanism that induces this change remains unknown, but these results present exciting promise for omentin's use as a biomarker and role in understanding the relationship between obesity-related factors and endometrial carcinoma.

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Background and Introduction

Endometrial Adenocarcinoma

Endometrial cancer is a malignant process that arises from the inner lining of the uterus (endometrium). It is the fourth most common cancer among women in the United States and the most frequently diagnosed gynecologic malignancy. In 2014, more than 52,000 American women will be diagnosed with the disease.[1]

Endometrial cancer is most commonly diagnosed in postmenopausal women, with a median age at diagnosis of 62 years. While white women are most likely to develop the disease compared to other races, black women are most likely to die from the disease (Table 1).[2] Reasons for this disparity are unclear.

Race	New Cases	Deaths
White	25.4	4.0
Black	23.2	7.5
Asian	19.1	2.7
Native American	20.0	3.4
Hispanic	19.8	3.4

Table 1: Endometrial Cancer Cases and Deaths per 100,000

People[2]. Number of cases and deaths by race in 2014.

Histologically, endometrial cancer is classically divided into two categories: type I and type II. Type I tumors account for up to 85% of endometrial cancers and are defined as grade 1 or grade 2 endometrioid endometrial cancers. Most of these tumors are diagnosed at an early stage and therefore confer a favorable prognosis. Of interest, type I endometrial cancers are estrogen-dependent and often develop in the setting of complex atypical hyperplasia of the endometrium. Aberrations in PTEN and K-ras are commonly found in type I tumors.[3] In contrast, type II endometrial cancers are high grade and are more likely to be diagnosed at an advanced stage. They have no known precursor lesion. As such, type II endometrial cancer often has a poor prognosis. Mutations in p53 are frequently identified in these tumors.[4]

The strongest risk factor for type I endometrial cancer is unopposed estrogen therapy, with a relative risk of 10-20. While unopposed estrogen may be due to exogenous estrogen, such as hormone replacement therapy or tamoxifen, it is more commonly due to excess endogenous estrogen. Several factors can increase endogenous estrogen, including chronic anovulation (such as in patients with polycystic ovarian syndrome or PCOS), an estrogen-producing tumor, or obesity. Of these, however, obesity confers the greatest risk.[5] Studies have found that obesity confers up to a five-fold risk of developing endometrial cancer when compared to normal-weight women.[6] Other risk factors for type I tumors include advanced age, diabetes, and hypertension, and some genetic syndromes, such as Lynch Syndrome. The risk factors for type II tumors are not well understood.

Early stage endometrial cancer is highly curable, with 5-year survival rates approaching 90% for patients with stage I malignancies.[7] In fact, many women with early stage, grade 1 or 2, endometrioid, endometrial cancer will be cured with surgical resection alone. However, the majority of patients who recur are incurable, with an average survival of only six to eighteen months.[8]

There are currently no prognostic biomarkers for endometrial cancer consistently utilized in clinical practice. However, multiple markers have been associated with prognosis in studies. For example, expression of EGFR in endometrioid tumors has been found to predict a decrease in survival by up to 20% from patients without EGFR expression.[9] Other potential markers of poor prognosis include p53 overexpression, DNA methylation, and increased Ki-67 staining. Expression of estrogen and progesterone receptors has been associated with improved prognosis.[10]

Endometrial Hyperplasia

Endometrial hyperplasia is a benign overgrowth, or proliferation, of glands in the endometrium. It is also most commonly diagnosed in postmenopausal women. As the precursor lesion to type I endometrial cancers, it has similar risk factors with obesity as the most significant risk factor.

In an attempt to classify hyperplasia, the World Health Organization (WHO) has divided endometrial hyperplasia into four categories based upon the glandular and stromal pattern of the endometrium and the presence or absence of nuclear atypia. These four categories are simple hyperplasia without atypia,

complex hyperplasia without atypia, simple hyperplasia with atypia, and complex hyperplasia with atypia.[11] Though all patients with endometrial hyperplasia have a risk of developing endometrial cancer, some histologic subtypes have a greater risk than others. The WHO classification system correlates well with this risk (Table 2).[12]

WHO Classification	Incidence of Endometrial Cancer
Simple Hyperplasia without Atypia	1%
Simple Hyperplasia with Atypia	3%
Complex Hyperplasia without Atypia	8%
Complex Hyperplasia with Atypia	29%

Table 2: Incidence of Endometrial Cancer by WHO Hyperplasia

Category[12]. Categories of hyperplasia ranked by risk of endometrial cancer development.

Of all subtypes of endometrial hyperplasia, complex atypical hyperplasia confers the greatest risk of developing cancer. Prior observational studies have noted that it takes several years for women who have complex atypical hyperplasia to develop cancer.[13] However, up to 35% of women with complex atypical hyperplasia will have an underlying malignancy in their hysterectomy specimen.[14] For this reason, the definitive treatment for complex hyperplasia with atypia is hysterectomy.

As the risk for developing endometrial cancer in women with hyperplasia without atypia is low, it can be managed with progestin therapy. This may

include oral progesterone or progesterone-impregnated intrauterine devices (IUD). Studies have noted regression of hyperplasia without atypia in up to 80% of women treated with oral progesterone.[15, 16] Of interest, studies have noted an increased rate of regression of complex hyperplasia without atypia in women who utilize the IUD than in women treated with oral progesterone. However, there was no significant difference in the rate of regression among women with simple hyperplasia without atypia.[17] Figure 1 illustrates the difference between benign endometrium, endometrial hyperplasia, and endometrial adenocarcinoma under low power.

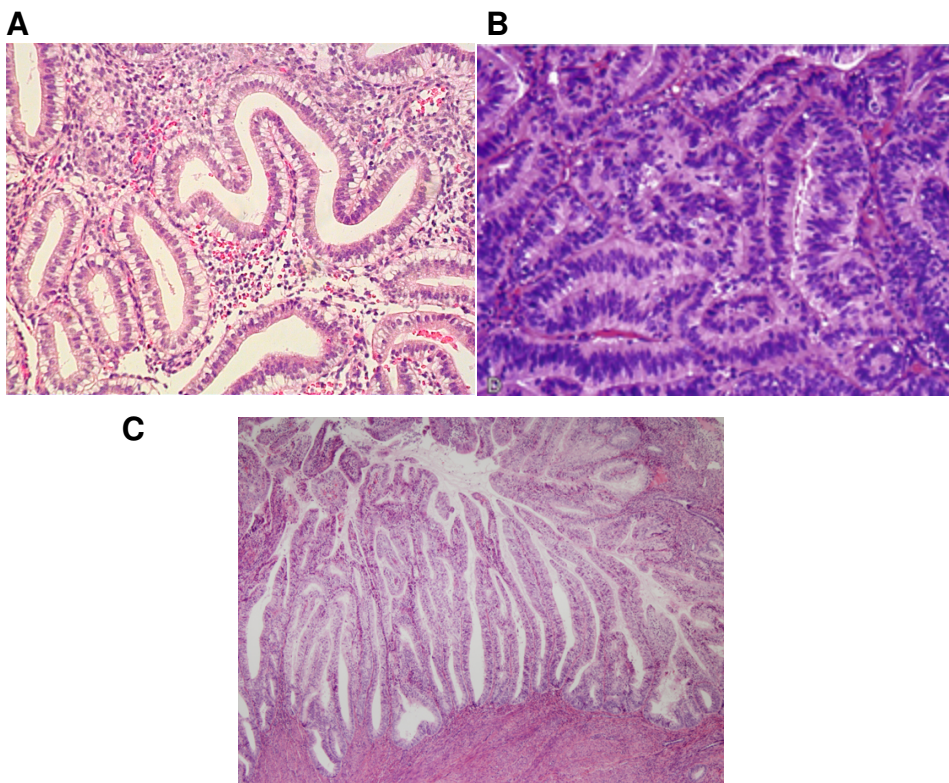


Figure 1: H&E Comparing Normal Endometrium to Hyperplasia and Cancer. A) Proliferative endometrium. B) Complex atypical hyperplasia. C) Endometrial Adenocarcinoma

Obesity and Development of Endometrial Hyperplasia and Cancer

Obesity is one of the greatest risk factors for developing endometrial hyperplasia or cancer. In fact, almost 50% of endometrial cancer cases may be related to obesity.[18] Furthermore, increasing body mass index (BMI) leads to increased risk. Lu and colleagues demonstrated that overweight women were twice as likely and obese women up to five times as likely to develop endometrial cancer as their normal-weight counterparts.[6] In fact, for each 5 kg/m² increase in BMI, there is a significant increase in risk of developing endometrial cancer.[19]

In addition to being at increased risk for developing endometrial cancer, obese women are at increased risk for dying from the disease. Calle and colleagues found that obese women were approximately 2.5 times more likely and morbidly obese women were 6 times more likely than normal-weight women to die from endometrial cancer.[20] Additionally, obese endometrial cancer patients are almost 3 times as likely to die from causes other than endometrial cancer.[21] This is likely secondary to the association of obesity with other medical conditions, such as diabetes mellitus, hypertension, and cardiac disease.

The Role of Estrogen in the Development of Endometrial Cancer

Unopposed estrogen, whether exogenous or endogenous, is a well-known risk factor for the development of endometrial hyperplasia and cancer. In the 1960's, the first cases of endometrial cancer related to hormone replacement therapy were reported.[22] This observation has been validated in multiple

studies since that time.[23-25] Furthermore, the cancer risk has been found to be associated with both dose and duration of estrogen use.[26, 27]

It has long been hypothesized that unopposed endogenous or exogenous estrogen leads to the development of endometrial cancer. Though premenopausal women make estrogen mostly in the ovaries, postmenopausal women form estrogen only in peripheral tissue, including adipose tissue. In adipose tissue, aromatase converts androgens to estrone and estradiol (Figure 2). Aromatase is an enzyme produced in the adipose tissue and levels are increased in obesity. The increased aromatase levels in combination with decreased sex hormone binding globulin levels lead to increased circulating estrogen levels in obese women. In fact, studies have demonstrated up to 40% higher estrogen levels in obese as compared with normal-weight postmenopausal women.[28] Furthermore, studies have shown an association between elevated estrogen concentration and endometrial cancer.[29, 30]

The mechanism by which excess unopposed estrogen leads to endometrial hyperplasia and cancer is complex. In essence, however, estrogen leads to increased mitotic activity in endometrial cells. This can cause DNA replication errors and eventually mutations that result in the development of

carcinoma.[31, 32] Estrogen is also involved in activation of kinase signaling

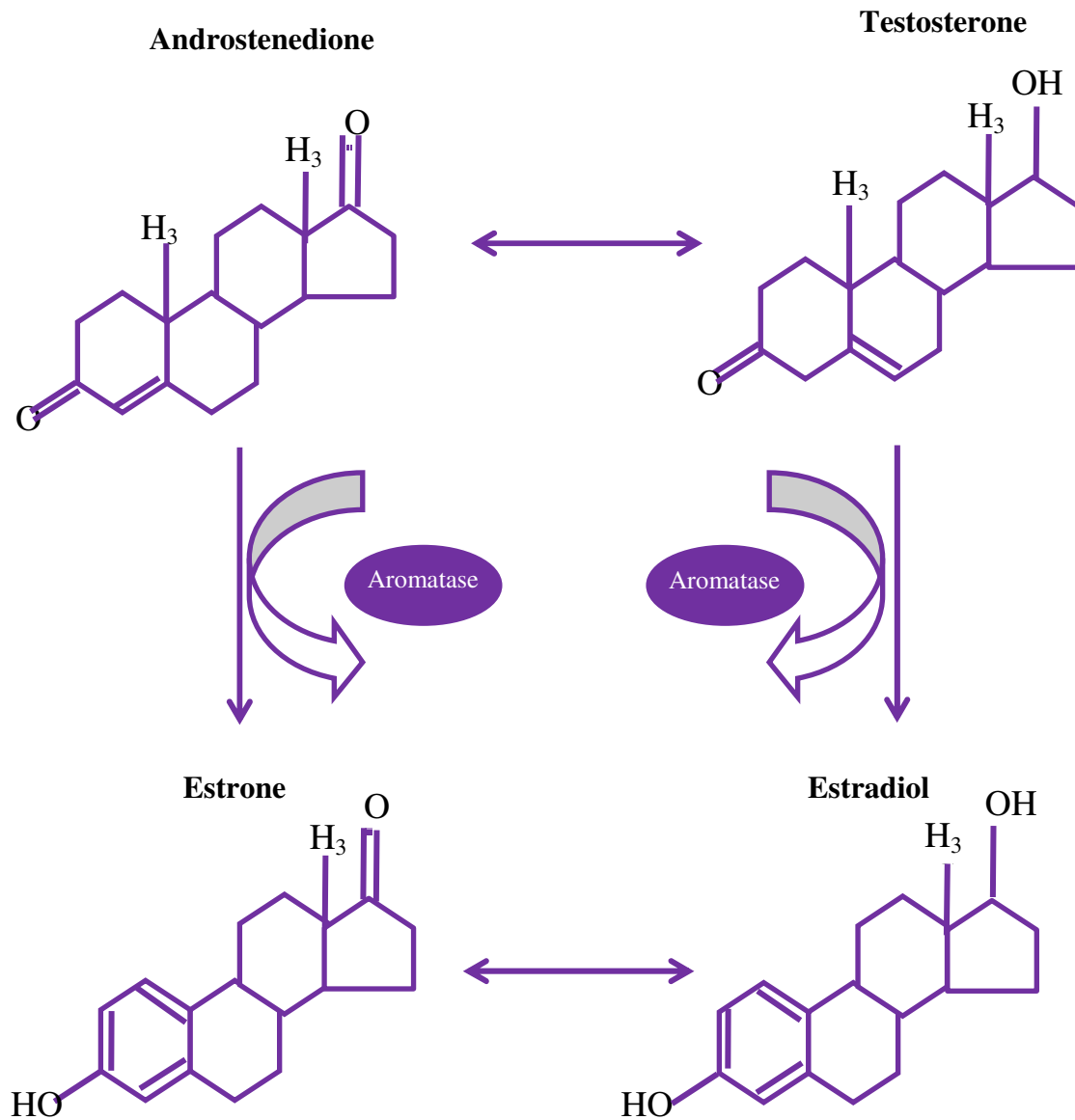


Figure 2: Conversion of Androgens to Estrogens in Adipose Tissue.

Mechanism by which testosterone is converted to estrogen by the enzyme aromatase in peripheral tissue.

cascades, including the PI3 and MAP kinase pathways, via association between estrogen receptors and cell surface receptors.[33] These pathways lead to cellular proliferation and aberrations in them are frequently found in endometrial cancer.[34]

A model for the role of estrogen in the pathogenesis of endometrial cancer has been proposed by Rizner in 2013.[35] In this hypothesis, estrogens are synthesized in peripheral tissue (mainly adipose tissue), leading to increased circulating levels of estrogens. These estrogens are transported into the endometrial cells, where they are metabolized into 4-hydroxyestrogens and 3,4-quinones due to upregulation of the enzyme CYP1B1. The 3,4-quinones produced by this reaction induce formation of DNA adducts and reactive oxygen species, which can cause mutations in tumor suppressor genes or oncogenes, such as PTEN and K-RAS. These mutations lead to the development of cancer. In these cancer cells, estrogen receptor is over-expressed (specifically, estrogen receptor α), leading to more estrogen binding and proliferation of the mutated cells. Of note, the Dr. Rizner is careful to point out that this hypothesis is based on interpretation of the available literature and that further studies are warranted to fully evaluate its accuracy.

Insulin Resistance and the Development of Endometrial Cancer

While the prevailing hypothesis is that unopposed estrogen is the driver of endometrial cancer development, there is increasing evidence that insulin resistance may also play a role. Insulin resistance is decreased sensitivity to

insulin leading to increased blood glucose levels.[36] Patients with insulin resistance develop hyperinsulinemia to compensate for the hyperglycemic state. This increased insulin concentration has been correlated with the development of malignancies, including endometrial cancer. Several studies have associated insulin resistance with endometrial cancer, even when controlling for obesity or estrogen levels.[37-39]

Inflammatory mediators are believed to be some of the common regulatory factors in both insulin resistance and the development of endometrial cancer. In insulin resistance, inflammation recruits of macrophages to adipose tissue via secretion of monocyte chemoattractant protein-1. By releasing tumor necrosis factor- α , the macrophages activate the extracellular signal-related kinase and c-Jun-N-terminal-kinase pathways, thereby inhibiting insulin signaling. The macrophages also secrete interleukin-6, which is believed to play a role in insulin resistance, though the exact mechanism is unknown.[36, 40-42] Interleukin-6 is also known to lead to secretion of C-reactive protein, which is a marker of inflammation and has been associated with endometrial cancer risk.[43]

The hyperinsulinemia associated with insulin resistance has been associated with the development of endometrial cancer. For example, *in vitro*, endometrial cancer cell lines treated with insulin lead to decreased apoptosis and increased cellular proliferation in a dose-dependent fashion. This finding is likely secondary to activation of insulin receptor upon insulin binding, leading to activation of the PI3K and MAPK pathways.[36, 44] The activated PI3K and

MAPK pathways can subsequently activate estrogen receptor, thereby leading to unregulated cell growth and division.[45] In fact, these pathways have been associated with a large percentage of endometrial cancers.[46]

Hyperinsulinemia may also lead to endometrial cancer development through indirect mechanisms. For example, insulin inhibits production of sex hormone binding globulin, thereby causing increased blood levels of free sex hormones, such as estrogens and androgens.[47] Furthermore, insulin promotes androgen production by the ovaries. This allows for more free androgen to be converted to estrogen in the peripheral tissues.

Insulin resistance can be difficult to study as there are many different methods to measure it. These approaches include the QUICKI ($1/[\log \text{fasting insulin} + \log \text{fasting glucose}]$), the insulin resistance index ($\text{fasting plasma glucose} \times \text{fasting insulin} / 25$), and the HOMA-IR index ($\text{fasting insulin} \times \text{fasting glucose} / 22.5$).[37-39] Unfortunately, there is no standard method to measure insulin resistance and no universally accepted way to measure the relationship between insulin resistance and endometrial cancer.

Given the association between insulin resistance and endometrial cancer, there has been increasing interest in insulin-sensitizing agents as potential therapies for endometrial cancer. Metformin is one of the agents that is being investigated for this purpose. *In vivo*, metformin has been found to inhibit endometrial cancer cell growth through mTOR inhibition and AMPK activation.[48] Other *in vivo* and *in vitro* studies have found that metformin may

have the greatest effect in cells with K-Ras mutations.[49] Recently, a phase II trial of everolimus and letrozole in patients with recurrent endometrial cancer found a significantly higher response rate in patients who were also taking metformin.[50] This unexpected finding led to the development of a phase II trial of everolimus, letrozole, and metformin among women with recurrent endometrial cancer, which is currently accruing patients (ClinicalTrials.gov identifier: NCT01797523).

Adipokines

Historically, adipose tissue has been thought of as only insulation and a storage site for energy. However, it is now known that adipose tissue is also an endocrine organ that is involved in bone remodeling, immunity, and energy homeostasis.[51] Adipose tissue also secretes a class of cytokines called adipokines. Multiple adipokines with varied functions that can act either locally or systemically have been described (Table 3).

Adipokines have been associated with the development and progression of multiple cancers, including breast, colon, pancreas, and endometrial.[52] This is believed to occur through the activity of pro-inflammatory adipokines, which stimulate cancer stem cells. This produces a feedback loop whereby there is increased secretion of pro-inflammatory adipokines leading to tumor growth.[53] Alternatively, some adipokines have been proposed as protective against cancer development with decreased serum levels in patients with cancer.

Adipokine	Proposed Function(s)
Adiponectin	Increases insulin sensitivity, Anti-inflammatory
Adipsin	Activates alternative complement pathway
Angiotensin II	Regulation of blood pressure
Apelin	Aid in control of energy stores
Appetite-Regulating Hormone	Aid in distribution and rate of energy use
Complement C1q TNF-Related Protein 1	Increases insulin sensitivity, Anti-inflammatory
Chemerin	Aids with adipocyte differentiation and glucose uptake
IL-6	Proinflammatory, Multiple functions
IL-8	Proinflammatory, Multiple functions
Leptin	Signals CNS regarding energy stores
Lipocalin 2	Promotes insulin resistance
Monocyte Chemoattractant Protein 1	Proinflammatory, Promotes insulin resistance
Omentin	Anti-inflammatory
Plasminogen Activator Inhibitor 1	Inhibits plasminogen activation
Resistin	Increases insulin resistance
TNF-α	Reduces insulin sensitivity, Increases energy consumption
Vaspin	Increases insulin sensitivity
Visfatin	Increases insulin sensitivity

Table 3: Select Adipokines and Proposed Functions [52, 54]. Selection of some of the most commonly studied adipokines with their proposed function.

Adiponectin, an adipokine produced exclusively by adipocytes, has been associated with insulin resistance and in the pathogenesis of endometrial cancer.

For example, in insulin resistance pathways, adiponectin has been noted to activate AMP-activated protein kinase and peroxisome proliferator-activated receptor- α . These enzymes decrease serum glucose concentration and increase the expression of adiponectin and adiponectin receptors, leading to a positive feedback loop.[36, 55] Interestingly, serum adiponectin levels have been inversely correlated with insulin levels.[56, 57] Serum adiponectin levels have also been found to be lower in endometrial cancer patients than in women with endometrial hyperplasia or normal endometrium.[58] Other studies have confirmed that adiponectin levels are decreased in endometrial cancer patients as compared with controls and that elevated adiponectin levels are associated with a lower risk of endometrial cancer.[59-61] Of note, adiponectin receptors are expressed in both benign and malignant endometrial cancer cell lines, but treatment with adiponectin in the malignant cell lines led to apoptosis.[62, 63] This suggests a possible therapeutic application for adiponectin, but further studies are needed.

Insulin resistance also appears to be mediated by leptin. This adipokine inhibits insulin receptor and promotes the activity of other proteins that have been associated with insulin resistance.[64, 65] The leptin receptor is also expressed on multiple endometrial cancer cell lines and leptin may lead to increased proliferation and invasion of these cells.[66, 67] The prognostic implications of leptin are unclear as findings from clinical studies have been contradictory. Small studies of serum leptin levels have found no difference between endometrial cancer patients and controls.[68] However, a meta-analysis that

included approximately 3,000 patients found elevated serum leptin to be an independent risk factor for endometrial cancer.[69]

Omentin

Omentin, also known as intelectin, is a recently discovered adipokine that is expressed by multiple tissues, including the heart, lungs, and ovary.[70] However, omentin is preferentially secreted by the mesothelial cells of visceral adipose tissue. There are two isoforms of omentin: omentin-1 and omentin-2. However, omentin-1 is the major circulating isoform in human plasma. Omentin is believed to be an insulin-sensitizing adipokine and aids with glucose uptake in adipocytes as well as AKT signaling.[71]

It has been hypothesized that omentin has a role as an anti-inflammatory agent since levels are generally decreased in patients with pro-inflammatory conditions (Figure 3). For example, serum omentin levels are decreased among patients with inflammatory bowel disease.[72] Furthermore, there is some evidence that decreased omentin levels may correlate with disease activity in these patients.[73] Li and colleagues recently demonstrated that omentin levels in synovial fluid of osteoarthritis patients are negatively correlated to disease severity.[74] Type 1 and type 2 diabetes mellitus are also associated with decreased plasma omentin levels.[75, 76] This negative correlation remains true when controlling for BMI.[76] Furthermore, treating omental adipose tissue with insulin and glucose dose-dependently decreases omentin expression in this tissue.[75]

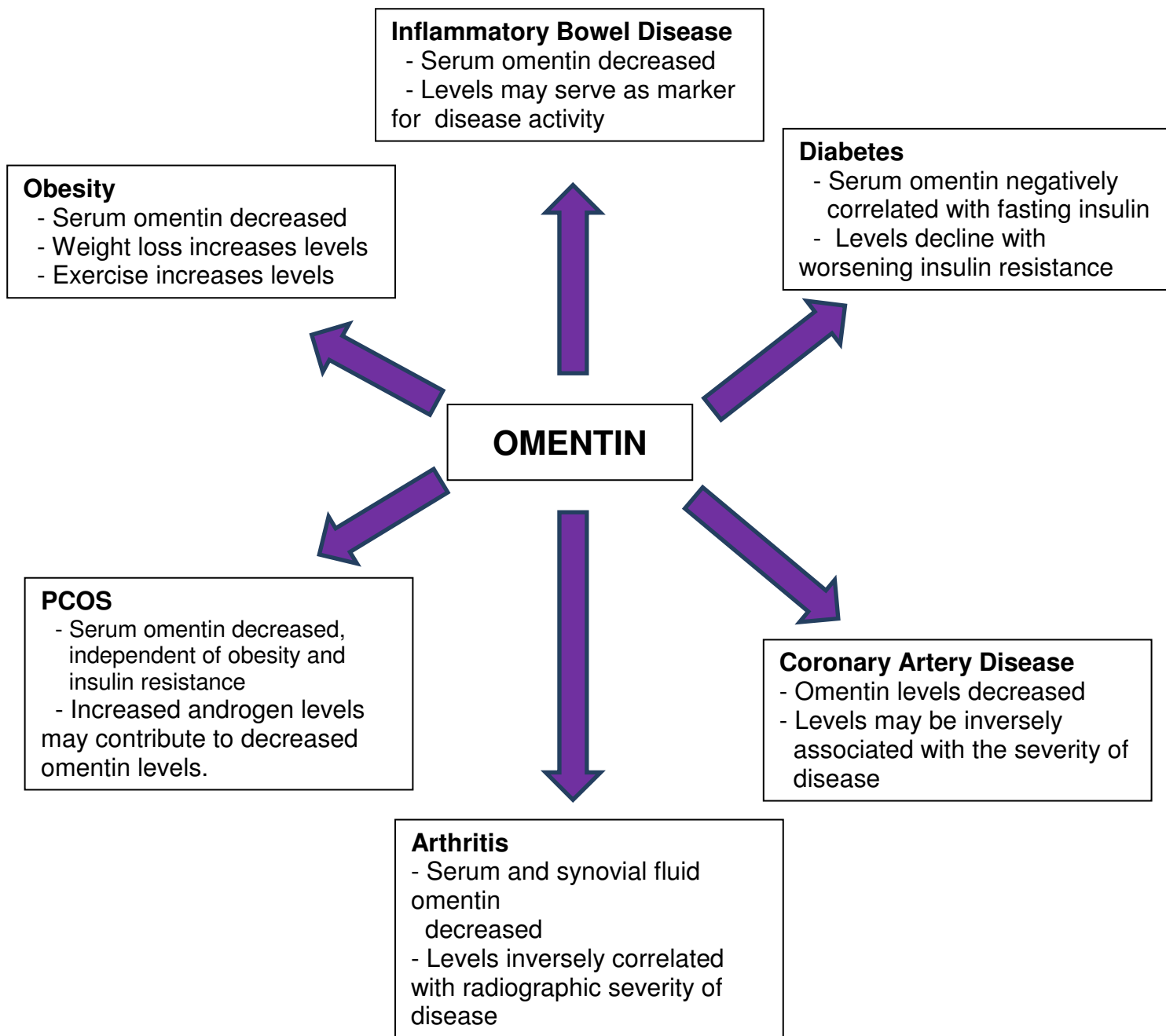


Figure 3: Omentin in Other Disease Processes. Many pro-inflammatory diseases have found an inverse correlation with omentin, including obesity, diabetes, arthritis, PCOS, and inflammatory bowel syndrome.

Omentin levels have also been inversely correlated to BMI, waist circumference, and other markers of obesity.[77] Interestingly, omentin levels have been noted to increase with weight loss and aerobic exercise.[78, 79] Tan and colleagues also demonstrated an increase in serum omentin levels after treatment with metformin in women with polycystic ovarian syndrome (PCOS).[80] However, this has not been evaluated in obesity.

The mechanism of omentin's anti-inflammatory function is poorly understood, though there is evidence that it may be via activation of AMPK and inhibition of TNF- α . For example, Yamawaki and colleagues demonstrated that omentin inhibits TNF- α -induced COX-2 expression in vascular endothelial cells. It has been hypothesized that omentin is able to do this by inhibiting JNK via activation of the AMPK/eNOS/NO pathway.[81, 82]

Omentin and Cancer

Cancer is also an inflammatory disease state, but few studies have attempted to examine the relationship between omentin and cancer. In their study of tissue from 196 patients with gastric cancer, Zheng and colleagues found that more than 72% of tumors expressed intelectin (omentin). Additionally, intelectin positivity of these tumors was inversely correlated with depth of invasion, lymph node metastases, distant metastases, and clinical stage.[83] Omentin levels were also noted to be decreased in patients with colorectal cancer and the findings of one small study suggested that omentin may be able to aid with prognosis in advanced stage colorectal cancer patients.[84, 85]

Furthermore, omentin levels appear to be elevated after these patients complete their therapy as compared with healthy controls.[86] Of interest, a recent study of prostate cancer patients found that they had higher omentin levels than patients with benign prostate disease. However, BMI and other confounders were not controlled for and these results have not yet been validated.[87] Unpublished work from our lab has noted that circulating omentin levels are lower in women with ovarian cancer and higher levels have been correlated with improved overall survival. These findings provide exciting promise for the use of omentin as a potential prognostic biomarker in malignancies.

Hypotheses and Specific Aims

There are currently no studies evaluating omentin in endometrial hyperplasia or endometrial cancer. Given that these are both inflammatory states associated with obesity and insulin resistance, it would be prudent to study the role of omentin in this disease process.

The present study was intended to better understand the relationship between omentin and endometrial hyperplasia and endometrial cancer with the following specific aims:

- Specific Aim 1: To evaluate the serum omentin levels of women with complex atypical hyperplasia of the endometrium and endometrioid endometrial adenocarcinoma as compared with controls.
 - Hypothesis: Serum omentin levels will be decreased in women with complex atypical hyperplasia of the endometrium or endometrial adenocarcinoma, independent of BMI or menopausal status.
- Specific Aim 2: To assess the difference in serum omentin levels among BMI groups in women with complex atypical hyperplasia of

the endometrium and endometrioid endometrial adenocarcinoma as compared with controls.

- Hypothesis: Serum omentin levels will be lower among obese women with complex atypical hyperplasia of the endometrium or endometrial adenocarcinoma as compared with normal-weight women with these diseases.

Methods

Approvals

Approval for relevant studies was obtained from the University of Texas at MD Anderson Cancer Center Institutional Review Board (IRB).

Statistical Considerations

Clinical characteristics and demographics were summarized with descriptive statistics. Because this was a matched case-control study and data were paired, the paired difference in omentin was measured between cancer cases and their controls. Descriptive statistics were then utilized to summarize these differences. A t-test was performed on the differences from zero within each BMI category. The Wilcoxon rank sum test was then used to compare BMI groups with respect to medians of these differences. A similar analysis was performed on the paired difference in omentin between hyperplasia cases and controls. A p -value of <0.05 was considered statistically significant.

Clinical Analysis

A retrospective, laboratory-based case-control study was conducted to compare the levels of omentin in women with endometrial adenocarcinoma or endometrial hyperplasia (cases) to women with no history of cancer (controls). After IRB approval was obtained, cases and controls were identified in the University of Texas MD Anderson gynecologic oncology tumor bank. All women with samples stored in the tumor bank provided informed consent to have their

blood, urine, and tissue samples collected at the time of surgery and be stored for future research.

To be included in the study, cases must have a confirmed diagnosis of grade 1 endometrioid endometrial adenocarcinoma or complex atypical hyperplasia with serum available in the tumor bank. Patients with type II tumors, inadequate banked serum, or without available data in the clinical record were excluded from the study. Women included in the control group must have no history of endometrial hyperplasia or carcinoma with serum available in the tumor bank. Additionally, controls were matched to the cases with respect to menopausal status and BMI category (Table 4). Patients were excluded from the control group if they had active cancer of any kind at the time of serum collection, inadequate banked serum, or no available data in the clinical record.

Category	BMI Range (kg/m ²)
Underweight	< 18.5
Normal Weight	18.5 – 25
Overweight	26 – 30
Obese	>30

Table 4: BMI Categories. Standardized classification of BMI.

In total 140 cancer cases and 72 hyperplasia cases were matched 1:1 to controls by BMI and menopausal status. Clinical data for both cases and controls was then obtained from the medical record, including age, ethnicity, menopausal status, medical history, and pathology.

ELISA

Patient serum samples were initially obtained from centrifuged whole blood and stored in a freezer at -80° Celsius before being thawed for the purpose of this study. Serum omentin was measured in using a commercially available enzyme-linked immunoadsorbent assay (ELISA) kit (BioVendor, Human Omentin-1 ELISA, Czech Republic). The assay was performed in triplicate for each sample and the mean concentration of omentin for each patient determined.

Prior to beginning the assay, serum samples were diluted 1:40 using a dilution buffer provided in the kit. The standards were prepared according to the kit instructions to obtain omentin concentrations of 64 ng/mL, 32 ng/mL, 16 ng/mL, 8 ng/mL, 4 ng/mL, and 2 ng/mL. Quality controls were also prepared with dilution buffer and assayed to ensure that the results were acceptable. All samples, standards, and quality controls were mixed with a vortex prior to pipetting 100 μ L of each into a 96-well plate.

After all samples were plated, the plate was incubated at 37° Celsius for two hours without shaking. The wells were then removed from the incubator, aspirated, and washed with 300 μ L of wash solution (provided by the kit) three times. After this wash, 100 μ L of a biotin-labeled antibody solution (provided by the kit) was pipetted into each well. The plate was then incubated at 37° Celsius for 30 minutes without shaking. The plate was again removed from the incubator, aspirated, and washed three times with 300 μ L of wash solution. Next,

100 μ L of streptavidin-HRP conjugate was pipetted into each well. The plate was again incubated without shaking at 37⁰ Celsius for 30 minutes. Upon removal from the incubator, the plate was aspirated and washed three times with 300 μ L of wash solution. After completion of this wash, 100 μ L of substrate solution was added to each well. The plate was covered with aluminum foil and incubated at room temperature for 10 minutes. After the incubation period, 100 μ L of stop solution was added to halt the color change.

Upon completion of the ELISA, the plate was read by a microplate reader set to 450 nm with a reference wavelength of 630 nm. A standard curve was calculated by subtracting the absorbance values for the wavelengths, plotting the difference of absorbencies on the y-axis against the known concentration of the standards on the x-axis. The x-axis was then set to a logarithmic scale. The omentin concentration of the samples was obtained using the standard curve and multiplying values by 40 to adjust for the dilution of the sample.

Results

As demonstrated in table 5, cancer cases, hyperplasia cases, and controls had similar demographic and clinical factors. Overall, almost three-fourths of women were white, and this proportion was similar between groups. The mean age was 58, though women in the hyperplasia group were slightly younger. The BMI of each group was very similar, as expected given that the groups were matched based upon BMI.

Characteristic	Cancer	Hyperplasia	Controls	Total
Mean Age (SD)	60 (± 11.44)	53 (± 12.29)	59 (± 11.62)	58 (± 11.62)
Ethnicity (n)				
White	68.6 (96)	76.4 (55)	77.9 (120)	74 (271)
Black	4.3 (6)	2.8 (2)	7.8 (12)	5.5 (20)
Hispanic	20 (28)	16.7 (12)	10.4 (16)	15.3 (56)
Asian	7.1 (10)	4.2 (3)	3.9 (6)	5.2 (19)
Diabetes (n)	22.1 (31)	18.1 (13)	12.3 (19)	17.2 (63)
Metformin Use (n)	15.7 (22)	9.7 (7)	8.4 (13)	11.5 (42)
Breast Cancer History (n)	2.9 (4)	23.6 (17)	15.6 (24)	12.3 (45)

Table 5: Participant Characteristics. Characteristics are summarized by group – cancer, hyperplasia, and controls. The hyperplasia group is younger with less diabetes and metformin use. Ethnicity is similar across groups.

As anticipated, the mean serum omentin levels were significantly higher in lean women (673 ng/mL) as compared to overweight (481 ng/mL) and obese women (475 ng/mL) with $p < 0.000$. This is demonstrated in figure 4.

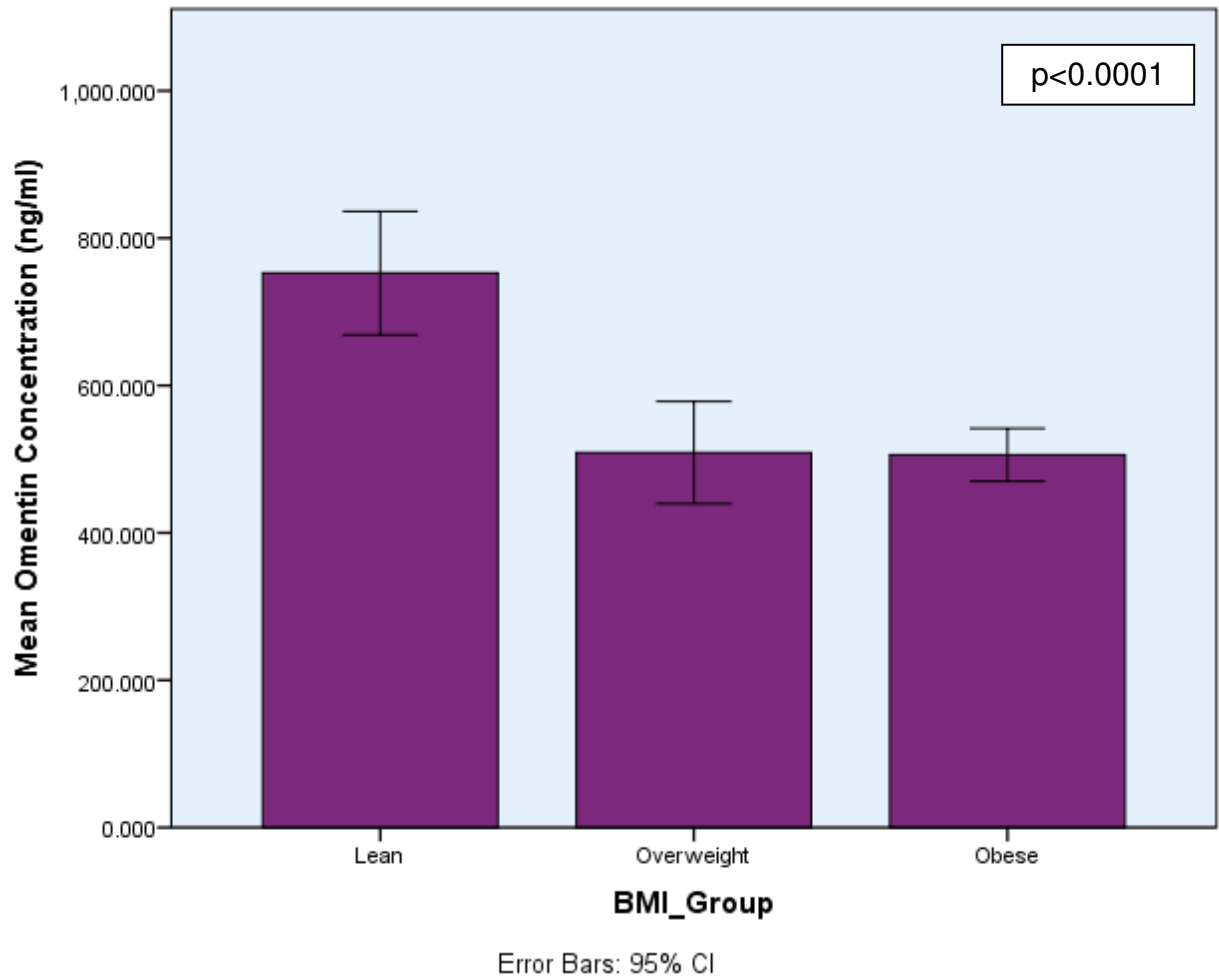


Figure 4: Mean Omentin Concentration by BMI Group. Omentin levels were higher in lean patients as compared with overweight and obese women.

As seen in figure 5, mean serum omentin levels were significantly lower in women with endometrial cancer as compared with matched controls (576 ng/mL vs 673 ng/mL, $p=0.02$). Furthermore, among women with endometrial hyperplasia, the mean serum omentin concentration was significantly lower than matched controls (359 ng/mL vs 723 ng/mL, $p<0.001$).

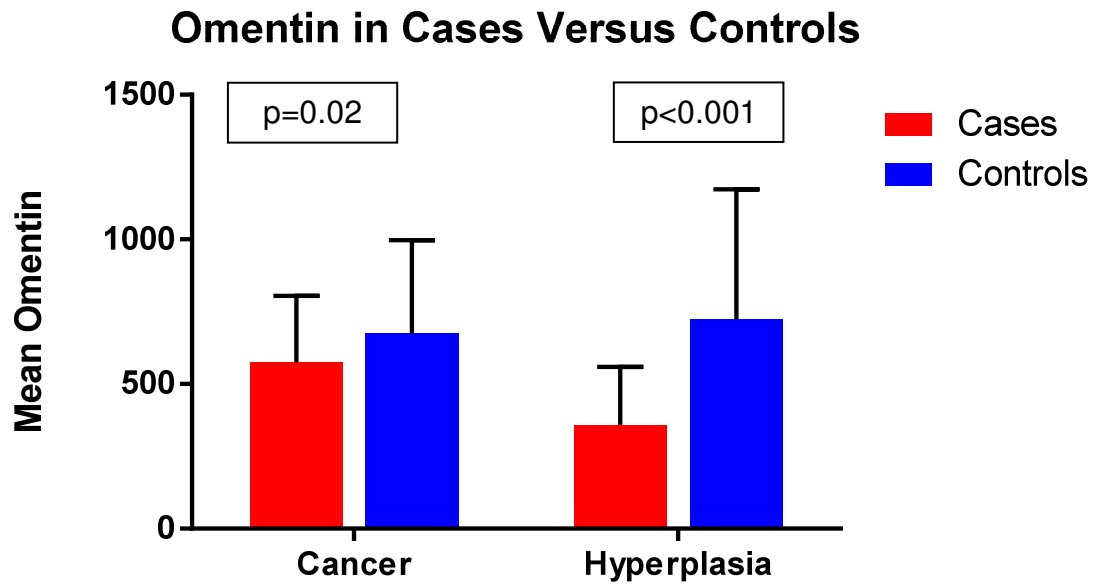


Figure 5: Omentin Concentration in Cases versus Controls. Women with endometrial cancer and hyperplasia had significantly lower omentin than their matched controls.

When stratified by BMI, lean women with endometrial cancer had a statistically lower serum omentin level than lean controls. However, there was no difference in omentin concentration between overweight and obese endometrial cancer patients and controls. When women with hyperplasia were evaluated, there was a significantly lower serum omentin level in lean and obese cases when compared with controls. However, there was no difference between overweight hyperplasia cases and controls (table 6).

Endometrial Cancer				Controls			p-value
	N	Omentin (Mean)	SD	N	Omentin (Mean)	SD	
Total	74	575.76	229.47	74	673.77	324.87	0.0204
BMI (kg/m ²)							
< 25	21	667.73	241.60	21	954.06	317.46	<0.001
25 to 30	15	474.31	153.79	15	593.30	250.35	0.177
> 30	38	564.98	233.01	38	550.64	259.33	0.802
Endometrial Hyperplasia				Controls			p-value
	N	Omentin (Mean)	SD	N	Omentin (Mean)	SD	
Total	74	359.57	199.64	74	723.32	449.80	<0.0001
BMI (kg/m ²)							
< 25	17	438.32	251.20	17	995.94	523.52	0.002
25 to 30	16	354.79	212.33	16	652.42	575.61	0.068
> 30	41	328.78	164.24	41	637.96	307.06	<0.001

Table 6: Mean Serum Omentin in Cases vs Controls Stratified by BMI. Only lean endometrial cancer patients have lower serum omentin than controls. Lean and obese hyperplasia cases had lower omentin than controls.

Discussion

Prior studies have consistently shown an inverse relationship between omentin and obesity. De Souza Batista and colleagues demonstrated that serum omentin levels are negatively correlated with BMI and waist circumference.[77] It has also been noted that serum omentin is decreased in patients with metabolic syndrome. In a population of Japanese men, omentin levels were significantly lower in patients with large waist circumference, dyslipidemia, elevated blood pressure, and impaired glucose tolerance.[88] Interestingly, weight loss has been shown to increase serum omentin levels and improve insulin sensitivity.[78]

The present study is consistent with other researchers, confirming that serum omentin concentration is inversely related to BMI. Interestingly, there is not a strict negative correlation between omentin levels and BMI. For example, while lean women were noted to have a significantly higher serum omentin than overweight and obese women, there was no difference in omentin levels between overweight and obese women. The reasons for this are unclear. However, many factors others than BMI may influence omentin levels (i.e. waist circumference, amount of visceral vs peripheral fat, etc) and these confounders may account for the results in our study.

There are currently no published studies evaluating the relationship between omentin and endometrial cancer. In the present study, women with endometrial adenocarcinoma have decreased serum omentin levels when

compared with controls, as hypothesized. However, when stratified by BMI, there was only a difference in omentin levels between lean cases and controls. Reasons for this finding are not clear. However, it may be related to the relatively small numbers in each BMI group and other confounders that affect omentin levels.

The etiology of the relationship between omentin and endometrial cancer has not yet been investigated. However, it is likely multifactorial and may be indirectly related to the inhibition of omentin's anti-inflammatory effects. Multiple studies in other disease processes have demonstrated omentin's anti-inflammatory properties. In patients with juvenile arthritis, for example, omentin levels are lower among patients with active disease as compared with those with disease in remission.[89] Patients with Crohn's disease have also been found to have lower omentin levels, and it appears that these lower levels are important to disease activity.[90] In vascular endothelial cells, omentin has been demonstrated to inhibit COX-2 expression by activating the AMPK and eNOS pathways, thereby decreasing inflammation.[82]

Other adipokines may also be indirectly involved in the process of endometrial cancer development. Leptin was the first adipokine to be discovered and is known to be associated with regulating appetite, weight, and metabolism.[91] Elevated serum leptin levels have been associated with obesity and endometrial carcinoma.[92] Another adipokine, adiponectin, has similar characteristics to omentin and has been shown to be inversely related to endometrial cancer.[59, 93] An evaluation of patients with diabetes, impaired

glucose tolerance, and normal glucose tolerance noted a positive correlation between serum omentin levels and serum adiponectin levels. Furthermore, serum adiponectin was an independent predictor of serum omentin levels.[76] The role of adipokines in the pathogenesis of endometrial cancer appears to be complex and has not been completely elucidated.

How decreased levels of omentin directly affect endometrial cancer development has not yet been studied. However, the mechanism may be extrapolated from work in other malignancies. For example, there is evidence that omentin may activate apoptosis pathways. In hepatocellular carcinoma cell lines, administration of omentin led to activation of the caspase-3 and JNK pathways.[94] Other studies have suggested that omentin may play a role in tumor suppression. In neuroblastoma, administration of intelectin-1 (omentin) led to suppression of cell growth, invasion, and metastasis. This finding occurred both *in vitro* and *in vivo*. [95] In ovarian cancer, unpublished data from our lab has demonstrated that omentin can aid with decreased cell proliferation, motility, and invasion potential.

The present study was the first to evaluate the relationship between omentin and complex atypical hyperplasia of the endometrium. We found that women with endometrial hyperplasia possessed significantly lower serum omentin levels than controls. When evaluated by BMI, lean and obese hyperplasia cases had lower omentin levels than controls. As complex atypical hyperplasia represents a precancerous condition, these results suggest that

omentin may be related to the early changes in the endometrium that ultimately lead to the development of cancer.

Of interest, the mean serum level of omentin in women with endometrial hyperplasia was lower than that of women with endometrial cancer. The reasons for this are unclear, as it would be expected that omentin levels in women with endometrial hyperplasia would be lower than that of controls, but higher than levels in women with endometrial cancer. However, it should be noted that with a case-control design, the present study was not designed to directly compare omentin levels between women with endometrial cancer and women with hyperplasia. Each group was matched to its own set of controls but not one another. Therefore, it is difficult to assess if a true difference in omentin levels exists between the hyperplasia and cancer groups.

Our results further illuminate the intricate relationship between adipokines, obesity, and endometrial cancer. While we have confirmed an inverse relationship between serum omentin levels and endometrial hyperplasia and carcinoma, the etiology for the lower omentin levels in these patients is unknown. It may be postulated that many endometrial cancer patients possess other risk factors associated with decreased omentin, such as obesity, diabetes, and PCOS. In these cases, it is likely that comorbidities led to decreased serum omentin levels. This, in turn, contributed to the development of endometrial cancer via absence of the tumor suppressor mechanisms of omentin. However, presumably many women with decreased omentin due to other risk factors never develop endometrial cancer. The etiology of this phenomenon should be

evaluated but may be related to downregulation of omentin expression by the cancer cells. For example, unpublished work from our lab has noted that ovarian cancer leads to decreased omentin mRNA expression in the mesothelial cells. However, it should be remembered that endometrial cancer development is a complex process and unlikely to be solely dependent on low serum omentin levels. Further research is warranted to investigate this.

The clinical implications of our study findings are currently only theoretical. For example, there is potential use of omentin as a biomarker for endometrial hyperplasia and its progression to cancer. This may be especially true in lean women, as this was the only group noted to have significantly lower omentin levels in both hyperplasia and cancer cases when compared to controls. Also, given that controls had higher serum omentin levels than cases, omentin may have utility in endometrial cancer prevention trials. For example, it is known that serum omentin levels increase with weight loss and exercise.[78] Additionally, in studies of PCOS, administration of metformin led to increased serum omentin levels.[80] These findings provide for exciting possibilities for clinical trials if they hold true in women at high risk for endometrial cancer. Furthermore, there is unpublished data from our lab demonstrating that serum omentin levels are inversely correlated with survival in ovarian cancer patients. This finding is difficult to extrapolate to the low grade or early stage endometrial cancer population as these women typically have excellent overall survival at baseline. However, omentin may be a biomarker to predict survival or risk of recurrence in women with high grade or advanced stage endometrial cancer. Additional

studies are warranted to explore this possibility. Finally, given that administration of omentin to cancer cell lines leads to increased cell apoptosis and tumor suppression, it may be that omentin itself has a therapeutic benefit. However, the role of omentin administration in preventing or reversing endometrial hyperplasia or cancer is unknown and needs to be further evaluated.

One of the main limitations of this study is its retrospective nature. Furthermore, serum samples were obtained from an institutional tumor bank and in some cases had been frozen for several years. Though omentin is believed to be a very stable protein, determining omentin from fresh rather than frozen samples may provide more consistent results. The relatively small number of samples that we evaluated may also limit this study, though our study was powered to detect a sufficient difference in omentin levels. Finally, there is a wide number of clinical factors that may affect omentin levels other than obesity (diabetes, cardiovascular disease, arthritis, etc). As it would be impossible to control for each of these, it is likely that our results are limited by these confounders.

Although it is clear that omentin is related to endometrial cancer and hyperplasia regardless of BMI, the etiology of this relationship is unknown. As such, it is difficult to determine what clinical implications the association between endometrial cancer and omentin will provide. Our results present exciting promise for omentin's role in further understanding the relationship between obesity-related factors and endometrial cancer.

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Vita

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