Molecular aspects of the link between obesity and endometrial cancer.

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MOLECULAR ASPECTS OF THE LINK BETWEEN OBESITY AND ENDOMETRIAL CANCER

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DECLARATION

The experimental work and results presented in this thesis except where accordingly acknowledged, are entirely my own.

Patricia Elizabeth Ellis

ABSTRACT

Five percent of all cancers in postmenopausal women are attributable to being overweight and the incidence rises to 51% in women with endometrial cancer (EC). EC patients that are obese tend to have a poorer outcome, shorter survival rates and more co-morbidities than their non-obese counterparts. However, the role of excess adipose tissue in increasing cancer risk is not well understood. More specifically, the exact role of intracellular signalling factors within adipose tissue, in the development of endometrial cancer, is still unclear.

This project aimed to partially address this lack of understanding by carrying out a systematic review / meta-analysis of primary studies related to the role of circulating adipokines and inflammatory molecules in EC. Cochrane Central Register of Controlled Trials (CENTRAL), Cinahl, Web of Science, Medline and Embase databases were searched using key words: endometrial cancer, obesity, adiponectin, leptin, interleukin-6 (IL-6), tumour necrosis factor alpha (TNFα), insulin-like growth factor I (IGF-I), and insulin-like growth factor II (IGF-II).

Obese, overweight and normal weight women with EC (n=25 including one patient with endometrial hyperplasia) were recruited into a small clinical study and blood samples taken for analysis of the above-mentioned inflammatory markers and adipokines.

Results from the meta-analysis suggest that participants with circulating adiponectin levels in the highest tertile had 0.51 times decreased EC risk compared to women with levels in the lowest tertile (summary of odds ratio (SOR): 0.51, 95% confidence interval (CI): 0.38-0.069, $p<0.00001$). Women with circulating leptin concentrations in the highest tertile had 2.19 times increased EC risk compared to the women with concentrations in the lowest tertile (SOR: 2.19, 95% CI: 1.45-3.30, $p<0.0002$). Pooled results demonstrated no significant differences in TNFα and IL-6 circulating levels between participants with the highest levels compared to the lowest levels.

In this small clinical study, higher concentrations of leptin, TNFα, IL-6, IGF-I and IGF-II were observed in obese patients with EC compared to overweight

and normal weight patients with EC. Adipocytokines may act synergistically to increase the risk and development of EC in women with different BMI.

Keywords: endometrial cancer, obesity, adipokines, adiponectin, leptin, tumour necrosis factor α (TNFα), interleukin-6 (IL-6), insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II)

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ABBREVIATIONS

BAT	Brown adipose tissue
BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
DM	Diabetes mellitus
ELISA	Enzyme linked immunosorbent assay
ERP	Enhanced recovery programme
FIGO	The International Federation of Gynecology and Obstetrics
HNPCC	Hereditary non-polyposis colorectal cancer
HOMA-IR	Homeostasis model of assessment of insulin resistance
HRP	Horse radish peroxidase
HRT	Hormone replacement therapy
IGF	Insulin like growth factor
IGFBP	Insulin like growth factor binding protein
IL	Interleukins
MHL1	MutL homolog 1
MHL2	MutL homolog 2
MHL6	MutL homolog 6
MMR	Mismatched repair
MOOSE	Meta-analyses of observational studies in epidemiology
MSI	Microsatellite Instability
NF	Necrosis factor
NW	Normal weight
OR	Odds ratio
OW	Overweight
PBS	Phosphate buffered saline
QUICKI	Quantitative insulin sensitivity check index
RIA	Radioimmunoassay
RR	Relative risk
SHBG	Sex hormone binding globulin
SOR	Summary odds ratio
TNFα	Tumour necrosis factor alpha
TNM	Tumour Node Metastasis

WAT	White adipose tissue
WHR	Waist–to-hip-ratio
WCC	White cell count

PUBLICATIONS ARISING FROM THESIS

Full publication

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CHAPTER 1: INTRODUCTION

1.1 Endometrial cancer

The number of women diagnosed with endometrial cancer continues to rise each year. In 2010, there were 8,475 new cases and, in 2014, this rose to 9,324 in the United Kingdom (UK): 65% increase in the incidence of endometrial cancer has been reported since 1980 (Cancer Research UK, 2018). The American Cancer Society (2018) estimated 61,880 new cases of endometrial cancer will be diagnosed in 2019, in the United States of America. The risk of developing endometrial cancer has been shown to be increased in overweight (a body mass index (BMI) of 25-29.9 kg/m^2) and obese (a BMI of 30 kg/m^2 and above) women (Calle and Kaaks, 2004). Renehan and colleagues (2008) demonstrated, in their meta-analysis and systematic review of prospective studies a 1.6-fold increase in endometrial cancer risk with each extra 5 kg/m^2 BMI. Endometrial cancer is most common in post-menopausal women; the average age women reach the menopause in the UK, is 51 years of age. The incident of endometrial cancer is highest in the 75-79-year age group, increasing steeply from around ages 45-49 years (Cancer Research UK, 2018). Women usually present with abnormal vaginal bleeding or discharge. However, the bleeding can be heavy and irregular in pre-menopausal women. The diagnosis of endometrial cancer is usually confirmed on an endometrial biopsy with approximately 80% of women requiring surgery in the form of a hysterectomy bilateral salpingo-oophorectomy and dependent on the stage and grade of the tumour may require removal of pelvic and para-aortic lymph nodes as treatment. A further 21% will require radiotherapy and 16% chemotherapy (Cancer Research UK, 2018). Figures 1.1 and 1.2 are schematic diagrams of the female reproductive system and of the location of the endometrium, respectively. The International Federation of Gynecology and Obstetrics (FIGO) staging is the method used to stage and, assess the extent of the disease, in endometrial cancer (Table 1.1).

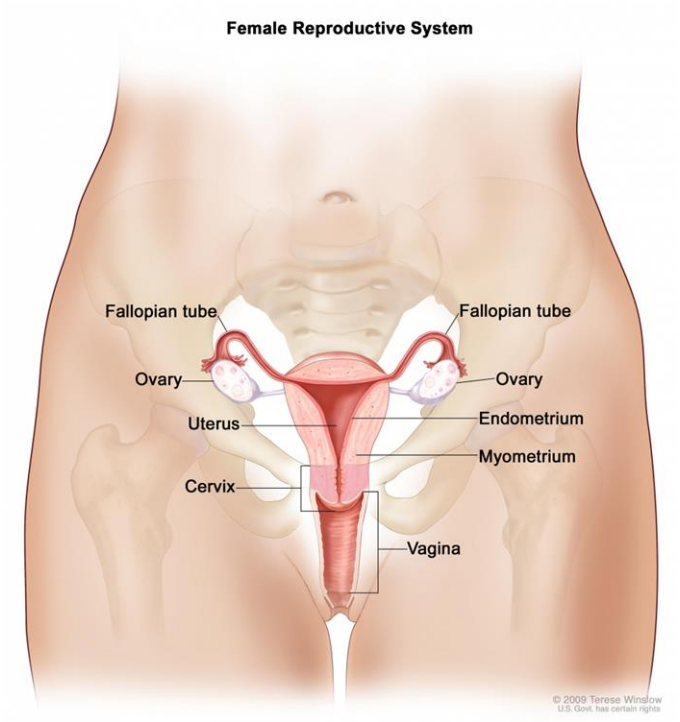

Figure 1.1 Female reproductive system. Terese Winslow. Reproduced with permission (2019).

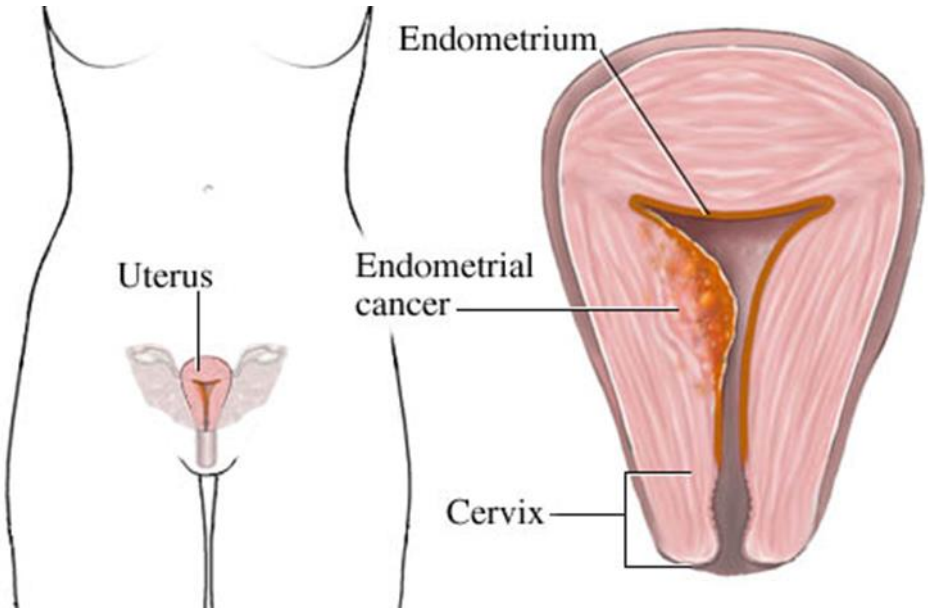

Figure 1.2 The development of endometrial cancer from the endometrium. Adapted from Encognitive.com (2018).

Table 1.1 TNM (8th Edition) FIGO staging for endometrial cancer (2009)

		Description
Primary Tumour (T)		
TNM	FIGO	
TX		Primary tumour cannot be assessed
T0		No evidence of primary tumour
T1	I	Tumour is confined to the uterus
T1a	1A	Tumour involves the endometrium and invades less than 50% of the myometrium
T1b	1B	Tumour involves the endometrium and invades more than 50% of the myometrium
T2	II	Tumour extends to the cervical stroma but is still confined to the uterus.
T3	III	Regional spread of the tumour beyond the uterus
T3a	IIIA	Tumour has spread to the serosa of the uterus and/or to the fallopian tubes and/or ovaries
T3b	IIIB	Tumour extends to the vagina and or parametrium
T4	IVA	Tumour invades bladder or bowel mucosa
		Positive pelvic lymph nodes
Regional Lymph Nodes (N)		
NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastasis
N1,N2	IIIC	Metastases to the pelvic and or para-aortic lymph nodes
N1	IIIC1	Positive pelvic lymph nodes
N2	IIIC2	Para-aortic lymph nodes involved with or without positive pelvic lymph nodes
Distant Metastasis (M)		
M0		No distant metastasis
M1	IVB	Distant metastasis

Source: Percorelli *et al.* 2009 /Endometrial cancers (FIGO/TNM8) www.iccr-cancer.org

1.1.1 Histopathology of endometrial cancer

There are two distinct types of endometrial cancer: Type 1 and Type 2; based on their distinct clinical/pathological and molecular identities (Bohkman, 1983) (Table 1.2).

Table 1.2 Characteristics of Type 1 and Type 2 endometrial carcinomas

Characteristics	**Type1**	**Type 2**
Unopposed oestrogen	Present	Absent
Growth	Slow growing	Rapid progression
Precursor	Atypical hyperplasia	Endometrial intraepithelial carcinoma
Histology	Endometrioid	Serous, Clear cell
Grade	Low	High
Molecular genetic changes	PTEN, KRAS gene and P13K/AKT pathway mutations; microsatellite instability,	p53 mutation

Source: *Chu et al*., 2008.

Type 1 endometrial cancers are known as endometrioid type because of their similarity to normal endometrial glands. They are usually preceded by endometrial hyperplasia and are oestrogen related because of unbound oestrogen diffusing into the uterine cavity and stimulating the endometrium. They account for approximately 80% of all endometrial cancers and are diagnosed in both pre and post-menopausal women.

Type 2 endometrial cancers are of a different histological type to Type 1 and usually are of the serous or clear cell type. Carcinosarcomas and undifferentiated tumours are also classified as Type 2 endometrial cancers. Type 2 endometrial cancers account for approximately 20% of endometrial cancers and are often diagnosed in post-menopausal women. They are high grade in nature and biologically more aggressive than Type 1. They can be preceded by endometrial intraepithelial carcinoma (Ambrose *et al.* 1995). They have a high recurrence rate and poor overall survival rate. Fifty percent of

relapses occur in women with Type 2 endometrial cancers (Santin *et al*., 2005). Unlike Type 1 endometrial cancers, they are not associated with oestrogen stimulation as the endometrium is usually atrophic in women with Type 2 endometrial cancers. The women can also be of normal weight because Type 2 endometrial cancers are non-oestrogen dependent. It has been postulated that the development of Type 2 endometrial cancers is more likely to be related to mutations in oncogenic signalling pathways. Abnormalities in the tumour suppressor gene, p53, and the p53 protein in endometrial cancers, have been reported in several studies (Lax *et al*., 2000, Yamazawa *et al*., 2007, Horree *et al*., 2008). An increase in the over-expression of the p53 protein from normal endometrium to hyperplasia through to endometrial carcinoma has been demonstrated, suggesting a possible role in disease progression in endometrial carcinoma (Horree *et al*., 2008).

Approximately 90% of endometrial cancers are sporadic and 10% hereditary (Bansal *et al*., 2009). Hereditary non-polyposis colorectal cancer (HNPCC), also known as lynch syndrome, is an inherited autosomal dominant syndrome due to defective mismatch repair (MMR) genes MLH1 (MutL homolog1), MSH2 (MutL homolog 2) and MSH6 (MutL homolog 6). The MMR genes are responsible for repairing errors during DNA replication. Mutations in these genes will generate microsatellite instability (MSI). MSI have been well documented in endometrial cancers and are more common in Type 1 than Type 2 endometrial cancers (Mackay *et al.*, 2010). After colorectal cancers, endometrial cancer is the second most common cancer to develop in patients with HNPCC, with approximately 30-60% of women developing endometrial cancer. Endometrial cancer occurs 10 years earlier in women with HNPCC, than the general population, with peak incidence between the ages of 40-60 years. Up to 3% of women with endometrial cancer will have mutations in the MMR genes (Ellis and Ghaem-Maghami, 2010).

The drive to develop strategies to reduce the risk of endometrial cancers and treatment options, have led to the identification of genomic alterations in endometrioid and serous endometrial cancers. Genomic classification of endometrial cancers has characterised them in to 4 molecular subgroups: pole ultramutated, microsatellite instability hypermutated, copy number low, copy number high (The Cancer Genome Atlas Research Network, 2013). Their work

has demonstrated that 25% of high grade endometrioid tumours have a molecular phenotype similar to serous endometrial carcinomas. These tumours have increased mutations in the p53 gene and also increased somatic copy alterations. This discovery has implications with regards to the treatment options available for these tumours. Chemotherapy for these high grade endometrioid tumours with a similar phenotype to serous endometrial carcinomas may be more appropriate than radiotherapy.

1.2 Endometrial cancer and obesity

The number of people classified as being overweight and obese has dramatically increased over the last few years in the UK. Obesity, which is defined as a BMI of greater than 30 kg/m^2 and characterised by an excessive accumulation of fat in adipose tissue, is thought to be a factor in the development of several cancers including breast, renal, oesophageal, colon and endometrial (Basen-Enquist and Chang, 2011).

The association between endometrial cancer and obesity is well documented at epidemiological level (Calle *et al.*, 2003, Courneya *et al*., 2005). Five percent of all cancers in post-menopausal women are attributable to being overweight (BMI ≥25 kg/m^2). For women with endometrial cancer, this rises to 51% (World Cancer Research Fund 2009, 2013). Women of normal weight have a 3% lifetime risk of endometrial cancer compared to a 9-10% lifetime risk in obese women (Modesitt *et al.*, 2012). This risk is also present in pre-menopausal women. Moreover, patients who are obese tend to have a poorer outcome and more co-morbidities (e.g. hypertension, respiratory disease and type 2 diabetes) than their non-obese counterparts: mortality rates have been reported to be significantly higher with increasing BMI (Secord *et al*., 2016). In addition to the poorer outcome, Type 1 endometrial cancers tend to be more common in obese women. This is possibly due to the theory that the aetiology of Type 1 endometrial cancer is linked to oestrogen. Obese women will produce more oestrogen due to the conversion of androstenedione to oestrone by the enzyme aromatase in the adipose tissue (Felix *et al*., 2010).

Several mechanisms have been put forward to explain the molecular link between obesity and endometrial cancer; however, further research is required

to better define such mechanisms, due to the complexity of the changes occurring to adipose tissue in an obese state.

1.3 Adipose tissue

There are three types of adipose tissue: brown adipose tissue (BAT), beige adipose tissue and white adipose tissue (WAT) and, each type differs in their location and species. BAT has an important function in thermoregulation in newborn infants and hibernating mammals. The amount of BAT decreases as a person ages (Virtanen *et al*., 2009). The BAT colour is derived from containing a higher number of iron containing mitochondria than WAT. Beige adipose tissue is also involved in thermoregulation and like BAT, the amount and function of beige adipose tissue decreases with age (Zoico *et al*., 2019). WAT contains trigylcerides and is the main site for energy storage (Saley *et al*., 2012). WAT can be further classified into visceral adipose tissue and subcutaneous adipose tissue. As a result of increased WAT, in an obese individual, the function of the adipose tissue can deteriorate resulting in a state of chronic inflammation. The abnormal inflammatory environment occurs as a result of at least 3 mechanisms: an increased production of inflammatory factors, increased tissue inflammation and adipose tissue remodelling (Ghigliotti *et al*., 2014).

In this inflammatory state, adipocytes and macrophages secrete several molecules, adipokines and inflammatory cytokines, which may promote tumour development and angiogenesis and stimulate adhesions and migration of cells (Nieman *et al*., 2013).

WAT (Figure 1.3) is an active endocrine gland which produces and secrete several hormones such as adiponectin, leptin, tumour necrosis factor alpha (TNFα), interleukin 6 (IL-6) and insulin-like growth factors (IGF).

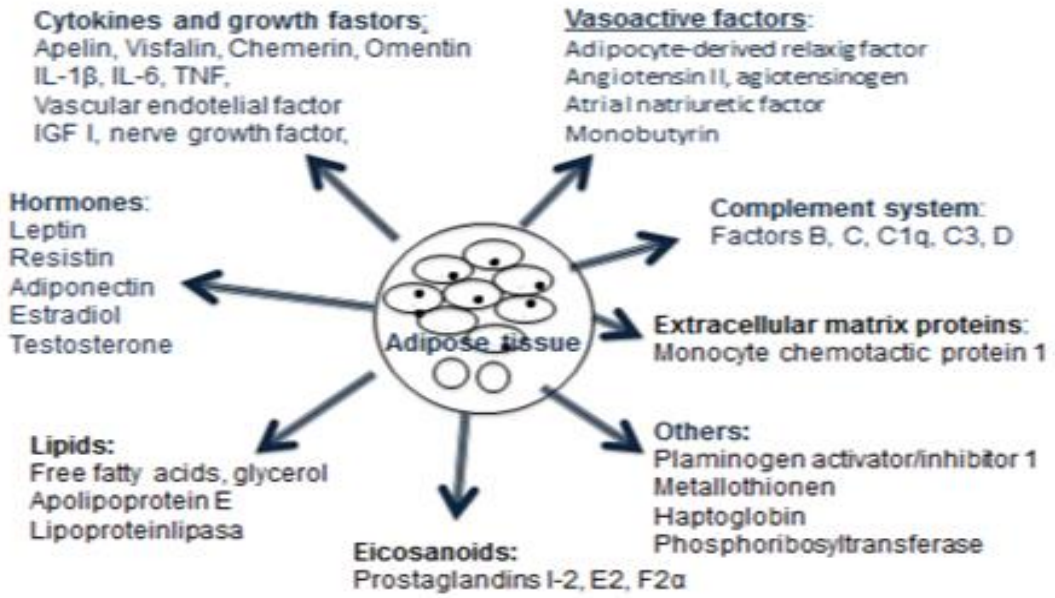

Figure 1.3 Adipose tissue and its secretion of different factors (Cordido *et al.*, 2014)

Adiponectin is involved in the control of glucose metabolism and fatty acid oxidation. Its plasma levels are generally lower in obese individuals (Kishida *et al*., 2014) and hypoadiponectinemia has been associated with diabetes and cancer. Moreover, losing weight through healthy eating, exercise or surgery increases adiponectin levels (Christiansen *et al*., 2010). Even though the mechanisms underlying the inverse relationship between body weight and adiponectin levels are still unclear, adiponectin has been shown to decrease blood glucose and insulin concentrations, and has anti-inflammatory and antiangiogenic properties (Dallal *et al*., 2013).

Leptin is also predominantly produced by adipocytes and has an insulin sensitising effect contributing to insulin resistance. In the obese state, leptin levels are raised which in turn promote inflammation by stimulating the production of IL-6, TNFα as well as IL-1 and IL-12 (Carbone *et al*., 2012; Strong *et al*., 2015). Several studies have demonstrated an inverse relationship between adiponectin and leptin in endometrial cancer (Ashizawa *et al*., 2010; *Ma et al.*, 2013; Wang *et al.*, 2014). Higher adiponectin levels appear to have a protective effect in endometrial cancer (Gong *et al.*, 2015, Tong *et al*., 2015). This contrasts with leptin which has been reported to be an independent risk factor for endometrial cancer (Wang *et al.* 2014). Another study, conducted by Cymbaluk and colleagues (2008) analysed the serum

concentrations of leptin in obese post-menopausal women to determine whether it differed from concentrations in patients with a normal endometrium. They found, in their case control study of 86 obese post-menopausal women (40 with endometrial cancer/hyperplasia and 46 with normal endometrium), that the mean serum concentration of leptin in endometrial cancer/hyperplasia to be 16737.1 pg/ml as opposed to 9048.7 pg/ml in patients without endometrial pathology ($p<0.0001$). Serum leptin levels in women with endometrial hyperplasia with atypia was 1524 pg/ml and in women with endometrial cancer, it was 17,879 pg/ml compared to 9048.7 pg/ml in women with a normal endometrium. They concluded that leptin appears to participate in proliferative processes of the endometrium.

The levels of TNFα, a pro-inflammatory cytokine produced by adipocytes, are raised in the obese state. Originally, described as a cytokine with anti-tumour properties, it was later found to have the opposite effect; promoting the development of cancer (Trayhurn and Wood, 2004). It has been shown to be involved in all stages of cancer development including progression and metastasis (Trayhurn *et al*., 2008). TNFα is a regulator of necrosis factor (NF)-κB, which subsequently controls a complex of proteins involved in cell growth and proliferation (Aggarwal, 2004). Choi and colleagues (2009) demonstrated the role of TNFα in activating signalling pathways which are essential for migration of endometrial cancer cells.

Another adipocyte inflammatory marker, IL-6, is increased in endometrial cancer and is associated with a poor prognosis (Bellone *et al*., 2005; Slater *et al*., 2006). It stimulates the release of acute phase proteins such as C-reactive protein (CRP) and is involved in the retention and proliferation of CD3+ and B lymphocytes respectively (Himbert *et al*., 2017). IL-6 has been shown to stimulate aromatase in the adipose tissue (Zhao *et al*., 1995). This can increase oestrogen production resulting in further stimulation of the endometrium and increase the risk of Type 1 endometrial cancers. Zemlyak and colleagues (2012) undertook a prospective study examining the expression of inflammatory cytokines in adipose tissue from patients with endometrial cancer. They assessed the gene expression of TNFα, IL-6, IκB (an inhibitor of NF-κB), CD68 (glycoprotein expressed on macrophages) and leptin in samples of adipose tissue from individuals with endometrial cancer *versus* patients with

benign conditions. Omental tissue was harvested from the patients. The total number of patients was 56: 24 patients with endometrial cancer and 32 controls. The gene expression of IκB TNFα, and IL-6 increased as BMI increased in the control group. There was no correlation of IκB TNFα, IL-6 or CD-68 gene expression levels with cancer status of the patients. Leptin had a weak protective effect against endometrial cancer. They concluded that obesity was associated with increased expression of certain inflammatory cytokines in the adipose tissue. However, increased levels of these inflammatory markers in adipose tissue of the omentum are not associated with the presence of endometrial cancer.

Insulin and IGF levels are also increased in obesity (Wang *et al.,* 2016). Insulin production from the pancreas is raised in individuals with insulin resistance. IGF-I is produced predominantly by the liver and plays a key role in normal growth and development. IGF-I and IGF-II are involved in mediating steroid hormone actions in the endometrium through autocrine and paracrine mechanisms. Both IGF-I and IGF-II are associated with endometrial differentiation. Insulin and IGF increased expression have been linked to endometrial cancer (Pavelic *et al.*, 2007). The authors examined the expression of IGF-II and its receptors in 59 endometrial cancers, 10 hyperplasia and 7 normal tissues. They found that levels of IGF-I and IGF-II receptors were much higher in the neoplastic tissue of Stage III and IV endometrial cancers compared to Stage I and II cancers and hyperplastic or normal endometrium. This correlated with a decreased apoptosis rate and IGF2R expression. They concluded that IGF-I, IGF-II and their receptors are involved in the progression of endometrial adenocarcinomas.

Oestrogen has been implicated in the link between obesity and endometrial cancer (Vicennat *et al*., 2015). The synthesis and levels of circulating oestrogen change throughout the female reproductive years. In pre-menopausal women, the ovaries are the main source of oestrogen. This changes once a woman reaches the menopause; the ovaries are no longer the main source of oestrogen but, it is secreted from other sites such as the adrenal glands and adipose tissue. Adipose tissue is the main source of oestrogen in obese post-menopausal women (Cleary and Grossmann, 2009). Obesity increases oestrogen levels by various processes; aromatisation of C19 steroids in the

adipose tissue and the skin results in increased levels of oestrogen synthesis. Aromatisation is performed by the enzyme aromatase, which is found abundantly in adipose tissue. Aromatase converts androstenedione to oestrone. Increased circulating oestrogen levels have a stimulatory effect on the endometrium which can induce neoplastic transformation (Navaratnarajah *et al.*, 2008). Obesity is also associated with the decreased production of the sex hormone binding globulin (SHBG) which results in the increase bioavailability of oestrogen. Obese pre-menopausal women tend to have more frequent anovulation leading to progestogen deficiency which increases unopposed oestrogen exposure (Pilay *et al*., 2006). Additional studies have also looked at the link between the amount of adiposity, levels of circulating adipokines and increased risk of endometrial cancer. In particular, the study by Mihu and colleagues, (2013) assessed the relationship between abdominal adiposity through adipocyte secretion products and the risk in developing endometrial cancer. The authors sought to identify a correlation between abdominal obesity, plasma adipokine levels (leptin and adiponectin) and endometrial cancer. This case-controlled analysis assessed two groups of patients; Group 1: 44 patients diagnosed with endometrial cancer and Group 2: 44 patients without gynaecological pathology or inflammatory disorders. Group 1 had an average BMI of 32.49±4.66 kg/m^2 and Group 2 average BMI of 24.55±4.0 kg/m^2. Abdominal fat was assessed by dual X-ray absorptiometry and plasma adiponectin and leptin levels were measured using ELISA. A significantly higher value for abdominal fat and leptin was found in Group 1 ($p<0.0001$), whilst the plasma adiponectin level was significantly lower in Group 1 ($p<0.00001$) compared to the control group (Group 2) ($p<0.00001$). Abdominal fat had a negative linear correlation with plasma adiponectin levels and a positive linear correlation with plasma leptin levels. They concluded that measurement of adiponectin and leptin levels associated with the determination of abdominal adipose tissue can be a useful predictor factor for endometrial cancer.

1.4 Literature review of secondary research

The literature currently available is quite fragmented and a systematic search of the literature was performed to capture secondary research investigating the association between adipokines, produced from WAT, with the

development and progression of endometrial cancer. A systematic literature search was, therefore, conducted within MEDLINE and Embase for articles that met the inclusion criteria by using the following key words: endometrial cancer, obesity, adipose tissue, adipocytes, adipokines, adiponectin, leptin, growth factors. The outcome of this search identified 4 meta-analysis of the published literature which are summarised below.

Lin and colleagues (2015) performed a meta-analysis to assess the association between serum levels of adiponectin and the risk of endometrial cancer. Studies were identified by searching PubMed and the Web of Knowledge in January 2015 and had to include the following parameters: 1. case-control or cohort studies; 2. adiponectin levels as exposure of interest; 3. endometrial cancer as end-point result; 4. sufficient data generated to calculate relative risk (RR) or odds ratio (OR) with 95% Confidence Interval (CI) and 5. provision of each category of adiponectin levels for dose-response analysis. A random-effect meta-analysis was carried out to assess the dose-response relationship between serum adiponectin levels and endometrial carcinoma using the methods suggested by Greenland and Longnecker (1992) and Orsini and Bellocco (2006). Twelve studies which included 5 prospective studies and 7 case-control studies involving 1916 endometrial carcinoma cases were included in this meta-analysis. The results demonstrated the risk of endometrial carcinoma was found to be decreasing by 3% for every 1 µg/ml increase in adiponectin levels. The authors concluded that higher serum adiponectin levels may have a protective effect against endometrial carcinoma, especially in post-menopausal women.

A similar meta-analysis by Zeng and colleagues (2015) also assessed the association between serum adiponectin concentrations and the risk of endometrial cancer. PubMed, Embase, the Chinese Biomedical Literature Database and the Science Citation Index were searched. Eight case-control studies, which included 1257 endometrial cancer patients and 1398 controls and four nested case-control studies including 659 endometrial cancer patients and 1398 controls were included. They reported serum adiponectin levels were inversely correlated with the risk of endometrial cancer development after pooling the case-control studies (OR=0.50, 95% CI: 0.39-0.60; $p<0.001$). The meta-analysis of the nested case-control studies did not support the link

between serum adiponectin level and endometrial cancer, although a correlation may exist in the subgroup of post-menopausal women (OR=0.81, 95% CI: 0.65-1.00; p=0.060) and particularly in post-menopausal women without current hormone replacement therapy (OR=0.62, 95% CI: 0.44-0.86; p=0.004). The meta-analyses on the association between adiponectin and endometrial cancer risk appear to support a positive effect; higher levels of adiponectin suggest a lower risk for endometrial cancer.

Wang and colleagues (2014) performed a meta-analysis to assess whether high leptin levels were an independent risk factor for endometrial cancer. PubMed, Web of Science and Embase databases were searched for suitable studies published up to June 2014. The pooled RR with 95% CI was used to assess the association between leptin levels and risk of endometrial cancer (RR=2.55, 95% CI: 1.91-3.41). The results found that a high leptin level was associated with an increased risk of endometrial cancer (RR=1.59, 95% CI: 1.27-1.98, p<0.001) and was an independent risk factor for endometrial cancer.

In addition, Gong and colleagues (2015) also conducted a meta-analysis into the associations between circulating adiponectin and leptin, the adiponectin-leptin ratio and endometrial cancer risk. PubMed and ISI Web of Science databases were searched for relevant studies. Eight case-control studies and 5 nested case-control studies involving a total of 1,963 endometrial cancer cases and 3,503 controls were included in the meta-analysis. The results from the meta-analysis supported the above findings of Wang *et al.* (2014); increased circulating levels of leptin concentrations were associated with an increased risk of endometrial cancer. They also concurred with the Zeng *et al.* (2015) study: increased circulating adiponectin and adiponectin/leptin ratio were associated with a decreased risk of endometrial cancer.

1.5 Research aims

Despite the current studies available in the literature on adiponectin and leptin, the role of intracellular signalling factors, such as TNFα, IL-6, IGF-I, IGF-II and insulin in the development of endometrial cancer, is still unclear. It is apparent that obesity is a risk factor for endometrial cancer; however, the molecules

that link obesity with its development are still to be defined. It is also known that not all women who are diagnosed with endometrial cancer are obese. This highlights the need for further studies to identify the role that intracellular signalling within the adipose tissue have, in the initiation and progression of endometrial cancer. Identification of these pathways may allow us to possibly intervene to prevent cancer development. This project aims therefore, to partially address this lack of understanding by carrying out, in the first instance, a systematic review/meta-analysis of primary studies related to the role of adipokines and inflammatory molecules in the development of endometrial cancer. Subsequently a clinical study was performed in a cohort of patients with endometrial cancer and different BMI to assess the correlation(s) between these molecular markers and obesity. Blood samples were taken and analysis of inflammatory molecules and adipokines was carried out.

CHAPTER 2: ADIPOCYTOKINES AND THEIR RELATIONSHIP TO ENDOMETRIAL CANCER RISK: A SYSTEMATIC REVIEW AND META-ANALYSIS (AS PUBLISHED IN GYNECOLOGIC ONCOLOGY, SEE APPENDIX)

2.1 Introduction

The exact biological mechanism underlying the development of endometrial cancer is still poorly understood. In the UK, endometrial cancer is the 4th most common female cancer; approximately 9000 women were diagnosed with endometrial cancer in 2015 (Cancer Research UK, 2018). Worldwide, 320 000 new cases of endometrial cancer were diagnosed in 2012 (Endometrial Cancer Report, World Cancer Research Fund, 2013).

Obesity is a well-recognised risk factor for endometrial cancer; however, the relationship between obesity and endometrial cancer is complex and likely to involve multiple biological pathways. Sex steroid and insulin pathways, chronic inflammation and alterations in circulating levels of adipokines have all been suggested as potential mechanisms affecting endometrial cancer risk (Berstein *et al*., 2003, Slater *et al*., 2006, Choi *et al*., 2009). Elevated levels of endogenous oestrogens cannot justify alone the correlation between obesity and endometrial cancer. Moreover, experimental studies have shown that adipokines, associated with hyperinsulinemia and insulin resistance, and inflammatory cytokines, associated to increased adiposity, are also thought to be involved in the development of endometrial cancer (Soliman *et al*., 2006).

Adiponectin, leptin, tumour necrosis factor alpha (TNFα), interleukin 6 (IL-6), insulin-like growth factor I and II (IGF-I and IGF-II), also collectively termed adipocytokines, are hormones and cytokines secreted from adipocytes; the major cell type of adipose tissue. Several studies have attempted to demonstrate an association between the risk of endometrial cancer and these circulating adipocytokines (Modugno *et al*., 2005, Wu *et al*., 2014). Adiponectin has several important functions; one of which is the regulation of insulin and glucose metabolism. Often described as an insulin-sensitising adipocytokine (Berg *et al.*, 2001, Yamauchi *et al*., 2001), adiponectin promotes insulin secretion from the β cells in the pancreas (Kharroubi *et al*., 2003) and

facilitates the up-take of insulin in the liver by increasing peripheral tissue sensitivity to insulin. This is a result of increased fatty acid oxidation and the inhibition of glucose production from the liver (Lihn *et al*., 2005). Adiponectin also has anti-proliferative properties; it exerts its anti-tumour effects by activating AMP activated protein kinase (AMPK) which inhibits cell growth, angiogenesis and promotes apoptosis in malignant cells (Barb *et al*., 2007).

Leptin affects the activity of several cell types and its main function involves regulating energy intake and expenditure (Daley-Brown *et al*., 2015). It has a role in glucose metabolism, as well as in the immune system. Leptin is also secreted by cancer cells and its levels have been reported to be increased in endometrial cancer and hyperplasia compared to controls with normal endometrium (Cymbaluk *et al*., 2006).

TNFα and IL-6 are pro-inflammatory cytokines released by macrophages within adipose tissue and have been implicated in tumourigenesis. TNFα is an activator of NFκB, which promotes cellular proliferation and prevents apoptosis (Philip *et al*., 2004), whereas IL-6 initiates tumour development and progression through several pathways (Ara *et al*., 2010). Both cytokines have been reported to be increased in endometrial cancer (Bellone *et al*., 2005). IL-6 was found to be overexpressed in the stroma of endometrial cancer cells and TNFα was associated with poor survival (Smith *et al*., 2013, Uzan *et al*., 2017) However, other studies have not reported such an increase. Chopra and colleagues (1997) found no difference in the expression of IL-6 in endometrial cancer and at the various clinical stages.

IGF-I and IGF-II are growth factors involved in growth and development (Agrogiannis *et al*., 2014). They are expressed in the normal development of the endometrium and also stimulated by oestrogen in the uterus (Murphy *et al*., 1990). IGF is thought to play a role in the initiation of endometrial cancer due to oestrogen increasing the synthesis and expression of IGF-I which stimulates cell proliferation thereby initiating endometrial cancer (Majchrzak-Baczmanska and Malinowski, 2006). An association between increased IGF-II expression and endometrial cancer have been reported by Pavelic and colleagues (2007). In their study, they found an increase in IGF-II expression in advanced endometrial cancer compared to early stage endometrial cancer.

Although evidences from *in vitro* and *ex vivo* studies for a causal role of adipocytokines in endometrial cancer are available, results from epidemiological studies are inconsistent. Meta-analyses allow for better estimation of the relation that exists in the population than single studies mainly because of the increased amount of data and statistical power. Evidence from epidemiological studies investigating the relationship between circulating adiponectin and leptin concentrations and endometrial cancer risk have been previously summarized in a number of meta-analyses (Gong *et al*., 2015, Zeng *et al*., 2015 and Lin *et al*., 2017), however, to date, no meta-analysis has been performed in to the relationship between the pro-inflammatory cytokines, TNFα and IL-6, or growth factors, IGF-I and IGF-II, and the risk of endometrial cancer. This study further clarifies the association between circulating levels of leptin and adiponectin, and endometrial cancer, and aimed to systematically assess the relationship between cytokines (IL-6 and TNFα) and the growth factors (IGF-I and IGF-II) levels with endometrial cancer risk *via* a meta-analysis of observational studies.

2.2 Methods

2.2.1 Literature search

Meta-analysis was performed and reported by adopting the Meta-analyses of Observational Studies in Epidemiology (MOOSE) guidelines (Stroup *et al*. 2000). English-language manuscripts published between January 2000 and August 2018 were searched from the databases: Medline, Web of Science, Embase, Cochrane and CINAHL. The timeline chosen reflect the increase in number of publications on endometrial cancer and obesity available in the databases and when the searches were carried out, respectively. The following string of words was used for the literature search in all databases – cancer and endometrial and (obesity or BMI) and (adiponectin or TNF* or IGF-I or IGF-II or IL-6 or leptin).

2.2.2 Selection of studies and exclusion criteria

Published studies were included if they met the following criteria: i) the study used an epidemiologic study design (e.g. case–control, case-cohort, nested case-control and cohort study); ii) the study provided information on circulating adiponectin, leptin, TNFα, IL-6, IGF-I, IGF-II concentrations as exposure of interest; iii) the study reported endometrial cancer as the outcome of interest; and iv) the study reported usable risk estimates (e.g. odds ratio (OR), risk ratio or relative risk (RR) with 95% confidence intervals (CI) between circulating adipocytokines levels and endometrial cancer risk). In addition, if more than one study was conducted in the same population, the most recent report or the report with the most applicable estimates was selected for analysis.

Published studies were omitted by the following exclusion criteria: i) non epidemiological studies, reviews without original data, ecological studies, editorials and case reports; ii) the study reported the risk estimates that could not be summarized (i.e. reported the risk estimates without 95% CIs); and iii) the study reported exclusively on endometrial cancer mortality. All study selection and exclusion procedures were carried out by two independent investigators (P.E.E and G.B). If there was discordance, a third independent reviewer, G.A.B would make the final decision *(authors' initials as per publication)*.

2.2.3 Data Extraction

The following key data were extracted from each included study: first author's name, publication year, study region, study design, number of cases/controls, assay methods, risk estimates, and matched or adjusted factors including age, body mass index (BMI), menopausal status, whether they have had diabetes mellitus (DM) or hypertension, hormone replacement therapy (HRT) usage, parity or whether they smoked.

2.2.4 Statistical analysis

Review Manager software, Version 5.0, was used to perform the meta-analysis: inverse variance, odds ratio and random effect were chosen as statistical

method, effect measure and analysis model, respectively. The risk estimates were analysed as an estimation of OR or RR for simplicity. People with the levels of exposure in the top tertile were compared with those in the bottom tertile. If the highest tertile (T3) and the lowest tertile (T1) were not available from the individual studies (Petridou *et al.,* 2002, 2003; Cust *et al.,* 2007; Dossus *et al*., 2010, 2011; Wang *et al*., 2011; Friedenreich *et al*., 2012 and Ohbuchi *et al.,* 2014) then a scaling method similar to Danesh *et al*. (1998) and used by Gong *et al*. (2015) was used: a scaling factor of 2.18 divided by 2.54 times the log OR for comparison of the top and bottom quartiles, or a scaling factor of 2.18 times the log OR for 1 standard deviation difference in the baseline levels of adiponectin or leptin. In addition, some of the studies (Soliman *et al*., 2006; Erdogan *et al*., 2013) used the highest category of adiponectin rather than the lower category as comparison: an effective count method described by Hamling and colleagues (2008) was therefore used to transform the comparison to the lowest tertile (T1). To assess the relationship between circulating adipocytokines and the risk of endometrial cancer, the summary of odds ratio (SOR) with 95% CI was estimated. This was performed using a random effect model of analysis. Chi-Squared test was used to assess the variation across the studies which were included in the forest plots. Heterogeneity across the studies was analysed using the I^2 statistics (Higgins *et al*. 2002) and results were defined as heterogenous for an $I^2 > 50\%$. All statistical tests were two-sided. $P<0.05$ was considered to be statistically significant.

Heterogeneity of the study results was explored by using stratified analyses and subgroup analyses. These analyses included design of the study, fasting status for the collection of the blood samples and the type of assay method used. Subgroup analyses to identify potential confounders included BMI, hypertension, diabetes and menopausal status. A variable was considered confounding if there were found to be significantly associated with endometrial cancer $p<0.05$ on the univariate analysis. Sensitivity analysis was performed to assess the influence of individual studies on the pooled OR and 95% CI by excluding each study in turn.

2.3 Results

2.3.1 Search Results and publication characteristics

The database searches identified 473 publications. A total of 427 studies were excluded on title and abstract review as they did not meet the inclusion criteria as shown in Figure 2.1. The remaining 46 studies were reviewed for further details and full text retrieved. Twenty-six studies were excluded for not containing OR values, risk ratio or relative risk with 95% CI. Therefore, a total of 20 articles were included in this meta-analysis, which corresponded to 18 studies involving 2921 endometrial carcinoma cases and 5302 controls. Seven articles reported circulating levels for leptin, 14 for adiponectin, 3 for TNFα, 3 for IL-6 and 1 for IGF-I. No article reported values for IGF-II. The characteristics of these studies, all published between 2002 and 2015, are presented in Tables 2.1 and 2.2.

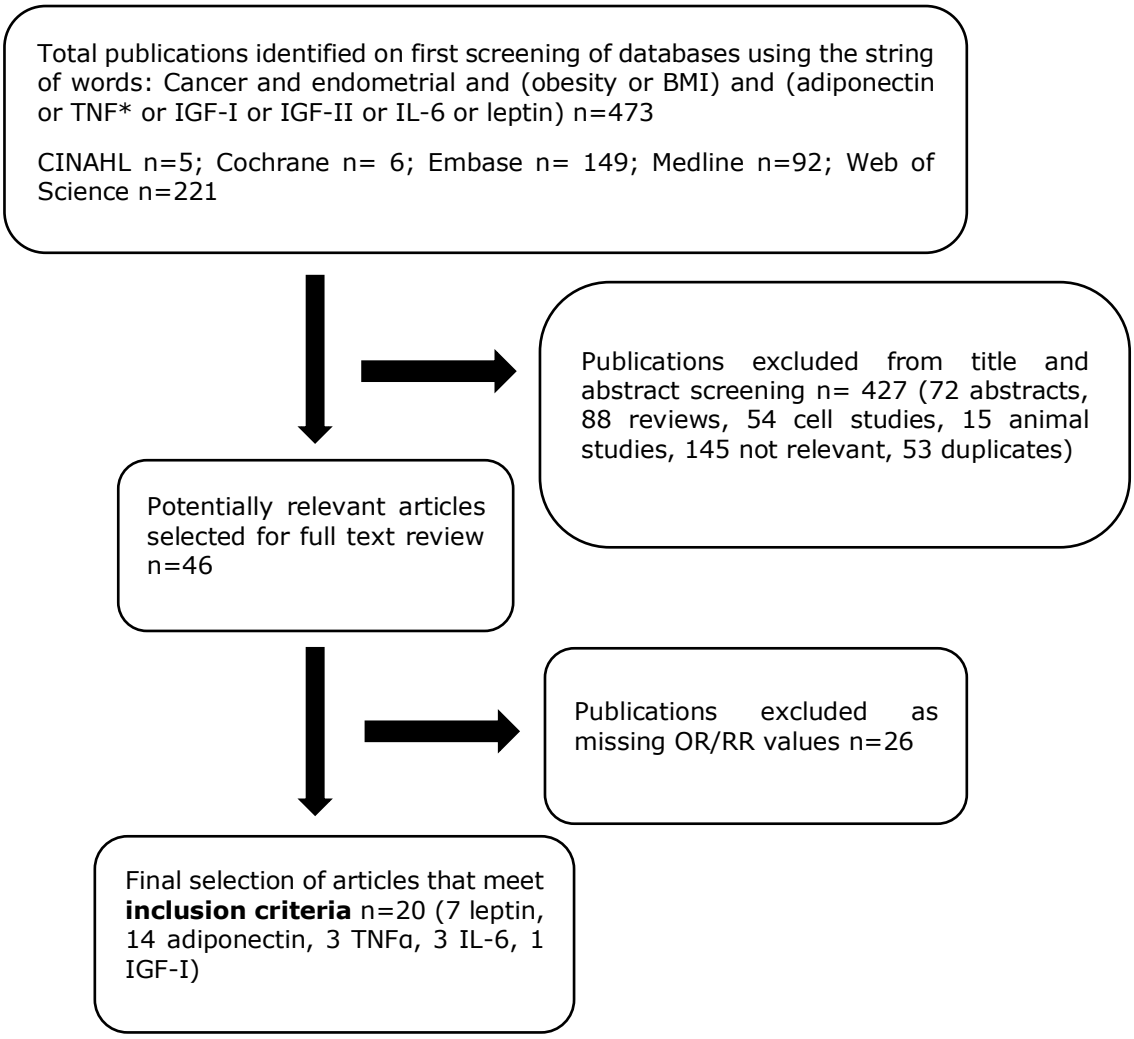

Figure 2.1 Flow diagram of screened, excluded and analysed publications

Table 2.1. Characteristics of included articles (n=20)

First author, Year, Study Country	Study design	No. of cases/controls	Biomarkers (assay method)	Risk Estimates (95% CI) Exposure categories	Adjusted factors
Retrospective studies					
Zhang, 2015 China	Case control	88/90	Adiponectin (ELISA)	OR 0.822 (0.759-0.889) Not specified in text	Age, BMI, WHR, diabetes, hypertension
Ohbuchi, 2014 Japan	Case control	43/62	Adiponectin (ELISA)	OR 1.987 (0.290-13.617) Q1 vs Q2	Age, BMI, diabetes, hypertension
Erodogan, 2013 Turkey	Cross sectional controlled study	60/70	Adiponectin (ELISA)	OR 10.64 (3.61-31.40) T1 vs T3	Age, BMI, HOMA-IR, QUICKI
Friedenreich, 2013 Canada	Case control	519/964	TNF-α (ELISA) IL-6 (ELISA)	OR 1.00 (0.84-1.18) OR 1.15 (0.89-1.48) Not specified in text	Age, BMI, nulliparity, physical activity, hypertension, alcohol consumption, hormone usage
Ma, 2013 China	Case control	206/310	Adiponectin (ELISA) Leptin (ELISA)	OR 0.52 (0.32-0.83) OR 2.05 (1.28-3.29) T3 vs T1	Age, BMI, glucose, cholesterol, triglycerides, HDL cholesterol, insulin, adiponectin (for leptin), leptin (for adiponectin)

First author, Year, Study Country	Study design	No. of cases/controls	Biomarkers (assay method)	Risk Estimates (95% CI) Exposure categories	Adjusted factors
Friedenreich, 2012 Canada	Case control	514/961	Adiponectin (ELISA) Leptin (ELISA)	OR 0.55 (0.37-0.80) OR 1.14 (0.73-1.77) Q4 vs Q1	Age, weight, waist to hip ratio, nulliparity, HRT, hypertension, glucose, insulin, adiponectin (for leptin), leptin (for adiponectin)
Ashizawa, 2010 Japan	Case control	146/150	Adiponectin (ELISA) Leptin (RIA)	OR 0.6 (0.3-1.2) OR 2.6 (1.4-4.9) T3 vs T1	Age, BMI, hypertension, diabetes
Soliman, 2006 USA	Case control	117/238	Adiponectin (ELISA)	OR 10.5 (4.18-26.35) T1 vs T3	Age, BMI, diabetes, hypertension,
Dal Maso, 2004 Italy	Case control	87/132	Adiponectin (RIA)	0.30 (0.14-0.68) T3 vs T1	Age, BMI, parity, education, HRT use, smoking status
Petridou, 2003 Greece	Case control	84/84	Adiponectin (RIA)	OR 0.78 (0.56-1.10) 1SD increment	Age, BMI, height, education, age at menarche, pregnancy, IGF-I, IGF-II, IGFBP-3 and leptin
Petridou, 2002 Greece	Case control	84/84	Leptin (IRMA)	OR 1.13 (0.70-1.81) 1SD increment	Age, education, height, age at menarche, menopausal status, history of pregnancy by outcome, alcohol and coffee consumption, smoking status
Prospective studies					
Wu, 2014 Taiwan	Nested case control	20/120	Adiponectin (ELISA) Leptin (ELISA)	OR 0.07 (0.01-0.62) OR 10.68 (2.09-54.67) T3 vs T1	Age, BMI, years of estrogen exposure

First author, Year, Study Country	Study design	No. of cases/controls	Biomarkers (assay method)	Risk Estimates (95% CI) Exposure categories	Adjusted factors
Soliman, 2011 USA	Nested case control	146/377	Adiponectin (ELISA)	OR 0.98 (0.57-1.68) T3vs T1	Age, BMI, parity, diabetes
Dallal, 2013 USA	Nested case control study	62/124	Adiponectin (ELISA) Leptin (ELISA)	OR 0.87 (0.39-1.94) OR 3.29 (1.41-7.69) T3 vs T1	Age, estradiol, C-peptide and BMI, diabetes
Luhn, 2013 USA	Nested case control	167/327	Adiponectin (RIA) Leptin (RIA)	OR 0.48 (0.29-0.80) OR 2.77 (1.60-4.79) T3 vs T1	Age, HRT, current smoking status, family history of breast and endometrial cancer, education, parity, diabetes, oral contraception use
Dossus, 2011 Europe	Nested case control	270/518	TNFα (ELISA)	OR 1.73 (1.09-2.73) Q4 vs Q1	Age, BMI, nulliparity, age at menopause, HRT use
Wang, 2011 USA	Case cohort	151/299	IL-6 (ELISA) TNFα (multiplex assay)	OR 0.70 (0.29-1.68) OR 1.65 (0.77 - 3.54) Q4 vs Q1	Age, BMI, Free IGF-I, estradiol, insulin
Dossus, 2010 Europe	Nested case control	305/574	IL-6 (ELISA)	OR 1.66 (1.08-2.54) Q4 vs Q1	BMI, C-peptide, estrone
Cust, 2007 Europe	Nested case control	284/548	Adiponectin (ELISA)	OR 0.63 (0.36-1.10) Q4 vs Q1	Age, BMI, C-peptide, IGFBP-1, IGFBP-2, SHBG, estrone, free testosterone

First author, Year, Study Country	Study design	No. of cases/controls	Biomarkers (assay method)	Risk Estimates (95% CI) Exposure categories	Adjusted factors
Lukanova, 2004 USA, Sweden, Italy	Case control	166/315	IGF-1(RIA)	OR 0.90 (0.44-1.82) Q5 vs Q1	Age, menopausal status, day of menstrual cycle for pre-menopausal women

BMI, body mass index; WHR, waist-to-hip-ratio; ELISA ,enzyme linked immunosorbent assay; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; IGF, insulin like growth factor; IGFBP, insulin like growth factor binding protein; SHBG, sex hormone binding globulin; HRT, hormone replacement therapy; OR , odds ratio; RIA, radio-immuno assay.

Table 2.2 Summary of characteristics of endometrial cancer cases and controls for included articles

	Cases								Controls							
Study ID	**N**	**AGE**	**BMI** (kg/m^2)	**Leptin** (ng/ml)	**Adiponectin** (µg/ml)	**IL-6** (pg/ml)	**TNF-α**(pg/ml)	**IGF-I** (mg/ml)	**N**	**AGE**	**BMI** (kg/m^2)	**Leptin** (ng/ml)	**Adiponectin** (µg/ml)	**IL-6** (pg/ml)	**TNF-α** (pg/ml)	**IGF-I (mg/ml)**
Retrospective studies																
Zhang, 2015	88	64.7±10.1b	n/a	n/a	8.29±4.07b	n/a	n/a	n/a	90	58.7±8.6d	n/a	n/a	12.43±5.34b	n/a	n/a	n/a
Ohbuchi, 2014	43	61.2±9.8 b	26.1±4.5b	n/a	4.91 (1.55-13.47)g	n/a	n/a	n/a	62	58.1±8.3d	23.3±3.8d	n/a	7.03 (2.86-26.06)	n/a	n/a	n/a
Erodogan, 2013	60	56.57±9.05b	31.12±4.18b	n/a	4.09 (0.67-43.55)e	n/a	n/a	n/a	70	49.7±7.59d	27.49±3.22d	n/a	17.13(2.59-108.69)e	n/a	n/a	n/a
Friedenreich, 2013	519	58.7	32.3	53.8	13.5	2.6	4.3	n/a	964	58.3	28.1	37.5	17.0	2.2	3.7	n/a
Ma, 2013	206	53.2 (26-81)d	n/a	28.8±2.2b	2.33±0.18b	n/a	n/a	n/a	310	53.3 (27-82)d	n/a	19.8±1.4b	2.58±0.15b	n/a	n/a	n/a
Friedenreich, 2012	514	59 (53, 65)a	31.0 (26.4, 36.8)a	44.2 (23.5, 72.4)a	11.6 (7.7, 17.3)a	n/a	n/a	n/a	962	59 (52, 66)a	27.2 (24.1, 30.9)a	28.2 (18.1, 47.5)a	14.6 (10.0, 21.5)a	n/a	n/a	n/a
Ashizawa, 2010	146	59.9±8.9b	23.7±4.5b	8.2±0.5h	6.2±0.4h	n/a	n/a	n/a	150	57.5±7.4b	22± 3.3b	4.5±0.5h	9.0±0.4h	n/a	n/a	n/a
Soliman, 2006	117	66.6(25-88)d	33.2	n/a	88.8±63.3 ng/mL b	n/a	n/a	n/a	238	61.2(50-80)d	28.0	n/a	148.2±68.3 ng/mL b	n/a	n/a	n/a
Dal Maso, 2004	87	62(34-78) g	27.8 (25.4-32) e	n/a	11.4(6.5-17.1)e	n/a	n/a	n/a	132	61(29-72) g	25.1(22.3-27.9) e	n/a	16.0 (8.4-22.5)e	n/a	n/a	n/a
Petridou, 2003	84	n/a	n/a	n/a	m/e	n/a	n/a	n/a	84	n/a	n/a	n/a	13.53±5.26b	n/a	n/a	n/a
Petridou, 2002	84	63.3±9.69b	29.2±5.72b	36.7±25.7b	n/a	n/a	n/a	n/a	84	62.6±11.3b	26.5±3.43b	26.9±19.8b	n/a	n/a	n/a	n/a

	Cases								Controls							
Study ID	**N**	**AGE**	**BMI** (kg/m^2)	**Leptin** (ng/ml)	**Adiponectin** (µg/ml)	**IL-6** (pg/ml)	**TNF-α**(pg/ml)	**IGF-I** (mg/ml)	**N**	**AGE**	**BMI** (kg/m^2)	**Leptin** (ng/ml)	**Adiponectin** (µg/ml)	**IL-6** (pg/ml)	**TNF-α** (pg/ml)	**IGF-I (mg/ml)**
Prospective studies																
Wu, 2014	38	44.3±8.5 b	n/a	22.53 (19.47-29.05) e	4.71 (3.95-6.62) e	n/a	n/a	n/a	11190	46.6±9.8 b	n/a	9.81 (6.16-14.56) e	8.92 (6.66-11.28) e	n/a	n/a	n/a
Soliman, 2011	146	57(47-67) d	27.2	n/a	12.88	n/a	n/a	n/a	377	57(47-67) d	25.5	n/a	12.85	n/a	n/a	n/a
Dallal, 2013	62	67.4±5.5b	29.5±6.9b	42.4 (12.5, 92.7)c	14.3 (6.7, 26.0)c	n/a	n/a	n/a	124	67.5±5.1b	26.8±4.7b	25.1 (8.3, 65.7)c	14.6 (11.0, 30.6)c	n/a	n/a	n/a
Luhn, 2013	167	66.4±5.7b	n/a	19.82 (7.55, 57.44)c	12.16 (6.79, 22.92)c	n/a	n/a	n/a	327	n/a	n/a	16.69 (5.98, 41.08)c	14.77 (7.49, 25.93)c	n/a	n/a	n/a
Dossus, 2011	270	57.0 (6.9) b	28.1(5.9)b	n/a	n/a	n/a	n/a	n/a	518	57.0(6.9)b	26.3(4.5) b	n/a	n/a	n/a	n/a	n/a
Wang, 2011	151	65.2 (7.1) b	29.7(7.8) b	n/a	n/a	2.2 (2.0) b	3.6 (5.2) b	n/a	299	63.5(7.5) b	27.5(5.8) b	n/a	n/a	2.1(2.0) b	3.3 (5.4) b	n/a
Dossus, 2010	305	56.9(7.3)b	27.5 (5.5) b	n/a	n/a	n/a	n/a	n/a	574	57.1(7.4) b	26.0(4.3) b	n/a	n/a	n/a	n/a	n/a
Cust, 2007	284	56.9(45.4-67.9) i	28.1(20.9-37.60) i	n/a	8.4(8.0-8.9) i	n/a	n/a	n/a	548	56.9(45.0-68.0) i	26.5(20.2-34.8) i	n/a	9.9(9.5-10.3) i	n/a	n/a	n/a
Lukanova, 2004	166	61(±7.8) b	27.3(26.5-28.0) f	n/a	n/a	n/a	n/a	169 (154.5-183.5)f	315	n/a	25.3(24.7-25.9) f	n/a	n/a	n/a	n/a	176.6 (164.4-188.8)f

a: median (25^{th}, 75^{th} percentile); b: mean ± SD; c: median (10^{th}, 90^{th} percentile); d: mean (range); e: median (interquartile range); f: mean (95% confidence interval); g: median (range); h : mean± SE; i: mean (5^{th}-95^{th} percentiles); n/a: not available

2.3.2 Adiponectin and its relationship to endometrial cancer risk

In this current meta-analysis, fourteen studies evaluated adiponectin and its relationship to endometrial cancer. Two thousand and twenty-four endometrial cancer cases and 3,593 controls were assessed in 9 retrospective studies (8 case control studies and, 1 cross sectional-controlled study) and 5 prospective studies (nested case control studies) (Table 2.1). Our combined data noted a significant relationship between adiponectin levels and endometrial cancer risk. The results suggest highest adiponectin levels compared to the lowest levels were significantly associated with a decreased risk of endometrial cancer. In particular, participants with adiponectin concentration levels in the highest tertile had a 0.51 times decreased risk of endometrial cancer compared to those women with adiponectin concentration levels in the lowest tertile, SOR 0.51, 95% CI: 0.38-0.69. There was significant heterogeneity, I^2=77% p<0.00001 (Figure 2.2).

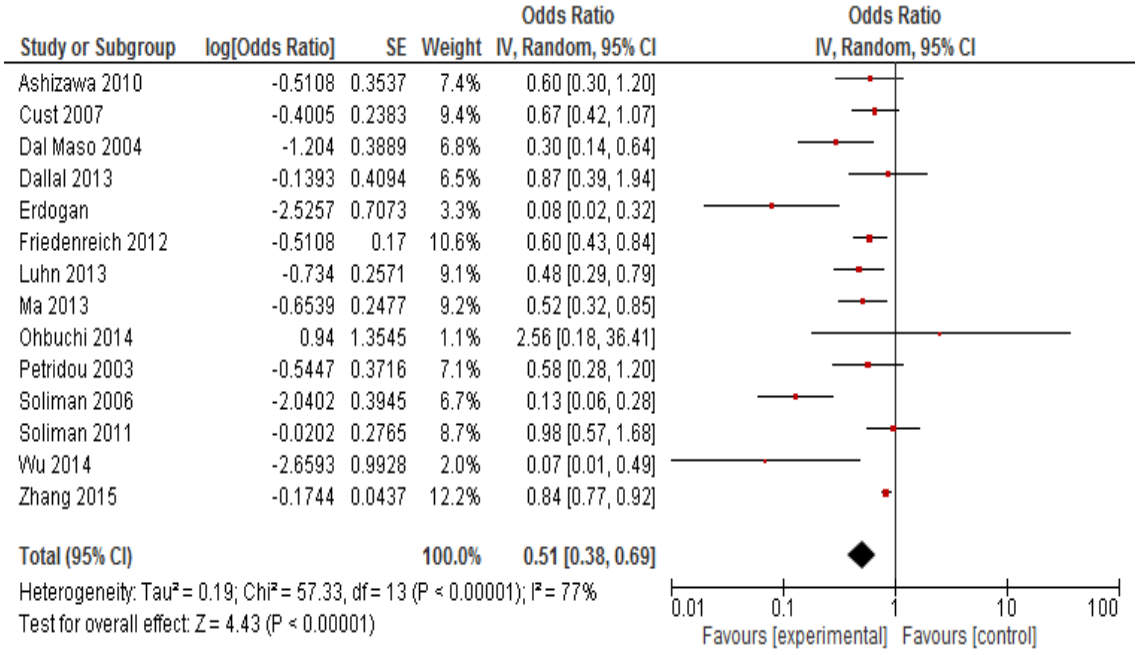

Figure 2.2 Forest plots of meta-analysis on the relationship between circulating adiponectin concentrations and endometrial cancer risk. The red squares represent the OR of the individual studies and the horizontal lines through the boxes represent the 95% CI. The overall treatment effect is represented by the black diamond.

Sensitivity analysis was performed to determine whether any particular study had a greater degree of influence between the association of the adiponectin and the risk of endometrial cancer. Omitting each study one at a time and analysing the SOR of the rest of the studies, the SOR ranged from 0.48 (95%

CI: 0.35-0.66, I^2=79%, p<0.00001) when omitting Soliman *et al*. (2011) study to 0.81 (95% CI: 0.75-0.87, I^2=85% p<0.00001) when omitting Cust *et al*. (2007). No one single study had a larger influence over the other studies when assessing the association between adiponectin and endometrial cancer risk. Stratifying by study design revealed a SOR of 0.64 (95% CI: 0.41-0.99, p=0.06) for prospective studies and a SOR of 0.45 (95% CI: 0.29-0.68, p<0.00001) for the retrospective studies (Table 2.3). The heterogeneity was lower for the prospective studies (56%) compared to the retrospective studies (83%). There were variations in the type of blood samples used as well as the method used to measure the concentration of adiponectin. Six studies used fasting samples to measure adiponectin and in the other 8, it was not clear whether the blood samples were fasted or postprandial. The point estimate for studies using fasting samples was 0.56 and for the non-fasting studies, it was 0.48. Eleven studies used an ELISA to measure adiponectin concentrations and 3 studies used RIA/IMRA. The point estimate of SOR in the studies using the ELISA method was similar to the studies using RIA/IMRA (0.53 *vs* 0.45). When comparing prospective studies *versus* retrospective studies, there was significant difference in the retrospective studies (p<0.00001 *vs* p=0.06). Similarly, when comparing fasting blood samples *versus* non-fasting blood samples, the non-fasting samples were significant (p<0.00001 *vs* p=0.31). The studies using ELISA demonstrated statistical significance (p<0.00001 *vs* p=0.45).

Raised BMI, hypertension, diabetes and menopause are all risk factors for endometrial cancer. Sub-analyses were performed to assess for potential confounding factors (Table 2.3). When considering BMI, the association between adiponectin levels and endometrial cancer risk is maintained (SOR 0.49, 95% CI: 0.34-0.71, I^2=79%, p=0.00001) but not for post-menopausal status. When considering hypertension or diabetes, a statistically significant association with endometrial cancers was maintained in the groups with diabetes and hypertension and in those without these conditions (Table 2.3).

Table 2.3 Summary of OR of the association between adiponectin and endometrial cancer stratified by study characteristics

	Adiponectin				
	No of study	**SOR**	**95% CI**	**I^2**	**p value**
Overall	14	0.51	0.38-0.69	77%	p<0.00001
Subgroup analyses					
Study design					
Prospective	5	0.64	0.41-0.99	56%	p=0.06
Retrospective	9	0.45	0.29-0.68	83%	p<0.00001
Fasting status					
Fasting blood samples					
Yes	6	0.56	0.42-0.74	16%	p=0.31
No	8	0.48	0.30-0.74	84%	p<0.00001
Assay method					
ELISA	11	0.53	0.38-0.57	79%	P<0.0001
RIA/IMRA	3	0.45	0.31-0.65	0%	p=0.45
BMI					
Yes	12	0.49	0.34-0.71	79%	p=0.00001
No	2	0.56	0.42-0.74	0%	p=0.47
Hypertension					
Yes	5	0.50	0.22-1.12	75%	p=0.003
No	9	0.50	0.35-0.75	62%	p=0.0008
Diabetes					
Yes	7	0.6	0.38-0.94	79%	p=0.03
No	7	0.44	0.30-0.65	60%	p=0.02
Menopausal status					
Post-menopausal	3	0.68	0.48-0.97	0%	p=0.78

2.3.3. Leptin and its relationship to endometrial cancer risk

A total of seven studies; four retrospective and three prospective, assessed the association between circulating leptin concentrations and the risk of endometrial cancer (Table 2.4). Three studies were nested case controls, one was a hospital-based control study, one population-based case control study and two were case control studies. One thousand, one hundred and ninety-nine endometrial cancers cases and 2076 control participants were assessed in the seven studies. The forest plot of the combined data (Figure 2.3) demonstrated a summary of OR of 2.19, 95% CI: 1.45-3.30, p<0.0002. These results suggest a significant difference between the risk of developing endometrial cancer in individuals with the highest leptin levels *versus* the lowest levels. Women with leptin concentrations in the highest tertile had 2.19 times increased risk of endometrial cancer compared to the women with leptin concentrations in the lowest tertile. There was variation between the studies, which was considered heterogenous, I^2=64%, p=0.01.

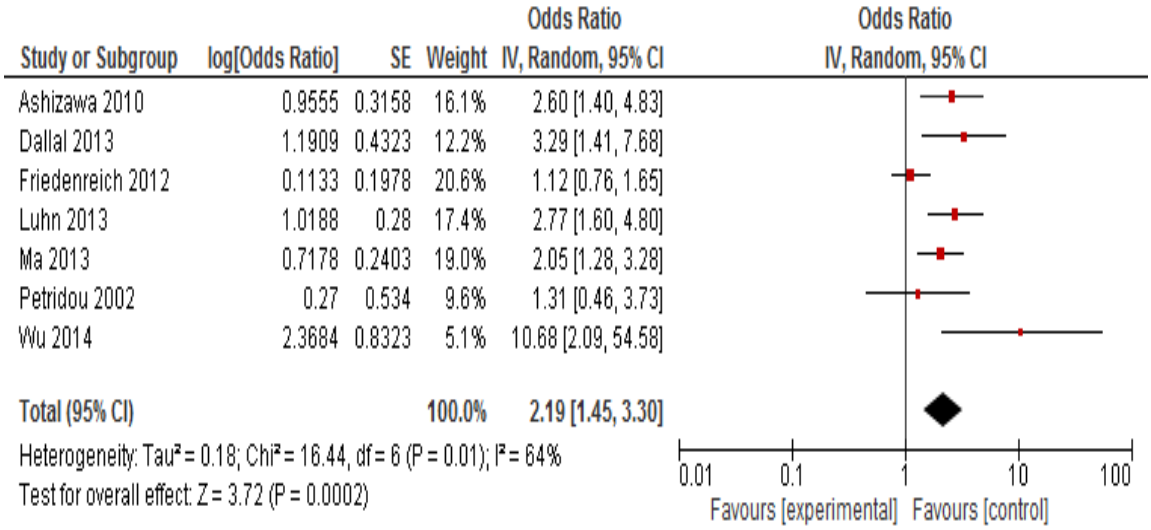

Figure 2.3 Forest plots of meta-analysis on the relationship between circulating leptin concentrations and endometrial cancer risk. Red squares represent the OR of the individual studies and the horizontal lines through the boxes represent the 95% CI. The overall treatment effect is represented by the black diamond.

Sensitivity analysis was performed to determine whether any particular study had a greater degree of influence between the association of leptin and the risk of endometrial cancer. Omitting one study at a time and analysing the SOR of the rest of the studies, the SOR ranged from 1.99 (95% CI: 1.37-2.91, I^2=58%, p<0.0003) when omitting Wu *et al*. (2014) to 2.48 (95% CI: 1.89-3.27, I^2=9% p<0.00001) when omitting Friedenreich *et al*. (2012). No single study had a larger influence over the other studies when assessing the association between leptin and endometrial cancer risk.

When stratifying by study design, the prospective studies had a higher SOR of 3.32 (95% CI: 1.98-5.56, I^2=15%, p=0.31) compared to the retrospective studies SOR of 1.67 (95% CI: 1.09-2.57, I^2=56%, p=0.08). There were variations between the type of samples used and the measurement of leptin concentration; 4 studies used fasting blood samples and 3 used a post prandial sample. When comparing the type of samples, the point estimate of SOR for studies using non-fasting blood samples was higher (3.32 *vs* 1.67) than the SOR for studies using fasting blood samples. The concentration of leptin was either measured using an ELISA or RIA in 4 and 3 studies, respectively. In a further study, leptin was measured using an IMRA. The point estimate of SOR for studies using ELISA was 2.27 and for the studies using RIA/IMRA was 2.45. Both pre and post-menopausal women were included in these studies. Other

factors that were matched/adjusted included BMI (n=4), hypertension (n=3) and a history of diabetes (n=4). Table 2.4 describes the stratified analyses by study characteristics. When BMI is not considered, the overall association between leptin levels and the risk of developing endometrial cancer is not lost, p=0.03 (SOR 1.61, 95% CI: 0.84-3.11, I^2=72%). When considering patients with hypertension, the overall association between leptin levels and the risk of endometrial cancer is maintained. The OR is not affected in comparison with the overall SOR (Figure 2.3) when considering patients with hypertension, p=0.02 (SOR 1.99, 95% CI: 0.98-4.04) and also patients with diabetes, p=0.01 (SOR 2.15, 95% CI: 1.23-3.76). There was significant heterogeneity (I^2=76%) in those studies that recorded hypertension compared to those studies that did not (I^2=42%). For those studies that recorded the presence or absence of diabetes in its participants, the heterogeneity was higher (I^2=74%) compared to those studies that did not record diabetes (I^2=57%).

Table 2.4 Summary of OR of the association between leptin and endometrial cancer stratified by study characteristics

	Leptin				
	No of study	**SOR**	**95% CI**	**I^2**	**P value**
Overall	7	2.19	1.45-3.30	64%	p=0.01
Subgroup analyses					
Study design					
Prospective	3	3.32	1.98-5.56	15%	p=0.31
Retrospective	4	1.67	1.09-2.57	56%	p=0.08
Fasting blood samples					
Yes	4	1.67	1.09-2.57	56%	p=0.08
No	3	3.32	1.98-5.56	15%	p=0.31
Assay method					
ELISA	4	2.27	1.16-4.42	75%	p=0.07
RIA/IMRA	3	2.45	1.67-3.59	0%	p=0.45
BMI					
Yes	4	2.69	1.76-4.12	27%	p=0.25
No	3	1.61	0.84-3.11	72%	p=0.03
Hypertension					
Yes	3	1.99	0.98-4.04	76%	p=0.02
No	4	2.42	1.47-3.97	42%	p=0.16
Diabetes					
Yes	4	2.15	1.23-3.76	74%	p=0.01
No	3	2.43	1.03-5.69	57%	p=0.10
Menopausal status					
Post-menopausal	3	2.80	1.93-4.05	0%	p=0.91

2.3.4 TNFα, IL-6 and IGF-I and their relationship to endometrial cancer risk

The paucity of studies analysing TNFα, IL-6 and IGF-I and their association with the risk of endometrial cancer is evident (Tables 2.1 and 2.2). Two studies (one prospective and one retrospective) assessed both TNFα and IL-6 in a single cohort and a further 2 studies (both prospective) assessed TNFα and IL-6 only. There was only one study (prospective) investigating the role of IGF-I and the risk of endometrial cancer. The total number of endometrial cancer and control cases for TNFα was 940 and 1781 respectively, and for IL-6, it was 975 and 1837, respectively. The prospective study assessing IGF-I and its correlation with endometrial cancer risk had 166 cases and 315 controls. From the meta analyses, there appeared to be no association between circulating levels of TNFα and IL-6 and overall risk of developing endometrial cancer (SOR=1.30, 95% CI: 0.87-1.95, SOR=1.21 95% CI: 0.85-1.72 respectively) (Figures 2.4 and 2.5).

Heterogeneity was present for TNFα studies but not for IL-6 studies (p=0.06 and I^2=65% for TNFα and p=0.14 and I^2=48% for IL-6).

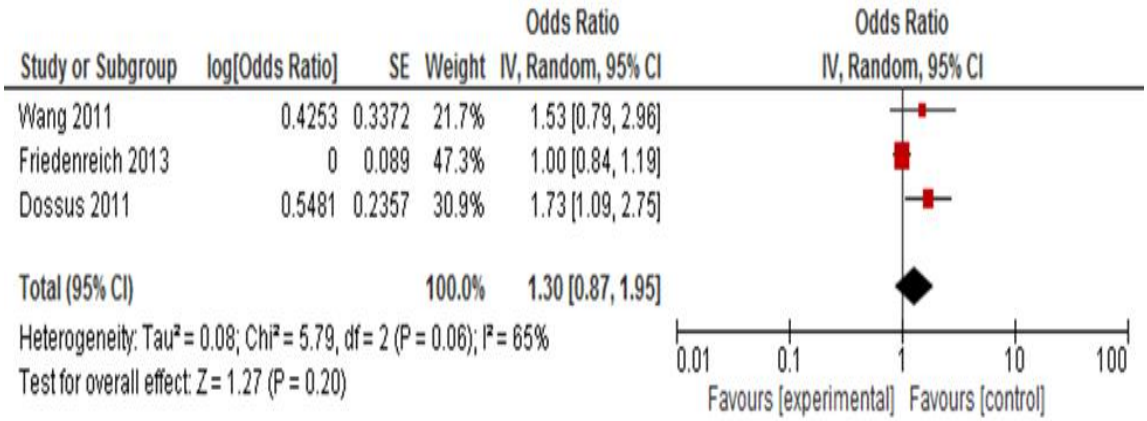

Figure 2.4 Forest plots of meta-analysis on the relationship between TNFα concentrations and endometrial cancer risk. The red squares represent the OR of the individual studies and the horizontal lines through the boxes represent the 95% CI. The overall treatment effect is represented by the black diamond.

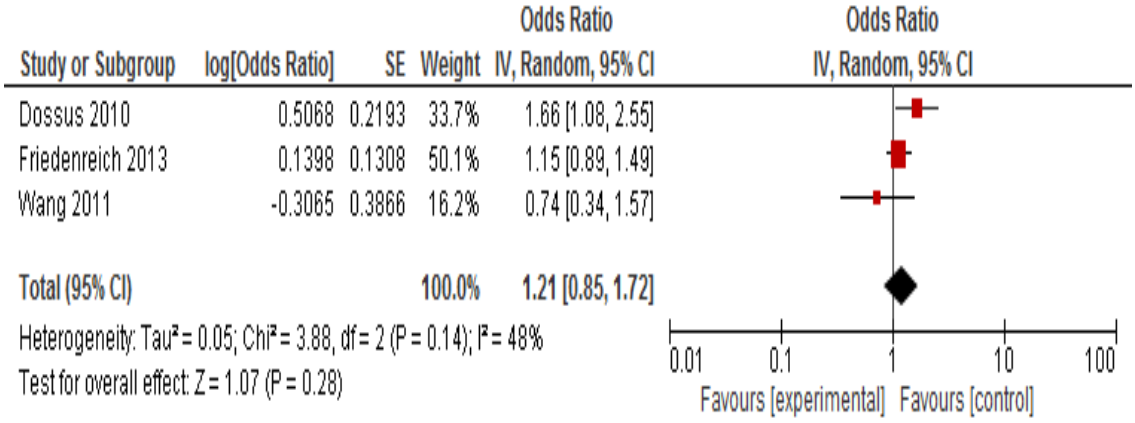

Figure 2.5 Forest plots of meta-analysis on the relationship between IL-6 concentrations and endometrial cancer risk. The red squares represent the OR of the individual studies and the horizontal lines through the boxes represent the 95% CI. The overall treatment effect is represented by the black diamond.

Sensitivity analysis was performed to determine whether any single study had a greater degree of influence between the association of TNFα and the risk of endometrial cancer. When Wang *et al*. (2011) was excluded, the SOR was 1.26 (95% CI: 0.74-2.14, I^2=79%, p=0.03); excluding the study performed by Freidenreich *et al*. (2013), the SOR was 1.66 (95% CI: 1.14-2.43, I^2=0%, p=0.77) and finally excluding the study performed by Dossus *et al*. (2011), the SOR was 1.09 (95% CI: 0.78-1.53, I^2=33%, p=0.27). There are differences between the 3 studies which could explain the change in SOR; the Wang *et al*. study was a prospective study and the studies by Friedenreich *et al.* (2013) and Dossus *et al*. (2011) were retrospective and prospective studies, respectively. The participants in the Wang *et al*. (2011) study were postmenopausal women who were not using any hormone treatments. Both pre- and post–menopausal women were included in the other 2 studies and some participants in these 2 studies were also noted to be using hormones.

Sensitivity analysis was also performed to determine whether any single study had a greater degree of influence between the association of IL-6 and endometrial cancer risk. The SOR ranged from 1.08 (95% CI: 0.8-1.46, I^2=10%, p=0.290, when omitting the Dossus *et al*. (2010) to a SOR of 1.32 (95% CI: 0.93-1.88, I^2=52% p=0.15), when excluding the Wang *et al*. (2011) study. Excluding the Friedenreich *et al*. (2013) study, the SOR was 1.19 (95% CI: 0.54 - 2.59, I^2=69%, p=0.07).

2.4. Discussion

Inflammation, an important factor in the development and progression of cancer has been implicated in the link between obesity and cancer (Arendt *et al.,* 2013; Bowers *et al.,* 2015). Adiponectin, leptin, TNFα, IL-6 and IGF-I are biological factors that are involved in different stages of the inflammatory pathway. The aim of the present meta-analysis was to further clarify the link between adiponectin and leptin and endometrial cancer risk, and to assess the association between TNFα, IL-6, IGF-I and IGF-II and endometrial cancer risk by conducting a systematic review and meta-analysis of the published literature. The paucity of studies reported in the literature investigating the link between the adipocytokines and endometrial cancer is evident; between 2000 and 2018, only 20 publications were found in the literature that met the inclusion criteria set. Undertaking a systematic review and meta-analysis increased the population size which would enhance the accuracy and precision of the findings from the various studies. This would allow a greater understanding of the association between adipocytokines and endometrial cancer risk. Our analysis concurred with other reported studies (Luhn *et al*., 2013; Ma *et al*., 2013) on the association between adiponectin and leptin concentration levels and endometrial cancer risk: increased adiponectin serum levels and decreased leptin levels are associated with an overall decreased risk of endometrial cancer. It was found that women with higher levels of adiponectin had the risk of developing endometrial cancer decreased by half compared to those women with lower levels of adiponectin. Women with high levels of leptin had a two-fold increased risk of developing endometrial cancer compared to women with low levels of leptin. The studies reported by Dallal *et al.* (2013) and Soliman *et al*. (2011) did not find an association between adiponectin serum levels and endometrial cancer risk. Due to the increased numbers of cases and controls obtained in our meta-analysis, our findings are more robust compared to the single studies performed by Dallal *et al*. (2013) and Soliman *et al*. (2011). In addition, both studies were prospective, and slight differences between the prospective and retrospective studies were highlighted by the sub analyses carried out (Table 2.3). For adiponectin, the SOR was 0.64 for prospective studies compared to 0.45 for retrospective with statistical difference for retrospective studies ($p<0.00001$) and for leptin, the

SOR for prospective studies was 3.32 compared to 1.67 for retrospective studies.

The meta-analysis of the studies assessing IL-6 and TNFα did not demonstrate an association with endometrial cancer risk. Despite the limited number of studies, the number of endometrial cancer cases and controls were relatively high; 975 *vs* 1837 and 940 *vs* 1781 cases vs controls, respectively. However, when assessing the individual studies, Dossus *et al.* (2011), reported elevated levels of TNFα was significantly associated with an increased risk of endometrial cancer amongst women in the highest quartile compared to the lowest quartile. Similarly, Dossus *et al*. (2010) reported an increased risk of endometrial cancer with elevated levels of IL-6 whereas the studies conducted by Wang *et al.* (2011) and Friedenreich *et al.* (2013) did not find an association between TNFα and IL-6 and the risk of endometrial cancer. It is unclear why the findings of these studies were inconsistent in relation to the risk of endometrial cancer and levels of TNFα and IL-6. All the studies used ELISA to measure TNFα and IL-6. Both the studies conducted by Dossus *et al.* (2010; 2011) had pre-menopausal women (25% and 18.5% in the 2010 and 2011 studies, respectively) in their endometrial cancer cases. The average BMI of the cases in the studies was 27.5 kg/m^2 and 28.1 kg/m^2, respectively. The number of cases in the studies with a history of diabetes was very small; 4.6% and 3.4%, respectively. A history of hypertension was not recorded in either studies. The study conducted by Wang *et al*. (2011), included only post-menopausal women and non-users of hormones. The average BMI was 30 kg/m^2. A history of hypertension or diabetes was not recorded in the study. Friedenreich *et al*. (2013) consisted mainly of perimenopausal and post-menopausal women (89.4%) and 44.5% users of HRT. In their study, the average BMI was 32.3 kg/m^2 and 32% of the cases had a history of hypertension. A history of diabetes was not recorded. It appears that BMI, menopausal status or a history of diabetes/hypertension does not influences the association of TNFα or IL-6 and endometrial cancer risk.

Further sub-analyses were performed to identify any other factors that could affect the risk of endometrial cancer. Tables 2.3 and 2.4 summarises the OR of the association between circulating adiponectin, leptin and endometrial cancer stratified by study characteristics. BMI appeared to affect the

association between circulating adiponectin levels and endometrial cancer risk, but not with circulating leptin levels and endometrial cancer risk. Hypertension and diabetes appear to affect the association between circulating leptin levels and increased endometrial cancer risk. The presence or absence of hypertension and diabetes appeared to affect the association between circulating adiponectin levels and endometrial cancer risk. Adiponectin and leptin may act synergistically and increase the risk of endometrial cancer. This is not the case for TNFα and IL-6.

The strength of this meta-analysis is that it presents a relatively comprehensive review of the existing evidence on the association of various adipocytokines and endometrial cancer. In particular, stratified analysis using a variety of selected variables has strengthened the results against the influence of confounding factors. There were limitations to the meta-analysis; the number of cases in each study was relatively small, however the overall number of endometrial cancer cases in the meta-analysis was high, 2921, and the timeline selected for the searches could have been increased in order to include more studies. Retrospective studies were included and therefore, there is always a risk of potential bias in the form of recall bias.

Our meta-analysis was the first to assess 5 adipocytokines in relation to endometrial cancer risk. Larger prospective studies assessing the 5 adipocytokines are required to investigate further the association between adipocytokines and endometrial cancer. This would allow us to elucidate in more details, the exact mechanisms underlying the link between adipocytokines and endometrial cancer.

In chapter 3, 6 adipocytokines in blood samples taken from a cohort of patients with different BMI were assessed.

CHAPTER 3: CIRCULATING LEVELS OF ADIPOCYTOKINES IN WOMEN WITH ENDOMETRIAL CANCER

3.1 Introduction

Chronic low-grade inflammation is generally accepted to initiate the development of cancer due to the genetic damage caused by the release of reactive oxygen and nitrogen species. These species induce the formation of mitogenic adherent DNA lesions. This theory forms part of the intrinsic pathway mechanism where tumour cells stimulate the release of local inflammatory mediators promoting tumour growth and progression (Mantovani *et al.,* 2009). Inflammatory mediators can concomitantly affect endometrial cancer risk by promoting insulin resistance, hyperglycaemia and increase the production of oestrogen within the adipose tissue and the endometrium (Greenberg *et al*., 2002; Purohit and Reed, 2002; Dossus *et al*., 2012; Shaw *et al*., 2016).

Adipocytokine related signalling pathways are important in the development of an inflammatory microenvironment for tumours. This process is thought to increase the risk of endometrial cancer by inducing cell proliferation and the production of free radicals (Shaw *et al*., 2016). It has also been observed that adipocytokines can indirectly inhibit the DNA damage repair system and apoptosis (Pucino *et al*., 2014). Several studies have demonstrated an association between the risk of endometrial cancer and single circulating adipocytokines (Lukanova *et al.,* 2004; Friedenreich *et al*., 2013; Gong *et al*., 2015). However, no study to date has been carried out to assess the association of several adipocytokines together in the same patient population and in relation to their obesity status.

3.2 Aim

To assess the circulating levels of adiponectin, leptin, tumour necrosis factor alpha (TNFα), interleukin 6 (IL-6), insulin-like growth factor I (IGF-I) and II (IGF-II) in serum from women with endometrial cancer and to determine whether there was a correlation between serum adipocytokine levels and BMI.

3.3 Materials and Methods

3.3.1 Blood specimens

In 2018, patients from the Royal Surrey County hospital, with a new diagnosis of confirmed endometrial cancer, were invited to take part in the retrospective study titled "Discovering new cancer biomarkers from patient's blood, urine and DNA" (study Number 08/H1306/115). Ethical approval was granted by the Royal Surrey Hospital. Patient information sheet and consent form are documented in the appendix.

Blood samples were taken on the morning of surgery from patients undergoing a Da Vinci robotic assisted hysterectomy and bilateral salpingo-oophorectomy with or without pelvic lymph node sampling. The blood samples were immediately processed by centrifugation and serum stored at – 80°C at the University of Surrey. Serum samples from twenty-four patients with a diagnosis of endometrial cancer, one with endometrial hyperplasia and 2 control participants without endometrial cancer or hyperplasia, were collected and analysed for circulating levels of adiponectin, leptin, TNFα, IL-6, IGF-I and IGF-II.

Patient history and clinical examination were recorded for all patients. Data collected on clinical characteristics included age, BMI, type of endometrial cancer, menopausal status and any medical conditions, such as, hypertension or diabetes. Where possible, white cell counts (WCC) and C-reactive protein (CRP) levels were also assessed to determine signs of inflammation.

3.3.2 ELISA

Sandwich ELISA (enzyme-linked immune-sorbent assay) is a very useful tool in quantifying antigens and requires the use of a capture antibody, specific for an antigen, bound to a microplate well. The protein mixture is added to the well and the specific antigen binds to the capture antibody. After several washes to remove any nonspecific binding, a detection antibody, linked to biotin, is added. Following the removal of the excess detection antibody through repeated washes, Streptavidin-Horse Radish Peroxidase (HRP) conjugate is then added that binds to the detection antibody. Subsequently, a substrate is added, which is then converted by the HRP enzyme to form a colour signal. The intensity of the colour signal within the final product is proportional to the concentration of antigen present in the original sample and is measured using a microplate reader.

Adipocytokines were measured using a standard ELISA protocol, following manufacture guidelines: human Adiponectin (DY1065); human Leptin (DY398), human TNFα (DY210), human IL-6 (DY206), human IGF-I (DY291) and human IGF-II (DY292) (Biotechne, UK).

A capture antibody specific to each adipocytokine was first dissolved in phosphate buffer saline (PBS) without carrier protein and then diluted to a final working concentration (Table 3.1). One hundred µl of diluted capture antibody were then added per well in a 96-well microplate. The plate was sealed and incubated overnight at room temperature. Each well was aspirated and washed 3 times with 300 µl wash buffer (0.05% Tween 20 in PBS) using a multichannel pipette. At each step, the wash was completely removed by tapping the plate and blotting it on paper towels. Reagent diluent (300 µl) was added to each well to block any non-specific binding and left at room temperature for 1 hour. A further 3 washes were performed as before with wash buffer in preparation for the addition of samples or standards (Table 3.1).

One hundred µl of standard (Table 3.1) or samples were added to each well, covered and incubated for 2 hours at room temperature. The standards and samples were aspirated and the wells washed (x3) as previously stated. This was followed by the addition of the detection antibody (100 µl), diluted in reagent diluent (Table 3.2), to each well, covered with a new plate cover and

incubated for two hours. A repeated aspiration and wash (x3) were performed. One hundred µl of Streptavidin–HRP (Table 3.2) was added to each well and incubated for 20 minutes at room temperature, covered in foil to avoid exposure to light.

Table 3.1 Details of the capture antibodies, the concentration range of standards and sample dilution used for determination of adipocytokine concentrations by ELISA

	Capture antibody stock concentration	Capture antibody working concentration	Standard stock concentration	Standard range	Sample dilution
Adiponectin	360 µg/ml	2 µg/ml	340 ng/ml	4000-62.5 pg/ml	1:800
Leptin	480 µg/ml	4 µg/ml	200 ng/ml	2000-31.3 pg/ml	1:100
TNFα	720 µg/ml	4 µg/ml	370 ng/ml	1000-15.6 pg/ml	Neat
IL-6	360 µg/ml	2 µg/ml	120 ng/ml	600-9.4 pg/ml	Neat
IGF-I	480 µg/ml	4 µg/ml	110 ng/ml	2000-31.2 pg/ml	1:5
IGF-II	240 µg/ml	2 µg/ml	85 ng/ml	1500-23.4 pg/ml	1:5

Table 3.2 Details of the detection antibodies concentration, Streptavidin HRP and reagent diluent for the determination of adipocytokine concentrations by ELISA

	Detection antibody stock concentration	Detection antibody working concentration	Streptavidin HRP dilution	Reagent diluent
Adiponectin	180 µg/ml	1 µg/ml	1:200	1% BSA in PBS*
Leptin	1.5 µg/ml	25 ng/ml	1:40	1% BSA in PBS*
TNFα	90 µg/ml	500 ng/ml	1:200	1% BSA in PBS*
IL-6	9 µg/ml	50 ng/ml	1:200	1% BSA in PBS*
IGF-I	9 µg/ml	150 ng/ml	1:40	5% Tween20 in PBS*
IGF-II	12 µg/ml	200 ng/ml	1:40	5% Tween20 in PBS*

*PBS pH 7.2-7.4

This was followed by a repeat aspiration and wash (x3), and then the addition of 100 µl substrate solution (substrate solution A and B). Plate was covered with foil and incubated again for 20 minutes at room temperature. Finally, 50 µl Stop Solution was added to each well. The optical densities of the standards and samples were obtained using a microplate reader (BioTek, UK) set to 450 nm and with 540 nm for correction.

3.3.3 Statistical analysis

A two-tailed t test, using EXCEL 2016, was used to assess the differences between the groups. A $p<0.05$ was considered statistically significant. The percentage change in adipocytokine levels was described and calculated by comparing the average adipocytokine concentration levels in the obese and OW groups and comparing it to the adipocytokine levels in the NW groups. The formula: % change = change divided by the original value x100 was used.

3.4. Results

3.4.1 Participant characteristics

Twenty-four patients with a diagnosis of endometrial cancer, one with endometrial hyperplasia and 2 patients without endometrial cancer were included in this study. Participant characteristics were recorded for obese, overweight (OW) and normal weight (NW) cases with endometrial cancer and endometrial hyperplasia (Table 3.3) and control participants without endometrial cancer (Table 3.4). A history of hypertension or diabetes and the use of HRT were also recorded. The average age of the women with endometrial cancer was 69 ± 2 years old.

Seventy-two percent of the women with endometrial cancer had Type 1 (endometrioid) endometrial cancer. All except one case had stage I or II disease. The average age of the obese and OW cases was 71 ± 2 years and 70 ± 2 years respectively and the average BMI was 37.1 ± 1.7 kg/m^2 and 27.3 ± 0.6 kg/m^2, respectively. The NW cases were younger compared to the other weight categories with an average age of 59 ± 9 yeas and average BMI of 21.8 ± 1.1 kg/m^2. There was a significant difference between the age of the obese cases and the NW cases ($p=0.025$) but not when comparing the OW cases with the NW cases ($p=0.225$). One explanation for the increasing age in the obese group could be due to the fact that, as we become older, most people gain weight which in turn increase the incidence of obesity related cancers in both

men and women and also chronic illnesses such hypertension, diabetes and cardiovascular disease (Zhen *et al*., 2017). The control cases had an average age of 66.5 years and BMI of 28.1 kg/m^2 (n=2).

Within the obese category, 4 out of 16 cases had hypertension and 3 out of 16 had diabetes. One of the obese cases had both co-morbidities. In the overweight group, 3 out of 5 patients had hypertension and 2 out of 5 had diabetes. Two of the cases described in the overweight group had both co-morbidities. The 4 NW cases had neither hypertension nor diabetes. One out of the 2 control cases had both co-morbidities. WCC and CRP levels were analysed in each patient to assess signs of inflammation and thus linking chronic inflammation to the development of endometrial cancer. Two out of the 16 obese cases had a raised CRP of 14 mg/L and 32 mg/L, respectively (normal level <10 mg/L) and one of the 5 NW cases also had a raised CRP of 20 mg/L. The OW cases and the control cases did not have raised CRP levels and none of the cases in any of categories or control cases had raised WCC (normal range =4-11 mg/L). Twenty three patients did not use HRT. One patient used HRT in the form of local oestrogens in the vagina and for the remaining patient, we were unable to locate the clinical notes.

Table 3.3 Clinical characteristics of women with endometrial cancer and endometrial hyperplasia

	Age years	**BMI**	**Grade and Type of EC**	**CRP** mg/L	**WCC** 10*9	**Hypertension**	**Diabetes**	**M status**	**Use of HRT within last 3 years**
Obese cases n = 16									
Case 1	63	31.2	Grade 2 Type 1	5.6	4.3	No	Yes	PM	No
Case 2	73	32.5	Grade 3 Type 2	<4	7.4	No	Yes	PM	No
Case 3	71	45.9	Grade Type 1	<4	9	Yes	Yes	PM	No
Case 4	64	42.7	Grade 1 Type 1	X	7.8	No	No	PM	No
Case 5	51	32	Grade 2 Type 1	X	6.4	No	No	PM	No
Case 6	75	31.2	Hyperplasia	X	8.5	No	No	PM	Vaginal oestrogen
Case 7	74	47.9	Grade1 Type 1	<4	7.8	Yes	No	PM	No
Case 8	71	30.9	Grade 2 Type 1	<4	8.6	No	No	PM	No
Case 9	73	40.5	Grade 2 Type 1	14	5.9	No	No	PM	No
Case 10	78	31.6	Grade 3 Type 2	<4	5.8	No	No	PM	No
Case 11	76	30	Grade 3 Type 1	32	9.9	No	No	PM	No
Case 12	74	36.8	Grade 3 Type 2	<4	6.8	No	No	PM	No
Case 13	75	48.3	Grade 3 type 1	<4	10.3	Yes	No	PM	No
Case 14	78	30	Grade 1 Type 1	<4	6.3	No	No	PM	No
Case 15	66	38	Grade 3 Type 2	<4	6.8	No	No	PM	No
Case 16	74	43.6	Grade 3 Type 2	X	7.8	Yes	No	PM	No
Overweight cases n = 5									
Case 17	70	27.7	Grade 1 Type 1	4	6.6	Yes	No	PM	No
Case 18	77	25.2	Grade1 Type 1	9.7	5	No	No	PM	No
Case 19	71	27.3	Grade 2 Type 1	4	8.6	Yes	Yes	PM	No
Case 20	65	29.2	Grade 3 Type 2	8	6.4	No	No	PM	No
Case 21	68	27.3	Grade 2 Type 1	4	8.1	Yes	Yes	PM	No
Normal weight cases n = 4									
Case 22	36	19.2	Grade 1 Type 1	20	8.5	No	No	Pre-M	No
Case 23	55	23.7	Grade 1 Type 1	X	6.6	No	No	PM	No
Case 24	65	21	Grade 3 Type 1	4	7.2	No	No	PM	Unknown
Case 25	80	23.4	Grade 1 Type 1	4	5.1	No	No	PM	No

CRP: C-reactive Protein (normal range = <10); WCC: White cell count (normal range = 4-11); X not measured; M status: menopausal status; Pre-M: pre-menopausal and PM: post-menopausal.

Table 3.4 Clinical characteristics of women without endometrial cancer

	Age Years	**BMI**	**WCC** 10*9	**CRP** mg/L	**Hypertension**	**Diabetes**	**M Status**	**Use of HRT within 3 years**
Controls without EC n = 2								
Control 1	65	32	10.9	4	Yes	Yes	PM	No
Control 2	68	24.2	5.1	8	No	No	PM	No

3.4.2 Circulating levels of adipocytokines

The concentration levels of the adipocytokines in the obese, OW and NW patients are presented in Figures 3.1 to 3.6. Serum levels of the individual adipocytokines are compared between each BMI category.

The mean adiponectin concentration was 2.30 ± 0.15 µg/ml for the obese cases, for the OW cases, the mean concentration was 2.2 ± 0.12 µg/ml and for the NW cases patients, it was 2.26 ± 0.14 µg/ml (Figure 3.1).

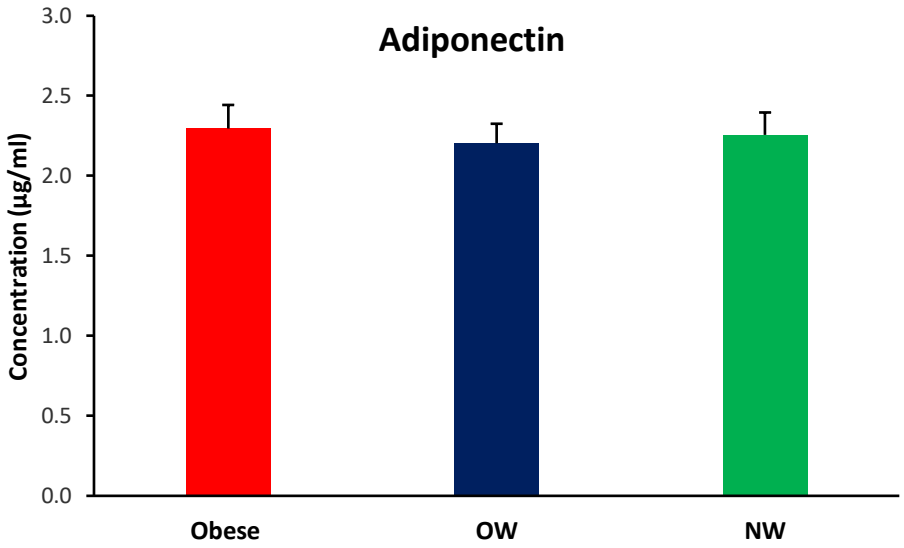

Figure 3.1 Adiponectin levels in different weight categories in cancer patients. Results are presented as mean±standard deviation. Obese group n=16, overweight group (OW) n=5, normal weight group (NW) n=4.

When comparing these results with the adiponectin level in the NW cases, there was a 1.8% increase in the obese cases and a 2.6% decrease in the OW cases. There was no significant difference (p=0.63) between the adiponectin

concentration levels in the obese category and the NW category and between the OW group and the NW group (p=0.56).

Leptin concentration was statistically significantly higher (p=0.0065) in the obese group compared to the NW group and when compared to the OW group (p=0.0039). No significant differences were observed when comparing the OW group to the NW group (p=0.55). In the obese group, leptin concentration level was 59.41 ± 28.72 ng/ml, 16.23 ± 5.19ng/ml in the OW group and 14.30 ± 3.67 ng/ml in the NW group (Figure 3.2).

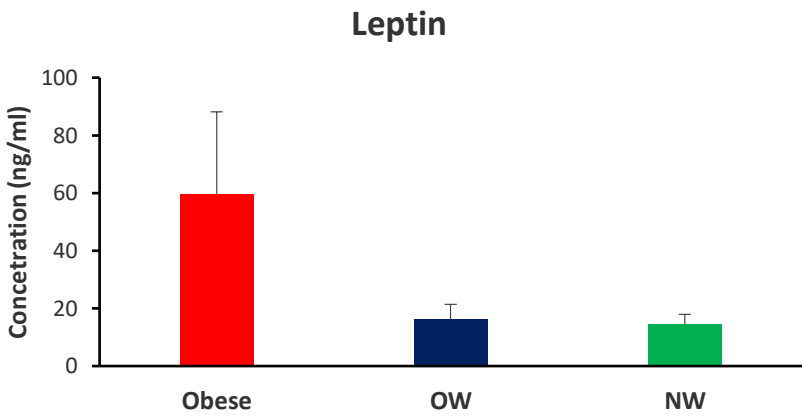

Figure 3.2 Leptin levels in different weight categories in cancer patients**.**
Results are presented as mean± standard deviation. Obese group n=16, overweight group (OW) n=5, normal weight group (NW) n=4. p= 0.0065 in the obese group compared to the NW group. p=0.0039 in the obese group compared to the NW group.

This equated to an increase of 315% in leptin concentration levels in the obese cases and 13.6% increase in the OW group when compared to the NW group.

TNFα levels were significantly higher in the obese category compared to OW group (p=0.01). In the obese cases, the mean TNFα level was 704 ± 459.7 pg/ml, in the OW group, it was 78.54 ± 15.85 pg/ml. There was only one value obtained for the NW group, which was 216 pg/ml (Figure 3.3). When comparing the change in TNFα mean concentrations between the obese and the NW groups, there was 226% increase in the concentration levels but a decrease of 63.4% in the TNFα levels between the OW and NW.

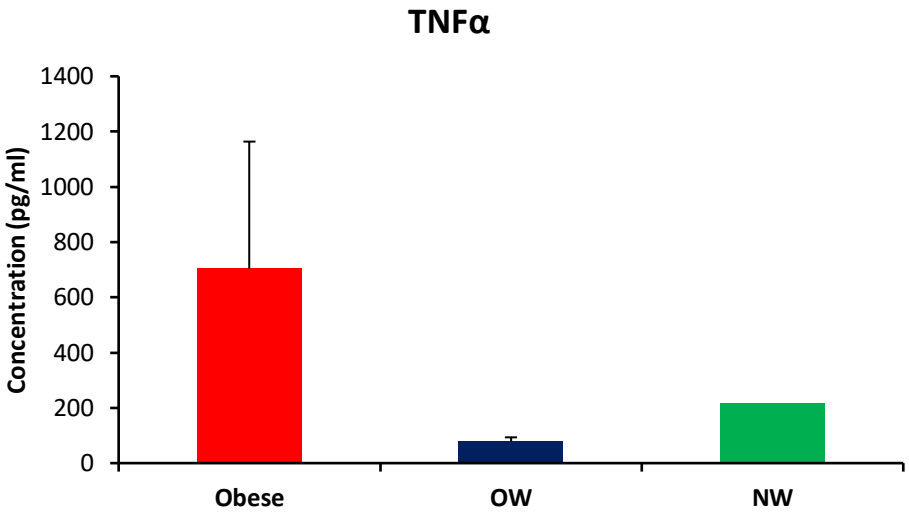

Figure 3.3 TNFα levels in different weight categories in cancer patients**.**
Results are presented as mean± standard deviation. Obese group n=16, overweight group (OW) n=5, normal weight group (NW) n=4.

IL-6 mean concentration levels in the obese category were also higher compared to the other groups but no statistical comparison could be made as only one value was available in the OW and NW groups respectively. The concentrations were 182.45 ± 188.99 pg/ml in the obese group, 2.42 pg/ml and 0.6 pg/ml in the OW and NW groups, respectively (Figure 3.4).

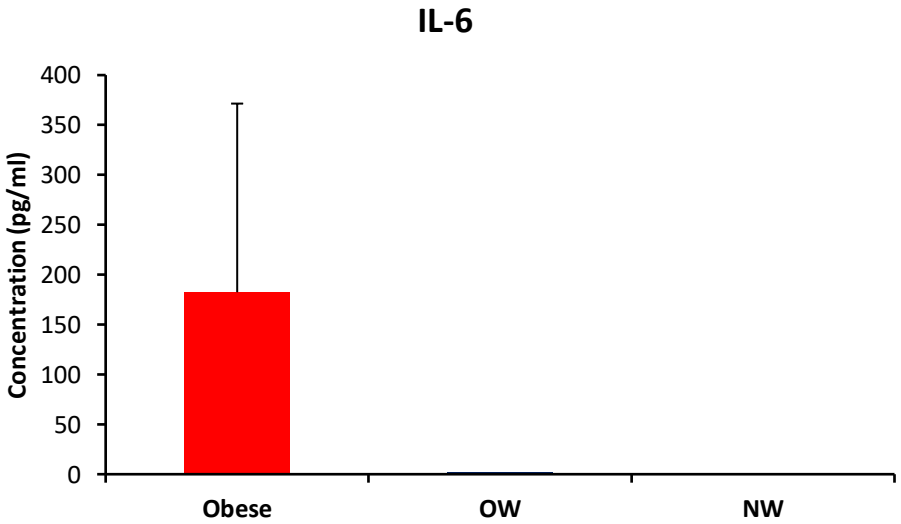

Figure 3.4 IL-6 levels in different weight categories in cancer patients.
Results are presented as mean± standard deviation. Obese group n=16, overweight group (OW) n=5, normal weight group (NW) n=4. IL-6 concentrations were below detection levels in serum from some of the OW and NW groups.

IGF-I mean concentration levels in the obese and OW groups demonstrated an increase when compared to the mean concentration of IGF-I in the NW group. In the obese and OW groups, the IGF-I concentrations were 948.57±984.58 ng/ml and 376.25 ± 150.26 ng/ml respectively. There was a statistical difference (p=0.04) between the IGF-I levels in the obese cases compared to the OW group. There was only one value (11.5 ng/ml) in the NW group and therefore no statistical comparison could be made (Figure 3.5).

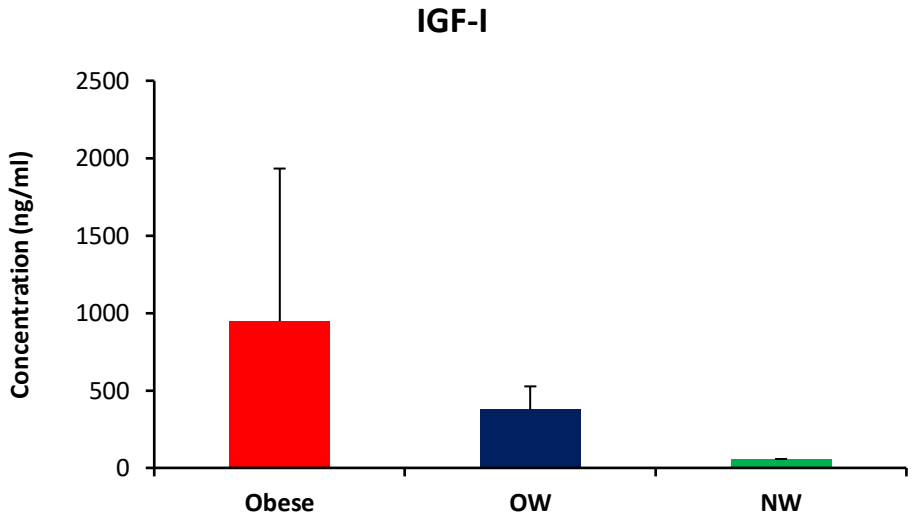

Figure 3.5 IGF-I levels in the different categories in cancer patients**.**
Results are presented as mean± standard deviation. Obese group n=16, overweight group (OW) n=5, normal weight group (NW) n=4. p=0.04 between the obese category and OW group.

As demonstrated for IGF-I, a similar increase in IGF-II concentrations were also noted in the obese and OW groups when comparing the concentration levels to the single value of 13.21 ng/ml in the NW group. In the obese and OW groups, the IGF-II concentrations were 4200 ± 3745 ng/ml and 733 ± 943 ng/ml, respectively. The difference between IGF-II levels in the obese group and the OW group were statistically significant (p=0.049) (Figure 3.6).

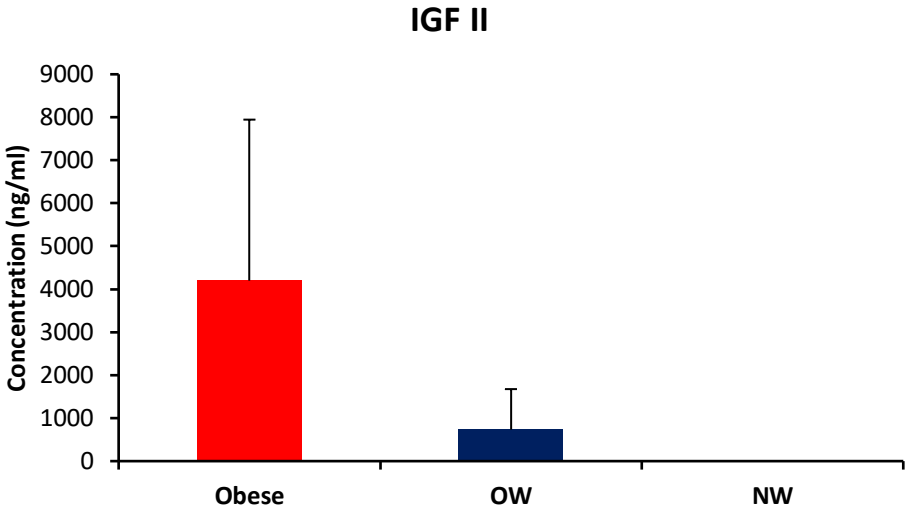

Figure 3.6 IGF-II levels in the different weight categories in cancer patients**.** Results are presented as mean± standard deviation. Obese group n=16, overweight group (OW) n=5, normal weight group (NW) n=4. IGF-II concentrations were below detection levels in serum of NW patients. p=0.049 between the obese category and OW group.

3.5 Discussion

Women with endometrial cancer have been reported to have a higher incidence of cardiovascular disease risk factors compared to women in the general population (Felix *et al*., 2010). Hypertension and diabetes are independent risk factors for endometrial cancer and women with diabetes have a 2–fold increased risk of developing endometrial cancer compared to women without diabetes (Kitson *et al.,* 2018). In the current study, 28% and 20% of women with endometrial cancer/ hyperplasia had hypertension and diabetes, respectively. It was also noted that 63% of the obese patients had Type 1 endometrial cancer which increased to 80% of patients in the OW group and 100% in the NW group.

This observation is in contradiction to the general theory that suggests the aetiology of Type 1 (endometrioid) endometrial cancers is the result of excess oestrogen production in the adipose tissue, stimulating neoplastic growth in the endometrial lining (Britton *et al*., 2016), however, the numbers (25) are too small to draw any clear conclusions from these findings.

It has been reported that there is an association between CRP concentrations and the development of cancer (Heikkila *et al*., 2007; Allin *et al*., 2009). Most

evidence can be drawn from studies on colorectal cancer (Tsilidis *et al*., 2008; Pendyala *et al*., 2011). Zhou and colleagues (2014) reported, in their meta-analysis of 18 studies, a higher risk of colon cancer with increasing concentration of CRP. However, they did not assess the association between colon cancer CRP and different BMI. To our knowledge, there has been no study which has assessed the relationship between CRP, WCC concentrations and endometrial cancer risk. In our study, the highest CRP level above the normal threshold of 10 mg/L, were higher in the obese category (32 mg/L compared to 9.7 mg/L and 20 mg/L in the OW and NW groups, respectively).

In the obese state, hypertrophic adipocytes secrete abnormal levels of pro inflammatory cytokines, which include TNFα and IL-6, with a decrease production of anti-inflammatory markers such as adiponectin (Smitka *et al*., 2015). The anti-inflammatory properties of adiponectin are well characterised; it promotes the secretion of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist and reduces the production of TNFα (Wolf *et al*., 2004). Adiponectin levels are reduced in the obese state, which in turn further increase the pro-inflammatory cytokines, TNFα and IL-6. Adiponectin levels are also reduced in postmenopausal women as well as in chronic illnesses such as hypertension, diabetes and coronary artery disease (Mather *et al*., 2014; Riestra *et al*., 2015; Lai *et al*., 2015).

In this current study, adiponectin levels in the obese patients did not correlate with the results observed in other studies in the literature (Cust *et al*., 2007; Ashizawa *et al*., 2010). There was no significant difference ($p=0.63$ in the obese *vs* NW and in the OW *vs* NW, $p=0.56$) in adiponectin levels amongst the different weight categories in the endometrial cancer patients. Increasing BMI was associated with decreasing adiponectin levels ($p<0.0001$) which was also associated with endometrial cancer cases in the study conducted by Cust *et al*. (2007). In their study, the mean adiponectin concentration was 8.4 µg/ml in the endometrial cancer cases and 9.9 µg/ml in the control cases. In their obese cancer group, adiponectin levels were 6.6 µg/ml, 8.6 µg/ml in the OW and 10.4 µg/ml in the NW groups. In the current study, the mean adiponectin concentration levels in the endometrial cancer cases was 2.27 µg/ml, 2.3 µg/ml in the obese group, 2.2 µg/ml in the OW and 2.26 µg/ml in the NW categories. Ashizawa *et al*. (2010), demonstrated similar findings to Cust *et al*. (2007);

women with endometrial cancer had significantly lower adiponectin levels (6.2 ± 0.4 µg/ml) in the endometrial cancer cases, than in the control (9.0 ± 0.4 µg/ml), ($p<0.0001$). Adiponectin levels significantly correlated with BMI ($p<0.05$). In the current study, the adiponectin levels in the endometrial cancer cases were low which supports the hypothesis that lower adiponectin levels are associated with increased risk of endometrial cancer. It is worth noting that, in different cancers, studies have reported findings similar to our results i.e. no differences in the levels of adiponectin and different categories of BMI in cancer patients. Serratta *et al*. (2019) did not find any differences in the association between BMI and circulating levels of adiponectin and leptin in prostate cancer. The lack of differences between levels in different BMI categories could be due to a number of factors; 84% of our patients were in the obese and OW groups. Our findings may be due to a relatively homogenous group of patients underpowering the study for a relatively small number of NW patients. Also, except for one patient, all the women with endometrial cancer had reached menopause and an average age of 69 ± 2 years. The oldest endometrial cancer cases were in a study conducted by Dallal *et al.* (2013), mean age 67.4 ± 5.5 years and the youngest endometrial cancer cases were in the study by Wu *et al*. (2014), mean age 44.3 ± 8.5 years. Adiponectin levels are known to be reduced in postmenopausal women due to the aging process (Mathers *et al.,* 2014). The different assay techniques used for determination of adiponectin concentration compared to other studies may have also influenced the results: Petridou *et al*. (2003) and Dal Maso *et al*. (2004) used RIA to measure adiponectin concentrations. Interestingly, Ma *et al.* (2013) also had the same adiponectin levels in their endometrial cancer cases. Their mean adiponectin levels in the endometrial cancer cases were 2.33 µg/ml. In the current study, it was also 2.3 µg/ml. Both studies used an ELISA to measure adiponectin levels and the study design was retrospective in each study. However, the mean age of the endometrial cancer patients was different; 53.2 years in the Ma *et al*. (2013) study and 69 years in the current study.

In the present study, adiponectin levels were measured after the diagnosis of endometrial cancer was made rather than prior to diagnosis. The effect of the endometrial cancer on circulating levels of adiponectin, reverse causation, is

unknown. Moreover, all patients participated in the enhanced recovery programme (ERP) before surgery. ERP is an evidence based modern approach that all patients undergoing major surgery take part in. It helps people to recover more quickly after surgery. It requires all patients undergoing major surgery, taking a preloaded glucose drink the night before and on the morning of their surgery. It is well recognised that a person's nutritional status and or body weight can influence the serum concentration levels of adiponectin (Kopp *et al*., 2005; Agra *et al*., 2018). The glucose preload that patients are required to drink may have an indirect effect on the serum levels of adiponectin. It is known that adiponectin reduces glucose production in the liver by decreasing the levels of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase gene expression (Lihn *et al.,* 2005). What is not clear is the effect of a sudden surge of high levels of glucose in the form of the pre-load that patients are required to take before surgery, has on adiponectin levels. As ERP is a relatively new approach in surgery, it is unlikely patients in previous studies, would have participated in this programme.

Leptin plays a dynamic role in the regulation of appetite and energy expenditure and its secretion is dependent on the status of both these metabolic processes. It is widely accepted that leptin levels are increased in the obese state (Fredman and Halaas, 1998). Increased leptin levels promote low grade inflammation by elevating levels of IL-6 and TNFα within the adipose tissue microenvironment (Cabone *et al*., 2012 Strong *et al*., 2015). IL-6 has been shown to promote the release of CRP by the liver, further enhancing a state of chronic low-grade inflammation (Pasceri *et al.,* 2000). Due to this effect of promoting an inflammatory state, raised leptin levels indirectly increase the risk of endometrial cancer by promoting cell proliferation and the release of free radicals that cause DNA damage (Coussens *et al*., 2002). Our results correlate with the findings of Cymbaluk *et al*. (2008); leptin levels were higher in the obese endometrial cancer patients compared to the OW and NW patients. In their study, Cymbaluk and colleagues assessed leptin serum levels in 86 obese post-menopausal women with endometrial cancer and hyperplasia and 40 controls. The obese women were subdivided into different BMI categories. The mean serum leptin level was 16737.1 pg/ml in the endometrial cancer group and 9048.7 pg/ml in the control group. They found a positive

correlation between serum leptin concentration and BMI. Significantly higher concentration levels of leptin ($p<0.005$) was reported with increasing BMI compared to the control group.

Increasing IL-6 levels have been reported to correlate with increasing BMI (Yudkin *et al*., 1999). IL-6 is also a stimulator of aromatase activity which increases the production of oestrogen in the adipose tissue (Zhao *et al*., 1995) and may increase and promote the development of endometrial cancer. In the current study, IL-6 circulating levels increased with increasing weight. It was higher in the obese category compared to the OW and NW groups. Similarly, TNFα was also higher in the obese category compared to the OW group. Leptin, TNFα and IL-6 were all increased in the obese cases which would support the evidence that leptin promotes low grade inflammation by increasing TNFα and IL-6 levels within the adipose tissue (Cabone *et al*., 2012 Strong *et al*., 2015). However, Wang *et al*. (2011), in their prospective study, did not report similar results; TNFα and IL-6 did not correlate with increasing BMI or endometrial cancer risk. It is unclear why there are conflicting results. Both the current study and the one by Wang *et al*. (2011) had endometrial cancer cases of a similar average age range and similar menopausal status. Both studies used ELISA to measure TNFα and IL-6 concentrations. One difference noted was that the Wang *et al*. (2011) study was a prospective study and the current study was a retrospective one which could explain the different results in each study. It is known that prospective studies reduce the occurrence of "reverse causation bias" and therefore the cancer cells could have modified the levels of the TNFα and IL-6 in the current study.

The overall number of studies assessing the associations between TNFα and IL-6 with obesity and endometrial cancer is small. Increasing the number of prospective studies, on assessing the association between obesity related endometrial cancer and these two pro-inflammatory cytokines, would enhance our knowledge further.

There have been conflicting reports in the literature regarding IGF-I and IGF-II levels in the obese state (Frystyk *et al*., 1995; Ricart *et al*., 2001; Gomez *et al*., 2004; Nam *et al*., 2014). IGF-I and IGF-II have important roles in growth metabolism and reproduction (Wolfe *et al*., 2014). The underlying mechanism

between IGF-I and IGF-II, endometrial cancer risk and the development of endometrial cancer is not well understood. IGF-I and IGF-II have been reported to be involved in the progression of endometrial cancer (Pavelic *et al*., 2007). Liang *et al.* (2012) reported higher IGF-I and IGF-II mRNA levels in tumour tissues and tumour adjacent tissues than in the control cells. Increased production of IGF-I in the liver is a result of hyperinsulinemia in the obese state. IGF-I is dependent on growth hormone secretion, which in turn is reduced in obesity; this would imply a reduction in IGF-I levels. However, it has been reported that total IGF-I levels remain the same due to increased hepatic sensitivity to growth hormones (Lewitt *et al*., 2014). Lukanova *et al*. (2004) reported no association between endometrial cancer risk and IGF-I levels. Their IGF-I levels in the endometrial cancer cases were lower: 169 ng/ml compared to our cases, which was 202.85 ng/ml. The study design of Lukanova *et al.* (2004) was prospective with an average age of the endometrial cancer cases of 61 years and a mean BMI of 27.3 kg/m^2. In the current study, the average age was 69 years and the mean BMI 32.7 kg/m^2. The mean concentration levels of IGF-I were raised in the obese and overweight categories compared to the normal weight and control. There was significant difference ($p=0.04$) between the obese and the OW groups. This could be due to the presence of hyperinsulinemia in the obese and OW groups, which would result in an increased production of IGF-I. This would account for the raised levels of IGF-I; however, only 3 of the 16 obese patients had diabetes and 2 out of the 4 patients in the OW group. None of the NW patients had diabetes. It could be argued that this is the result of the patients ingesting a glucose pre-load in the evening prior to surgery and in the morning of their operations. However, as the mean concentration levels of IGF-I in the NW group was similar to the control participants; this explanation for the higher levels of IGF-I is unlikely. The relationship between IGF-II and obesity and endometrial cancer risk is less clear. To our knowledge, there is no study in the literature that assesses the relationship between IGF-II and endometrial cancer in different weight categories. Frystyk *et al.* (1995) conducted a study which involved 92 (56 males, 36 females) healthy subjects and measured free serum and total IGF-II and IGF-I levels in obese cases. The participants were allocated to 3 groups depending on body BMI: 31 controls (BMI<25 kg/m^2), 33 subjects with moderate obesity (25<BMI<30 kg/m^2) and 28 subjects with severe

obesity (BMI >30 kg/m^2). Total IGF-II levels were increased in the obese subject ($p>0.05$) but the serum free IGF-II levels were unaltered in the obese state. In the current study, IGF-II levels were found to be increased in the obese state. There was a significant difference ($p=0.04$) between the obese group and the OW group.

Work conducted by the Bermano group (unpublished work) at Robert Gordon University, assessed the adiponectin, leptin, TNF-α and IL-6 concentration levels in twenty-two female volunteers: 6 obese, 6 OW and 10 NW cases. The mean age and BMI in the obese cases was 30.2 ± 5.4 years of age and the BMI 40.5 ± 2.5 kg/m^2; for the OW cases, it was 26.7 ± 5.6 years of age and the BMI 27.1 ± 1.6 kg/m^2; and the NW cases, 23.9 ± 4.8 years of age and the BMI 22.2 ± 1.5. The average adiponectin level in the obese cases was 9.98 ± 5.60 µg/ml; for the OW group it was 10.7 ± 6.18 µg/ml and for the NW group, it was 12.07 ± 8.27 µg/ml. Comparing these data to the current study findings, adiponectin levels were lower in the obese cancer cases (2.3 ± 0.15 µg/ml). The lower levels of adiponectin in the obese, OW and NW endometrial cancer cases compared to the healthy volunteers adds further to the argument that lower levels of adiponectin is associated with obesity and endometrial cancer. It is noted that the average age in the endometrial cancer cases, in the current study, is 69 years of age and it is known that adiponectin levels are reduced in postmenopausal women due to the process of ageing (Mathers *et al*., 2014). However, a recent study by Wang *et al.* (2019) assessed the clinical significance of serum adiponectin in 53 endometrial cancer patients with an average age of 54.93 ± 9.59 years and mean BMI of 25.68 ± 4.08 kg/m^2. Their mean adiponectin level was 2.09 ± 1.24 µg/ml, which was similar to our findings suggesting adiponectin levels may be affected by other factors, apart from the process of aging in women with endometrial cancer. Leptin levels followed similar levels of expression in the current study compared to the ones in the study from the Bermano group (unpublished work); in the healthy volunteers, leptin levels were highest in the obese category (78.81 ± 45.4 ng/ml) compared to the OW (21.95 ± 25.94 ng/ml) and NW (6.38 ± 4.08 ng/ml) categories. It is noted that the obese healthy volunteers have higher leptin levels than the obese endometrial cancer cases (78.81 ng/ml *vs* 59.4 ng/ml, $p=0.0125$). This result could be due to difference in age between the

obese healthy volunteers and the obese endometrial cancer group (average age 30 *vs* 69 years). Isidori and colleagues (2000) reported decreasing leptin levels with increasing age in women, with a consistent fall after the menopause (-21% $p<0.001$). Leptin concentrations fluctuate with the levels of oestrogen and progestogen, particularly in pre-menopausal women. Adiponectin, on the other hand, do not fluctuate in relation to the sex hormone levels during the menstrual cycle (Asimakopoulos *et al.*, 2009).

TNFα concentration levels in the Bermano group study (unpublished work) were raised in the obese compared to the OW and NW (90.81 ± 93.57 pg/ml for the obese group; 63.29 ± 109.29 pg/ml for the OW and 19.28 ± 24.10 pg/ml for the NW group). In the current study, TNFα levels were higher in the obese cases and in the NW participant (704 ± 459.7 pg/ml for the obese group and 216 pg/ml for the NW participant) compared to the results of the healthy volunteers. There was only one value for TNFα levels in the NW group. TNFα is raised generally in the obese state, however, the findings in the endometrial cancer cases, may suggest that, the higher levels promote the development of endometrial cancer. In contrast to our study findings, there was no difference in IL-6 concentration levels in the different weight categories in the healthy volunteers (obese group 74.03 ± 98.12 pg/ml, the OW 68.12 ± 92.39 pg/ml and the NW group, 85.54 ± 104.90 pg/ml) of the Bermano group study. In the current study IL-6 levels were 182.45 ± 188.89 pg/ml in the obese group, 2.42 pg/ml in the OW group and 0.6 pg/ml in the NW group. The IL-6 levels in the obese case was higher than in the obese healthy volunteer but did not reach statistical significance ($p=0.25$) but lower in the OW and NW cases compared to the OW and NW healthy volunteers. It is not immediately apparent why there are inconsistencies between IL-6 concentration levels in the study from the Bermano group and the current study. Park *et al*. (2004) assessed IL-6 levels in 46 obese subject and 54 non obese subject (both groups with a mean age of 36 years) and demonstrated that the median concentration of IL-6 were significantly higher ($p<0.05$) in the obese group compared to the non-obese group. IL-6 was measured using the ELISA method in the current study and by Park *et al.* (2005). It was not used to measure IL-6 levels in the Bermano group study. This may account for the difference in the results.

In conclusion, except for adiponectin, higher concentrations of leptin, TNFα, IL-6, IGF-I and IGF-II were observed in the obese patients with endometrial cancer compared to OW and NW patients. Our study is the first to assess 6 adipocytokines in a single sample of women of different weight categories, diagnosed with endometrial cancer. These preliminary results suggest differences in adipocytokines serum concentration levels between obese, overweight and normal weight women, which may play a role in the development of endometrial cancer, although further larger studies are required to elucidate the potential molecular pathways involved.

CHAPTER 4: DISCUSSION AND CONCLUSION

Altered cell metabolism and dysfunctional signalling pathways are a common occurrence in obesity and is an accepted hallmark of cancer (Hanahan and Weinberg, 2011). Adipocytokines are mediators in the initiation of neoplastic development and progression and may exert their cancer risk as a result of different biological effects. These include (1) altered plasma or serum concentrations of adipocytokines in cancer patients; (2) their contrasting expression of adipocytokines in cancerous and non-cancerous lesions; (3) their up-regulation of adipocytokines in malignant tissues; (4) the association of genetic polymorphism of adipocytokines genes with increased risk to certain cancers (Spyrou *et al*., 2018). The exact role of adipocytokines associated intracellular signalling pathways within adipose tissue, and the development of endometrial cancer, remains to be still unclear.

This project aimed to partially address this lack of understanding by carrying out a systematic review/ meta-analysis of primary studies related to the role of adipokines and inflammatory molecules in the development of endometrial cancer. By undertaking such a review/ meta-analysis it was hoped that further clarification into the relationship between endometrial cancer and circulating levels of adiponectin and leptin, and also the assessment into the relationship between exposure to cytokines (IL-6 and TNFα) and growth factors (IGF-I and IGF-II) with endometrial cancer risk could be determined.

A small clinical study was also undertaken to examine blood concentration levels of adiponectin, leptin, TNFα, IL-6, IGF-I and IGF-II in women with endometrial cancer and to assess whether CRP and WCC were elevated in women with endometrial cancer. It was also important to investigate whether there was a correlation between levels of the serum adipocytokines and BMI.

Association of circulating levels of adiponectin and leptin with endometrial cancer risk

A meta-analysis of fourteen studies evaluated adiponectin levels and its association to endometrial cancer risk. Two thousand and twenty-four

endometrial cancer cases and 3,593 controls were assessed in 14 studies. Our combined data noted a significant inverse relationship between adiponectin levels and endometrial cancer risk; SOR 0.51, 95% CI: 0.38-0.069, $p<0.00001$. The results suggest highest adiponectin levels compared to the lowest levels were significantly associated with reduced risk of endometrial cancer. Participants with adiponectin concentrations in the highest tertile had a 51% decreased risk of endometrial cancer compared to those women with adiponectin concentration levels in the lowest tertile. There was significant heterogeneity, $I^2=77\%$ $p<0.00001$.

Stratified and sub analyses were performed, and these did not alter the findings of increased adiponectin levels and a reduced risk of endometrial cancer.

Seven studies; four retrospective and three prospective, assessed the association between circulating leptin concentrations and the risk of endometrial cancer. One thousand, one hundred and ninety-nine endometrial cancers cases and 2076 controls were assessed in the seven studies. Our meta-analysis demonstrated increased levels of leptin was associated with endometrial cancer risk; SOR 2.19, 95% CI: 1.45-3.30, $p=0.01$. Women with leptin concentrations in the highest tertile had a 219% times increased risk of endometrial cancer compared to the women with leptin concentrations in the lowest tertile. There was variation between the studies, which was considered heterogenous, $I^2=64\%$, $p=0.002$. Stratified and sub analyses were also performed, and these results did not alter the findings that higher leptin levels were associated with a risk of endometrial cancer.

The meta-analysis results correlated with the clinical study with regards to adiponectin levels but not for leptin. In the clinical study, only 2 healthy control values were available for adiponectin and leptin measurement (mean control value for adiponectin 2.45 µg/ml and for leptin 21.3 ng/ml). However, when evaluating the individual studies in the meta-analysis, the control group generally had higher adiponectin levels than the endometrial cancer cases. Lower levels of adiponectin levels were found in the endometrial cancer cases compared to the healthy controls and therefore reinforced the association between low adiponectin levels and endometrial cancer. Moreover, when assessing adiponectin levels in plasma from endometrial cancer patients with

different BMI in the clinical study, there was no difference in adiponectin levels across the different weight categories, making it difficult to distinguish if it is the low adiponectin levels associated to cancer status which play a more important role in the initiation of endometrial cancer or the increase in BMI. Low circulating adiponectin levels result in reduced anti-proliferative effects thereby increasing tumour proliferation and decreased cell apoptosis. Higher leptin levels were noted in the obese endometrial cancer cases (59.41 ng/ml) compared to the healthy controls (mean value 21.3 ng/ml) but not in the OW and NW groups (16.23 ng/ml and 14.23 ng/ml, respectively) Higher leptin levels in the obese endometrial cancer cases suggest leptin may also be involved in the initiation and development of endometrial cancer.

Association of circulating levels of TNFα, IL-6, IGF-I and IGF-II with endometrial cancer risk.

There have been very few studies analysing the circulating levels of TNFα, IL-6, IGF-I and IGF-II and their association with the risk of endometrial cancer. Two studies assessed both TNFα and IL-6 in a single cohort and a further 2 studies assessed TNF-α and IL-6 only. There was only one study investigating the role of IGF-I and the risk of endometrial cancer. The total number of endometrial cancer and control cases for TNF-α was 940 and 1781 respectively and for IL-6, 975 and 1837, respectively. The prospective study assessing IGF-I and its correlation with endometrial cancer risk had 166 cases and 315 controls. The pooled results from the meta-analyses on TNFα and IL-6 studies demonstrated no significant association to endometrial cancer risk in patients with high levels of TNFα and IL-6 when compared to low levels; SOR 1.30 95% CI: 0.87-1.95, p=0.06 for TNFα and SOR 1.21 95% CI: 0.85-1.72, p=0.14 for IL-6.

In our clinical study, TNFα concentration levels were lower in the obese endometrial cancer cases compared to the single healthy control value that was available (704 pg/ml *vs* 826 pg/ml) and IL-6 concentration levels higher in the endometrial cancer cases compared to the healthy controls (182.45 pg/ml *vs* 75.91 pg/ml). However, when assessing TNFα and IL-6 circulating levels in the different weight categories, TNFα and IL-6 were both raised in the obese group compared to the OW and NW groups in the endometrial cancer

cases. Leptin can stimulate the production of TNFα and IL-6; higher leptin levels were found in the obese endometrial cancer cases compared to the OW and NW group. These results suggest that leptin may act synergistically with TNFα and IL-6 to increase the risk and development of endometrial cancer in women of different weight categories. Hyperleptinaemia, as a result of obesity, promotes low grade inflammation by increasing TNFα and IL-6 levels. The higher the BMI, the more TNFα and IL-6 is present to initiate the changes necessary for the development of endometrial cancer.

Assessment of blood concentration levels of adiponectin, leptin, TNFα, IL-6, IGF-I, IGF-II in women with endometrial cancer

Twenty-four patients with a diagnosis of endometrial cancer, one with endometrial hyperplasia and 2 patients without endometrial cancer were included in this study. There was no significant difference in the adiponectin serum concentration between the obese, OW and NW cancer patients.

Leptin concentration was significantly higher ($p=0.0065$) in the obese group compared to the NW group but not when statistically different comparing the OW group to the NW group ($p=0.55$).

TNFα levels were significantly higher in the obese category compared to OW group ($p=0.01$). IL-6 levels in the obese group were also higher (182.45 ± 188.99 pg/ml) than compared to the other groups; however, only one value was available in the OW and normal group respectively. Leptin may act synergistically with TNFα and IL-6 to increase the risk and development of endometrial cancer in women of different weight categories.

IGF-I concentration levels in the obese and OW categories demonstrated a dramatic increase when compared to the NW group. There was a statistical difference ($p=0.04$) between the IGF-I levels in the obese category compared to the OW category. The IGF-II levels between the obese group and the OW group was also statistically significant ($p=0.049$). Low adiponectin levels in the obese endometrial cancer case result in a decrease in IGFBPs. This may result in an increase in circulating IGF-I and IGF-II levels in the endometrial cancer cases. These results suggest that adipocytokines may act synergistically in the development of endometrial cancer.

Are CRP and WCC elevated in women with endometrial cancer and is there a correlation between levels of serum adipocytokines and BMI?

Only 2 out of the 16 obese patients had a raised CRP of 14 mg/L and 32 mg/L respectively (normal range ≤10 mg/L) and one of the 5 normal weight patients had a raised CRP of 20 mg/mL. These results suggest that CRP and WCC count are not routinely elevated in women with endometrial cancer. We assessed whether there was a correlation between levels of serum adipocytokines and BMI and apart from adiponectin, leptin, TNFα, IL-6, IGF-I and IGF-II were all raised in the obese group compared to the other weight categories (NW and OW). IL-6 stimulates the release of CRP and also the enzyme, aromatase, in the adipose tissue, which increases the production of oestrogen through its conversion of androstenedione to oestrone. It is apparent from the results that IL-6 in the endometrial cancer cases may not stimulate the release of CRP. However, IL-6 may be responsible for elevating the circulating oestrogen levels and therefore be involved in the development of endometrial cancer.

This study is the first to assess the serum concentration levels of 6 adipocytokines simultaneously and their relationship with obesity and endometrial cancer in a single project. The interaction of these adipocytokines (present in the serum most probably secreted by adipose tissue) with other molecular signalling pathways may contribute to the development of endometrial cancer. Large prospective studies will need to be undertaken to identify the molecular mechanisms involved and the individual roles of each of these adipocytokines.

There were limitations to the study due to time constraints: there were fewer cases and age match controls in the clinical study and therefore the sample size was very small. The inherent disadvantage of bias in retrospective case control studies was also a factor that limited the study. There were also limitations when performing the systematic review and meta-analysis. Only manuscripts published in English were included in our meta-analysis. No unpublished studies were included in our meta-analysis and therefore this could possibly misrepresent the results. There were bias risks in the primary studies such as heterogeneity in the methodology and variations in the characteristics of the populations within the individual studies. Subgroup and

stratified analysis were performed to explore the reasons for these heterogeneities.

FUTURE WORK

Future work should focus on assessing the relationship between adipocytokines in the serum, visceral and subcutaneous adipose tissue and endometrial cancer and its association to different weight categories. The meta-analysis has provided a good foundation for the design of larger prospective studies which will assess a variety of adipocytokines in the same cohort of patients in order to investigate further the association between adipocytokines and endometrial cancer; especially studies considering circulating levels of TNFα, IL-6 and IGF-I and II, would allow elucidating, in more details, the exact mechanisms underlying the link between adipocytokines and endometrial cancer. A prospective study which measures serum adiponectin, leptin, TNFα, IL-6 IGF-I and IGF II concentration levels in obese and normal weight healthy volunteers, and assesses their risk for endometrial cancer over time, could be carried out in the future. Meanwhile blood samples from patients with EC and different BMI could be collected in addition to blood from healthy, obese and normal weight volunteers in order to be able to dissect the relationship between obesity and EC aetiology/progression. Finally, the correlation between the presence of adipocytokines in endometrial tumour cells, serum and the cancer stage should be investigated, by collecting tissue biopsies from the cancer tissue and the normal tissue.

APPENDIX

School of
Biosciences and Medicine

Leggett Building
Daphne Jackson Road
Manor Park
Guildford, Surrey GU2 7WG UK

Patient Information Sheet

Study title: Discovering new cancer biomarkers from patients' blood, urine and DNA.

1. **Introduction**

You are being invited to take part in a research project currently running at the University of Surrey and the Royal Surrey County Hospital, Before you decide it is important for you to understand why the research is being done and what it will involve. This information sheet will help you decide if you wish to participate in this research. Please take time to read the following information carefully.
We are dedicated to the treatment and research of diseases such as cancer. This involves a co-operation between laboratory and clinical investigations and procedures. Research undertaken at the University of Surrey aims to advance the understanding of the causes of cancer and develop new methods of detection and treatment.

2. **What is the purpose of the study?**

Many cancers could be cured if found early. Some cancers produce so called tumour markers or biomarkers which are helpful in diagnosis of cancer and in detecting cancer recurrence. Some of the known cancer markers are also helpful in deciding the best treatment for cancer. Unfortunately very few cancers have specific markers. Even the ones we know such as PSA or CA125 are not ideal. Some of the tumour markers can be raised in conditions other that cancer and cause great distress and unnecessary investigations and tests. Discovering new cancer markers is extremely important for future treatment and diagnosis of all types of cancer. Cancer markers are usually made of miniscule proteins that can be detected in blood, urine and other bodily fluids. It is possible to analyse very small amounts of fluid and tissue and detect various proteins and potential cancer markers. We would like to analyse blood, urine, DNA , and if the cancer is being removed during surgery –a small piece of the tumour and fat tissue.

3. **Who is organizing and funding the research?**

The study is being sponsored by the University of Surrey. The blood, tissue and tumour collection is conducted by the staff at the Royal Surrey County Hospital,. The samples will be stored in a tissue bank at the Leggett Building , University of Surrey and analysed by scientists who specialise in research on cancer markers.

4. **Why have I been chosen?**

You have been referred for investigations of possible cancer or you may have been diagnosed with cancer. We would like to analyse your blood to try and identify cancer markers. If you are going to have an operation to remove suspected cancer we will ask you to offer a small piece of the tissue removed during surgery.

5. **Do I have to take part?**

Your participation in the study is entirely voluntary. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw or not take part will not affect the standard of care you will receive.

6. **What will happen to me if I take part?**

If you give your consent, we will simply obtain a blood and urine sample, make it anonymous and store it in a tissue bank. If you are going to have surgery as part of your treatment we will obtain a small piece of the tumour removed during surgery. This will not compromise your treatment in any way. Blood, urine and tissue samples will be used later for research to discover new cancer markers.... We would also like to collect information about your diagnosis and treatment. This information will be strictly confidential and only the study researchers will have access to this information.

7. **Expenses and payments:**

There will be no payment to you. The blood and tissue samples will be considered a donation for scientific research.

8. **What are the side effects of this procedure?**

There are no side effects of a blood test, you may feel a minimal discomfort during blood taking but it we will aim to minimise it. The tissue would only be taken as part of a routine procedure planned as part of your treatment therefore it would not cause any side effects other than the ones described to you by your surgeon.

9. **What are the benefits of this procedure?**

There is no direct benefit for you as a result of this procedure. However, the information gained from the research may result in discovery of new cancer markers, which may be helpful to other cancer patients in the future.

10. Will my taking part in the study be kept confidential?

All information which is collected about you during the course of this study will be kept strictly confidential: blood , urine and tissue sample will be anonymised. Any information about you which leaves the hospital will have your name and address removed, so you cannot be recognised from it. We will not give your name or address to anyone outside the hospital,
Your medical records may be seen by hospital staff involved in your care, Staff at the Oncology Department at the University, Ethics Committee, and the Regulatory Authorities that control research. They will respect your confidentiality.
The research team may write a report on the results and might write an article to be published in a medical journal or presented at a conference. If the results are published, we will make sure that you cannot be recognised from the article or presentation. Under the Data Protection Act 1998, you are entitled to see your study records and to request additions or corrections.

11. What if there is a problem?

The University of Surrey, the sponsor of the study, has indemnity arrangements in place, which allows it to provide compensation without your needing to prove negligence. This could apply if you suffered a significant and enduring injury (including illness or disease) which is directly attributable to any clinical intervention or procedure for which the University is responsible.

If you have any complaint about the way you have been dealt with during the study or any other concerns you can speak to Dr Agnieszka Michael or the research team on 01483688572
If you have concerns about the NHS involvement in the study the normal NHS complaints mechanisms are available to you, and if you are harmed by taking part in this research project and you believe this is due to the negligence of the NHS then you may have grounds for legal action against the NHS but you may have to pay for it.

12. What will happen to any samples I give?

Samples will be anonymized and code numbers will be given. Participants' age and sex go onto a database. This database is password protected and access is restricted to Principal Investigator and Research Team.
Samples will be kept frozen in the laboratory of the Oncology Department, Leggett Building, University of Surrey. The DNA will be isolated form the blood and kept separately in a secure place. Only the research team will have access to the samples. The samples will then be used for cancer research by scientists with interest and expertise to conduct such research.

13. What will happen to the results of the research study?

Research will be published in a scientific/medical journal and therefore available to all. Your sample will not be identifiable in any study report. All publications appear on the PubMed website and will be accessible to all.

14. Who has reviewed the study?

The Study has been reviewed by the Leeds (East) Research Ethics Committee

15. Contact Details:

Oncology Department. Leggett Building, Manor Park. University of Surrey.
Research Nurses01483 688572
Principal Investigator (Dr Agnieszka Michael) 01483 688546

Thank you very much for considering taking part and taking time to read this sheet.

School of
Biosciences and Medicine

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Guildford, Surrey GU2 7WG UK

Consent Form

Study Title

Discovering new cancer biomarkers from patients' blood, urine and DNA.

	Initial
• I have read and understood the Information Sheet provided. I have been given a full explanation by the investigators of the nature, purpose and likely duration of the study, and of what I will be expected to do. I have been advised about any discomfort and possible ill-effects on my health and well-being which may result. I have been given the opportunity to ask questions on all aspects of the study and have understood the advice and information given as a result.	☐ ☐
• I understand that all personal data relating to volunteers is held and processed in the strictest confidence, and in accordance with the Data Protection Act (1998). I agree that I will not seek to restrict the use of the results of the study on the understanding that my anonymity is preserved.	☐
• I understand and agree that NHS staff, Ethics Committee, authorised representatives of the University of Surrey (the sponsor of this study), and the Regulatory Authorities that control clinical research may review my records to check on details about my health relevant to the study	☐
• I understand that I am free to withdraw from the study at any time without needing to justify my decision and without my medical care being affected.	☐
• I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation and agree to comply with the instructions and restrictions of the study.	☐

Name of patient (BLOCK CAPITALS) Name of the researcher

Signed ....

Date ....

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Review Article

Adipocytokines and their relationship to endometrial cancer risk: A systematic review and meta-analysis

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HIGHLIGHTS

- Patients with lower adiponectin levels are more likely to develop endometrial cancer despite BMI, hypertension or diabetes.
- When considering BMI or a history of diabetes, increased leptin levels confer a greater risk of endometrial cancer.
- Larger studies are required to establish the role of TNFα and IL-6 in the development of endometrial cancer.

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ABSTRACT

Objective. To investigate the association between circulating levels of adipocytokines (adiponectin, leptin, tumour necrosis factor alpha (TNFα), interleukin 6 (IL-6)) and growth factors (insulin-like growth factor I (IGF-I) and II (IGF-II)), and the risk of endometrial cancer.

Methods. Cochrane, CINAHL, Embase, Medline and Web of Science were searched for English-language manuscripts published between January 2000 and August 2018 using the following string of words: cancer and endometrial and (obesity or BMI) and (adiponectin or TNF* or IGF-I or IGF-II or IL-6 or leptin).

Results. Twenty articles were included in this meta-analysis, which corresponded to 18 studies involving 2921 endometrial carcinoma cases and 5302 controls. Fourteen articles reported circulating levels for adiponectin, seven for leptin, three for TNFα, three for IL-6 and one for IGF-I. No article reported values for IGF-II.

Patients with circulating adiponectin levels in the highest tertile had decreased endometrial cancer risk compared to women with levels in the lowest tertile, (summary of odds ratio (SOR) 0.51, 95% confidence interval (CI): 0.38–0.69, $p < 0.00001$). Women with circulating leptin concentrations in the highest tertile had increased endometrial cancer risk compared to women with concentrations in the lowest tertile (SOR 2.19, 95% CI: 1.45–3.30, $p = 0.0002$). There was no difference in cancer risk between participants with the highest TNFα and IL-6 levels compared to the lowest levels (SOR 1.27, 95% CI: 0.88–1.83, $p = 0.20$ and SOR 1.20, 95% CI: 0.89–1.63, $p = 0.23$, respectively).

Conclusions. Endometrial cancer risk is inversely affected by adiponectin and leptin levels. There appears to be no relationship between TNFα and IL-6 and the overall risk of endometrial cancer.

Contents

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1. Background

The exact biological mechanism underlying the development of endometrial cancer is still poorly understood. In the UK, endometrial cancer is the 4th most common female cancer; approximately 9000 women were diagnosed with endometrial cancer in 2015 [1]. Worldwide, 320,000 new cases of endometrial cancer were diagnosed in 2012 [2].

Obesity is a well-recognised risk factor for endometrial cancer; however, the relationship between obesity and endometrial cancer is complex and likely to involve multiple biological pathways. Sex steroid and insulin pathways, chronic inflammation and alterations in circulating levels of adipokines have all been suggested as potential mechanisms affecting endometrial cancer risk [3–5]. Whilst elevated levels of endogenous oestrogens cannot justify alone the correlation between obesity and endometrial cancer, experimental studies have shown that adipokines, associated with hyperinsulinemia and insulin resistance, and inflammatory cytokines, associated to increased adiposity, may be also involved in the development of endometrial cancer [6].

Adiponectin, leptin, tumour necrosis factor alpha (TNFα), interleukin 6 (IL-6), insulin-like growth factor I and II (IGF-I and IGF-II), collectively termed adipocytokines, are hormones and cytokines secreted from adipocytes, and potentially key circulating molecules associated to endometrial cancer risk [7,8].

Adiponectin, the most abundant circulating adipocytokines, plays an important role in regulating insulin and glucose metabolism, by promoting insulin secretion from pancreatic β cells and facilitating insulin up-take in the liver [9–12]. Moreover, adiponectin has antiproliferative properties and, by activating AMP activated protein kinase (AMPK), inhibits cell growth, angiogenesis and promotes apoptosis in malignant cells [13]. Because of its properties and the fact that adiponectin is decreased in obesity, insulin resistance and type 2 diabetes, all independent risk's factors for endometrial cancer, circulating adiponectin levels may be an important factor in endometrial cancer.

Leptin affects the activity of several cell types and its main function involves regulating energy intake and expenditure [14]. It has a role in glucose metabolism, as well as in the immune system. Leptin is also secreted by cancer cells and its levels have been reported to be increased in endometrial cancer and hyperplasia compared to controls with normal endometrium [15].

TNFα and IL-6 are pro-inflammatory cytokines released by macrophages within adipose tissue and have been implicated in tumourigenesis. TNFα promotes cellular proliferation and prevents apoptosis by activating NFκB, [16], whereas IL-6 initiates tumour development and progression through several pathways [17]. Both cytokines have been reported to be increased in endometrial cancer and their pro-inflammatory actions play a role in cancer growth and metastasis by inducing reactive oxygen species and subsequent DNA damage and DNA repair inhibition [18]. IL-6 was found to be overexpressed in the stroma of endometrial cancer cells and TNFα was associated with poor survival [19,20]. However, other studies have not reported such an increase and found no difference in the expression of IL-6 in endometrial cancer and at the various clinical stages [21].

IGF-I and IGF-II are growth factors involved in growth and development [22]. They are expressed in the normal development of the endometrium and also stimulated by oestrogen in the uterus [23]. Epidemiological, clinical and experimental data have identified IGF-I and II as important players in endometrial cancers. IGFs are thought to play a role in the initiation of endometrial cancer due to oestrogen increasing the synthesis and expression of IGF-I which stimulates cell proliferation thereby initiating endometrial cancer [24]; whereas IGF-II expression was increased in advanced endometrial cancer compared to early stage endometrial cancer [25]. Relative few studies have assessed the correlations between endometrial cancer risk and circulating levels of IGF axis components: however a large degree of variability between studies and results was reported probably reflecting the complexity of this hormonal system and the involvement of additional (hormonal or other) factors that can either positively or negatively impinge upon IGF axis components.

Although evidence from *in vitro* and *ex-vivo* studies for a causal role of adipocytokines in endometrial cancer are available, results from epidemiological studies are inconsistent. A number of meta-analyses [26–28] have previously summarised epidemiological studies investigating the relationship between circulating adiponectin and leptin concentrations and endometrial cancer risk, however, to date, no meta-analysis has been performed to assess the relationship between circulating levels of the pro-inflammatory cytokines, TNFα and IL-6, or growth factors, IGF-I and IGF-II, and the risk of endometrial cancer. This study further clarifies the association between circulating levels of leptin and adiponectin, and endometrial cancer, and aimed to systematically assess the relationship between cytokines (TNFα and IL-6) and growth factors (IGF-I and IGF-II) levels with endometrial cancer risk via a meta-analysis of observational studies.

2. Methods

2.1. Literature search

Meta-analysis was performed and reported by adopting the Meta-analyses of Observational Studies in Epidemiology (MOOSE) guidelines [29]. English-language manuscripts published between January 2000 and August 2018 were searched from the databases: Cochrane, CINAHL, Embase, Medline and Web of Science. The following string of words was used for searches in all databases – cancer and endometrial and (obesity or BMI) and (adiponectin or TNFα or IGF-I or IGF-II or IL-6 or leptin).

2.2. Selection of studies and exclusion criteria

Published studies were included if they met the following criteria: the study i) used an epidemiologic study design (e.g. case–control,

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case-cohort, nested case-control and cohort study); ii) provided information on circulating adiponectin, leptin, TNFα, IL-6, IGF-I, IGF-II concentrations as exposure of interest; iii) reported endometrial cancer as the outcome of interest; and iv) reported usable risk estimates (e.g. odds ratio, risk ratio or relative risk with 95% confidence intervals (CI) between circulating adipocytokines levels and endometrial cancer risk). In addition, if more than one study was conducted in the same population, the most recent report or the report with the most applicable estimates was selected for analysis.

Published studies were excluded by the following exclusion criteria: i) non epidemiological studies, reviews without original data, ecological studies, editorials and case reports; ii) the study reported the risk estimates that could not be summarised (i.e. reported the risk estimates without 95% CIs); and iii) the study reported exclusively on endometrial cancer mortality. All study selection and exclusion procedures were carried out by two independent investigators (PEE and GB). If there was discordance, a third independent reviewer, GAB would make the final decision.

2.3. Data extraction

The following key data were extracted from each included study: first author's name, publication year, study country, study design, number, ages and BMI of cases/controls, assay methods, risk estimates, and matched or adjusted factors including age, body mass index (BMI), menopausal status, whether they have had diabetes mellitus (DM) or hypertension, hormone replacement therapy (HRT) usage, parity or whether they smoked.

2.4. Statistical analysis

Review Manager software, Version 5.3, was used to perform the meta-analysis: inverse variance, odds ratio and random effect were chosen as statistical method, effect measure and analysis model, respectively. The risk estimates were analysed as an estimation of odds ratio (OR) or relative risk (RR) for simplicity. People with the levels of exposure in the top tertile were compared with those in the bottom tertile. If the highest tertile (T3) and the lowest tertile (T1) were not available from the individual studies [30–37], a scaling method similar to Danesh et al. [38] and used by Gong et al. [26] was applied: a scaling factor of 2.18 divided by 2.54 times the log OR for comparison of the top and bottom quartiles, or a scaling factor of 2.18 times the log OR for 1 standard deviation difference in the baseline levels of adiponectin or leptin. In addition, some of the studies [6,39] used the highest category of adiponectin rather than the lower category as comparison: an effective count method described by Hamling and colleagues [40], was therefore used to transform the comparison to the lowest tertile (T1). To assess the relationship between circulating adipocytokines and the risk of

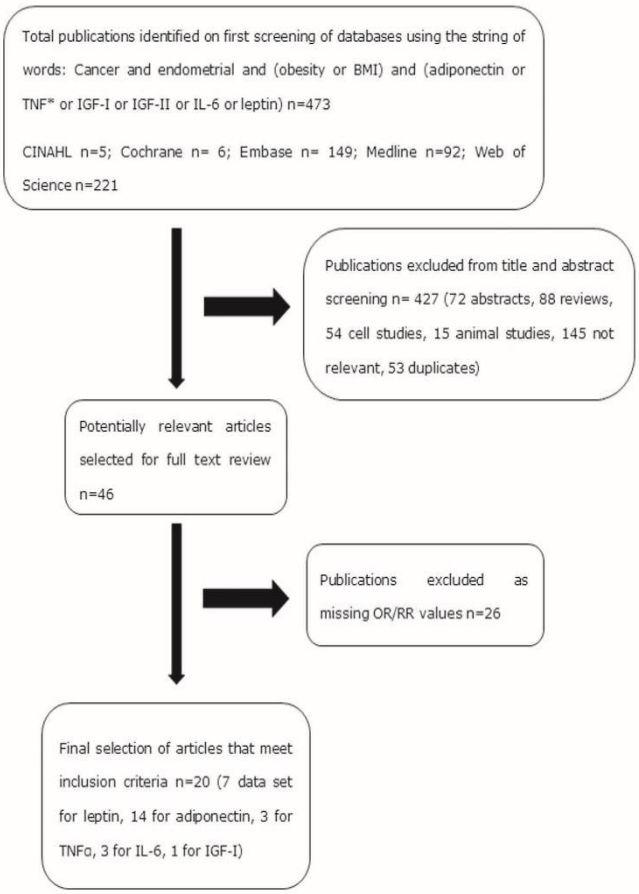

Fig. 1. Flow diagram of screened, excluded and analysed publications.

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endometrial cancer, the summary of odds ratio (SOR) with 95% CI was estimated. This was performed using a random effect model of analysis. Chi-Squared test was used to assess the variation across the studies, which was included in the forest plots. Heterogeneity across the studies was analysed using the I^2 statistics [41] and results were defined as heterogenous for an $I^2 > 50\%$. All statistical tests were two-sided. $p < 0.05$ were considered to be statistically significant.

Sensitivity analysis was performed to assess the influence of individual studies on the pooled OR and 95% CI by excluding each study in turn.

Heterogeneity of the study results were explored by using stratified analyses and subgroup analyses. These analyses included design of the study, fasting status for the collection of the blood samples and the type of assay method used. Subgroup analyses to identify potential confounders included BMI, hypertension, diabetes and menopausal status. A variable was considered confounding if they were found to be significantly associated with endometrial cancer $p < 0.05$ on the univariate analysis.

3. Results

3.1. Search results and publication characteristics

The database searches identified 473 publications. A total of 427 studies were excluded on title and abstract review as they did not meet the inclusion criteria as shown in Fig. 1. The remaining 46 studies were reviewed for further details and full text retrieved. Twenty-six studies were excluded for not containing OR values, risk ratio or relative risk with 95% CI. Therefore, a total of 20 articles were included in this meta-analysis, which corresponded to 18 studies involving 2921 endometrial carcinoma cases and 5302 controls. Fourteen articles reported circulating levels for adiponectin [6,8,31,36,37,39,42–49], 7 for leptin [8,30,36,43,44,47,48] 3 for TNFα [34,35,50], 3 for IL-6 [33,35,50] and 1 for IGF-I [51]. No article reported values for IGF-II. The characteristics of these studies, all published between 2002 and 2015, are presented in Table 1.

3.2. Adiponectin and its relationship to endometrial cancer risk

In this current meta-analysis, fourteen studies evaluated adiponectin and its relationship to endometrial cancer [6,8,31,36,37,39,42–49]. Two thousand and twenty-four endometrial cancer cases and 3593 controls were assessed in 9 retrospective studies (8 case control studies [6,31,36,37,42–45] and 1 cross sectional-controlled study [39]) and 5 prospective studies (nested case control studies) [8,46–49] (Table 1). Combined data showed a significant difference between the risks of developing endometrial cancer in women with the highest adiponectin levels compared to the lowest levels. Women with adiponectin concentrations in the highest tertile had a reduced risk (~0.5 times) of endometrial cancer compared to women with adiponectin concentration levels in the lowest tertile (SOR 0.51, 95% CI: 0.38–0.69 $p < 0.00001$). There was significant heterogeneity, $I^2 = 77\%$ $p < 0.00001$ (Fig. 2).

Sensitivity analysis was performed to determine whether any particular study had a greater degree of influence between the association of adiponectin's levels and the risk of endometrial cancer. Omitting each study one at a time and analysing the SOR of the rest of the studies, the SOR ranged from 0.48 (95% CI: 0.35–0.66, $I^2 = 79\%$, $p < 0.00001$) when omitting Soliman et al. [46] study to 0.58 (95% CI: 0.45–0.75, $I^2 = 67\%$ $p = 0.0002$) when omitting Soliman et al. [6]. No single study had a larger influence over the other studies when assessing the association between adiponectin and endometrial cancer risk.

Stratifying by study design revealed a SOR of 0.64 (95% CI: 0.41–0.99, $p = 0.05$) for prospective studies [8,46–49] and a SOR of 0.45 (95% CI: 0.29–0.68, $p = 0.0001$) for the retrospective studies (Table 2) [6,31,36,37,39,42–45]. The heterogeneity was lower for the prospective studies (56%, $p = 0.06$) compared to the retrospective studies (83%, $p < 0.00001$). There were variations in the type of blood samples used as well as the method used to measure the concentration of adiponectin. Eight studies [8,31,36,37,39,43,44,47] used fasting samples to measure adiponectin and in the other six [6,42,45,46,48,49], it was not clear whether the blood samples were fasted or postprandial. The point estimate for studies using fasting samples was 0.51 (95% CI: 0.34–0.76, $p = 0.0009$) and for the non-fasting studies, it was 0.51 (95% CI: 0.32–0.81, $p = 0.004$). Eleven studies used an ELISA to measure adiponectin concentrations [6,8,36,37,39,42–44,46,47,49] and 3 studies used RIA/IMRA [31,45,48]. The point estimate of SOR in the studies using the ELISA method was similar to the studies using RIA/IMRA (SOR 0.53 vs 0.45). Within prospective studies, there was no significant heterogeneity ($I^2 = 56\%$ $p = 0.06$), whereas there was within retrospective studies ($I^2 = 83\%$ $p < 0.00001$). Within studies using fasting or non-fasting blood samples, there was significant heterogeneity ($p = 0.04$ and $p < 0.00001$, respectively). The studies using ELISA demonstrated statistically significant heterogeneity (79% $p < 0.00001$) whereas the one using RIA/IMRA did not ($p = 0.45$). However there was no evidence of significant heterogeneity between subgroups detected by meta-regression analyses (Table 2).

Raised BMI, hypertension, diabetes and menopause are all risk factors for endometrial cancer. Sub-analyses were performed to assess for potential confounding factors. When considering BMI [6,8,31,37,39,42–46,49], the association between adiponectin levels and endometrial cancer risk is maintained (SOR 0.46, 95% CI: 0.31–0.69, $p = 0.0002$, $I^2 = 81\%$, $p < 0.00001$). When considering hypertension [6,36,37,42,44,47], diabetes [6,37,42,44,46–48], or menopause status [8,31,36,37,39,42,44,47,48], a statistically significant association with endometrial cancers was maintained in the groups with hypertension (SOR 0.57, 95% CI: 0.36–0.91, $p = 0.02$, $I^2 = 81\%$ $p < 0.0001$), diabetes (SOR 0.6, 95% CI: 0.38–0.94, $p = 0.03$, $I^2 = 79\%$ $p < 0.001$) and post menopause (SOR 0.56, 95% CI: 0.40–0.80, $p = 0.001$, $I^2 = 70\%$ $p = 0.0007$). This association was not lost in those without these conditions (Table 2).

3.3. Leptin and its relationship to endometrial cancer risk

A total of seven studies [8,30,36,43,44,47,48]; four retrospective [30,36,43,44] and three prospective [8,47,48], assessed the association between circulating leptin concentrations and the risk of endometrial cancer. Three studies were nested case controls [8,47,48], and four were case control studies [30,36,43,44]. One thousand, one hundred and ninety-nine endometrial cancers cases and 2076 control participants were assessed in the seven studies. The forest plot of the combined data (Fig. 3) demonstrated a summary of OR of 2.19 (95% CI: 1.45–3.30, $p = 0.0002$). These results suggest a significant difference between the risks of developing endometrial cancer in individuals with the highest leptin levels versus the lowest levels. Women with leptin concentrations in the highest tertile had 2.19 times increased risk of endometrial cancer compared to women with leptin concentrations in the lowest tertile. There was variation between the studies, with significant heterogeneity, $I^2 = 64\%$, $p = 0.01$.

Sensitivity analysis was performed to determine whether any particular study had a greater degree of influence between the association of leptin and the risk of endometrial cancer. Omitting one study at a time and analysing the SOR of the rest of the studies, the SOR ranged from 1.99 (95% CI: 1.37–2.91 $p = 0.0003$, $I^2 = 58\%$, $p = 0.03$) when omitting Wu et al. [8] to 2.50 (95% CI: 1.84–3.40 $p < 0.00001$, $I^2 = 13\%$ $p = 0.330$) when omitting Friedenreich et al. [36]. No single study had a larger influence over the other studies when assessing the association between leptin and endometrial cancer risk.

When stratifying by study design (Table 2), the prospective studies [8,47,48] had a higher SOR of 3.32 (95% CI: 1.98–5.56 $p < 0.00001$, $I^2 = 15\%$, $p = 0.31$) compared to the retrospective studies' SOR of 1.67 (95% CI: 1.09–2.57 $p = 0.02$, $I^2 = 56\%$, $p = 0.08$) [30,36,43,44]. There were variations between the type of samples used and the measurement of leptin concentration; 6 studies used fasting blood samples [8,30,36,43,44,47] and 1 used a post prandial sample [48]. When

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Table 1
Characteristics of included articles (n = 20).

First author, Year, Study country	Study design	No. of case/control	Age of case/control	BMI of case/control	Biomarkers (Assay method)	Risk estimates (95% CI) Exposure categories	Adjusted factors
Retrospective studies							
Zhang, 2015 China	Case control	88/90	64.7 ± 10.1a 58.7 ± 8.6b	n/a	Adiponectin (ELISA)	OR 0.822 (0.759–0.889) Not specified in text	Age, BMI, WHR, diabetes, hypertension
Ohbuchi, 2014 Japan	Case control	43/62	61.2 ± 9.8a 58.1 ± 8.3b	26.1 ± 4.5a 23.3 ± 3.8b	Adiponectin (ELISA)	OR 1.987 (0.290–13.617) Q1 vs Q2	Age, BMI, diabetes, hypertension
Erodogan, 2013 Turkey	Cross sectional controlled study	60/70	56.57 ± 9.05a 49.7 ± 7.59b	31.12 ± 4.18a 27.49 ± 3.22b	Adiponectin (ELISA)	OR 10.64 (3.61–31.40) T1 vs T3	Age, BMI, HOMA-IR, QUICKI
Friedenreich, 2013 Canada	Case control	519/964	58.7 58.3	32.3 28.1	TNF-α (ELISA) IL-6 (ELISA)	OR 1.00 (0.84–1.18) OR 1.15 (0.89–1.48) Not specified in text	Age, BMI, nulliparity, physical activity, hypertension, alcohol consumption, hormone usage
Ma, 2013 China	Case control	206/310	53.2 (26–81)b 53.3 (27–82)b	n/a	Adiponectin (ELISA) Leptin (ELISA)	OR 0.52 (0.32–0.83) OR 2.05 (1.28–3.29) T3 vs T1	Age, BMI, glucose, cholesterol, triglycerides, HDL cholesterol, insulin, adiponectin (for leptin), leptin (for adiponectin)
Friedenreich, 2012 Canada	Case control	514/961	59 (53, 65)c 59 (52, 66)c	31.0 (26.4, 36.8)c 27.2 (24.1, 30.9)c	Adiponectin (ELISA) Leptin (ELISA)	OR 0.55 (0.37–0.80) OR 1.14 (0.73–1.77) Q4 vs Q1	Age, weight, waist to hip ratio, nulliparity, HRT, hypertension, glucose, insulin, adiponectin (for leptin), leptin (for adiponectin)
Ashizawa, 2010 Japan	Case control	146/150	59.9 ± 8.9a 57.5 ± 7.4a	23.7 ± 4.5a 22 ± 3.3a	Adiponectin (ELISA) Leptin (RIA)	OR 0.6 (0.3–1.2) OR 2.6 (1.4–4.9) T3 vs T1	Age, BMI, hypertension, diabetes
Soliman, 2006 USA	Case control	117/238	66.6 (25–88)b 61.2 (50–80)b	33.2 28.0	Adiponectin (ELISA)	OR 10.5 (4.18–26.35) T1 vs T3	Age, BMI, diabetes, hypertension,
Dal Maso, 2004 Italy	Case control	87/132	62 (34–78)d 61 (29–72)d	27.8 (25.4–32)e 25.1 (22.3–27.9)e	Adiponectin (RIA)	0.30 (0.14–0.68) T3 vs T1	Age, BMI, parity, education, HRT use, smoking status
Petridou, 2003 Greece	Case control	84/84	n/a	n/a	Adiponectin (RIA)	OR 0.78 (0.56–1.10) 1SD increment	Age, BMI, height, education, age at menarche, pregnancy, IGF-I, IGF-II, IGFBP-3 and leptin
Petridou, 2002 Greece	Case control	84/84	63.3 ± 9.69a 62.6 ± 11.3a	29.2 ± 5.72a 26.5 ± 3.43a	Leptin (IRMA)	OR 1.13 (0.70–1.81) 1SD increment	Age, education, height, age at menarche, menopausal status, history of pregnancy by outcome, alcohol and coffee consumption, smoking status
Prospective studies							
Wu, 2014 Taiwan	Nested case control	20/120	44.3 ± 8.5a 46.6 ± 9.8a	n/a	Adiponectin (ELISA) Leptin (ELISA)	OR 0.07 (0.01–0.62) OR 10.68 (2.09–54.67) T3 vs T1	Age, BMI, years of oestrogen exposure
Soliman, 2011 USA	Nested case control	146/377	57 (47–67)b 57 (47–67)b	27.2 25.5	Adiponectin (ELISA)	OR 0.98 (0.57–1.68) T3vs T1	Age, BMI, parity, diabetes
Dallal, 2013 USA	Nested case control study	62/124	67.4 ± 5.5a 67.5 ± 5.1a	29.5 ± 6.9a 26.8 ± 4.7a	Adiponectin (ELISA) Leptin (ELISA)	OR 0.87 (0.39–1.94) OR 3.29 (1.41–7.69) T3 vs T1	Age, estradiol, C-peptide and BMI, diabetes
Luhn, 2013 USA	Nested case control	167/327	66.4 ± 5.7a n/a	n/a	Adiponectin (RIA) Leptin (RIA)	OR 0.48 (0.29–0.80) OR 2.77 (1.60–4.79) T3 vs T1	Age, HRT, current smoking status, family history of breast and endometrial cancer, education, parity, diabetes, oral contraception use
Dossus, 2011 Europe	Nested case control	270/518	57.0 (6.9)a 57.0 (6.9)a	28.1 (5.9)a 26.3 (4.5)a	TNFα (ELISA)	OR 1.73 (1.09–2.73) Q4 vs Q1	Age, BMI, nulliparity, age at menopause, HRT use
Wang,	Case cohort	151/299	65.2 (7.1)a	29.7 (7.8)a	IL-6	OR 0.70	Age, BMI, Free IGF-I, estradiol, insulin

(continued on next page)

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Table 1 (*continued*)

First author, Year, Study country	Study design	No. of case/control	Age of case/control	BMI of case/control	Biomarkers (Assay method)	Risk estimates (95% CI) Exposure categories	Adjusted factors
2011 USA			63.5 (7.5)a	27.5 (5.8)a	(ELISA) TNFα (multiplex assay)	(0.29–1.68) OR 1.65 (0.77–3.54) Q4 vs Q1	
Dossus, 2010 Europe	Nested case control	305/574	56.9 (7.3)a 57.1 (7.4)a	27.5 (5.5)a 26.0 (4.3)a	IL-6 (ELISA)	OR 1.66 (1.08–2.54) Q4 vs Q1	BMI, C-peptide, estrone
Cust, 2007 Europe	Nested case control	284/548	56.9 (45.4–67.9)f 56.9 (45.0–68.0)f	28.1 (20.9–37.60) f 26.5 (20.2–34.8) f	Adiponectin (ELISA)	OR 0.63 (0.36–1.10) Q4 vs Q1	Age, BMI, C-peptide, IGFBP-1, IGFBP-2, SHBG, estrone, free testosterone
Lukanova, 2004 USA, Sweden, Italy	Case control	166/315	61 ± 7.8a n/a	27.3 (26.5–28.0) g 25.3 (24.7–25.9)g	IGF-1 (RIA)	OR 0.90 (0.44–1.82) Q5 vs Q1	Age, menopausal status, day of menstrual cycle for pre-menopausal women

BMI, body mass index; WHR, waist-to-hip-ratio; ELISA, enzyme linked immunosorbent assay; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; IGF, insulin like growth factor; IGFBP, insulin like growth factor binding protein; SHBG, sex hormone binding globulin; HRT, hormone replacement therapy; OR, odds ratio; RIA, radio-immuno assay.
a: mean ± SD; b: mean (range); c: median (25th, 75th percentile); d: median (range); e: median (interquartile range); f: mean (5th–95th percentiles); g: mean (95% confidence interval); n/a: not available.

comparing the type of samples, the point estimate of SOR for studies using non fasting blood samples was higher than the SOR for studies using fasting blood samples (2.77 vs 2.10). The concentration of leptin was either measured using an ELISA [8,36,43,47] or RIA [44,48], in 4 and 2 studies, respectively. In a further study, leptin was measured using an IMRA [30]. The point estimate of SOR for studies using ELISA was 2.27 (95% CI: 1.16–4.42 p = 0.02, I^2 = 75%, p = 0.007) and for the studies using RIA/IMRA was 2.45 (95% CI: 1.67–3.59 p < 0.00001, I^2 = 0%, p = 0.45). Within prospective or retrospective studies there was no significant heterogeneity (I^2 = 15% p = 0.31, I^2 = 56% p = 0.08, respectively), however there was evidence of significant heterogeneity (p = 0.04) between subgroups detected by meta-regression analyses (Table 2). Within studies using fasting blood samples [8,30,36,43,44,47] and measuring leptin levels by ELISA [8,36,43,47], there was significant heterogeneity (I^2 = 65% p = 0.01 and I^2 = 75% p = 0.007, respectively), whereas the one using non-fasting blood samples and RIA/IMRA did not (n/a and p = 0.45). There was no evidence of significant heterogeneity between the two subgroups detected by meta-regression analyses (Table 2).

Both pre- and post-menopausal women were included in these studies. Other factors that were matched/adjusted included BMI (n = 4) [8,30,43,44], hypertension (n = 3) [36,44,47], a history of diabetes (n = 3) [44,47,48] and post-menopausal status (n = 5) [8,30,44,47,48]. When BMI is not considered [36,47,48], the overall association between leptin levels and the risk of developing endometrial cancer is reduced to borderline levels, p = 0.05 (SOR 2.05, 95% CI 0.99–4.25, I^2 = 80% p = 0.007). When considering patients with hypertension [36,44,47], the overall association between leptin levels and the risk of endometrial cancer is borderline (p = 0.06, SOR 1.99, 95% CI 0.98–4.04), whereas the overall association is increased when considering patients with diabetes [44,47,48], (p < 0.00001, SOR 2.80, 95% CI 1.93–4.05). When post-menopausal status is not considered [36,43], the overall association between leptin levels and the risk of developing endometrial cancer is lost, p = 0.18 (SOR 1.49, 95% CI: 0.83–2.70). There

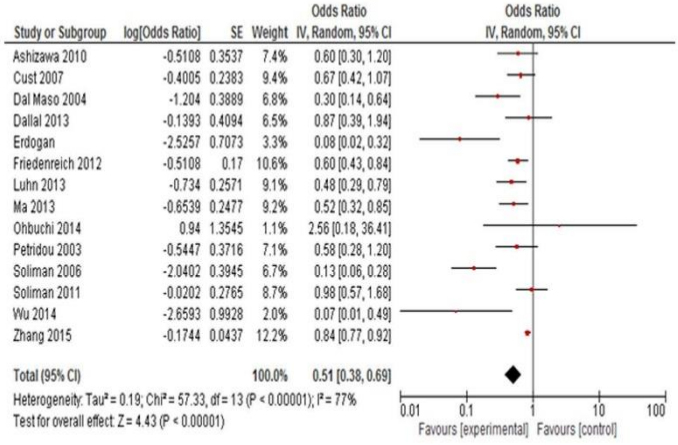

Fig. 2. Forest plots representing the association between circulating levels of adiponectin and the risk of endometrial cancer risk. The red squares represent the OR of the individual studies and the horizontal lines through the boxes represent the 95% confidence interval. The overall treatment effect is represented by the black diamond. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Table 2
Summary of OR of the relationship between adiponectin or leptin and possible risk factors for endometrial cancer.

Adiponectin							Leptin					
No of study		SOR	95% CI	I^2	p value[1]	p value[2]	No of study	SOR	95% CI	I^2	p value[1]	p value[2]
Study design												
Prospective	5	0.64	0.41–0.99	56%	p = 0.06	p = 0.27	3	3.32	1.98–5.56	15%	p = 0.31	p = 0.04
Retrospective	9	0.45	0.29–0.68	83%	p < 0.00001		4	1.67	1.09–2.57	56%	p = 0.08	
Fasting blood samples												
Yes	8	0.51	0.34–0.76	52%	p = 0.04	p = 1	6	2.10	1.31–3.38	65%	p = 0.01	n/a
No	6	0.51	0.32–0.81	85%	p < 0.00001		1	2.77	1.60–4.80	n/a	n/a	
Assay method												
ELISA	11	0.53	0.38–0.75	79%	p < 0.00001	p = 0.52	4	2.27	1.16–4.42	75%	p = 0.007	p = 0.85
RIA/IMRA	3	0.45	0.31–0.65	0%	p = 0.45		3	2.45	1.67–3.59	0%	p = 0.45	
BMI												
Yes	11	0.46	0.31–0.69	81%	p < 0.00001	p = 0.31	4	2.35	1.43–3.88	39%	p = 0.18	p = 0.76
No	3	0.59	0.45–0.76	0%	p = 0.46		3	2.05	0.99–4.25	80%	p = 0.007	
Hypertension												
Yes	6	0.57	0.36–0.91	81%	p < 0.0001	p = 0.54	3	1.99	0.98–4.04	76%	p = 0.02	p = 0.66
No	8	0.47	0.31–0.70	64%	p = 0.007		4	2.42	1.47–3.97	42%	p = 0.16	
Diabetes												
Yes	7	0.6	0.38–0.94	79%	p < 0.001	p = 0.31	3	2.80	1.93–4.05	0%	p = 0.91	p = 0.23
No	7	0.44	0.30–0.65	60%	p = 0.02		4	1.80	0.97–3.35	68%	p = 0.02	
Menopausal status												
Yes	9	0.56	0.40–0.80	70%	p = 0.0007	p = 0.47	5	2.75	1.87–4.05	16%	p = 0.31	p = 0.09
No	5	0.44	0.25–0.81	81%	p = 0.0004		2	1.49	0.83–2.70	73%	p = 0.05	

[1] p value for heterogeneity within each subgroup.
[2] p values for heterogeneity between subgroups with meta-regression analysis.

was significant heterogeneity (I^2 = 76%, p = 0.02) in those studies that recorded hypertension compared to those studies that did not (I^2 = 42%, p = 0.16). For those studies that did not adjust for the presence of diabetes in its participants, the heterogeneity was higher (I^2 = 68%, p = 0.02) compared to those studies that considered diabetes as confounding factor (I^2 = 0%, p = 0.91). Similarly, for those studies that did not adjust for post-menopausal status, the heterogeneity was higher (I^2 = 73%, p = 0.05) compared to those studies that considered it as confounding factor (I^2 = 16%, p = 0.31). No evidence of significant heterogeneity between BMI, hypertension, diabetes and post-menopausal status subgroups was detected by meta-regression analyses (Table 2).

3.4. TNFα, IL-6 and IGF-I and their relationship to endometrial cancer risk

The paucity of studies analysing TNFα, IL-6 and IGF-I and their association with the risk of endometrial cancer is evident (Table 1) [33–35,50,51]. Two studies (one prospective [35] and one retrospective [50]) assessed both TNFα and IL-6 in a single cohort and a further 2 studies (both prospective) assessed TNFα [34] and IL-6 [33] only. There was only one study (prospective) investigating the role of IGF-I [51] and the risk of endometrial cancer. The total number of endometrial cancer and control cases for TNFα was 940 and 1781 respectively, and for IL-6, it was 975 and 1837, respectively. The prospective study assessing IGF-I and its correlation with endometrial cancer risk had 166 cases and 315 controls.

From the meta-analyses, there appeared to be no association between circulating levels of TNFα or IL-6 and overall risk of developing endometrial cancer (SOR = 1.27, 95% CI: 0.88–1.83 p = 0.20, SOR = 1.20, 95% CI: 0.89–1.63, p = 0.23, respectively) (Fig. 4A and B). Heterogeneity was not present for either TNFα studies or IL-6 studies (I^2 = 65% p = 0.06 for TNFα, and I^2 = 42% p = 0.18 for IL-6).

Sensitivity analysis was performed to determine whether any single study had a greater degree of influence between the association of TNFα and the risk of endometrial cancer. When Wang et al. [35] was excluded,

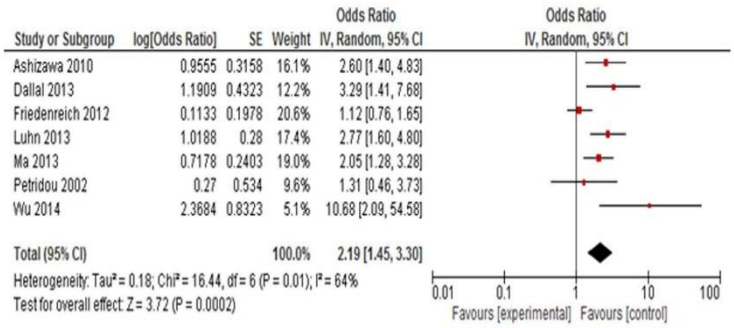

Fig. 3. Forest plots representing the association between circulating levels of leptin and the risk of endometrial cancer risk. Red squares represent the OR of the individual studies and the horizontal lines through the boxes represent the 95% confidence interval. The overall treatment effect is represented by the black diamond. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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the SOR was 1.22 (95% CI: 0.77–1.93 p = 0.39, I^2 = 78% p = 0.030); excluding the study performed by Freidenreich et al. [50], the SOR was 1.58 (95% CI: 1.13–2.22 p = 0.007, I^2 = 0%, p = 0.92) and finally excluding the study performed by Dossus et al. [34], the SOR was 1.10 (95% CI: 0.77–1.56 p = 0.59, I^2 = 36%, p = 0.21). There are differences between the 3 studies which could explain the change in SOR; the Wang study [35] was a prospective study and the studies by Friedenreich et al. [50] and Dossus et al. [34] were retrospective and prospective studies, respectively. The participants in the Wang et al. [35] study were postmenopausal women who were not using any hormone treatments. Both pre- and post-menopausal women were included in the other 2 studies and some participants in these 2 studies were also noted to be using hormones.

Sensitivity analysis was also performed to determine whether any single study had a greater degree of influence between the association of IL-6 and endometrial cancer risk. The SOR ranged from 1.06 (95% CI: 0.76–1.49 p = 0.72, I^2 = 18%, p = 0.27), when omitting the Dossus et al. [33] to a SOR of 1.29 (95% CI: 0.97–1.70 p = 0.08, I^2 = 37% p = 0.21), when excluding the Wang et al. [35] study. Excluding the Friedenreich et al. [50] study, the SOR was 1.15 (95% CI: 0.56–2.34 p = 0.71, I^2 = 66%, p = 0.09).

Only one prospective study [51] investigated the association of prediagnostic blood concentrations of IGF-I and other factors associated to hyperinsulinemia with endometrial cancer risk. While increased circulating C-peptide levels were associated with increased endometrial cancer risk, the risk was unrelated to IGF-I levels (OR 0.90, 95% CI 0.44–1.82, p = 0.54) when case-control pairs were matched for study cohort, age at recruitment into the study, menopausal status, and adjusted for BMI and HRT use.

4. Discussion

Inflammation, an important factor in the development and progression of cancer, has been implicated in the link between obesity and cancer [52,53]. Adiponectin, leptin, TNFα, IL-6 and IGF-I are biological factors that are involved in different stages of the inflammatory pathway. To the best of our knowledge, this study is the most updated meta-analysis examining the relationship between circulating levels of adiponectin and leptin, and endometrial cancer; and the first one to assess the association between TNFα, IL-6, IGF-I and IGF-II and endometrial cancer risk. Our findings indicated that decreased circulating levels of adiponectin and increased levels of leptin are associated with increased endometrial cancer risk, whereas no difference in cancer risk were observed between participants with the highest TNFα and IL-6 levels.

The paucity of studies reported in the literature investigating the link between the adipocytokines and endometrial cancer is evident; between 2000 and 2018, only 20 publications were found in the literature that met the inclusion criteria set. Undertaking a systematic review and meta-analysis increased population size enhancing the accuracy and precision of the findings from the various studies and allowing a greater understanding of the association between adipocytokines and endometrial cancer risk. Our analyses concurred with other reported studies [43,48] on the association between adiponectin and leptin concentration levels and endometrial cancer risk: increased adiponectin serum levels and decreased leptin levels are associated with an overall decreased risk of endometrial cancer. It was found that women with higher levels of adiponectin had the risk of developing endometrial cancer decreased by half compared to those women with lower levels of adiponectin. Women with high levels of leptin had a two-fold increased risk of developing endometrial cancer compared to women with low levels of leptin. Similarly to the findings in this meta-analysis, low serum adiponectin levels and high serum leptin levels have been associated to increased risk of other types of cancer (e.g. colon and breast cancer) [54,55]. In colorectal cancer patients, the association between TNFα, adiponectin and leptin has also been assessed concluding that leptin levels correlated with TNFα levels and that TNFα levels were an independent predictor of increased leptin levels [54]. Such association may be present in endometrial cancer, and leptin and TNFα may act synergistically to promote the development of endometrial cancer due to evidence that leptin promotes low grade inflammation by elevating levels of TNFα [56].

The studies reported by Dallal et al. [47] and Soliman et al. [46] did not find an association between adiponectin serum levels and endometrial cancer risk, possibly due to the limited numbers of cases and controls Moreover, both studies were prospective, and slight differences between the prospective and retrospective studies were highlighted by the sub analyses carried out (Table 2). For adiponectin, the SOR was 0.64 for prospective studies compared to 0.45 for retrospective with statistical difference for retrospective studies (p = 0.0001) and for leptin, the SOR for prospective studies was 3.32 (p < 0.00001) compared to 1.67 (p = 0.02) for retrospective studies.

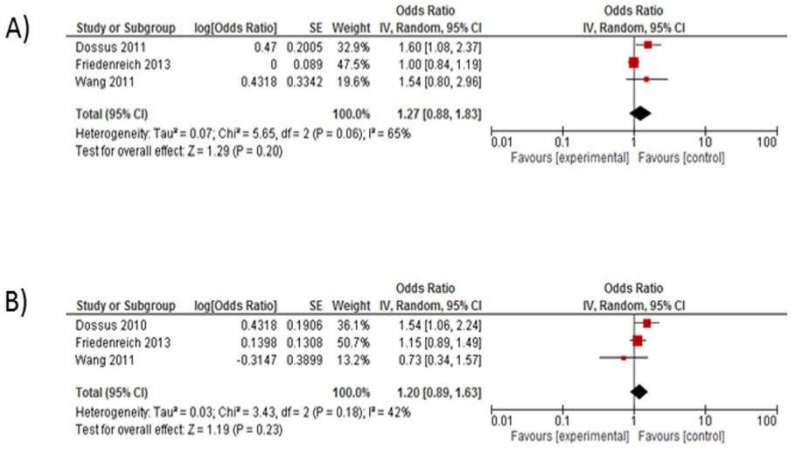

Fig. 4. Forest plots representing the association between circulating levels of TNFα (A) or IL-6 (B) and the risk of endometrial cancer risk. The red squares represent the OR of the individual studies and the horizontal lines through the boxes represent the 95% confidence interval. The overall treatment effect is represented by the black diamond. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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There have been limited studies assessing TNFα and IL-6 and its risk with endometrial cancer. TNFα and IL-6 play an important role in promoting carcinogenesis through the activation of various transcription factors and multiple oncogenic pathways. However, no significant associations between these two markers and risk of cancers were observed in the current meta-analysis. Despite the limited number of studies, the number of endometrial cancer cases and controls were relatively high; 940 vs 1781 and 975 vs 1837 cases vs controls, respectively. When assessing the individual studies, Wang et al. [35] and Friedenreich et al. [50] did not find an association between TNFα and IL-6 which is in contrast to the studies conducted by Dossus et al. [33,34]. The risk of endometrial cancer appears not to be initiated by TNFα and IL-6, but may develop through other inflammatory pathways, such as genetic aberrations in PTEN or NFκB genes and the increased production of other mediators of inflammation [57]. Similar results related to the association of increased risk of endometrial cancer with TNFα and IL-6 were also found in a recent systematic review and meta-analysis on circulating adipokines and their risk to obesity related cancers including breast, colorectal, kidney pancreatic, prostate, endometrial, and multiple myeloma cancers [58].

The only study considering the association of circulating levels of IGF-I with endometrial cancer, showed no association, in agreement with a study by Petridou et al. [59] which showed that endometrial cancer was positively associated with IGF-II and inversely with IGF-I. This study adds to the gradually developing consensus that components of the IGF system play a central role in human carcinogenesis, and that IGF-II, rather than IGF-I, may be closely linked to the aetiology of endometrial cancer, one of the types of cancer most strongly associated with obesity.

Different study populations have differing characteristics, including BMI levels and presence of hypertension and diagnosis of diabetes. Further sub-analyses were performed to identify any other factors that could affect the risk of endometrial cancer. Table 2 summarises the OR of the association between circulating adiponectin, leptin and endometrial cancer stratified by study characteristics. BMI appeared to affect the association between circulating leptin levels and endometrial cancer risk, but not with circulating adiponectin levels and endometrial cancer risk. Hypertension and diabetes appear to affect the association between circulating leptin levels but not between adiponectin levels and increased endometrial cancer risk. Adiponectin and leptin may act synergistically and increase the risk of endometrial cancer. This is not the case for TNFα and IL-6.

The strength of our research is that this study presents a relatively comprehensive review of the existing evidence on the association of various adipocytokines and endometrial cancer. In particular, stratified analysis using a variety of selected variables has strengthened our results against the influence of confounding. There were also limitations to the meta-analysis; the number of cases in each study was relatively small, however the overall number of endometrial cancer cases in the meta-analysis was high, 2921. Retrospective studies were included and therefore, there is always a risk of potential bias in the form of recall bias.

This meta-analysis is the first to assess multiple adipocytokines in relation to endometrial cancer risk. Larger prospective studies assessing a variety of adipocytokines in the same cohort of patients are required to investigate further the association between adipocytokines and endometrial cancer, especially studies considering circulating levels of TNFα, IL-6 and IGF-I and II. This would allow elucidating in more details, the exact mechanisms underlying the link between adipocytokines and endometrial cancer.

Ethics approval and consent to participate

In this meta-analysis, we used only previously published data. Because no unpublished data were used, we did not seek ethics committee approval. The study is in accordance with the tenets of the Declaration of Helsinki.

Consent for publication

Not applicable.

Data availability

Not applicable.

Authors' contributions

PEE, GAB, and GB conceptualized this study, developed the protocol, and wrote the manuscript. PEE and GB selected articles for full-text review, extracted data from the included studies, and performed all statistical analyses.

Declaration of competing interest

None.

Acknowledgements

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Levels of adipocytokines in women with endometrial cancer and obesity

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Background

The association between endometrial cancer and obesity is well documented[1]: women of normal weight have a 3% lifetime risk of endometrial cancer compared to a 9-10% lifetime risk in obese women[2]. However, the relationship between obesity and endometrial cancer is complex and likely to involve multiple biological pathways.

In obese individuals, excess adipose tissue has been associated to increased cancer risk and to a state of chronic inflammation. Nevertheless, the exact role of intracellular signalling factors within adipose tissue, in the development of endometrial cancer, is still unclear.

In this inflammatory state, adipocytes and macrophages secrete several molecules, which may promote tumour development. Several studies have demonstrated an association between the risk of endometrial cancer and single circulating adipocytokines, however no study has been carried out to assess such association for several adipocytokines in the same patient population.

Aim

This study aims to examine serum concentration levels of adiponectin, leptin, tumour necrosis alpha (TNFα), interleukin 6 (IL-6), insulin growth factor 1 and 2 (IGF-I, IGF-II) in women with endometrial cancer.

Participants/Methods

- In 2018, patients with a new diagnosis of confirmed endometrial cancer were invited to take part in the study titled 'Discovering new cancer biomarkers from patient's blood, urine and DNA'.
- Blood samples were taken on the morning of surgery from the patients undergoing a Da Vinci robotic assisted hysterectomy and bilateral Salpingo oophorectomy, with or without pelvic lymph node sampling.
- The blood samples were immediately processed by centrifugation and serum stored at – 80° C at the University of Surrey. Serum samples from 24 patients with a diagnosis of endometrial cancer and 1 with endometrial hyperplasia were analysed for circulating levels of adiponectin, leptin, TNFα, IL-6 , IGF I and IGF-II using commercially available Enzyme-linked immunosorbent assays (ELISA) (Biotechne, UK).
- A history and clinical examination were also taken from all of the patients and data collected on the clinical characteristics included age, body mass index (BMI, kg/m²), menopausal status and any medical conditions such as hypertension or diabetes (Table 1).
- White cell counts (WCC) and C-reactive protein (CRP) were also assessed, where possible, to see if there were any signs of inflammation.

Table 1. Clinical characteristics of women with endometrial cancer and endometrial hyperplasia

	Age	BMI	Type of EC	WCC	CRP	Hypertension	Diabetes
Obese cases n = 16							
Case 1	63	31.2	grade 2 type 1	4.3	5.6	no	yes
Case 2	73	32.5	grade 3 type 2	7.4	<4	no	yes
Case 3	71	45.9	grade 2 type 1	9	<4	yes	yes
Case 4	64	42.7	grade 1 type 1	7.8	x	no	no
Case 5	51	32	grade 2 type 1	6.4	x	no	no
Case 6	75	31.2	hyperplasia	8.5	x	no	no
Case 7	74	47.9	grade1 type 1	7.8	<4	yes	no
Case 8	71	30.9	grade 2 type 1	8.6	<4	no	no
Case 9	73	40.5	grade 2 type 1	5.9	14	no	no
Case 10	78	31.6	grade 3 type2	5.8	<4	no	no
Case 11	76	30	grade 3 type 1	9.9	32	no	no
Case 12	74	36.8	grade 3 type 2	6.8	<4	no	no
Case 13	75	48.3	grade 3 type 1	10.3	<4	yes	no
Case 14	78	30	grade 1 type1	6.3	<4	no	no
Case 15	66	38	grade 3 type2	6.8	<4	no	no
Case 16	74	43.6	grade 3 type2	7.8	x	yes	no
Overweight cases n = 5							
Case 17	70	27.7	grade1 type1	6.6	4	yes	no
Case 18	77	25.2	grade1 type 1	5	9.7	no	no
Case 19	71	27.3	grade2 type1	8.6	4	yes	yes
Case 20	65	29.2	grade 3 type2	6.4	8	no	no
Case 21	68	27.3	grade 2 type1	8.1	4	yes	yes
Normal weight cases n = 4							
Case 22	36	19.2	grade 1 type 1	8.5	20	no	no
Case 23	55	23.7	grade 1 type 1	6.6	x	no	no
Case 24	65	21	grade 3 type 1	7.2	4	no	no
Case 25	80	23.4	grade 1 type 1	5.1	4	no	no

WCC: white cell count (normal= 4-11 *10⁹/l); CRP: C-reactive protein (normal = <10 mg/l); x not measured

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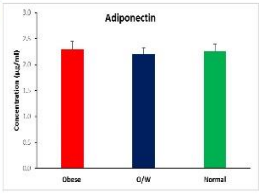
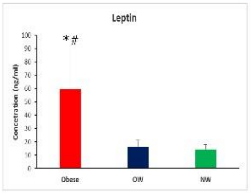
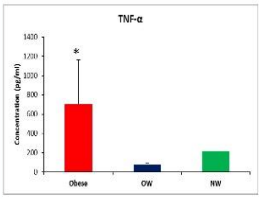
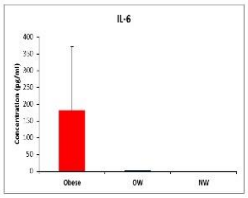
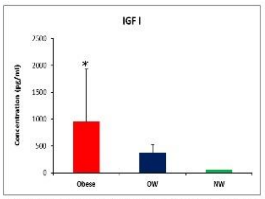
Results

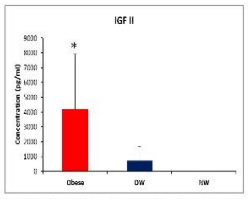












Results are presented as mean±standard deviation. Obese group n=16, overweight group (OW) n=5, normal weight group (NW) n=4. TNFα, IL-6 , IGF I and IGF-II concentrations were below detection levels in serum from some of the OW and NW participants.. * p<0.05 compared to OW; # p<0.05 compared to NW

- White cell counts were similar between groups, however CRP levels were higher in the obese group suggesting a certain degree of inflammation was present in this group.
- No differences in adiponectin levels were observed among weight categories in cancer patients; however mean values were lower compared to levels in a group of obese female healthy volunteers (6.5 ±1.9 µg/ml).
- Leptin concentration was higher in the obese group compared to OW and NW and similar to levels in a group of obese female healthy volunteers (61.1 ng/ml)
- TNFα and IL-6 levels were higher in obese patients compared to OW and NW, even if difference did not reach statistical significance for IL-6. Mean values were much higher than levels in a group of obese female healthy volunteers (33.7 and 24.6 pg/ml, respectively).
- IGF I and IGF-II levels were higher in obese patients compared to OW group.

Conclusions

- Except for adiponectin, higher serum concentrations of leptin, TNF-α, IL-6, IGF-I and IGF-II were observed in obese patients with endometrial cancer compared to overweight and normal weight patients.
- These results are comparable to what is present in the literature.
- These preliminary results suggests differences in adipocytokines serum concentration levels between obese, overweight and normal weight women may play a role in the development of endometrial cancer, although further studies are required to elucidate potential molecular pathways involved.

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