Endogenous hormones in the etiology of ovarian and endometrial cancers

Annekatrin Lukanova

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To the women from the

New York University Women’s Health Study, New York, USA
Northern Sweden Health and Disease Study, Umeå, Sweden
ORDET cohort, Milan, Italy
ABSTRACT

Endogenous hormones in the etiology of ovarian and endometrial cancers

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The main purpose of this thesis was to examine the relationship of pre-diagnostic circulating levels of sex-steroids (androgens and estrogens), sex hormone binding globuline (SHBG), insulin-like growth factor-I (IGF-I), IGF binding proteins (BP) and C-peptide (as a marker of pancreatic insulin secretion) with risk of ovarian and endometrial cancer. Additionally, the interrelationships of body mass index (BMI), sex-steroids, IGF-I and IGFBP-3 were examined.

Two case-control studies were nested within 3 prospective cohort studies centered in New York (USA), Umeå (Sweden) and Milan (Italy). The ovarian study included 132 cancer cases. The endometrial study included 166 cancer cases in the IGF-I and C-peptide component and 124 postmenopausal cases in the sex-steroids component. For each case, two controls matching the case for cohort, age, menopausal status and date at recruitment were selected. In total 286 and 315 controls were included in the ovarian and endometrial cancer studies, respectively. Odds ratios (OR) and their 95% confidence intervals (CI) for cancer risk associated with increasing hormone concentrations were estimated by conditional logistic regression. The cross-sectional analysis was based on anthropometric and hormonal data from 620 controls selected for the two nested case-control studies.

There was no association of prediagnostic androstenedione, testosterone, DHEAS, SHBG or estrone with ovarian cancer risk in the whole study population or in women who were pre- or postmenopausal at blood donation. In the premenopausal group, risk appeared to increase with increasing androstenedione (OR (95% CI) for the highest tertile: 2.35 (0.81-6.82), p=0.12). There was no association of IGF-I, IGFBP-1, 2, 3 or C-peptide concentrations with risk of ovarian cancer risk in the study group as a whole. In analyses restricted to subjects who had developed ovarian cancer at an early age (<55), circulating IGF-I was directly and strongly associated with risk (OR (95% CI): 4.74 (1.20-18.7), p<0.05 for the highest IGF-I tertile).

In the endometrial study, previous observations were confirmed that elevated circulating estrogens and androgens and decreased SHBG increase risk of developing endometrial malignancy after menopause. Multivariate ORs (95% CI) for endometrial cancer for quartiles with the highest hormone levels were: 4.13 (1.76-9.72), p<0.001 for estradiol; 3.67 (1.71-7.88), p<0.001 for estrone; 2.15 (1.05-4.40), p<0.04 for androstenedione; 1.74 (0.88-3.46), p<0.06 for testosterone; 2.90 (1.42-5.90), p<0.01 for DHEAS and 0.46 (0.20-1.05), p<0.01 for SHBG. Prediagnostic IGF-I, IGFBP-1, -2 and -3 were not related to risk of endometrial cancer in the whole study population. In postmenopausal women, levels of IGFBP-1 were inversely related to risk with an OR for the highest quartile of 0.36 (0.13-0.95), p<0.05. Endometrial cancer risk increased with increasing levels of C-peptide (p<0.01), up to an OR of 4.40 (1.65-11.7) for the highest quintile after adjustment for BMI and other confounders.

The cross-sectional analyses showed that in both pre- and postmenopausal women SHBG decreased with increasing BMI. In the postmenopausal group, estrogens, testosterone and androstenedione increased with BMI, while the association with IGF-I was non-linear, the highest mean IGF-I concentration being observed in women with BMI between 24 and 25. In postmenopausal women, IGF-I was positively related to androgens, inversely correlated with SHBG, and was not correlated with estrogens.

In conclusion, elevated pre-diagnostic sex-steroids, IGF-I or C-peptide increase risk of developing ovarian and endometrial cancer. BMI influences the circulating levels of these hormones, especially after menopause.

Key words: ovarian cancer, endometrial cancer, sex-steroid hormones, sex-hormone binding globulin, insulin-like growth factor-I, insulin-like growth factor binding proteins, C-peptide.
This thesis is based on the publications listed below, which are referenced in the text by their Roman numerals:


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ABBREVIATIONS

AR Androgen receptor
BMI Body mass index
CEPRT Combined estrogen-progestin replacement therapy
CI Confidence intervals
COC Combined oral contraceptives
DHEA Dehydroepiandrosterone
DHEAS Dehydroepiandrosterone sulfate
DSL Diagnostic System Laboratories
ER Estrogen receptor
ERT Estrogen only replacement therapy
GH Growth hormone
GLM Generalized Linear Models
FSH Follicle-stimulating hormone
HRT Hormone replacement therapy
IARC International Agency for Research on Cancer
IGF-I Insulin-like growth factor-I
IGFBP Insulin-like growth factor binding protein
IRMA Immunoradiometric assays
LH Luteinizing hormone
NSHDS Northern Sweden Health and Disease Study
NYUWHS New York University Women’s Health Study
OC Oral contraceptive
OR Odds ratio
ORDET Hormones and Diet in the Etiology of Breast Cancer cohort
OSE Surface epithelium of the ovary
PCOS Polycystic ovary syndrome
POC Progestogen only oral contraceptives
RIA Radioimmunoassays
RR Relative risk
SAS Statistical Analysis System software program
SEPRT Sequential estrogen-progestin replacement therapy
SHBG Sex-hormone binding globulin
SOC Sequential oral contraceptives
WHO World Health Organization
WHR Waist-to-hip ratio
VIP Västerbotten Intervention Program
INTRODUCTION

Tumors of the ovary and corpus uteri comprise more than 70% of all gynaecological malignancies \(^1\,^2\). There are large international variations in the occurrence of these cancers \(^1\). The age-standardised incidence rates are up to ten-fold higher in women from North America, Northern and Western Europe than in women from most parts of Africa and Eastern Asia (Figure 1)\(^1\,^2\). Increases in the incidence rates of these cancers in migrants moving from low-risk to high-risk (Western) areas \(^3\,^4\,^6\) strongly suggests that environmental, i.e. non-genetic risk factors, related to westernization of lifestyle play an important etiological role. It is believed that differences in reproductive characteristics, prevalence of obesity and in the level of physical activity are largely responsible for the international variations in cancer incidence \(^7\,^11\). Physiological mechanisms that might mediate the effects of a western-lifestyle on cancer risk include alterations in the synthesis of endogenous hormones, such as sex-steroids, insulin, insulin-like growth factor-I (IGF-I) and IGF-binding proteins (IGFBP). A number of prospective studies have now demonstrated that elevated circulating androgens, estrogens, insulin or IGF-I are directly related to

![Incidences of Ovary etc. cancer: ASR (World) (All ages)](image)

![Incidences of Corpus uteri cancer: ASR (World) (All ages)](image)

**Figure 1**: Age-standardized incidence rates of ovarian and endometrial cancers world-wide.
increased risk of several cancers, including breast, colorectal and prostate cancers \(^8,12-18\). The available epidemiological and experimental data suggest that endogenous hormones are involved in the pathogenesis of ovarian and endometrial cancers, but, so far, only a few, small studies have reported on the association of pre-diagnostic endogenous hormone levels with risk of these gynaecological malignancies \(^19-22\).

The main objective of the studies included in this work was to examine the association of prediagnostic levels of several sex-steroid and peptide hormones with risk of developing ovarian or endometrial cancer. The identification of potentially modifiable life-style or hormonal determinants of IGF-I and sex-steroid hormone concentrations is important in light of the increasing epidemiological evidence linking these hormones to cancer risk. For this reason we examined the role of BMI as a determinant of circulating levels of androgens, estrogens, sex hormone binding globuline (SHBG), IGF-I and IGFBP-3 and the interrelationship between these hormones.

In the following section, a summary is presented of the available epidemiological evidence for ovarian and endometrial cancers, with a special emphasis on the role of endogenous hormones. A brief outline of well-established risk factors for these two cancer types is followed by discussion of the involvement of endogenous sex-steroids (androgens, estrogens and progesterone), insulin and insulin-like growth factor-I in the etiology of these cancers, in the light of the available epidemiological evidence and experimental data. A brief review of the possible role of inclusion cysts and Müllerian epithelium differentiation in the pathology of epithelial ovarian cancer is also discussed.
BACKGROUND

1. OVARIAN CANCER

According to the cell types from which ovarian tumors are presumed to originate, they are divided into three major groups: surface epithelial-stromal tumors, germ cell tumors and sex cord-stromal tumors, each having distinct clinical and epidemiological characteristics 23-26 (Figure 2). Epithelial ovarian neoplasms account for 80 - 90% of ovarian malignancies in the USA and Western Europe 27 and usually dominate the data from cancer registries. The epithelial ovarian tumors are further divided into serous (about 50% of all ovarian malignancies), mucinous (5-10%), endometrioid (10-25%), clear cell (4-5%), Brenner, undifferentiated (5%) and mixed subtypes 24,28. Most epidemiological studies have focused on the group of epithelial ovarian cancers as a whole, although some studies were able to investigate and show possible differences in risk factors according to histological subtype 25,29-32. About 15% of epithelial ovarian neoplasms are classified as ‘borderline tumors’, which are mostly of serous and mucinous subtypes 24,33,35. They comprise a separate entity, usually with epidemiological characteristics similar to the frankly invasive tumors, but occur in younger women, present at an earlier stage and have a favourable prognosis 24,34,36.

Epithelial origin of most ovarian tumors and formation of inclusion cysts

Epithelial ovarian tumors are thought to arise from the surface epithelium of the ovary (OSE) and its inclusion cysts 27. The OSE is composed of modified peritoneal mesothelial cells which replicate as generative stem cells, i.e., the division of a surface epithelial cell yields two daughter cells with equal potential for further cell division 37. The OSE is separated from the hormone-producing ovarian stroma by a basement membrane and, underneath, a

Figure 2: Age-specific incidence of ovarian cancer according to histological sub-type. SEER (1993-1997), USA
collagenous tissue layer, the tunica albuginea.

OSE has the potential to differentiate either to stromal or to ectopic (aberrant) epithelial phenotypes. Auersperg et al. (2001) proposed that the capacity of OSE cells to undergo epithelio-mesenchymal conversion might be a homeostatic mechanism to accommodate OSE cells that become trapped within the ovary as stromal fibroblasts. An inability to undergo such conversion would preserve the epithelial forms within the ovarian stroma, which could lead to OSE aggregation and inclusion cyst formation. The epithelial phenotypes appear to be prone to metaplastic and dysplastic changes that might ultimately lead to tumorigenesis. It was proposed that differentiation of OSE cells specifically towards Müllerian types of epithelia, similar to those of the fallopian tube, endometrium or the endocervix, might confer a selective growth advantage through changes in hormone/growth factor receptors and responsiveness (reviewed in detail in ). Indeed, the most frequent type of epithelial ovarian malignancies—serous tumors—resemble the epithelium of the fallopian tube, while endometrioid tumors resemble the epithelium of the endometrium, and mucinous tumors that of the endocervix.

Although not conclusive, there is evidence to suggest that OSE inclusion cysts are more prone to malignant transformation than the surface epithelium itself (reviewed in ). First, most early carcinomas of the ovary appear to be confined within the organ without involvement of its surface. Second, tubal metaplasia has been observed more frequently in inclusion cyst OSE than in OSE itself. Third, OSE metaplasia has been found 2- to 3- times more frequently in inclusion cysts of ovaries contralateral to ovaries containing carcinomas than in ovaries of cancer-free subjects. Fourth, several ovarian carcinoma tumor markers, such as CA-125 or CA19-9, were identified immunohistochemically more often in the epithelium of the inclusion cysts than in the OSE itself. Epithelial tumors are also less frequent in the related pelvic peritoneal mesothelium, which has a much larger surface area.

It has been suggested that ovarian inclusion cysts form as a consequence of entrapment of OSE cells in the stromal tissue during repeated damage and remodelling of the OSE induced by ovulations. However, this may not invariably be the case, because the majority of cortical inclusion cysts are not, in fact, related to the repair of the ovulatory defect. Inclusion cysts can be found in ovaries from women of all ages, but their frequency increases with age, and they are commonly observed at late-reproductive and postmenopausal ages. Alternative possible origins of the inclusion cysts are inflammatory adhesions involving the ovarian surface, polycystic ovary syndrome (PCOS), or infoldings, which are typical of the normal surface of the ovary. The ovarian stromal-mesothelial interface is dynamic, and proliferation of either component may result in the pinching off of portions of the mesothelium to form small cysts. The epithelium covering the ovaries is avascular, and, therefore, its cells are more likely to be exposed to hormones and growth factors through paracrine than to endocrine mechanisms. OSE cells in inclusion cysts embedded in the ovarian
stroma and in immediate proximity to hormone and growth factor producing cell types, may be exposed through both endocrine and paracrine mechanisms.\textsuperscript{48,53,54}

**Basic epidemiological risk factors**

Well-established risk factors for ovarian cancer are age, family history of ovarian cancer and infertility, while increasing parity, oral contraceptive (OC) use, hysterectomy or tubal ligation decrease risk.\textsuperscript{23,32,48,55-57}

The incidence rates of ovarian cancer increase with age, but the rate of increase is lower after age 50-55 in countries with low incidence rates and after age 60-65 in countries with high incidence rates.\textsuperscript{1,2} The median age at ovarian cancer diagnosis is between 58 to 65 years in well-developed countries with high incidence rates.\textsuperscript{1} Women with relatives affected with ovarian cancer are at higher risk, depending on the number of affected first-degree relatives.\textsuperscript{58} Risk is substantially higher in carriers of BRCA1 or BRCA2 mutations with average cumulative risks by age 70 of about 40% and 10%, respectively.\textsuperscript{59}

The protective effect of pregnancy has been uniformly demonstrated in North American, European and Asian populations,\textsuperscript{55} with a 10-16% decrease in risk for each additional pregnancy.\textsuperscript{29,56,60} Some studies,\textsuperscript{29,30,61} but not all,\textsuperscript{25,31,32,62} have indicated that the protection may be restricted to non-mucinous tumors. Although not uniformly so, several studies have found a significant trend of decreasing ovarian cancer risk with increasing age at first birth.\textsuperscript{32,56,63-66} and two studies reported decrease of risk with greater age at last birth.\textsuperscript{64,66} These observations are in contrast to the protective effect of early age of first pregnancy for breast cancer.\textsuperscript{67}

It has been estimated that five or more years of OC use confers a 30-50% reduction in risk.\textsuperscript{56,68,69} The favourable effect has been observed for at least 10-15 years since last use\textsuperscript{56,68,69} and recent studies showed that the protection may persist for even longer (> 20-25 years).\textsuperscript{32,71,489} In contrast, use of exogenous hormones for menopause-related symptoms may even confer slightly higher risk, as suggested by two meta-analyses (including 9 and 15 studies respectively).\textsuperscript{74,75} Subsequently, 3 cohort\textsuperscript{76,78,79} and 1\textsuperscript{77} out of 3 large case-control studies\textsuperscript{77,490,491} have reported a direct association between use of HRT and ovarian cancer incidence or mortality. Prolonged periods of use (≥ 5-10 years) have been associated with about 1.5-2-fold increase in risk.\textsuperscript{76-79, 490,491} In the 3 recent cohort studies\textsuperscript{76,78,79} baseline intake of HRT was associated with higher risk and in two of these\textsuperscript{76,79} the effect was confined mainly to recent or baseline users. In 2\textsuperscript{77,78} out of 3 recent studies\textsuperscript{77,78,491} where separation by type of HRT formulations was possible, risk was elevated specifically among those who used estrogen unopposed by progesterone.\textsuperscript{77,78} However, the results of the Women’s Health Initiative randomized trial indicated that risk of epithelial ovarian cancer could be increased also in users of combined HRT [1.64 (0.78-3.45)]\textsuperscript{258} Some data suggest that the risk may be more pronounced for endometrioid and clear cell tumors and less so for mucinous tumors.\textsuperscript{32,80,81} Data regarding possible modification of the effect of HRT according to previous hysterectomy are largely inconclusive.\textsuperscript{77,78,258,491}

Investigating the effects of infertility on ovarian cancer beyond the elevated risk conferred by low parity and other factors, has proven to be problematic. Infertility appears to increase risk of ovarian cancer
among nulligravid/ nulliparous women with refractory infertility (as measured by unresponsiveness to fertility medications and long periods of unprotected intercourse without pregnancy) but not among parous women [55,56]. A recent study pooled the data from 8 case-control studies conducted between 1989 and 1999 in the United States, Denmark, Canada and Australia showed that, among nulligravid women, attempts for more than 5 years to become pregnant compared with attempts for less than 1 year conferred close to 3-fold higher risk of ovarian cancer [82]. Seeking medical attention for fertility problems was a modest risk factor for ovarian cancer, with the same odds ratio (OR) of 1.2 among ever and never pregnant women [82]. Fertility drug use is not clearly associated with risk of ovarian cancer [83-85]. In the pooled analysis of Ness et al. (2002) risk was somewhat elevated [1.60 (0.90-2.87)] among never pregnant women [82], but longer duration of fertility drug use did not significantly elevate ovarian cancer risk, even among nulligravid women, nor did longer duration of specific drugs use (i.e. clomiphene or human menopausal gonadotropin) [82].

Ovarian cancer risk is possibly decreased with increasing duration of breast-feeding [32,55] and twin pregnancies [86-88], possibly increased in women with endometriosis [89-91] or PCOS [92], but is not clearly related to ages at menarche or menopause [32,55,93,94]. Several recent studies have shown increased risk of ovarian cancer with tobacco smoking, overall [95,96], and specifically for mucinous tumors [96-99].

Excess body weight probably confers a small to moderate increase of risk of developing ovarian cancer risk (on average between 20 and 40%) [7,106-108], although in many studies the associations did not reach statistical significance [104-108], and even a decrease in risk has been observed [109,110]. No association with adult body mass index (BMI) was observed in a recent, very large prospective study including more than one million Norwegian women, among whom 7720 ovarian cancer cases occurred [111]. However, obesity (BMI ≥ 30) in early adulthood (from 20 to 29 years) was associated with about 45% increase in risk. In the same study, data on measured height and weight were also available for more than 110,000 adolescent girls among whom 260 incident ovarian cancers were identified (98 cases were included in both analyses) [111]. High or very high BMI during adolescence conferred about a 50% increased risk of developing ovarian cancer. Two more studies indicated that elevated BMI at age 18 may increase risk of ovarian cancer overall [112] or during premenopausal years specifically [104], but no association was observed in two others [105,113]. Generally, weight gain throughout adulthood has not been related to increased risk of ovarian cancer [104,107,112-114].

Only four studies have reported on the association of waist-to-hip ratio (WHR), as a measure of central (android) obesity, with ovarian cancer [104,106,107,115]. Despite the lack of association of BMI with ovarian cancer in one cohort [106] and one large case-control study [107], high WHR was associated with significantly increased risk of ovarian cancer in both of these studies, as well as in a small case-control study in premenopausal women [115]. No association was observed in a fourth (cohort) study [104].

Several recent studies have indicated a direct association of height with risk [100,110,111,113,114], and in some of these the association appeared stronger in younger women [110,111], whereas in other studies no association with height was found [105,107,112].
The reports on the association of physical activity and ovarian cancer have been contradictory and no clear tendency has yet become evident.\textsuperscript{7,106,116-118}

**Endogenous hormones and ovarian cancer development**

The epidemiological characteristics of ovarian cancer have given rise to several etiological hypotheses. Among those that do not postulate a direct involvement of endogenous hormones are the incessant ovulation\textsuperscript{119}, the inflammation\textsuperscript{120} and retrograde transport\textsuperscript{121} hypotheses. The most widely cited is the incessant ovulation hypothesis, proposed by Fathalla in 1971, which states that long periods of menstrual cycles increase the risk of ovarian cancer. Indeed, well-established epidemiological risk factors for ovarian cancer, such as the protective effect of OC use, parity and breast-feeding and the adverse effect of a high lifetime number of ovulatory cycles\textsuperscript{122,123} are consistent with the incessant ovulation hypothesis (Table 1).\textsuperscript{48} Fathalla’s hypothesis fails, however, to provide a rationale for other observations, such as the greater protective effect of both pregnancy and OC use than that expected simply on the basis of the number of suppressed ovulations, the protection associated with twin pregnancies and the lack of clear association with ages at either menarche or menopause (Table 1).\textsuperscript{23,48,56,60,86,87} Thus, the involvement of additional, probably hormonal, mechanisms have been proposed.

Hormones and growth factors play a central role in regulating cell proliferation, differentiation, and apoptosis. Dysregulation of these processes may allow cells that have harboured mutations in proto-oncogenes and tumor suppressor genes to survive and expand clonally.\textsuperscript{124-126} Pituitary gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)], androgens, estrogens, progesterone, and IGF-I have all been proposed to influence ovarian cancer development.\textsuperscript{48,127}

**Gonadotropins**

The “gonadotropin hypothesis” was the first hormonal hypothesis developed to explain ovarian cancer pathogenesis. It postulates that ovarian cancer develops as a consequence of excessive stimulation of ovarian tissue by pituitary gonadotropins (LH and/or FSH). The effect of gonadotropins may be exerted either directly, through activation of gonadotropin-responsive genes in those cells that would eventually undergo malignant transformation, or indirectly through stimulation of ovarian production of sex steroids that could influence malignant transformation through paracrine or endocrine mechanisms.\textsuperscript{48,54}

The existing experimental data on the effect of gonadotropin hormones on the proliferation of normal and malignant OSE-cell cultures is inconclusive, the majority of studies showing either an increase in proliferation\textsuperscript{128-132} or no effect\textsuperscript{130,133,134}. The generalisability of the animal models, from which the gonadotropin hypothesis originated, to human epithelial ovarian cancer has been questioned, as the tumors induced in rodents are mostly of non-epithelial origin (reviewed in).\textsuperscript{48,135}

Epidemiological evidence indirectly supporting the gonadotropin hypothesis includes the well-documented protective effects of pregnancies and oral contraceptive use, both of which suppress pituitary gonadotropin secretion (Table 1).\textsuperscript{48} Additional support comes from the increased ovarian cancer risk among...
Table 1. Agreement between observed association of some epidemiological factors with ovarian cancer in epidemiological studies and as predicted by several etiological hypotheses.

<table>
<thead>
<tr>
<th>Epidemiologic factor</th>
<th>Observed relationship with risk</th>
<th>Agreement between observed and predicted by etiological hormonal hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gonadotropin</td>
</tr>
<tr>
<td>↑ age at menarche</td>
<td>~</td>
<td>-</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>↓↓</td>
<td>+</td>
</tr>
<tr>
<td>Twin pregnancies</td>
<td>→</td>
<td>-</td>
</tr>
<tr>
<td>Breast feeding</td>
<td>→</td>
<td>+</td>
</tr>
<tr>
<td>OC use</td>
<td>↓↓</td>
<td>+</td>
</tr>
<tr>
<td>Cumulative # of ovulations</td>
<td>↑↑</td>
<td>+</td>
</tr>
<tr>
<td>↑ age at menopause</td>
<td>~</td>
<td>-</td>
</tr>
<tr>
<td>Estrogen only HRT</td>
<td>↑</td>
<td>-</td>
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<tr>
<td>Combined HRT</td>
<td>↑</td>
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<tr>
<td>Excess weight</td>
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<td>Diabetes</td>
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<tr>
<td>Endometriosis</td>
<td>↑</td>
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</tr>
<tr>
<td>PCOS</td>
<td>↓↑</td>
<td>-</td>
</tr>
<tr>
<td>Tubal ligation / hysterectomy</td>
<td>↓</td>
<td></td>
</tr>
</tbody>
</table>

↑ / ↓ / ↑↓ — increase / decrease / ~ - no clear or weak association with risk; + / - agreement /disagreement between observed association with risk and as predicted under an etiological hypothesis
women with PCOS (who have elevated circulating LH) \(^9\), and case reports of ovarian cancer following ovulation induction therapy \(^136\). In conflict with the gonadotropin hypothesis is the lack of increase in risk related to early age at menopause and with twin pregnancies (either of which are associated with an increase in gonadotropin levels \(^137,138\)), the absence of an elevation of the rate of increase of ovarian cancer incidence after menopause in spite of increasing LH and FSH levels \(^1,2\) and the moderately increased risks of ovarian cancer among HRT users \(^75,78\) or in obese women, who have lower circulating FSH and/or LH \(^139,140\).

Direct epidemiological evidence to support the gonadotropin hypothesis is scarce and inconclusive. Three prospective studies, all of which were relatively small, showed either no difference or even decreased blood levels of FSH and LH in ovarian cancer cases compared to control subjects \(^19,21,22\).

**Androgens**

In a seminal review paper, Risch (1998) proposed that ovarian cancer risk may be increased by excess androgenic stimulation of ovarian epithelial cells (the “androgen hypothesis”) \(^48\).

Androgens are produced by the ovaries, theca-interstitial cells surrounding the developing ovarian follicles secrete about 50% of circulating androstenedione (AD) and about 25% of testosterone (T) and dehydroepiandrosterone (DHEA) \(^141-143\). The adrenal glands contribute about 50% of circulating androstenedione and DHEA, up to 25% of testosterone and virtually all of DHEA-sulfate (DHEAS) \(^141-143\). The remaining 50 - 60% of circulating testosterone and 25-30% of DHEA are derived from peripheral conversion (e.g. in fat, liver, kidneys) from androstenedione and DHEAS \(^141-143\). After menopause and the loss of ovarian follicles (together with the associated theca-interstitial and granulosa cells), the elevated levels of gonadotropins stimulate the ovarian synthesis of androgens by the secondary interstitial cells (direct descendants of the theca-interstitial cells of atretic follicles) and by the hilar cells (equivalent to testicular Leydig cells) \(^143,144\). As a consequence, the ovarian contribution to circulating androstenedione falls to about 20%, while testosterone production remains largely unchanged (or even increases slightly) so that its relative contribution to circulating levels is about 40% \(^144-146\).

Androgen receptors (AR) have been found on ovarian epithelial cells from both normal ovaries and malignant tumors \(^127,147,148\). Several *in vitro* studies (although not all \(^149,150\)) have shown increased cell proliferation of normal OSE-cells or ovarian cancer cell lines after androgen administration \(^129,148,151-153\) and anti-androgens were shown to have a dose-dependent inhibitory effect on the growth of AR-positive ovarian cancer lines \(^154\) and primary cultures \(^127\). In an experimental study with guinea pigs, testosterone administration stimulated the growth of ovarian epithelial cells, resulting in formation of benign cyst and small adenomas in the ovarian parenchyma and papillomas on the ovarian surface \(^155\).

Epidemiological evidence in support of the androgen hypothesis includes the protective effect of OCS, which reduce androgen levels in both normo- and hyperandrogenic women \(^156-158\) and the increased risk in women with a previous diagnosis of
PCOS (Table 1) 

PCOS is a syndrome of ovarian hyperandrogenism, characterised by increased ovarian stromal cell mass, excessive androgen production and elevated pituitary LH secretion. Histological examinations of the ovaries of PCOS patients have shown an increased occurrence of epithelial inclusion cysts compared to ovaries of normo-androgenic women 

Mice genetically modified to produce excessive amounts of LH had increased plasma androgen concentrations, presented an ovarian morphology similar to that of PCOS patients and developed ovarian tumors at high frequency .

PCOS is often associated with obesity and usually develops during puberty , hence, it is tempting to relate recent findings of increased ovarian cancer risk in adolescents with high BMI to a higher prevalence of PCOS among obese girls . Nevertheless, PCOS is observed also among lean women, in whom the androgen excess is more strongly related to elevated LH secretion than in obese women .

Finally, patients treated with danazol (a synthetic androgen that binds to androgen receptors and SHBG, resulting in a 3-fold increase in free testosterone) had almost 3-fold higher risk of ovarian cancer in comparison with patients who took leuprolide /nafarelin (gonadotropin releasing hormone analogues) or no medication .

More direct epidemiological evidence for androgen involvement comes from the results of the first prospective study on endogenous hormones and ovarian cancer, which was nested within a population-based serum bank in Washington County, Maryland. It included 31 case and 62 control subjects, of which 13 case and 26 control subjects were premenopausal at blood donation .

In premenopausal women, case subjects had 44% higher androstenedione, 110% higher DHEA levels than control subjects, while no such difference was observed for levels of DHEAS. In postmenopausal women there were no statistically significant differences in mean androgen concentrations between case and control subjects.

**Estrogens**

Granulosa cells of the premenopausal ovary secrete both estradiol and estrone and, at ovulation, OSE cells are bathed in the estrogen-rich follicular fluid, which may have concentrations four orders of magnitude higher than circulating levels .

Estrogen receptors (ER) are expressed in normal ovarian epithelium, ovarian carcinoma cell lines and primary cultures . Recent studies have indicated that expression of ER subtypes – ERα and ERβ - may differ between cells of normal OSE, benign and malignant tumors .

Several *in vitro* and animal experiments (although not all ) have shown the potential of estrogens to stimulate cell proliferation and inhibit apoptosis of OSE and ovarian cancer cells and one study has reported higher estradiol levels in the fluid from malignant than from benign ovarian cysts in postmenopausal women .

OSE cells and stromal cells of several subtypes of epithelial ovarian neoplasms have been reported to express aromatase, the enzyme responsible for the synthesis of estrogens from precursor androgens. Patients with endometriosis are at increased risk to develop ovarian cancer and endometriotic lesions have been shown to harbour molecular aberrations that favour increased local production of estradiol, including higher expression of aromatase and deficiency of 17β-hydroxysteroid dehydrogenase type 2 (that
converts estradiol to estrone)\textsuperscript{191}. However, endometriosis has been linked with development of endometrioid and clear cell tumors, but not with the predominant serous type or with mucinous tumors (recently reviewed in \textsuperscript{35,91}). Along the same lines, about 15-20\% of endometrioid carcinomas of the ovary are associated with carcinoma of the endometrium \textsuperscript{192} and estrogens are a major risk factors for the development of endometrial malignancies. Treatment of premenopausal breast cancer patients with tamoxifen, which increases ovarian synthesis and circulating levels of estradiol \textsuperscript{182-184}, may be complicated by the development of persistent functional ovarian cysts \textsuperscript{185-187}, although, so far, tamoxifen treatment has not been found to increase risk of ovarian cancer \textsuperscript{188}.

From an epidemiological perspective, support for an adverse effect of elevated estrogens on ovarian cancer comes from the associations of OC and HRT use with risk (Table 1). The opposite effects of exogenous estrogens use before and after menopause can be explained by their differential effect on intra-ovarian and circulating estrogen levels. OC use decreases ovarian estrogen production and early to mid-follicular phase circulating estrogen levels are maintained during use \textsuperscript{48}. In contrast, the purpose of HRT supplementation after menopause is to increase the overall estrogenic background. Currently, the most consistent pattern of the role of HRT in ovarian cancer is that long term exposure to any type HRT increases risk \textsuperscript{76-79,258,490}, which would point to a direct etiological importance of persistently elevated estrogen concentrations. However, given the long latency of ovarian cancer \textsuperscript{48} and that in some studies the association was confined to recent users, would argue for a role of estrogens as a stimulator of the growth of pre-existing, undiagnosed ovarian cancer.

The weaker effect of combined estrogen and progestin than estrogen-only formulations might be due to the beneficial role of the progestin component. The increase in risk only in women without hysterectomy and the stronger effect of unopposed estrogen formulations observed in some studies was proposed to be mediated also through increased retrograde menstrual flow, more frequent in users of unopposed estrogen \textsuperscript{77,80,81}.

While some of the above evidence provides indirect support for the estrogen hypothesis, the relatively weak and inconsistent association of obesity with ovarian cancer, in contrast to the more pronounced relationship with cancers of the endometrium and breast \textsuperscript{7}, argues against a major effect of circulating estrogens on disease development, especially after menopause. Additionally, no difference in mean estradiol and estrone levels in postmenopausal case and control subjects were found in the first prospective study of Helzlsouer et al. and premenopausal ovarian cancer cases had even slightly lower estrone and estradiol levels than control subjects \textsuperscript{19}.

**Progesterone**

Ovarian surface epithelium cell contain progesterone receptors, indicating that they have the potential to respond to this hormone \textsuperscript{194}. It has been shown that subtypes of progesterone receptor (type A and B) expressed in normal and malignant ovarian epithelial cells may differ \textsuperscript{169}.

A number of observations led Risch (1998) to suggest that progesterone might protect against ovarian tumor development (Table 1) \textsuperscript{48}. Risch proposed that the protective effect of pregnancy, which exceeds that due to simple suppression of ovulation, may be due to the elevated
progesterone concentrations\textsuperscript{48}. Moreover, such a protective effect of progesterone could account for the protective effect of twin pregnancies, an observation which is in sharp contradiction with both the incessant ovulation and the gonadotropin hypotheses. Progesterone levels during multiple pregnancies are higher in comparison with singleton pregnancies\textsuperscript{195-198} and there is some evidence that mothers of dizygotic twins may also have higher follicular phase serum progesterone than women with single pregnancies\textsuperscript{199}.

Some of the protection conferred by OC use might be due to the strong potency of the synthetic progestins, which may more than compensate for the decrease in endogenous progesterone synthesis in pill users\textsuperscript{48,200}. A 3-year study in primates showed that the progestin component of the OC Triphasil (levonorgestrel) has a potent apoptotic effect on ovarian epithelium\textsuperscript{201}. Possible mechanisms mediating the pro-apoptotic effect of progestins include differential regulation of transforming growth factor-β\textsuperscript{202} and up-regulation of p53 expression\textsuperscript{203}. A pro-apoptotic effect of progesterone together with the observed protective effect of later age at first and last birth are in line with the suggestion of Adami et al. (1994) that pregnancy might clear from the ovaries cells that have already undergone malignant transformation\textsuperscript{63}.

The indication of a protective effect of progestin-only formulations is also intriguing\textsuperscript{204}, because their contraceptive effect is through thickening of the cervical mucus, making it relatively impenetrable to sperm, and through reduction of the endometrial receptivity to implantation, while suppression of ovulation occurs in about 40% of the users\textsuperscript{48,69}. Support for the protective effect of the progestin component in exogenous hormone preparations comes also from one study that showed that independently of their estrogen component, combination OC formulations with high progestin potency confer greater reduction in ovarian cancer risk than those with low-progestin potency\textsuperscript{72} and two other studies found that risk of ovarian cancer is higher in users of estrogen-only HRT than in combined estrogen-progestin HRT users\textsuperscript{77,78}. Nevertheless, epidemiological data about the effect of progestin-containing hormone preparations remains scarce and some studies do not support a preferential protective effect of the progestin formulations\textsuperscript{48,69,71}.

Under the progesterone hypothesis, obese premenopausal women, who have increased frequency of anovulatory cycles and consequently decreased progesterone production, should be at increased risk of ovarian cancer. Few studies have reported on the association of BMI with ovarian cancer specifically in premenopausal women or at a relevant age group (e.g. BM at age 30). In most of the studies, the association of BMI with ovarian cancer tended to be stronger or confined to the premenopausal group (although the difference of the effect of elevated BMI in pre- and postmenopausal women was far from reaching statistical significance)\textsuperscript{101,104,105,107,205,206}. One may also speculate that the protection conferred by progesterone is mostly evident after a prolonged exposure to high concentration of this hormone (such as observed during pregnancy and OC use), while the elevation of progesterone during the luteal phase of the menstrual cycle is counter-balanced by concomitant changes associated with ovulation, such as an increase in estrogen and androgen production by the ovaries.
Insulin has been shown to exert mitogenic and anti-apoptotic properties and has been directly implicated in the development of several cancer types. A number of tumor tissues, including ovarian tumors, have been shown to have increased insulin receptor content. At high concentrations, insulin may bind to the IGF-I receptor and activate intra-cellular signalling pathways under its control. Insulin might also enhance ovarian cancer development through its effects on the synthesis and metabolism of other hormones. It can stimulate LH-induced synthesis of androgens and is a powerful down-regulator of the synthesis of sex-hormone binding globulin and IGF-I binding protein (IGFBP)-1, and hence it is a determinant of the free, biologically active fraction of sex-steroid hormones and IGF-I. Insulin resistance is believed to play a central role in the development of PCOS, a hyperandrogenic syndrome, that was associated with increased risk of ovarian cancer in one study.

Despite the above-mentioned characteristics of insulin, that might link it to ovarian cancer pathogenesis, the available epidemiological evidence, so far, does not strongly support its involvement in ovarian cancer development (Table 1). Obesity, which is a widespread hyperinsulinaemic condition in affluent societies, appears to be weakly associated with ovarian cancer. Moreover, weight gain throughout adulthood does not seem to influence ovarian cancer risk. Regular physical activity improves insulin sensitivity, but the data relating physical activity to ovarian cancer are conflicting and do not show a clear-cut protective effect. Type-II diabetes, which is generally associated with a long history of insulin resistance and hyperinsulinemia before diagnosis and also for several years after diagnosis, also is not associated with an increased risk of ovarian cancer. However, insulin levels are more strongly correlated with WHR than with BMI and some initial data indicates that WHR may be related to risk of ovarian cancer.

IGF-I and IGFBPs

Recent epidemiological studies have related elevated circulating levels of IGF-I – measured as absolute concentrations, or relative to levels of IGFBP-3 – to increased risk of cancers of the breast, prostate and colon. The principal mechanisms by which IGF-I is believed to influence cancer risk involve increased cell proliferation and inhibition of apoptosis, effects that have been demonstrated in many cell types, including normal and neoplastic epithelial ovarian cells.

The biological activity of IGF-I is modulated by IGFBPs. IGFBPs are the transport proteins for IGFs in plasma and regulate the half-lives and the metabolic clearance rates of IGFs. Over 90% of circulating IGF-I is in a complex with IGFBP-3 and a leucine rich glycoprotein, acid labile subunit. IGFBP-3 has a very high affinity for IGF-I, and the large IGF-I/IGFBP-3/acid-labile subunit complex cannot pass through the capillary barrier to target tissues. IGFBP-5, which has even higher affinity than IGFBP-3 for IGF-I, may form similar IGF-I/IGFBP-5/acid-labile subunit complexes. Practically, almost all of the remaining IGF-I is bound to IGFBP-1, -2, -4 and -6, which have lower binding affinities for IGF-I (compared to IGFBP-3 and -5), do not form complexes with an acid-labile subunit, and are small enough to cross the endothelial barrier. A decrease in plasma IGFBP-3, with a transfer of IGF-I
to smaller IGFBPs not complexed with acid-labile subunit, is believed to increase IGF-I availability to its tissue receptors. Reduction in plasma concentrations of the smaller IGFBPs, and particularly IGFBP-1 and –2, is also thought to increase the bioavailability of circulating IGF-I.

At the tissue level, IGFBPs have been proposed mostly to inhibit receptor binding by complexing IGF-I. Nevertheless, results from in vitro studies suggest that, depending on the relative concentrations of IGF-I and IGFBPs, and perhaps depending also on the tissue type, certain IGFBPs (e.g. IGFBP-1, -2, -3 and -5) may actually enhance IGF-I binding to its receptors. Furthermore, it is possible that certain IGFBPs can also exert effects through their own specific receptors. For example, IGFBP-3 has been shown to stimulate apoptosis of breast, prostate and endometrial cancer cells in vitro through an IGFBP-3 specific binding site on the cell membrane.

The main determinant of the synthesis of IGF-I, IGFBP-3 and acid-labile subunit is growth hormone (GH). Insulin appears to be a central regulator of IGF-I bioactivity as a function of available dietary energy. Insulin enhances GH-stimulated IGF-I synthesis by increasing GH receptor content in the liver and by stimulating cellular uptake of amino acids and protein synthesis. Insulin may augment IGF-I bioactivity by inhibiting the production of IGFBP-1 and –2 in the liver and other tissues.

Initial data linking the IGF-I system to ovarian cancer come from observations that IGF-I levels are higher in cystic fluid from invasive malignant ovarian neoplasms than in cystic fluid from benign neoplasms. IGF-I receptors are present in surgical specimens from primary and metastatic ovarian tumors and carcinoma cells derived from fresh, untreated ovarian cancers express all major components of the IGF-I system – IGF-peptides, type-I IGF-I receptor, IGFBPs – and demonstrate functional responses to exogenous IGF.

II. ENDOMETRIAL CANCER

Endometrial tumors have been divided into two main subgroups according to their histopathological features. Type-I endometrial tumors are endometrioid carcinomas, which comprise about 80% of endometrial tumors. The predominant histology of type-II tumors is serous-papillary carcinoma, but it includes also clear-cell adenocarcinoma and squamous cell carcinoma. It is believed that endometrioid tumours (type-I) develop via a characteristic sequence of hyperplastic changes of the endometrium with increasing premalignant potential, consisting of simple, complex or mixed hyperplasia either with or without atypia. However, endometrial hyperplasia is not always present in the uteri of women with endometrioid carcinoma and further subdivision of type-I tumors to endometrioid carcinomas with or without uterine hyperplasia has been suggested.

Histopathological data suggest that the majority of type-II tumors (serous carcinoma) develop from a distinctive lesion termed ‘endometrial intraepithelial carcinoma’, which is a malignant transformation of atrophic surface endometrium. Further differences between the two major types of endometrial carcinoma are that endometrioid carcinomas strongly express estrogen and progestin receptors while serous carcinomas do not and that endometrioid carcinomas are associated with
mutations in ras, PTEN, and microsatellite instability, whereas up to 90% of the serous carcinomas are associated with p53 mutations. An important difference between type-I and type-II tumors is that, in endometrioid carcinoma, architectural and nuclear grade are nearly always concordant whereas in serous carcinoma, well-differentiated architecture is usually associated with high grade nuclear atypia.

Endometrioid carcinomas are often diagnosed in an early stage and have a very good prognosis while serous carcinomas are often diagnosed when there has been deep myometrial invasion, early lymph node or distant metastases, therefore having a poor prognosis.

Most epidemiologic studies have analyzed all types of endometrial carcinoma as a single entity, rather than considering the different histopathological types separately.

**Basic epidemiological risk factors**

Epidemiological research has identified several risk factors for endometrial cancer. These include age, obesity, postmenopausal estrogen replacement therapy, diabetes mellitus, early menarche, late menopause, nulliparity, ovarian dysfunction, infertility and tamoxifen use. Protective factors for endometrial cancer include combined estrogen and progestin OCs, parity, physical activity and possibly tobacco smoking.

In USA, Sweden and Italy age-standardised incidence rates of endometrial cancer increase steadily from age 35 to late 50ies with a lower rate of increase thereafter until they peak at 70-75 years, and subsequently decline (Figure 3). A direct association between body weight and endometrial cancer has been observed in the overwhelming majority of epidemiological studies, including studies conducted in North America, Europe and Asia. Overweight and obese women (BMI ≥ 25kg/m²) appear to be at a 2-3 fold increased risk of endometrial cancer, an association that has been observed in both pre- and postmenopausal women (reviewed in ).

Use of exogenous sex-steroids, either as oral contraceptives or hormonal replacement therapy, influence the risk of developing endometrial carcinoma. There are three types of oral contraceptives based on their estrogen and progestin content: sequential oral contraceptives (SOC), combined oral contraceptives (COC), and progestogen only oral contraceptives (POC). SOC formulations include estrogen only pills (used for up to 16 days), followed by estrogen and progestogen pills. COC pills contain an estrogen and a progestogen, given throughout the monthly cycle (usually for 22 days). Users of sequential oral contraceptives (SOC) before late 1970s, and specifically of a particular brand (“Oracon”) containing a long-duration, relatively potent estrogen (ethinylestradiol) and a short duration weak progestin (dimethisterone) were shown to be at increased risk of endometrial cancer, which led to withdrawal of
these formulations from the market \(^{69}\). Data relating other than Oracon SOC to endometrial cancer risk are very limited and do not allow definite conclusions to be drawn \(^{69}\). Use of COC has been consistently shown to decrease risk of endometrial cancer (by about 40-50%), the protection being stronger with increasing duration of use and persisting for many years after discontinuation of use \(^{69,253}\). Very few data on the effect of POC on endometrial cancer risk are available, but despite the small numbers the results of these studies indicate that POC use may be associated with reduced risk of endometrial cancer \(^{69,253}\).

In peri or postmenopausal women, three major types of hormone therapy are used – estrogen only replacement therapy (ERT), sequential estrogen-progesterin replacement therapy (SEPRT) and combined estrogen-progesterin replacement therapy (CEPRT). A meta-analysis of 30 studies showed more than two-fold increase in endometrial cancer risk for ever users of ERT compared to non-users and a rising risk with increasing dose of estrogen as well as with increasing duration of use \(^{254}\). It has been estimated that a woman’s risk of developing endometrial cancer increases approximately 120% for each 5 years of ERT use \(^{255}\). Since the 1980s, progestins were added to the ERT, either in a sequential fashion (between 5 and 15 days per month) or continuously with each daily dose of estrogens (CEPRT), to avoid an increase in endometrial cancer risk. Users of SEPRT regimens containing progesterone for less than 10 days are at increased risk of developing endometrial cancer, but at lower risk when compared to users of ERT, the reduction being proportional to the number of days of opposed estrogen \(^{255}\). SEPRT regimens that include progestin use for 10 or more days (usually 14-16) generally are not related to an increase in endometrial cancer risk \(^{252,255}\), and use of CEPRT, with continuous addition of progestins, has been associated with either a reduced risk of endometrial cancer or with no risk changes \(^{252,255,257,258}\).

Among the reproductive characteristics that have been shown to influence risk of endometrial cancer, an early age at
menarche and late age at menopause have been related to increased risk. Nulliparous women have been shown to have about two to three-fold higher risk than parous women and most studies have shown a decreasing risk with increasing number of children. Although age at first birth generally appears to be unrelated to risk of endometrial cancer, several studies have shown that late age at last birth reduces the risk of developing malignancy and that the risk increases with increasing time since last birth. Infertility has also been associated with increased risk of endometrial cancer. Specifically, women with PCOS and granulosa-thecal cell ovarian tumors have been found to be at increased risk. There have been frequent case reports of PCOS in women developing endometrial cancer, especially in young patients below the age of 40. Furthermore, several case-control and cohort studies have shown an increased risk of endometrial cancer either among women who have PCOS or among infertile women who were clinically characterised as having normal plasma estrogen levels but with a deficiency of progesterone, (which is characteristic of women with PCOS). Relative risk estimates in these studies varied from 3.1 to 9.4, with an average of about 5.

Tamoxifen is a non-steroidal compound that has estrogenic or anti-estrogenic effects according to the target tissue. It is the most widely prescribed anti-neoplastic agent for the adjuvant treatment of breast cancer. Several reports have documented increased incidence of endometrial hyperplasia and polyps in women treated with tamoxifen. A combined analysis of most randomised trials started before 1990 showed that the incidence of endometrial cancer was approximately doubled in women treated in trials lasting up to two years and approximately quadrupled in trials lasting five years. A recent study from the Finnish Cancer Registry database showed about 3-fold increase in endometrial cancer in women with breast cancer treated with tamoxifen (either as adjuvant or palliative treatment). In 1996, an expert panel at the International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence that tamoxifen increases the risk for endometrial cancer in humans and it has been classified as carcinogenic to humans (Group 1).

Diagnosis of diabetes mellitus is long known to be associated with increased risk of developing endometrial malignancy. However, diabetes, and specifically type II diabetes, is commonly accompanied by obesity and in some of the studies the association between diabetes and endometrial cancer lost statistical significance after adjustment for BMI and other confounders. It has also been suggested that the increased risk associated with diabetes may be restricted to overweight and obese women. Very few studies so far have reported on the association of type I diabetes with endometrial cancer. One large case-control study from Sweden found an odds ratio of 13.3 (3.1-56.4) for type I diabetes, while another case-control study from Italy found no association.

The results of epidemiological studies on the association between physical activity and risk of endometrial cancer are consistent and suggest about 20-40% decrease in risk for the highest levels of physical activity. Most of these studies have taken into consideration other known factors for endometrial cancer,
including BMI, making it unlikely that the observed associations were due to confounding.

Epidemiological studies have shown that cigarette smoking may be associated with a slight reduction of endometrial cancer, in contrast to the increased risks observed with many other non-respiratory tract cancers, including those of the bladder, pancreas and cervix uteri. Three prospective cohort studies showed no clear association between smoking and risk of endometrial cancer, however, some of them had very small numbers of current smokers among the case subjects, resulting in wide confidence intervals. Twelve population-based case-control studies showed slightly to moderately lower risk among current smokers than never smokers, but former smoking was not associated with development of endometrial cancer (reviewed in detail in 250). Hospital-based case-control studies have shown more pronounced (on average 30-40%) decrease in endometrial cancer risk among current smokers, however, the interpretation of these studies must be cautious, as the selection of hospital controls may have been related to their smoking habits.

Most of the risk and protective factors for endometrial cancer can be effectively explained within the framework of the unopposed estrogen hypothesis.

**Estrogens and progestins, the unopposed estrogens hypothesis**

The predominant theory describing the relationship between endogenous steroid hormones and endometrial cancer risk is known as the unopposed estrogen hypothesis. This hypothesis states that endometrial cancer risk is increased in women who have high plasma bioavailable estrogens and/or low plasma progesterone, so that mitogenic effects of estrogens are insufficiently counter-balanced by progesterone. This theory originated from at least two important observations, namely the dynamics of endometrial mitotic rate throughout the menstrual cycle and the effect of exogenous estrogen and progestin hormone formulations used for contraception or relief of menopausal symptoms.

During the follicular phase of the menstrual cycle, when the ovaries produce estradiol but virtually no progesterone, epithelial tissue and stromal fibroblasts in the upper two-thirds of the endometrium (“functional” layer) rapidly increase their mitotic rate. The high proliferation rates continue until ovulation and then decline rapidly during the luteal phase of the menstrual cycle in the face of the post-ovulation increase in progesterone production. The anti-proliferative action of progesterone in the endometrium is believed to be mediated mainly by reducing the concentration of estrogen receptors, by stimulating the local synthesis of enzymes that favour the conversion of estradiol into the less potent estrone (17β-hydroxysteroid dehydrogenase) or into estrogen sulphates (estrogen sulphotransferase) that are rapidly excreted from cells and from the body, and by stimulating the differentiation of endometrial cells to a secretory state.

As discussed, endometrial cancer risk is increased in women using exogenous estrogens (OC or HRT) without continuously added progestins and is decreased with use of combined estrogen plus progestin formulations. The greater risk in women using SOC may be explained by the fact that oral estrogens block ovulation and ovarian progesterone synthesis, thus increasing the number of
days in which women are exposed to unopposed estrogens. The protective effect of COC can be explained by the fact that this type of contraceptive pill keeps endogenous estradiol levels comparatively low (comparable to the early follicular phase of the menstrual cycle) while simultaneously providing a continuous supply of progesterone, for 21-28 days per cycle (i.e., more than during the natural menstrual cycle in non-users).

The increase in endometrial cancer risk conferred by early age at menarche, late age at menopause, obesity in postmenopausal women, type II diabetes and tamoxifen use is believed to be mediated through increasing estrogenic exposure. Alternatively, the protective effect of physical activity and smoking may be due to the anti-estrogenic effect on circulating estrogen concentrations by a reduction in relative body weight, later age at menarche (due to intense physical activity) or an earlier age at menopause (due to smoking) 7,250. Under the unopposed estrogen hypothesis, the protective effect of pregnancy may be due to the prolonged exposure to high progesterone concentrations. However, mechanical clearing of the uterine lining from cells that have undergone malignant transformation at partition has also been suggested as a mechanism that could contribute to the protective effect of parity.

Further support for the role of unopposed estrogens in endometrial cancer comes from studies directly relating levels of endogenous estrogens and SHBG levels to risk of endometrial cancer. Several case-control studies, although not all 302-310, have shown increased total 311-322 and bioavailable 317,318,320 estrogens and decreased plasma levels of SHBG 314,318, in postmenopausal women who developed endometrial cancer compared to cancer-free control subjects. Similar relationships were found recently in one prospective cohort study 20.

In premenopausal women, one large case-control study showed decreased total and bioavailable estradiol in endometrial cancer patients, although they also had lower levels of SHBG and higher levels of estrone 318. On first consideration, the decrease in estradiol levels among premenopausal women with endometrial cancer might seem to be at variance with the unopposed estrogen hypothesis. However, it has been argued that in premenopausal women low progesterone, rather than increased estrogen, is the predominant determinant of endometrial cancer risk 298. Proponents of this theory suggest that endometrial cancer risk is related to plasma estrogens only when estrogen concentrations are comparatively low (i.e., within the postmenopausal range of 5-20 pg/ml), and that neither endometrial mitotic activity nor cancer risk increase further at estradiol levels above a limit of 50 pg/ml 298. This upper limit was derived from observations that the maximal endometrial mitotic rate is reached in the early follicular phase of the menstrual cycle, when plasma estradiol concentrations are around 50 pg/ml, without any further increase in mitotic rate when estradiol levels rise during the late follicular and ovulatory phases. The limit was also approximately consistent with the estimated increase in endometrial cancer risk with estrogen exposures from ERT, or with obesity-related increases in plasma estradiol levels in postmenopausal women. The importance of low progesterone is also supported by observations that obesity, a major risk factor for endometrial cancer in both pre- and postmenopausal women,
does not increase total or bioavailable estrogens in premenopausal women, but in some women can cause chronic anovulation and strongly reduce progesterone synthesis. So far, no studies have been conducted to examine directly endometrial cancer risk in relation to measurements of endogenous progesterone, and such studies would indeed be difficult to design and conduct because of the wide variation in progesterone levels during the menstrual cycle.

**Androgens**

A number of endocrine similarities between endometrial cancer patients and women with PCOS suggest that excessive androgen production may also play an etiological role in endometrial cancer pathogenesis (Figure 4). First, experiments in vitro with ovarian stromal tissue obtained from both endometrial cancer patients and PCOS patients show increased responsiveness of androgen production to insulin stimulation, as compared to the stroma from normo-androgenic control subjects. Second, endometrial cancer patients have increased ovarian vein concentrations of testosterone and androstenedione, as well as increased 6-hour integrated plasma levels of LH, the key hormone stimulating ovarian androgen synthesis, which is generally elevated in women with PCOS. Third, increased risk of endometrial cancer has been reported in women with irregular menstrual cycles, an indication of chronic anovulation that is frequently related to ovarian hyperandrogenism.

Although endometrial tissue contains androgen receptors, androgens do not appear to have any direct stimulatory effect on endometrial cell proliferation; if anything, the results from in vitro studies suggest a reduction in proliferation rates. However, androgens may influence risk of endometrial cancer indirectly by increasing estrogens, unopposed by progesterone. In postmenopausal women, plasma androgen levels, especially andro-

**Figure 4:** Endogenous hormones and endometrial cancer development
stenedione, are key determinants of the amount of estrogens formed in the endometrium and adipose tissue. In premenopausal women, intra-ovarian androgen excess contributes to follicular atresia, and can lead to chronic anovulation and reduced levels of progesterone.

Elevated circulating androgens have been associated with hyperplasia of the endometrium, which generally precedes and accompanies the occurrence of type-I endometrial carcinomas. Several case-control studies, but not all, have shown that endometrial cancer risk is increased in both pre- and postmenopausal women with elevated plasma levels of androstenedione and testosterone. One large case-control study showed that the strong positive association of androstenedione with endometrial cancer persisted after adjustment for estrone, suggesting that androgens may have an independent of estrogens effect on cancer risk.

**Insulin**

Insulin may influence endometrial cancer risk by a number of mechanisms (Figure 4). Insulin is a key down-regulator of the hepatic synthesis and plasma levels of SHBG, and is thus a direct determinant of bioavailable estradiol unbound to SHBG, of particular relevance after menopause when no feed-back mechanisms regulate estrogen synthesis. Chronically elevated insulin concentrations are a major contributing cause of ovarian androgen excess, which in premenopausal women may cause chronic anovulation and progesterone deficiency. Indeed, as discussed, chronic hyperinsulinemia is a key feature of women who have PCOS, and PCOS has been related to increased risk of endometrial cancer. In women who have PCOS, plasma insulin concentrations correlate with levels of androstenedione and testosterone and more severe insulin resistance and hyperinsulinemia are related to more frequent anovulatory menstrual cycles. Insulin is also a major down-regulator of IGFBP-1 and in vitro experiments have shown that it suppresses IGFBP-1 gene expression and production not only in the liver, but also in other tissue types, including endometrium. Additionally, insulin has been shown to promote the growth of cancer cell lines in vitro, including endometrial cancer cells and an increased insulin receptor content in endometrial tumors has also been reported.

Epidemiological evidence that supports insulin involvement in endometrial cancer pathogenesis includes the increased risk observed in several conditions associated with hyperinsulinemia – obesity, type II diabetes and PCOS. In contrast, regular physical activity enhances insulin sensitivity and epidemiological studies suggest a 20-40% decrease in endometrial cancer risk for the highest levels of physical activity.

More direct evidence for the role of insulin comes from several case-control studies relating blood concentrations of insulin or markers of hyperinsulinemia to risk of endometrial cancer. In one large study, elevated levels of C-peptide (a marker of pancreatic insulin secretion) were related to an increase in endometrial cancer risk in women without diabetes, although this association did not persist after adjustment for BMI. In another large case-control study, where previous diabetes diagnosis was not an exclusion criterion, insulin concentrations were similarly directly related to risk of endometrial cancer.
cancer in women who never used HRT, but the association lost statistical significance after adjustment for BMI, diabetes, physical activity, menopausal status and OC use. Interestingly in the group of women who were former or current users of HRT, insulin was inversely related to risk of endometrial cancer, an association that persisted after adjustment for BMI and other confounders. Four other very small studies showed higher fasting, post-glucose challenge insulin or glycosylated haemoglobin levels in endometrial cancer patients than in control women.

**Insulin-like growth factor – I and IGF binding proteins**

IGF-I has been shown to exert mitogenic and anti-apoptotic properties on endometrial and other tissues. Tumor tissues, including endometrial tumors, generally have increased levels of IGF-I receptors. Estrogens -- the major established risk factor for endometrial cancer -- increase IGF-I and IGF-I receptor expression in the uterus, and IGF-I is proposed to mediate their mitogenic effect on the endometrium (“estromedin hypothesis”). IGF-I mRNA expression in uterine tissue of rats is markedly dependent on estradiol, and in humans the IGF-I gene is also expressed primarily during the follicular and early luteal phases of the menstrual cycle. Progesterone provides the key stimulus for the gene expression and for the synthesis of IGFBP-1 in the uterus and endometrial IGFBP-1 mRNA expression follows the pattern of progesterone synthesis during the menstrual cycle; it is minimal during the proliferative phase but reaches peak levels during late luteal phase. It was proposed that part of the effect of progesterone on the rate of mitotic activity of endometrial tissue is mediated through an increase in IGFBP-1 synthesis. Insulin, in contrast, reduces the synthesis of IGFBP-1 in the endometrium and other tissues.

Three case-control studies have reported on the association of IGFBP-1 with endometrial cancer risk. The results of the studies are conflicting, however, two of the studies were very small and the third one found a direct association of IGFBP-1 with risk of endometrial cancer only in women who had ever used HRT. Similarly conflicting are the results of the three case-control studies that reported IGFBP-3 levels in endometrial cancer patients and control women.
AIMS OF THE THESIS

1. To examine the association of prediagnostic circulating androstenedione, testosterone, DHEAS, estrone and SHBG with risk of ovarian cancer (Paper I).

2. To examine the association of prediagnostic circulating IGF-I, IGFBP-1, -2, -3 and C-peptide with risk of ovarian cancer (Papers II and III).

3. To examine the association of prediagnostic circulating androstenedione, testosterone, DHEAS, estradiol, estrone and SHBG with risk of endometrial cancer in postmenopausal women (Paper IV).

4. To examine the association of prediagnostic circulating IGF-I, IGFBP-1, -2, -3 and C-peptide with risk of endometrial cancer (Paper V).

5. To examine the association of BMI with circulating androstenedione, testosterone, DHEAS, estrone, estradiol, SHBG, IGF-I, and IGFBP-3, and the relationship between sex-steroids, IGF-I and IGFBP-3 (Paper VI).
SUBJECTS AND METHODS

The thesis is based on the data from two multi-centered nested case-control studies on ovarian and endometrial cancers (Figure 5).

DESCRIPTION OF THE COHORTS
Study subjects were selected among participants in three prospective cohorts, originally designed to address the role of endogenous hormones and diet in the etiology of cancer and other diseases. The three collaborating cohorts are:

The New York University Women’s Health Study (NYUWHS), New York, USA
Cohort members are 14,275 apparently healthy women, who were recruited between March 1985 and June 1991 at a mammography screening center in New York City. Eligibility was restricted to women who had not used hormonal medications and who had not been pregnant in the preceding six months. The age of the recruited women ranged between 34 and 65 years (Table 2). Seventy-six percent of the NYUWHS members provided information about ethnic origin: about 62% indicated that they were non-Hispanic Whites, 8% Black, 4% Hispanic and 2% other ethnicity. At the time of enrolment in the cohort, all subjects signed an informed consent indicating their willingness to complete questionnaires and donate blood for re

The 3 cohorts studies
NYUWHS (New York, USA), NSHDS (Umeå, Sweden), ORDET (Milan, Italy)

![Study scheme](image)

Figure 5: Study scheme.
Table 2. Characteristics of the 3 parent cohort studies.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Study setting</th>
<th>Recruitment period</th>
<th>Cohort size</th>
<th>Age range at enrolment</th>
<th>Last complete follow-up*</th>
<th>Exclusion criteria</th>
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<tr>
<td>NYUWHS New York USA</td>
<td>Mammographic screening clinic</td>
<td>1985-1991</td>
<td>14,275</td>
<td>34 - 65</td>
<td>Feb. 98</td>
<td>Women who had used hormonal medications or had not been pregnant in the preceding 6 months.</td>
</tr>
<tr>
<td>NSHDS Umeå Sweden</td>
<td>General population</td>
<td>1986-present</td>
<td>43,268</td>
<td>30 - 65</td>
<td>Dec. 00</td>
<td>None.</td>
</tr>
<tr>
<td>ORDET Milan Italy</td>
<td>Healthy volunteers and women attending breast cancer prevention unit</td>
<td>1987-1992</td>
<td>10,788</td>
<td>35-70</td>
<td>Jan 97</td>
<td>Women with history of malignant tumors or bilateral ovariectomy; pregnancy, nursing or hormonal treatment at the time of recruitment.</td>
</tr>
</tbody>
</table>

* for the ovarian and the endometrial cancer studies.
search purposes.

From each participant, 30 cc of venous blood was drawn by collection tubes without anticoagulant. With rare exceptions, blood was collected between 9.30 AM and 1.00 PM. Fasting was not required, but exact time at venipuncture was recorded as well as time since last meal. After blood withdrawal, tubes were kept covered at room temperature for 15 minutes, then at 4°C for 60 minutes to allow clot retraction and then centrifuged. Supernatant serum was partitioned into 1-cc aliquots in capped plastic vials within 2 hours after separation. Labelled aliquots were stored in closed cardboard freezer boxes for banking at –80°C. If cohort members returned to the clinic for subsequent annual breast screening, they were asked to donate additional blood specimens and 51% of the initially enrolled participants contributed 2 or more blood samples.

At enrolment, women were also asked to complete a self-administered baseline questionnaire to collect demographic, medical, life-style and reproductive information, to report their height and weight (and measurements of waist and hip, after July 1986), and to respond to a self-administered semi-quantitative food frequency questionnaire. If premenopausal, women were asked to return a pre-addressed postcard indicating the date of the beginning of the menstrual cycle that immediately followed blood donation. Information about the next menstrual cycle was provided by 76% of the eligible premenopausal subjects and date of previous cycle is available for most of the others. The baseline and diet questionnaires were completed by all study subjects and every two years follow-up questionnaires are sent to cohort members to update life-style, reproductive and medical history information. The reliability of repeated self-reported data for height and weight in the NYUWHS has been shown to be very high (intraclass correlations 0.98 for height and 0.95 for weight).

Ascertainment of vital status and disease incidence has been performed to identify all malignant tumors that occurred after enrolment in the cohort. Follow-up in the NYUWHS consists of periodic contact by mail and telephone, as well as record linkages with state-wide tumor registries (New York, New Jersey, Connecticut and Florida) and the U.S. National Death Index. A capture-recapture analysis showed the overall invasive cancer ascertainment rate to be 95%.

Once a new cancer is reported by a cohort member or by her contacts, the NYUWHS solicits written permission from each patient (or from the responsible family member) to request clinical and pathological information from hospitals / physicians for internal review. Permission is obtained from over 96% of known cases. International Classification of Diseases (ICDO-9) codes are assigned for tumor topography, morphology, TNM classification, stage and surgical procedures.

For the ovarian and endometrial cancer studies data were used from the last complete follow-up round in the NYUWHS, which was started in February 1998 and the last linkage with tumor registries, which was conducted in May 2000 (Table 2). In this round, 83% of the 13,543 cohort members presumed alive were successfully followed-up and completed the questionnaire by mail or telephone.
The Northern Sweden Health and Disease Study (NSHDS), Umeå, Sweden

The Northern Sweden Health and Disease Study includes subjects recruited through the Västerbotten Intervention Program (VIP), the Monica Project of the World Health Organization (WHO) and through a local mammographic screening project \(^{487}\). The age of the recruited women is mostly between 30 and 65 years (Table 2). The NSHDS population is stable; most participants are native Swedes with a small minority of Finnish descent.

The VIP is a long-term prospective cohort and intervention study intended for the health promotion of the county of Västerbotten in Northern Sweden with approximately 254,000 inhabitants. Since 1985, all individuals, 30, 40, 50 and 60 years of age in the county are invited by VIP for screening for primary prevention of cardiovascular disease and diabetes. The overall participation rate is about 60% and it is expected that within the next 10 years, the majority of the county population aged 30 to 69 years will be part of the project \(^{488}\). Since 1994, at 10 years intervals, a second blood sample and questionnaire data is collected from cohort members. In December 2000 the cohort included over 70,000 individuals, among whom 63,000 had donated blood.

The Monica project includes both men and women who were invited for cardiovascular screening in 1986, 1990, 1994 and 1999 as part of an international, WHO collaborative cardiovascular epidemiological study. The age range of the recruited subjects varied from 25 to 64 years. A total of 7,500 individuals were recruited, with a participation rate of 70-80% \(^{488}\). About 20% of Monica participants are also recruited in VIP \(^{487}\). The NSHDS also includes a sub-cohort of about 25,000 women who were recruited through a local mammographic screening project with a recruitment rate of about 7,000 subjects per year since 1995, and a participation rate of about 60%. Since 1997 blood samples are collected every second year from women aged 50-69. About half of the Mammary project members participate also in VIP.

At recruitment NSHDS members are asked to sign an informed consent form. VIP and Monica participants are asked to complete a life-style questionnaire (education, occupation, diet), while participants in the Mammary project complete a detailed reproductive history questionnaire. For all NSHDS participants a questionnaire with data about medication and smoking at baseline is available. Height and weight were measured for subjects recruited through VIP and Monica arms and self-reported for women recruited through the mammary project. Analysis in a sub-sample of the NSHDS, for which information on both self-reported and measured anthropometric characteristics were available, showed a very high correlation of self-reported with measured height \((r = 0.97)\) and a less strong correlation with weight \((0.75)\) \(^{379}\).

NSHDS participants are also asked to donate a sample of peripheral venous blood for research purposes. Blood collection, preparation and storage procedures were strictly standardized across sub-projects. Blood is collected in tubes containing EDTA or heparin as an anticoagulant. In the VIP and Monica studies, 80% of the participants provided a blood sample after an overnight fast, while the remaining 20% had fasted for 4 hours. Fasting was not required for participant in the mammary project, but time since last
meal was recorded. Blood samples were divided into 10 1.5 cc aliquots: 6 plasma, 2 buffy coat and 2 erythrocyte concentrates. All samples were rapidly frozen at –80°C for long term storage.

Ascertainment of vital status and disease incidence is performed through record linkages with regional and national cancer and all-cause mortality registries, using the unique national individual identification number as an identity link. The completeness of the National Cancer Registry is estimated to be 98%. Usually, the time from diagnosis to registration in the cancer registry is around one month.

In December 2000, the NSHDS had blood samples of 43,268 women (Table 2). The Hormones and Diet in the Etiology of Breast Cancer, ORDET cohort, Milan, Italy

The ORDET cohort includes 10,788 healthy volunteers, recruited in 1987-1992 among female residents of Varese Province in Northern Italy who were adhering to a campaign of free breast examination organised by the Italian National Tumor Institute in Milan or were invited by letters mailed to those listed in the general practitioners’ records of the local branch of the National Health Survive or contacted during a recruitment campaign held in collaboration with local employers and among teachers in public schools. Women with no history of malignant tumors or bilateral ovariectomy, who were not pregnant, nursing or under hormonal treatment were considered eligible. The age of the recruited ORDET participants ranged from 35 to 70 years (Table 2). All participants were Caucasian, 80% born in Northern Italy and the remaining 20% were immigrants from Southern or Central Italy.

Subject recruitment involved a two-step process. First, each woman was invited to the center, where she signed an informed consent form and had a detailed personal interview on demographic, lifestyle, medical and reproductive history, conducted by trained nurses. Anthropometric measurements (height, weight, waist and hip) were also taken by the nurses. Subsequently, each woman was invited again for blood collection and to deliver a self-administered dietary questionnaire. Forty cc of peripheral venous blood was drawn after an overnight fast and strictly between 8.00 and 9.00 AM. In premenopausal subjects, blood was drawn during the luteal phase of the menstrual cycle, between 20th and 24th day after last menstruation. After blood collection, the samples were kept in refrigerators, protected from light and were partitioned in 6 aliquots of serum, 6 aliquots of plasma, 3 of red blood cell cytoplasm, 3 of red blood cell membranes and 3 of lymphocytes (buffy coats). Specimens were stored at –80°C freezers.

The Varese Province is covered by the population-based Lombardy Cancer Registry. Cohort follow-up is performed by periodical linkage of the ORDET record file with the Lombardy Cancer Registry and direct reporting from the hospitals. The completeness of the Lombardy Cancer Registry is estimated to be about 95%. For each cancer case, the clinical and pathological records are retrieved and reviewed. The ORDET file is also linked with the records of the Lombardy Region residents to ascertain vital status. For those who are not traceable, vital status is ascertained at the town of last residence. As of June 1995, after 5.5 years of average observation, only 15 subjects were lost to follow-up. The last complete follow-up for the ovarian and endometrial cancer studies included cancer cases diagnosed up to
January 1997 (Table 2).

DATABASE PREPARATION
The common databases for the ovarian and endometrial studies were created centrally, at IARC, Lyon, France. The parent cohort studies provided data stripped of personal identifiers, copies of the original and follow-up questionnaires and description of the included variables. According to the available information in the three cohorts, common variables were created for the two nested case-control studies.

OVARIAN CANCER STUDY
Case subjects were cohort members with primary invasive epithelial ovarian cancer diagnosed at least 1 year after the initial blood donation, who had no previous cancer diagnosis, who did not use exogenous hormones at the time of blood donation and who were identified within the parent cohort by the date of the last complete follow-up. A total of 132 ovarian cancer cases were included for this study from the NYUWHS, the NSHD and the ORDET cohorts (Table 3). Seventy four (56%) case subjects were from the NYUWHS, 42 (32%) from the NSHD and 16 (12%) from the ORDET cohort.

Of the 115 malignancies with histological verification provided by the cohort studies, 55 (48%) were serous, 14 (12%) endometrioid, 13 (11%) mucinous and 8 (7%) clear cell types. Of the remaining, 20 (17%) were carcinomas not otherwise specified, 1 was mixed and 4 were undifferentiated. Among the 99 case subjects with information about stage, 38% (38 case subjects) were diagnosed with ovarian cancer stage 1 or 2 and 62% (61 case subjects) had stage 3 or 4 disease. The distribution of stages was similar among the three cohorts.

For each case subject, 2 control subjects were selected at random among appropriate risk sets. The risk set for a given case included all cohort subjects alive, free of cancer, who had not had a bilateral ovariectomy, and matched the case on cohort, menopausal status at enrolment, age (± 6 months) and date of recruitment (± 3 months). In the NYUWHS, premenopausal control subjects were matched to their index case for phase of menstrual cycle at blood donation, based on information on the reported date of the first menstrual cycle following blood donation. In the NSHD, a reproductive history questionnaire has been administered prospectively to 47% of the subjects and a similar questionnaire was sent out retrospectively to all women selected to participate in the study (95% response rate). Seventy-three women selected for the ovarian cancer study had filled in both a prospective and a retrospective questionnaire. There was a very good correspondence between prospectively and retrospectively collected data about ever having a full-term pregnancy (100%), number of full-term pregnancies, including still births (97%), ever use of OC (88%), age at start of OC use (0.84, n=23), height (r=0.99) and weight (r=0.96). The correlation between data from the two questionnaires was less strong for age at menarche (r=0.73), age at menopause (r=0.73, n=58) and duration of OC use (r=0.71, n=24). Data were also collected from medical records of the NSHD case subjects; for a few deceased women (n = 13) these records were the only available source of information about reproductive history and hormone use. Potential control subjects from the NSHD who reported use of exogenous hormones at the time of blood donation were not considered...
Table 3: Distribution of risk factors for ovarian cancer in cases and controls; mean (95% CI) or frequencies (%).

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 132)</th>
<th>Controls (n = 286)</th>
<th>p for case-control difference**</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m(^2)) *</td>
<td>25.0 (24.3 – 25.8)</td>
<td>26.0 (25.5 – 26.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>Age at menarche *</td>
<td>13.1 (12.8 – 13.3)</td>
<td>13.1 (12.9 – 13.4)</td>
<td>0.56</td>
</tr>
<tr>
<td>Full-term pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>40 (30)</td>
<td>52 (18)</td>
<td>0.01</td>
</tr>
<tr>
<td>Ever</td>
<td>90 (68)</td>
<td>231 (81)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>2 (2)</td>
<td>3 (1)</td>
<td></td>
</tr>
<tr>
<td>OC use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>82 (62)</td>
<td>155 (54)</td>
<td>0.29</td>
</tr>
<tr>
<td>Ever</td>
<td>31 (23)</td>
<td>89 (31)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>19 (14)</td>
<td>42 (15)</td>
<td></td>
</tr>
<tr>
<td>Age at menopause *</td>
<td>50.0 (48.8 – 51.3)</td>
<td>48.7 (47.7 – 49.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>67 (51)</td>
<td>114 (40)</td>
<td>0.15</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>31 (23)</td>
<td>71 (25)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>13 (10)</td>
<td>36 (13)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>21 (16)</td>
<td>65 (23)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (6)</td>
<td>11 (4)</td>
<td>0.44</td>
</tr>
<tr>
<td>No</td>
<td>113 (86)</td>
<td>239 (84)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>11 (8)</td>
<td>36 (13)</td>
<td></td>
</tr>
<tr>
<td>Family history of breast cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24 (18)</td>
<td>45 (16)</td>
<td>0.55</td>
</tr>
<tr>
<td>No</td>
<td>108 (82)</td>
<td>238 (83)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>0 (0)</td>
<td>3 (1)</td>
<td></td>
</tr>
</tbody>
</table>

* Mean adjusted for age and study cohort;
** General linear models or conditional logistic regression for categorical variables (never vs. ever).

eligible for the nested case-control studies. The matching for menopausal status was confirmed by FSH measurement and women were considered as postmenopausal if their FSH measurement was >12.75 IU/L. The FSH cut-off point was selected on the basis of data provided by the kit manufacturer [Diagnostic System Laboratories (DSL)] and an analysis of the distribution of FSH levels according to menopausal status as assessed by questionnaire, as well as according to age (< 42 and > 55 years), using data from more than 300 women from the 3 cohorts. Two hundred sixty-three control subjects were included in the IGF-I, IGFBPs and insulin component of the ovarian cancer study. For six case-control sets, the
matching for menopausal status did not hold. Twenty seven percent of the NYUWHS, 71% of the NSHDS and all of the ORDET subjects had donated a blood sample after at least four hours fast.

For the study on sex-steroids, for which precise matching on menopausal status was of greater importance, further efforts were made to improve the matching for menopausal status. Twenty-three control subjects were replaced and 5 subjects were excluded because of insufficient blood sample or following attempts to improve the matching for menopausal status, leaving a total of 258 control subjects. Overall, 286 control subjects were included in the ovarian cancer study (Table 3).

ENDOMETRIAL CANCER STUDY

For the endometrial cancer study, a special effort was made to update data on reproductive factors and especially on exogenous hormone use up to the index date (date of cancer diagnosis of the case subject in each matched case-control set) in the NYUWHS and in the NSHDS. In the NYUWHS, data on smoking, OC and HRT use were collected by telephone interviews from cases (88%) and matched controls (83%) or by using the most recent follow-up questionnaire for participants who did not complete the interview (resulting in data available on 93% of the cases and 96% of the controls). A reproductive history questionnaire was administered prospectively to 62% of the NSHDS subjects and a similar questionnaire was sent out retrospectively to all women selected to participate in the study (95% response rate). There was a very good correspondence between prospectively and retrospectively collected data about ever having a full-term pregnancy (100%, n=87), number of full-term pregnancies, including still births (100%, n=87), ever use of OC (94%, n=98), height (r=0.99, n=102) and weight (r=0.94, n=95). The correlation between data from the two questionnaires was less strong for age at menarche (r=0.75, n=100), age at menopause (r=0.61, n=72) and age at start of OC use (r=0.76, n=30).

IGF-I, IGFBPs and C-peptide component

Case subjects were cohort members with primary invasive endometrial cancer diagnosed 6 or more months after the initial blood donation, without preceding cancer diagnosis, who did not use exogenous hormones at the time of blood donation and who were identified within the parent cohort by the date of the last complete follow-up. A total of 166 endometrial cancer cases were included for the study from the 3 cohorts (Table 4). Ninety one (55%) cases were from the NYUWHS, 60 (36%) from the NSHDS and 15 (9%) from the ORDET cohort.

Of the 137 malignancies with histological verification provided by the cohort studies, 91 (66%) were endometrioid, 10 (7%) serous, 3 (2%) mucinous, 1 (1%) clear cell, 2 (1%) mixed type, and 30 (22%) were carcinomas or adenocarcinomas not otherwise specified. Among the 133 case subjects with information about stage, 118 (89%) were diagnosed with endometrial cancer stage 1 or 2 and only 15 (11%) case subjects had stage 3 or 4 disease. The distribution of stages was similar among the three cohorts.

For each case subject, 2 control subjects were selected at random from appropriate risk sets. The risk set for a given case included all cohort subjects alive and free of cancer, who had not had a hysterectomy at index date and who
Table 4: Distribution of risk factors for endometrial cancer in cases and controls; mean (95% CI) or frequencies (%).

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 166)</th>
<th>Controls (n = 315)</th>
<th>p for case-control difference**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)*</td>
<td>161.1 (160.1 - 162.2)</td>
<td>160.8 (160.0 – 161.6)</td>
<td>0.71</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>70.8 (68.7 - 73.0)</td>
<td>65.4 (63.7 - 67.1)</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI kg/m²**</td>
<td>27.3 (26.5 – 28.0)</td>
<td>25.3 (24.7 – 25.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age at menarche*</td>
<td>12.9 (12.7 – 13.2)</td>
<td>13.1 (12.9 – 13.3)</td>
<td>0.29</td>
</tr>
<tr>
<td>Full-term pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>39 (23)</td>
<td>54 (17)</td>
<td>0.06</td>
</tr>
<tr>
<td>Ever</td>
<td>123 (74)</td>
<td>251 (80)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>4 ( 2)</td>
<td>10 ( 3)</td>
<td></td>
</tr>
<tr>
<td>OC use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>117 (70)</td>
<td>204 (65)</td>
<td>0.06</td>
</tr>
<tr>
<td>Ever</td>
<td>44 (27)</td>
<td>106 (34)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>5 ( 3)</td>
<td>5 ( 2)</td>
<td></td>
</tr>
<tr>
<td>Age at menopause*</td>
<td>50.9 (50.0-51.7)</td>
<td>50.1 (49.4 – 50.7)</td>
<td>0.24</td>
</tr>
<tr>
<td>HRT use</td>
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</tr>
<tr>
<td>Never</td>
<td>107 (64)</td>
<td>227 (72)</td>
<td>0.22</td>
</tr>
<tr>
<td>Ever</td>
<td>53 (32)</td>
<td>83 (26)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>6 ( 4)</td>
<td>5 ( 2)</td>
<td></td>
</tr>
<tr>
<td>ERT use***</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>113 (80)</td>
<td>229 (85)</td>
<td>0.16</td>
</tr>
<tr>
<td>Ever</td>
<td>28 (20)</td>
<td>39 (15)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>74 (45)</td>
<td>133 (42)</td>
<td>0.99</td>
</tr>
<tr>
<td>Yes</td>
<td>25 (15)</td>
<td>47 (15)</td>
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<td>Ex-smoker</td>
<td>36 (22)</td>
<td>69 (22)</td>
<td></td>
</tr>
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<td>31 (19)</td>
<td>66 (21)</td>
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</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12 ( 7)</td>
<td>13 ( 4)</td>
<td>0.22</td>
</tr>
<tr>
<td>no</td>
<td>147 (89)</td>
<td>291 (92)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>7 ( 4)</td>
<td>11 ( 3)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean (95% confidence intervals) adjusted for age and study cohort;
** Paired t-test on log-transformed continuous data (case subject values vs. means of the matched controls subjects) or conditional logistic regression for categorical variables (never vs. ever);
*** ORDET subjects and those with missing values from the NYUWHS and NSHDS cohorts were not included.
matched the case on cohort, date (±3 months), age (±6 months) and menopausal status at enrolment. In the NYUWHs, premenopausal control subjects were matched to their index case for phase of menstrual cycle at blood donation, based on information on the day of the first menstrual cycle following blood donation. The matching for self-reported menopausal status was confirmed by FSH measurement in women younger than age 60, classifying women as premenopausal if their FSH measurement was > 12.75 IU/L. Thirty-two control subjects were replaced because they did not meet this criterion, however for 4 matched case-control sets control replacement was not feasible. A total of 315 control subjects were included in this study (Table 4). Twenty seven percent of the NYUWHs, 64% of the NSHDS and all of the ORDET subjects had donated a blood sample after at least four hours fast.

Sex-steroids and SHBG component

Case subjects were postmenopausal cohort members with primary invasive endometrial cancer diagnosed 6 or more months after the initial blood donation, who did not use exogenous hormones at the time of blood donation and who were identified within the parent cohort by the date of the last complete follow-up. A total of 124 endometrial cancer cases were eligible for the study from the 3 cohorts. The selection of control subjects was identical to that for the C-peptide and IGF-I component of the endometrial cancer study. A total of 236 control subjects were included.

CROSS-SECTIONAL STUDY

The hormonal data of 620 women who were selected as control subjects in either the ovarian (n=308) or the endometrial cancer (n=312) nested case-control studies were combined for a cross-sectional analyses. Only women whose samples were analysed at the Hormone Laboratory at IARC were included. The majority of women (53%) included in the cross-sectional study were from the NYUWHs, 37% were from the NSHDS and 10% from the ORDET cohort.

LABORATORY ANALYSES

The hormone analyses were performed on serum samples obtained from the NYUWHs subjects and heparinised plasma samples obtained from the NSHDS and ORDET subjects. Samples from case subjects and their matched control subjects were always analysed within the same batch, assay kit and on the same day. Laboratory personnel were unable to distinguish between case and control samples.

Analyses on sex steroids and SHBG

Androstenedione, testosterone, DHEAS and SHBG were measured in pre- and postmenopausal women from the ovarian and endometrial cancer studies; estrone was measured only in samples of women who were postmenopausal at recruitment; and estradiol only in postmenopausal women from the endometrial cancer study.

All blood analyses for the ovarian cancer study and 57% for the endometrial cancer study (endometrial cancer Study 2, Figure 5) were performed at the Hormone Laboratory at IARC, Lyon, France. Testosterone and DHEAS were measured by radioimmunoassays (RIA) with reagents from Immunotech, Marseille, France; androstenedione, estrone and estradiol by double antibody RIA with reagents from Diagnostic System Laboratories, Webster, Texas, USA; and SHBG by immuno-radiometric assays (IRMA) with reagents from Cis-Bio, Gif-sur-Yvette, France.

The mean intra-batch coefficients of
variation for the ovarian and endometrial study, respectively, were 9.6 and 8.2% for a testosterone concentration of 0.3 ng/ml, 7.0 and 6.3% for an androstenedione concentration of 0.5 ng/ml, 4.6% for DHEAS concentrations of 38 and 40 µg/dl, 6.8 and 5.3% for estrone concentrations of 15.6 and 20 pg/ml, 3.5 for an estradiol concentration of 30 pg/ml and 6.2 and 4.4% for SHBG concentrations of 28.7 and 40 nmol/l.

For the subjects included in the endometrial cancer Study 1 (43%) estradiol, estrone, androstenedione and testosterone were measured by the Clinical Studies Center of Quest Diagnostics Inc (Nichols Institute, San Juan Capistrano, CA, USA). Serum samples were subjected to organic extraction and celite chromatography and the appropriate fractions analysed by RIA. SHBG and DHEAS were measured in the laboratory of Dr. Levitz at NYU School of Medicine, using an immunometric chemiluminescent assay on an IMMULITE 2000 instrument (Diagnostic Products Corp., Los Angeles, CA). To assess laboratory precision, one aliquot from a common pool generated by using serum samples from a random sample of the healthy postmenopausal NYUWHS participants was included in every laboratory batch after labeling to prevent identification. Intra-batch coefficients of variation were 1.7% for testosterone, 8.9% for androstenedione, 4.6% for DHEAS, 4.8% for total estradiol, 9.5% for estrone and 4.2% for SHBG.

Three hundred eighty four postmenopausal NYUWHS subjects, who served as controls in studies of breast, endometrial and ovarian cancer, have provided a second blood sample from 12 to 60 months after the baseline blood donation. Sex steroid hormones and SHBG were measured in the initial and in the second blood sample of these women (a total of 768 samples) at the Hormone Laboratory at IARC, Lyon, France. Intra-class correlations between repeated hormone measurements were: 0.66 (95% confidence interval (CI) = 0.61-0.73) for estradiol, 0.58 (0.52-0.66) for estrone, 0.63 (0.57-0.70) for androstenedione, 0.64 (0.58-0.70) for testosterone, 0.92 (0.90-0.93) for DHEAS and 0.87 (0.85-0.90) for SHBG, indicating that hormone and SHBG levels are fairly stable over time in postmenopausal women and that a single measurement can be used to characterise an individual’s average level. Very similar results for the reproducibility of sex-steroid hormones in postmenopausal women over a 2-3-year period have been reported previously.

Analyses on IGF-I, IGFBPs, C-peptide and FSH

The laboratory analyses were performed by the Hormone Laboratory at IARC, Lyon, France. C-peptide and IGFBP-2 concentrations were measured by RIA and IGF-I, IGFBP-1, IGFBP-3 and FSH by IRMA, all reagents from Diagnostic System Laboratories (Webster, Texas, USA). The IGF-I assay included an acid-ethanol precipitation of IGFBPs.

To control the quality of the hormone measurements, samples from a pool of quality control plasma and 3 standard sera were inserted randomly in each batch. The mean intra-batch coefficients of variation for the ovarian and endometrial study, respectively, were 11.0 and 6.5% for a C-peptide concentration of 2 ng/ml, 1.5 and 1.8% for an IGF-I concentration of 150 ng/ml, 5.2 and 2.5% for an IGFBP-1 concentration of 15 ng/ml, 4.7 and 4.5% for an IGFBP-2 concentration of 400ng/ml, 4.8 and 1.7% for an IGFBP-3 concen-
tration of 3.8 and 4.0 µg/ml and 4.2% for an FSH concentration of 10 IUL.

One hundred and eight control subjects from the NYUWHS cohort, who were included in either the ovarian or endometrial cancer study, had a second blood sample taken 11 to 60 months after the first blood donation (mean duration between visits 18 months), intra-class correlations between repeated peptide measurements were 0.75 (0.68-0.84) for IGF-I, 0.65 (0.56-0.78) for IGFBP-1, 0.56 (0.45-0.73) for IGFBP-2, 0.84 (0.78-0.90) for IGFBP-3 and 0.56 (0.46-0.73) for C-peptide. These results confirm previous findings that single serum measurements of IGF-I or IGFBP-3 are representative of the peptide levels for a period of at least 1 year 16,17,381.

STATISTICAL METHODS

All statistical analyses were performed using the Statistical Analysis System software program (SAS Institute, Cary, NC, Release 8.2).

Hormonal data were log-transformed to reduce departures from the normal distribution when appropriate. To facilitate interpretations of relative risk (RR) for continuous variables log2 transformation was also used, as suggested by the Endogenous Hormones and Breast Cancer Collaborative Group 14.

Paired t-tests were used to compare mean hormone concentrations between cases and controls (case subject value vs. the mean of the matched control subjects) 392.

Multivariate regression models were used to calculate means and 95% CI and to compare mean hormone levels in subgroups of interest after adjusting for potential confounders. These analyses were performed using the Generalized Linear Models (GLM) SAS procedure. Spearman or Pearson partial correlations, adjusted for possible confounders (e.g. age, cohort study) were calculated between hormone variables, age, and BMI.

Odds ratios (OR) for disease by quantile (quintiles, quartiles or tertiles) levels of the hormone variables were estimated by conditional logistic regression models using the SAS ‘PHREG’ procedure. Quantile cut-off points were determined on the basis of the variable distributions of the case and control subjects combined (cohorts-wide) and separately for each of the three cohorts or sub-study to account for differences in hormone levels due to sample collection, processing and storage among the 3 cohorts and according to laboratory method for hormonal measurement. Likelihood ratio tests were used to assess linear trends in ORs over the quantiles, assigning quantitative scores of 1, 2, 3 etc. Ninety-five percent CI were calculated using the standard errors of the pertinent regression coefficients. Two-sided p-values were calculated and considered statistically significant when smaller than 0.05. The potential confounding effects of several factors (e.g. ages at menarche and menopause, parity, BMI, use of OC and HRT, smoking) were examined by including them in the conditional logistic regression models. When there were missing data for a covariate, analyses were run excluding subjects with missing values and by coding the missing values as a separate category. Missing values for BMI were also replaced by the cohort-specific median value of BMI, when BMI was not the main variable of interest.

Heterogeneity of RRs was assessed by chi-square tests and/or by including interaction term in the models. To explore in more detail the potential effect modi-
fication of a given factor (e.g. fasting status at blood donation), the risk of disease in subgroups of interest, as estimated by unconditional logistic regression models, adjusted for all matching factors was compared.

Intra-class correlations between repeated hormone measurements, available for a subset of NYUWHS subjects only, were calculated from variance components estimated by the SAS ‘Mixed’ procedure and their 95% CI were calculated as previously described.

In the cross-sectional analysis, the effect of BMI on sex steroid hormones, IGF-I and IGFBP-3 was studied across five BMI categories which were defined according to BMI distribution in pre- and postmenopausal women separately (BMI categories: ≤ 21.5, 21.5-23.5, 23.5-25, 25-27 and > 27 for premenopausal women; and ≤ 22.5, 22.5-24, 24-25, 25-30 and > 30 for postmenopausal women). Tests for linear trend were performed by scoring the categories according to the median BMI values.

Free testosterone and estradiol concent-

A validation study conducted at the Hormonal Laboratory at IARC compared measurements of free testosterone and free estradiol concentrations obtained by dialysis plus an in-house RIA after extraction and chromatographic purification (reference method) with those calculated from total serum concentrations of testosterone or estradiol and SHBG, as measured by direct, commercial RIAs. The study indicated that theoretical calculations are valid for the determination of free testosterone and estradiol concentrations.

ETHICAL APPROVAL

The Ethical Review Boards of New York University School of Medicine, the University of Umeå, Istituto Nazionale Tumori in Milan and the International Agency for Research on Cancer, in Lyon, periodically reviewed and approved the studies on ovarian and endometrial cancers.
RESULTS

OVARIAN CANCER STUDY

The results of the nested case-control study on ovarian cancer have been reported in papers I-III. The time between cohort recruitment and cancer diagnosis for the case subjects ranged from 12 months to 13.3 years, with an average of 5.7 years (median 5.4 years). Mean age at cancer diagnosis was 59.5 ± 8.8 years (median 61.1 years), which were very similar for the NYUWHS and NSHDS cases, but tended to be lower for the ORDET cases (p = 0.06). Eighty-six percent of the case subjects were diagnosed after more than 3 years. Forty-four case subjects (33%) were premenopausal at recruitment. About 50% of the included subjects had fasted for at least 4 hours before blood donation. Selected descriptive characteristics of the study subjects are shown in Table 3. Compared to the control subjects, the case subjects reported less frequently a history of full-term pregnancy (68% vs. 81%, p < 0.01), were leaner (BMI 25.0 vs. 26.0, p = 0.02), and had experienced menopause at a greater age (50.0 vs. 48.7 years, p = 0.04) (Table 3). There were no significant differences between the cases and the controls in mean age at menarche, frequency of OC use, smoking habits, diagnosis of diabetes or family history of breast cancer.

Results: Paper I

Mean levels of sex-steroid hormones varied somewhat between the three cohorts: mean androstenedione levels were lower in NYUWHS subjects when compared to those of NSHDS and ORDET participants, mean DHEAS levels were higher in NSHDS than in NYUWHS subjects, mean estrone levels were higher in NYUWHS than in ORDET subjects, but mean testosterone and SHBG levels were similar in the three cohorts.

Mean concentrations of steroid hormones were lower in postmenopausal than in premenopausal women (Table 3, paper I), but no significant differences in mean hormone values were observed according to ever-use of OC, smoking status at baseline or ever having had a full-term pregnancy, after adjustment for age at blood sampling, study cohort, menopausal and case-control status.

Mean sex steroid hormones and SHBG concentrations were similar in case and control subjects, both in the whole study population and in sub-groups according to menopausal status at blood donation (Table 3, paper I). There was no association of blood testosterone and DHEAS with ovarian cancer risk in the whole study population, either before or after adjustment for potential confounders (Table 4, paper I). There was a weak tendency for increase in risk with increasing SHBG concentrations, which was reduced by adjustment for ever having a full-term pregnancy and BMI. In women who were postmenopausal at recruitment, levels of testosterone, androstenedione, DHEAS or estrone were not related to ovarian cancer risk (Table 5, paper I). There was an approximately two-fold increase in risk between extreme quartiles of SHBG concentrations, but this association was weakened after adjustment for ever having a full-term pregnancy and BMI. In women who were premenopausal at recruitment, no significant association of sex steroid hormones and SHBG with ovarian cancer...
risk was observed, but risk tended to increase with increasing androstenedione levels (Table 5). Adjustments of the testosterone models for SHBG, to estimate the effect of the biologically active fraction of testosterone in blood, did not influence the point estimates.

Restricting the analyses to women diagnosed with ovarian cancer two or three years after blood donation did not change substantially the direction and strength of any of these associations. Very similar results were obtained when hormone level categories were defined by using study-wide cut-off points.

Results: Paper II

Mean IGF-I and IGFBP-3 levels differed between cohorts. After adjustment for age at blood donation, mean IGF-I and IGFBP-3 concentrations were lower in NSHDS in comparison with those of NYUWHS and ORDET.

Table 5: Odds ratios (95% CI) for ovarian cancer by quartiles of sex-steroids and SHBG in premenopausal women (44 case and 84 control subjects).

|                    | Tertile 1 | Tertile 2 | Tertile 3 | P for trend*
|--------------------|-----------|-----------|-----------|-------------
| **Testosterone**   |           |           |           |             |
| Crude model*       | 1.00      | 0.67 (0.24-1.87) | 1.54 (0.56-4.24) | 0.30        |
| Adjusted model**   | 1.00      | 0.67 (0.23-1.94) | 1.39 (0.48-4.07) | 0.43        |
| Number of cases/controls | 14 / 26 | 11 / 32 | 19 / 25 |
| **Androstenedione**|           |           |           |             |
| Crude model*       | 1.00      | 1.51 (0.55-4.18) | 2.19 (0.80-6.01) | < 0.13      |
| Adjusted model**   | 1.00      | 1.72 (0.60-4.99) | 2.35 (0.81-6.82) | < 0.12      |
| Number of cases/controls | 11 / 31 | 15 / 27 | 18 / 26 |
| **DHEAS**          |           |           |           |             |
| Crude model*       | 1.00      | 1.07 (0.42-2.74) | 1.74 (0.65-5.68) | < 0.27      |
| Adjusted model**   | 1.00      | 1.16 (0.39-3.41) | 1.52 (0.53-4.31) | < 0.43      |
| Number of cases/controls | 13 / 29 | 13 / 29 | 18 / 26 |
| **SHBG**           |           |           |           |             |
| Crude model*       | 1.00      | 1.81 (0.65-5.05) | 1.30 (0.47-3.59) | 0.74        |
| Adjusted model**   | 1.00      | 1.59 (0.53-4.73) | 1.16 (0.38-3.56) | < 0.95      |
| Number of cases/controls | 13 / 30 | 17 / 25 | 14 / 29 |

* Conditional logistic regression on case-control pairs matched for study cohort, age and date at recruitment into the study and day of menstrual cycle (for NYUWHS and ORDET subjects);
** Adjusted for ever full-term pregnancy (yes/no) and log BMI (missing values replaced by cohort-specific median values);
• Linear trends in ORs over tertiles by assigning quantitative scores (1, 2 and 3).
<table>
<thead>
<tr>
<th>Cohort</th>
<th>N cases / controls</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYUWHS</td>
<td>19/38</td>
<td>228 (204-253)</td>
<td>195 (177 - 213)</td>
<td>3.96 (3.71 - 4.22)</td>
<td>3.77 (3.59 - 3.95)</td>
</tr>
<tr>
<td>NSHDS</td>
<td>13/25</td>
<td>186 (153 - 218)</td>
<td>182 (159 - 205)</td>
<td>3.21 (2.47 - 3.94)</td>
<td>3.23 (2.74 - 3.716)</td>
</tr>
<tr>
<td>ORDET</td>
<td>9/18</td>
<td>264 (205 - 323)</td>
<td>216 (174 - 258)</td>
<td>3.97 (3.58 - 4.37)</td>
<td>3.72 (3.44 - 4.00)</td>
</tr>
<tr>
<td>All subjects</td>
<td>41/81</td>
<td>227 (207 - 247)</td>
<td>200 (185 - 215)</td>
<td>3.73 (3.47 - 3.98)</td>
<td>3.58 (3.40 - 3.76)</td>
</tr>
</tbody>
</table>

**Case and control subjects from sets where the cases subject was diagnosed before age 55**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N cases / controls</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYUWHS</td>
<td>55/110</td>
<td>163 (145 - 181)</td>
<td>165 (152 - 178)</td>
<td>3.92 (3.72 - 4.11)</td>
<td>3.88 (3.74 - 4.019)</td>
</tr>
<tr>
<td>NSHDS</td>
<td>29/57</td>
<td>138 (121 - 154)</td>
<td>133 (121 - 144)</td>
<td>3.80 (3.38 - 4.21)</td>
<td>3.82 (3.54 - 4.102)</td>
</tr>
<tr>
<td>ORDET</td>
<td>7/14</td>
<td>141 (86 - 196)</td>
<td>154 (115 - 192)</td>
<td>3.65 (3.03 - 4.27)</td>
<td>3.84 (3.40 - 4.282)</td>
</tr>
<tr>
<td>All subjects</td>
<td>91/181</td>
<td>148 (133 - 162)</td>
<td>149 (137 - 160)</td>
<td>3.83 (3.63 - 403)</td>
<td>3.83 (3.68 - 3.99)</td>
</tr>
</tbody>
</table>
ORDET subjects (Table 6). Mean hormone levels in sub-cohorts of the NSHDS were very similar.

After adjustment for age at sampling, study cohort and case-control status, mean IGF-I and IGFBP-3 levels showed no significant differences according to menopausal status at baseline, ever-use of OC, pre-existing diabetes diagnosis or family history of breast cancer. Women reporting a history of full-term pregnancy tended to have lower mean IGF-I (170 vs 185 ng/ml, \( p < 0.08 \)) and IGFBP-3 (3.75 vs 3.93 ng/ml, \( p < 0.08 \)) levels than nulligravid women. Smokers at baseline tended to have a slightly higher mean IGF-I level compared to non-smokers but the difference was not statistically significant (182 vs. 173, \( p < 0.28 \)).

In the three cohorts combined, mean IGF-I concentrations were only slightly higher (4.6%) in the case than in the control subjects (176 vs. 168 ng/ml IGF-I, \( p = 0.24 \)). However, for women who had developed cancer before age 55 or in those who were age 50 or less at recruitment this difference increased to 13% (227 vs. 200 ng/ml, \( p = 0.03 \)) and 18% (240 vs. 204 ng/ml, \( p < 0.004 \)), respectively. The difference in mean IGF-I levels between case and control subjects was evident for the NYUWHS and ORDET subjects, but not in the NSHDS subjects (Table 6). There were no significant differences in mean IGFBP-3 levels in case and control subjects in any of the cohorts (Table 6).

Overall, for all age groups combined, there were no significant associations between IGF-I and IGFBP-3 concentrations and ovarian cancer risk, either before or after adjustment for ever having a full-term pregnancy and BMI (multivariate OR (95% CI): 1.13 (0.59–2.16), 0.78 (0.40–1.52), 1.39 (0.69–2.80), \( p = 0.64 \) for quartiles of IGF-I and 1.23 (0.64–2.35), 1.22 (0.64-2.34), 0.89 (0.45–1.76), \( p = 0.74 \) for quartiles of IGFBP-3). Adjustment of the IGF-I models for IGFBP-3, and of the IGFBP-3 models for IGF-I, did not alter these results. A similar lack of association between peptide hormone levels and ovarian cancer risk was observed in the women who were 55 or older at ovarian cancer diagnosis (Table 7).

Among women who were younger than 55 when diagnosed with ovarian cancer (of whom 35 case and 62 control subjects where premenopausal at blood donation), there was a direct association between IGF-I concentration and ovarian cancer risk (Table 7). This increase in risk, however, was confined to the highest tertile of IGF-I concentrations. Further adjustments for levels of IGFBP-3 did not influence these results (Table 7). A similar direct association was observed in the group of women who were age 50 or younger at recruitment (multivariate OR (95% CI): 1.10 (0.31-3.95) and 4.26 (1.11-16.3), \( p = 0.03 \) for the second and top IGF-I tertile), although there was a significant (about 80%) overlap between these subgroups of young women. Restriction of these analyses to subjects diagnosed 2 or more years after blood donation did not influence the strength and the direction of the association between IGF-I levels and ovarian cancer diagnosed before age 55. Due to the small number of observations per cohort, the study lacked the statistical power to calculate meaningful tests for homogeneity of the association of IGF-I with ovarian cancer risk across cohort studies.

Among women who were younger than 55 when diagnosed with ovarian cancer, IGFBP-3 levels appeared to be directly related to an increase in ovarian cancer
Table 7: Odds ratios (95% CI) for ovarian cancer by tertiles of IGF-I and IGFBP-3 by age at diagnosis of the case subject (132 case and 263 control subjects).

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Tertile</th>
<th>P for trend•</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁</td>
<td>T₂</td>
</tr>
<tr>
<td>Case-control sets where the index case subject was diagnosed before age 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I</td>
<td>Unadjusted* OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Adjusted** OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Adjusted*** OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Number of cases/controls</td>
<td>9/29</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Unadjusted* OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Adjusted** OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Adjusted*** OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Number of cases/controls</td>
<td>6/31</td>
</tr>
<tr>
<td>Case-control sets where the index case subject was diagnosed at or after age 55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I</td>
<td>Unadjusted* OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Adjusted** OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Adjusted*** OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Number of cases/controls</td>
<td>28/60</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Unadjusted* OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Adjusted** OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Adjusted*** OR (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Number of cases/controls</td>
<td>26/58</td>
</tr>
</tbody>
</table>

*Conditional logistic regression on case-control pairs matched for study cohort, age and date at recruitment into the study, menopausal status and day of menstrual cycle for premenopausal women;
** Adjusted for ever full-term pregnancy and BMI (in categories ≤ 23, 23-25, 25-30, ≥ 30, 13 missing values were replaced by cohort specific medians);
*** Further adjustment of IGF-I models for levels of IGFBP-3 and IGFBP-3 models for levels of IGF-I;
• Linear trends in ORs over the tertiles by assigning quantitative scores (1, 2 and 3).
risk, but there was no evident trend of increase and the confidence intervals of the estimates for the top tertiles included unity. Adjustment of the IGFBP-3 models for levels of IGF-I considerably reduced all point estimates (Table 7).

Results: Paper III

There were no significant differences in mean C-peptide, IGFBP-1 and –2 levels according to ever-use of OC, parity, menopausal status at recruitment or family history of breast cancer. Current smokers had higher C-peptide levels (3.78 vs. 3.08 ng/ml, p = 0.05) than life-time non-smokers, but levels of IGFBP-1 were similar (33.0 vs. 35.2 ng/ml, p < 0.54). Nineteen women reported a history of diabetes diagnosis. After adjustment for cohort study, age at sampling, case-control and fasting status, women with diabetes compared with women not diagnosed as being diabetic had about two-fold higher mean levels of C-peptide (5.71 (4.62-6.80) vs. 3.08 (2.75-3.41) ng/ml, p < 0.0001) and lower levels of both IGFBP-1 (24.2 (14.2-34.2) vs. 35.4 (32.3-38.4) ng/ml, p < 0.03) and IGFBP-2 (193 (60-326) vs. 416 (375-457) ng/ml, p < 0.002). However, as the information about diabetes diagnosis was not complete and accurate for the NYUWHS and NSHDS cohorts, no adjustment was made for diabetes in the models of peptide hormones and ovarian cancer risk.

Mean C-peptide, IGFBP-1 and –2 levels were similar in case and control subjects in the whole study population and in analyses restricted to case-control sets for which only fasting or non-fasting samples were available (Table 3, paper III). Overall, for all age groups combined, there were no statistically significant associations between C-peptide, IGFBP-1 and –2 concentrations and ovarian cancer risk (Table 4, paper III). Adjustment for BMI only slightly increased the point estimates of the C-peptide - ovarian cancer model. Adjustment of IGFBP-1 and –2 models for BMI categories resulted in a decrease of the point estimates, most notably for the highest quartiles, but the confidence limits still included unity (Table 4, paper III). Further adjustments for C-peptide or IGF-1 did not have any effect. The tendency for a protective effect of increased IGFBP-1 and IGFBP-2 concentration was mostly confined to women for whom cancer was diagnosed before age 55, but the results did not reach statistical significance (OR (95% CI): 0.51 (0.18-1.49) and 0.53 (0.18-1.54) for the top IGFBP-1 and IGFBP-2 tertiles respectively).

To study in more detail the effect of C-peptide measurements in samples from fasting vs. non-fasting subjects, we also applied cohort and fasting-specific (for NYUWHS fasting, NYUWHS non-fasting, NSHDS fasting, NSHDS non-fasting and ORDET subgroups) and only fasting-specific (for fasting more than 4 hours and non-fasting subjects) cut off points in our analyses. The results remained very similar –ORs 0.83 (0.49-1.40), 0.71 (0.41-1.25), p = 0.24 when cohort and fasting specific tertile cut-off points were applied and 0.95 (0.57-1.57), 0.70 (0.40-1.22), p = 0.22 for fasting-specific tertiles of C-peptide. Unconditional regression models adjusted for all matching variables and splitting the population according to fasting status at baseline were also applied. The results showed a weak tendency for an increase in risk in the group of non-fasting women, but a protective effect of elevated C-peptide levels in the fasting group.

Restriction of the analyses to subjects
Table 8: Median (10th and 90th percentile) of sex-steroids and SHBG concentrations in endometrial cancer cases and controls, by study [Study I (Initial NYUWHS), Study II (new NYUWHS, NSHDS and ORDET) and combined] (124 case and 236 control subjects).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>N Cases</th>
<th>N Controls</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estradiol (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study I</td>
<td>53</td>
<td>103</td>
<td>0.05</td>
</tr>
<tr>
<td>Study II</td>
<td>69</td>
<td>127</td>
<td>0.0001</td>
</tr>
<tr>
<td>Combined</td>
<td>122</td>
<td>230</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Estrone (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study I</td>
<td>52</td>
<td>101</td>
<td>0.05</td>
</tr>
<tr>
<td>Study II</td>
<td>70</td>
<td>129</td>
<td>0.008</td>
</tr>
<tr>
<td>Combined</td>
<td>122</td>
<td>230</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Androstenedione (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study I</td>
<td>53</td>
<td>103</td>
<td>0.30</td>
</tr>
<tr>
<td>Study II</td>
<td>71</td>
<td>133</td>
<td>0.02</td>
</tr>
<tr>
<td>Combined</td>
<td>124</td>
<td>236</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Testosterone (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study I</td>
<td>53</td>
<td>103</td>
<td>0.81</td>
</tr>
<tr>
<td>Study II</td>
<td>71</td>
<td>133</td>
<td>0.03</td>
</tr>
<tr>
<td>Combined</td>
<td>124</td>
<td>236</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>DHEAS (µg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study I</td>
<td>53</td>
<td>103</td>
<td>0.14</td>
</tr>
<tr>
<td>Study II</td>
<td>71</td>
<td>133</td>
<td>0.002</td>
</tr>
<tr>
<td>Combined</td>
<td>124</td>
<td>236</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>SHBG (nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study I</td>
<td>53</td>
<td>103</td>
<td>0.05</td>
</tr>
<tr>
<td>Study II</td>
<td>71</td>
<td>133</td>
<td>0.05</td>
</tr>
<tr>
<td>Combined</td>
<td>124</td>
<td>236</td>
<td>0.006</td>
</tr>
</tbody>
</table>

* Paired t-test on log-transformed data (case subject values vs. means of matched control subjects).

Diagnosed 2 or more years after blood donation yielded very similar results. Applying study-wide cut-off points for defining hormone quartiles resulted in very similar results for C-peptide and IGFBP-2; however, the tendency for protective effect...
of high IGFBP-1 levels disappeared.

ENDOMETRIAL CANCER STUDY
The results of the nested case-control study on endometrial cancer were reported in papers IV and V. The time from recruitment and blood donation to cancer diagnosis for the case subjects ranged from 6.2 months to 14.4 years, with a median of 4.7 years. Mean age at cancer diagnosis was 61 ± 7.8 years (median 61.3 years). Ninety-six percent of the case subjects were diagnosed at least a year after cohort recruitment. At recruitment, 25% of the subjects (44 cases and 78 controls) were premenopausal. Forty-seven percent of the subjects included in the study had fasted for at least 4 hours before blood donation.

Selected descriptive characteristics of the study subjects are shown in Table 4. Both pre- and postmenopausal case subjects were substantially heavier and had a higher BMI than controls and tended to report less use of OCS and more nulliparity (Table 4). There were no significant differences between case and control subjects in the mean ages at menarche and menopause, use of HRT, ever smoking cigarettes or diabetes diagnosis. In the whole study population endometrial cancer risk increased across BMI categories (≤ 22.5, 22.5-25, 25-30, >30) with ORs of 0.73 [0.40-1.34], 1.49 [0.84-2.63] and 2.57 [1.37-4.80] for the 3 highest BMI categories, ptrend < 0.0003.

Results: Paper IV
Hormone levels did vary according to the assays used in the original (Study I) and second endometrial cancer study (Table 8). No significant differences in mean hormone values were observed according to ever-use of OC or HRT, smoking or ever having had a full-term pregnancy after adjustment for age at blood sampling, study cohort and case-control status.

Median hormone levels in case and control subjects are presented in Table 8. Case subjects had higher median levels of all sex-steroid hormones measured, the differences being more pronounced for estradiol, estrone and DHEAS. The differences in median levels of estradiol and circulating androgens were more pronounced in the newly added subjects, however, there was no statistically significant heterogeneity of the quantitative relationship of risk associated with hormone levels by laboratory method or study cohort. Case subjects had significantly lower levels of SHBG than control subjects.

Odds ratios for endometrial cancer (Table 9) were consistent with the differences in mean hormone levels in that an increase in risk was observed with higher levels of estradiol, estrone, DHEAS, androstenedione and testosterone, while SHBG levels were inversely related to risk. Adjustment for log-BMI, OC and HRT use reduced the strength of the associations of estradiol, estrone, DHEAS and SHBG with cancer risk, but they remained strong and significant, while the association with testosterone became marginally significant (Table 9). The association of androstenedione with cancer risk became only slightly stronger in the adjusted model. Among the adjusting variables, BMI had the greatest effect, reducing the strength of the associations of the hormone variables with endometrial cancer, while adjustment for HRT use increased the regression coefficients. Adjustment for OC use only slightly reduced the point estimates.

Restricting the analyses to the 101 women diagnosed two or more years after blood donation and their controls reduced
Table 9. Odds ratios (95% CI) for endometrial cancer by quartiles of steroid hormones and SHBG in postmenopausal women (124 case and 236 control subjects).

<table>
<thead>
<tr>
<th>Steroid Hormone</th>
<th>Quartile</th>
<th>P for trend•</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td></td>
<td></td>
<td>1.00</td>
<td>1.32 (0.65-2.69)</td>
<td>2.19 (1.07-4.47)</td>
<td>5.39 (2.50-11.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adjusted model**</td>
<td>1.00</td>
<td>1.24 (0.59-2.62)</td>
<td>1.88 (0.88-4.01)</td>
</tr>
<tr>
<td></td>
<td>No of cases/controls</td>
<td>17/63</td>
<td>25/65</td>
<td>35/63</td>
<td>45/39</td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td></td>
<td></td>
<td>1.00</td>
<td>1.46 (0.72-2.96)</td>
<td>2.10 (1.07-4.10)</td>
<td>4.55 (2.28-9.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adjusted model**</td>
<td>1.00</td>
<td>1.39 (0.66-2.93)</td>
<td>1.81 (0.88-3.71)</td>
</tr>
<tr>
<td></td>
<td>No of cases/controls</td>
<td>19/72</td>
<td>22/59</td>
<td>34/59</td>
<td>47/40</td>
<td></td>
</tr>
<tr>
<td>Androstenedione</td>
<td></td>
<td></td>
<td>1.00</td>
<td>1.24 (0.64-2.40)</td>
<td>1.47 (0.72-2.99)</td>
<td>2.04 (1.05-3.98)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adjusted model**</td>
<td>1.00</td>
<td>1.42 (0.69-2.94)</td>
<td>1.61 (0.75-3.45)</td>
</tr>
<tr>
<td></td>
<td>No of cases/controls</td>
<td>25/63</td>
<td>29/63</td>
<td>30/57</td>
<td>40/53</td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
<td></td>
<td>1.00</td>
<td>1.65 (0.85-3.20)</td>
<td>2.22 (1.17-4.23)</td>
<td>2.06 (1.06-4.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adjusted model**</td>
<td>1.00</td>
<td>1.62 (0.82-3.20)</td>
<td>2.30 (1.16-4.55)</td>
</tr>
<tr>
<td></td>
<td>No of cases/controls</td>
<td>21/67</td>
<td>30/59</td>
<td>38/56</td>
<td>35/54</td>
<td></td>
</tr>
<tr>
<td>DHEAS</td>
<td></td>
<td></td>
<td>1.00</td>
<td>1.55 (0.78-3.05)</td>
<td>2.29 (1.17-4.48)</td>
<td>3.03 (1.53-5.98)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adjusted model**</td>
<td>1.00</td>
<td>1.49 (0.73-3.02)</td>
<td>2.11 (1.05-4.24)</td>
</tr>
<tr>
<td></td>
<td>No of cases/controls</td>
<td>20/69</td>
<td>28/63</td>
<td>35/55</td>
<td>41/49</td>
<td></td>
</tr>
<tr>
<td>SHBG</td>
<td></td>
<td></td>
<td>1.00</td>
<td>0.65 (0.36-1.18)</td>
<td>0.38 (0.21-0.71)</td>
<td>0.35 (0.18-0.70)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adjusted model**</td>
<td>1.00</td>
<td>0.73 (0.38-1.39)</td>
<td>0.41 (0.21-0.81)</td>
</tr>
<tr>
<td></td>
<td>No of cases/controls</td>
<td>43/47</td>
<td>34/56</td>
<td>23/67</td>
<td>24/66</td>
<td></td>
</tr>
</tbody>
</table>

* Conditional logistic regression on case-control pairs matched for study cohort, age and date at recruitment into the study;
** Matched as above, plus adjusted for log BMI, OC and HRT use;
• Linear trends in ORs over the quartiles by assigning quantitative scores (1, 2, 3, 4).
somewhat the strength of the associations of estrogens and SHBG with endometrial cancer risk, but the associations remained strong and statistically significant (Table 6, paper IV). The test for homogeneity showed no evidence of effect modification by lag-time between blood donation and cancer diagnosis for estradiol ($p < 0.33$), estrone ($p < 0.80$) and SHBG ($p < 0.33$). The associations between endometrial cancer risk and androgens, although still positive, were weaker than in analyses including all cases and were no longer statistically significant for androstenedione and testosterone. The tests for homogeneity showed statistically significant stronger associations of androgens with endometrial cancer risk in the two years prior to diagnosis than in the preceding years for all three androgens ($p < 0.03$ for androstenedione, $p < 0.04$ for testosterone and $p < 0.02$ for DHEAS).

Adjustment of estrogen-endometrial cancer models for levels of circulating androgens or SHBG slightly reduced the strength of the associations, but all models remained statistically significant (Table 10). In contrast, adjustment for estrogens resulted in a decrease in the effect of androstenedione and testosterone (ranging from 17 to 24%) and a loss of statistical significance. Adjustment of DHEAS models for estrogens weakened the association with endometrial cancer risk, but the estimates remained significant after adjustment for estrone and very close to being significant after adjustment for estradiol (Table 11).

Restricting the analyses to women with endometrioid tumors (59 case-control sets), strengthened the associations of estradiol, estrone, androstenedione and testosterone with endometrial cancer risk, did not influence the association with SHBG and

### Table 10: Risk of endometrial cancer associated with a doubling of estrogen concentration, with or without adjustment for androgen or SHBG.

<table>
<thead>
<tr>
<th>Hormone or SHBG</th>
<th>Unadjusted</th>
<th>Androstenedione</th>
<th>Testosterone</th>
<th>DHEAS</th>
<th>SHBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>2.06 (1.47-2.89)</td>
<td>1.94 (1.37-2.76)</td>
<td>2.00 (1.39-2.88)</td>
<td>1.87 (1.32-2.66)</td>
<td>1.95 (1.39-2.75)</td>
</tr>
<tr>
<td>Estrone</td>
<td>1.84 (1.31-2.58)</td>
<td>1.69 (1.15-2.48)</td>
<td>1.78 (1.21-2.62)</td>
<td>1.52 (1.06-2.19)</td>
<td>1.69 (1.19-2.38)</td>
</tr>
</tbody>
</table>

### Table 11: Risk of endometrial cancer associated with a doubling of androgen and SHBG concentration, with or without adjustment for estrogen.

<table>
<thead>
<tr>
<th>Hormone or SHBG</th>
<th>Unadjusted</th>
<th>Adjusted for Estradiol</th>
<th>Adjusted for Estrone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione</td>
<td>1.52 (1.08-2.14)</td>
<td>1.22 (0.85-1.75)</td>
<td>1.19 (0.81-1.75)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1.26 (1.02-1.57)</td>
<td>1.05 (0.83-1.32)</td>
<td>1.04 (0.81-1.33)</td>
</tr>
<tr>
<td>DHEAS</td>
<td>1.51 (1.16-1.96)</td>
<td>1.30 (0.99-1.72)</td>
<td>1.40 (1.04-1.88)</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.66 (0.49-0.90)</td>
<td>0.74 (0.54-1.01)</td>
<td>0.71 (0.52-0.99)</td>
</tr>
</tbody>
</table>
abolished the statistical significance of the association with DHEAS.

Excluding subjects with FSH values below 30 IU/L (a more conservative cut-off point to define menopausal status) did not alter the observed associations.

**Results: Paper V**

Mean peptide and binding protein concentrations showed no significant differences between ever and never users of OC or HRT, smoking status at baseline, or ever having had a full-term pregnancy. Women who reported a diagnosis of diabetes had higher blood concentrations of C-peptide than women without such a diagnosis (4.36 vs. 3.03 ng/ml, \( p = 0.001 \)).

Mean hormone levels in case and control subjects are presented in Table 12. Case subjects had higher mean C-peptide and lower IGFBP-1 levels than control subjects, also observed in sub-groups defined according to fasting status at blood donation), but similar mean IGF-I and IGFBP-2 and -3 levels.

Endometrial cancer risk was not significantly related to blood levels of IGF-I, IGFBP-2 and IGFBP-3, either before or after adjustment for BMI, parity, OC and HRT use (Table 13). The molar ratio of IGF-I/IGFBP-3 was inversely related to risk of endometrial cancer (OR for the top quintile 0.51 (0.24-1.09), \( p_{\text{trend}} 0.03 \)), however, the association lost statistical

### Table 12: Mean (± 95% CI) of C-peptide, IGF-I, IGFBP-1, 2 and-3 concentrations adjusted for cohort study, age at sampling, menopausal and fasting (< 4 hours, 4-8 hours, > 8 hours) status at blood donation in endometrial cancer cases and controls, in the whole study population and by fasting status (166 case and 315 control subjects).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Cases</th>
<th>Controls</th>
<th>( p )-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (95% C.I.)</td>
<td>N</td>
</tr>
<tr>
<td>C-peptide (ng/ml)</td>
<td>166</td>
<td>3.38 (2.99-3.77)</td>
<td>313</td>
</tr>
<tr>
<td>C-peptide (fasting)</td>
<td>75</td>
<td>2.68 (2.40-3.00)</td>
<td>152</td>
</tr>
<tr>
<td>C-peptide (not fasting)</td>
<td>91</td>
<td>4.89 (4.24-5.54)</td>
<td>161</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>166</td>
<td>169 (155-184)</td>
<td>314</td>
</tr>
<tr>
<td>IGFBP-1 (ng/ml)</td>
<td>166</td>
<td>29.2 (25.5-32.8)</td>
<td>314</td>
</tr>
<tr>
<td>IGFBP-1 (fasting)</td>
<td>75</td>
<td>32.2 (27.1-37.4)</td>
<td>152</td>
</tr>
<tr>
<td>IGFBP-1 (non-fasting)</td>
<td>91</td>
<td>16.5 (12.0-20.9)</td>
<td>162</td>
</tr>
<tr>
<td>IGFBP-2 (ng/ml)</td>
<td>164</td>
<td>416 (332-500)</td>
<td>313</td>
</tr>
<tr>
<td>IGFBP-2 (fasting)</td>
<td>73</td>
<td>368 (290-445)</td>
<td>151</td>
</tr>
<tr>
<td>IGFBP-2 (non-fasting)</td>
<td>91</td>
<td>480 (348-613)</td>
<td>162</td>
</tr>
<tr>
<td>IGFBP-3 (µg/ml)</td>
<td>165</td>
<td>4.00 (3.87-4.14)</td>
<td>312</td>
</tr>
</tbody>
</table>

*Generalized linear model.
significance after adjustment for confounders (OR for the top quintile 0.62 (0.27-1.43), \( p_{\text{trend}} = 0.23 \)).

Levels of IGFBP-1 were inversely related to endometrial cancer risk, but the association was weakened and lost statistical significance after adjustment for BMI, ever having had a full-term pregnancy, use of OC or HRT. However, in women who were postmenopausal at recruitment, the association was stronger and remained significant after adjustments for BMI and other confounders (0.36 [0.13-0.95] for the top quintile, \( p_{\text{trend}} = 0.04 \)). Adjustment of the crude conditional logistic regression IGFBP-1 models for C-peptide did not abolish the significant inverse association of this peptide with endometrial cancer risk in the whole study population (OR of 0.36 [0.17-0.77] for the top quintile of IGFBP-1, \( p_{\text{trend}} < 0.02 \)) or in analyses restricted to women who were postmenopausal at blood donation (OR of 0.29 [0.12-0.71], \( p_{\text{trend}} < 0.01 \)).

Table 13: Odds ratios for endometrial cancer by quintiles of C-peptide, IGF-I, IGFBP-1, -2 and -3 (166 case and 315 control subjects).

<table>
<thead>
<tr>
<th></th>
<th>Quintile 1</th>
<th>Quintile 2</th>
<th>Quintile 3</th>
<th>Quintile 4</th>
<th>Quintile 5</th>
<th>( p ) for trend*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C-peptide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model*</td>
<td>1.00</td>
<td>1.14 (0.56-2.31)</td>
<td>1.00 (0.45-2.22)</td>
<td>2.52 (1.13-5.36)</td>
<td>4.76 (1.91-11.8)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Adjusted model**</td>
<td>1.00</td>
<td>1.12 (0.53-2.37)</td>
<td>0.99 (0.43-2.29)</td>
<td>1.98 (0.84-4.68)</td>
<td>4.40 (1.65-11.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>No of cases/controls</td>
<td>29/67</td>
<td>28/67</td>
<td>26/72</td>
<td>39/56</td>
<td>44/51</td>
<td></td>
</tr>
<tr>
<td><strong>IGF-I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model*</td>
<td>1.00</td>
<td>0.98 (0.55-1.75)</td>
<td>0.69 (0.37-1.30)</td>
<td>0.55 (0.28-1.07)</td>
<td>0.72 (0.38-1.37)</td>
<td>0.12</td>
</tr>
<tr>
<td>Adjusted model**</td>
<td>1.00</td>
<td>0.87 (0.46-1.66)</td>
<td>0.79 (0.40-1.53)</td>
<td>0.61 (0.30-1.26)</td>
<td>0.90 (0.44-1.82)</td>
<td>0.54</td>
</tr>
<tr>
<td>No of cases/controls</td>
<td>37/59</td>
<td>38/58</td>
<td>31/65</td>
<td>27/69</td>
<td>33/63</td>
<td></td>
</tr>
<tr>
<td><strong>IGFBP-1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model*</td>
<td>1.00</td>
<td>0.67 (0.37-1.21)</td>
<td>0.33 (0.17-0.65)</td>
<td>0.56 (0.30-1.07)</td>
<td>0.30 (0.15-0.62)</td>
<td>0.002</td>
</tr>
<tr>
<td>Adjusted model**</td>
<td>1.00</td>
<td>0.89 (0.46-1.74)</td>
<td>0.39 (0.19-0.79)</td>
<td>0.71 (0.35-1.43)</td>
<td>0.49 (0.22-1.07)</td>
<td>0.06</td>
</tr>
<tr>
<td>No of cases/controls</td>
<td>41/52</td>
<td>37/59</td>
<td>25/71</td>
<td>35/61</td>
<td>25/71</td>
<td></td>
</tr>
<tr>
<td><strong>IGFBP-2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model*</td>
<td>1.00</td>
<td>0.50 (0.26-0.96)</td>
<td>0.59 (0.32-1.09)</td>
<td>0.61 (0.32-1.18)</td>
<td>0.54 (0.27-1.06)</td>
<td>0.20</td>
</tr>
<tr>
<td>Adjusted model**</td>
<td>1.00</td>
<td>0.50 (0.25-0.99)</td>
<td>0.67 (0.34-1.30)</td>
<td>0.73 (0.36-1.50)</td>
<td>0.81 (0.38-1.74)</td>
<td>0.98</td>
</tr>
<tr>
<td>No of cases/controls</td>
<td>41/54</td>
<td>39/66</td>
<td>31/63</td>
<td>32/65</td>
<td>31/63</td>
<td></td>
</tr>
<tr>
<td><strong>IGFBP-3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model*</td>
<td>1.00</td>
<td>1.44 (0.78-2.66)</td>
<td>1.19 (0.63-2.36)</td>
<td>0.96 (0.49-1.90)</td>
<td>1.96 (0.94-4.10)</td>
<td>0.35</td>
</tr>
<tr>
<td>Adjusted model**</td>
<td>1.00</td>
<td>1.66 (0.85-3.22)</td>
<td>1.45 (0.72-2.94)</td>
<td>1.11 (0.53-2.34)</td>
<td>2.41 (1.07-5.45)</td>
<td>0.20</td>
</tr>
<tr>
<td>No of cases/controls</td>
<td>29/66</td>
<td>37/58</td>
<td>32/63</td>
<td>26/69</td>
<td>41/55</td>
<td></td>
</tr>
</tbody>
</table>

*Conditional logistic regression on case-control pairs matched for study cohort, age and date at recruitment into the study, menopausal status and day of menstrual cycle for premenopausal women; all C-peptide, IGFBP-1 and IGFBP-2 models were adjusted also for fasting (< 4 hours, 4-8 hours, ≥ 8 hours);
** Adjusted for BMI (in categories ≤ 22.5, 22.5-25, 25-30, ≥ 30, missing), parity, OC and HRT use;
* Linear trends in ORs over the quintiles by assigning quintile scores (1, 2, 3, 4, 5).
There was a strong direct dose-response relationship between C-peptide concentration and endometrial cancer risk that reached a more than four-fold increase in risk in the top quintile of C-peptide levels (Table 13). This increase in risk was attenuated after adjustment for BMI categories, but the dose-response pattern and the statistical significance of the test for trend and for odds ratio in the top quintile of C-peptide remained (Table 13). Similarly, adjustment for levels of IGFBP-1 reduced the point estimates, but the association of C-peptide with endometrial cancer risk remained significant.

The case and control subjects were not matched for fasting status and to explore the effect of fasting, unconditional logistic regression models were run with adjustment for all matching variables. The results were similar to the conditional regression models (ORs for the 2\textsuperscript{nd} to 5\textsuperscript{th} quintile: 1.14 [0.56-2.32], 1.03 [0.48-2.23], 2.31 [1.08-4.95] and 3.75 [1.63-8.65]). The study population was then divided according to the fasting status of the women at blood

### Table 14: Geometric mean (5th-95th percentile) of BMI, SHBG and sex hormones by menopausal status, adjusted for cohort study, age at blood donation and case-control sub-study.

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal Women</th>
<th>Postmenopausal Women</th>
<th>p-value*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Geometric Mean (5-95 %)</td>
<td>n</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>177</td>
<td>44.7 (36.9-50.9)</td>
<td>443</td>
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<tr>
<td>BMI</td>
<td>171</td>
<td>24.2 (20.0-30.2)</td>
<td>428</td>
</tr>
<tr>
<td>Weight</td>
<td>173</td>
<td>63.2 (49.0-85.5)</td>
<td>434</td>
</tr>
<tr>
<td>Height</td>
<td>172</td>
<td>162 (150-173)</td>
<td>432</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>167</td>
<td>0.27 (0.10-0.68)</td>
<td>315</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>166</td>
<td>12.3 (4.1-36.0)</td>
<td>314</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>168</td>
<td>1.31 (0.56-2.54)</td>
<td>320</td>
</tr>
<tr>
<td>DHEAS (µg/dl)</td>
<td>167</td>
<td>131.1 (47-303)</td>
<td>319</td>
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<tr>
<td>Estrone (pg/ml)</td>
<td>-</td>
<td>-</td>
<td>310</td>
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<tr>
<td>Estradiol (pg/ml)</td>
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<td>-</td>
<td>125</td>
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<tr>
<td>Free estradiol (pmol/l)</td>
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<td>-</td>
<td>125</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>167</td>
<td>50.1 (27.3-103)</td>
<td>319</td>
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<tr>
<td>IGF-I (ng/ml)</td>
<td>163</td>
<td>202.2 (99-359)</td>
<td>432</td>
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<tr>
<td>IGFBP-3 (µg/ml)</td>
<td>162</td>
<td>3.62 (2.31-4.78)</td>
<td>423</td>
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</table>

* General linear model.
donation. The OR for the top C-peptide tertile for the fasting subjects (2.38 [1.00-5.68]) was similar to that of the non-fasting subjects (2.16 [0.88-5.28]) and to that of the whole study population (1.95 [1.06-3.58]). The association in postmenopausal women appeared to be stronger than in premenopausal women, but a formal test for interaction was not significant.

Restricting the analyses to women diagnosed 2 or more years after blood donation or to those without history of diabetes did not influence substantially the direction and strength of any of the associations between levels of C-peptide, IGF-I, IGFBP-1, -2 and -3 and endometrial cancer risk. Very similar results were obtained when categories of hormone exposure, as used for the conditional logistic regression models, were re-defined according to the hormone distribution in each study cohort separately or when an FSH value of 30 IU/L (a more conservative estimate) was used as a cut-off point to classify women as postmenopausal.

CROSS-SECTIONAL STUDY

The cross-sectional analysis of the hormonal data of the control subjects of the ovarian and endometrial case-control studies are reported in paper VI. Only measurements that were performed at the IARC Hormonal Laboratory were included. In total, 595 measurements of IGF-I and IGFBP-3, 482 of testosterone, 488 of androstenedione, 486 of DHEAS, 486 of SHBG, 310 of estrone and 125 estradiol measurements were available. All laboratory assays were performed between December 2000 and February 2002.

Results: Paper VI

Selected descriptive characteristics of the control subjects included for these analyses by menopausal status are shown in Table 14. At recruitment, 29% of the study subjects were premenopausal (177 women; mean age 44.3 years) and 71% were postmenopausal (443 women; mean age 57.8 years). Pre- and postmenopausal women had similar mean height but postmenopausal women were slightly heavier and had higher BMI than premenopausal women.

Differences in mean hormone levels between pre- and postmenopausal women reached statistical significance for androstenedione, SHBG and IGFBP-3 (Table 14), but after adjustment for BMI only the difference in mean androstenedione levels remained significant [1.10 (0.96-1.25) in pre- versus 0.90 (0.83-0.99) in post-menopausal women, p < 0.04]. Estrogen levels in pre- and postmenopausal women could not be compared, as estrogen measurements were available only for postmenopausal women.

Spearman correlations between steroid and peptide hormones, BMI and age (adjusted for cohort study, age at recruitment and case-control sub-study) in pre- and postmenopausal women are presented in Table 15. Age was inversely correlated with all measured androgens in premenopausal women and with DHEAS, androstenedione and estrogens in postmenopausal women. In both pre- and postmenopausal women, age was inversely related to IGF-I, but not to IGFBP-3 or SHBG.

There were positive correlations between circulating concentrations of sex steroid hormones in both pre- and postmenopausal women. In general, the estrogen-androgen correlations (range from 0.24 to 0.48) were weaker than androgen-androgen (range from 0.43 to 0.92) or estrogen-estrogen correlations (range from
Table 15: Spearman partial correlation coefficients between hormonal variables, adjusted for cohort, study and age at blood sampling.
(Hormone measurements were available as follows: 163 for IGF-I, 162 for IGFBP-3, 167 for testosterone, 166 for free testosterone, 168 for androstenedione, 167 for DHEAS and 167 for SHBG in pre-menopausal women and 432 for IGF-I, 423 for IGFBP-3, 315 for testosterone, 314 for free testosterone, 320 for androstenedione, 319 for DHEAS, 310 for estrone, 125 for total and free estradiol and 319 for SHBG for post-menopausal women.)

<table>
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<tr>
<th></th>
<th>Testosterone</th>
<th>Free testosterone</th>
<th>DHEAS</th>
<th>Estrone</th>
<th>Estradiol</th>
<th>Free estradiol</th>
<th>SHBG</th>
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<th>IGFBP-3</th>
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<td>0.03</td>
<td>-0.04</td>
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</table>

* P < 0.0001; • P < 0.01; # P < 0.05.
Circulating levels of IGF-I correlated directly with levels of IGFBP-3 in pre- and post menopausal women ($r = 0.30$ and $0.47$, $p < 0.0001$, respectively).

BMI did not correlate with androgen levels in premenopausal women, but direct associations with androgens and estrogens were observed among postmenopausal women. In both pre- and postmenopausal women, BMI was inversely associated with SHBG and there was no monotonic correlation with IGF-I or IGFBP-3 (Table 15). A similar pattern of the variation of hormone levels with increasing BMI was observed when the hormonal data were presented as geometric mean concentrations across BMI categories (Figures 1 and 2 in paper VI). The only exceptions were IGF-I concentrations in postmenopausal women, which showed a clear bell-shape curve, with peak IGF-I levels in women who had a BMI between 24 and 25 and lower levels in women with BMI $< 22.5$ and BMI $> 30.0$ ($p_{\text{homogeneity}} < 0.01$). The observed variation of IGF-I levels across categories of BMI was not influenced by adjustments for either any of the androgens in pre- and postmenopausal women or for estrogens in postmenopausal women. The pattern of the associations between hormone levels and BMI did not differ in subgroups of subjects according to either measured or self-reported anthropometry data.

IGF-I correlated directly with all measured androgens. The associations were slightly stronger and reached statistical significance in postmenopausal women (range, 0.16 to 0.22; $P < 0.01$ for all correlations) than in premenopausal women (range, 0.11 to 0.21, $p < 0.01$ only for the correlation between androstenedione and IGF-I). The associations between estrogens and IGF-I were very weak (range, -0.11 to 0.05) and there was no significant variation in mean IGF-I levels across quintiles of estrogen concentrations, although mean IGF-I levels were somewhat lower in the higher two quintiles of total estradiol. IGFBP-3 was inversely correlated to estradiol levels ($r = -0.25$, $p < 0.01$), but its correlation with estrone and free estradiol did not reach statistical significance ($r = -0.14$ and 0.09, respectively). SHBG concentrations were inversely related to IGF-I and IGFBP-3 levels and declined across quintiles of IGF-I in post-, but not premenopausal women (Table 15).

As was to be expected, there was an inverse correlation of SHBG with the calculated free testosterone and free estradiol (Table 15).
GENERAL DISCUSSION

METHODOLOGICAL CONSIDERATIONS (PAPERS I-V)

Study populations

The two nested case-control studies on ovarian and endometrial cancers are part of an on-going collaboration between three prospective cohorts in New York (USA), Umeå (Sweden) and Milan (Italy). All three participating cohorts were designed to address the role of endogenous hormones and diet in the etiology of cancer and other diseases, which allowed pooling of data and other resources in a standardised manner. The age range of the recruited subjects in the three cohorts was similar, the majority of subjects included in the two nested case-control study from each cohort (>81%) being between 35 and 65 years old at enrolment. The follow-up in the three cohorts is based on high quality cancer and death registries and, in addition, active follow-up (by mail and telephone contact) is being conducted for the NYUWHS participants, assuring detection of more than 95% of the cancer cases among cohort members. Furthermore, the medical records of all cancer cases (with rare exceptions) are carefully reviewed by each parent cohort study to assure the quality of cancer cases ascertainment. So far, very few subjects (less than 1%) have been lost to follow-up in the NSHDS and the ORDET cohorts. In 2000, 83% of the NYUWHS members presumed to be alive, completed a questionnaire by mail or telephone.

The NYUWHS and the ORDET cohort recruited healthy volunteers, while the NSHDS is a population-based cohort with a participation rate of about 60%. A study in the VIP component of the NSHDS showed relatively small differences in the social characteristics of participants and non-participants in the study and the overall health status in the two groups was found to be similar rather than dissimilar (as measured by several risk factors including BMI and smoking). To some extent the low participation rate in the NSHDS is due to administrative difficulties. Only women who did not use exogenous hormones at blood donation were recruited in the NYUWHS and the ORDET cohorts, while subjects from NSHDS who used exogenous hormones at blood donation were not considered eligible for the ovarian and endometrial study. Thus, the women enrolled in the 3 cohorts are probably more health conscious and the included study subjects may have different pattern of hormone use than the underlying general population. However, it is plausible to assume that the qualitative association of endogenous hormone levels and risk of cancer (in terms of direction) observed in the ovarian and endometrial cancer studies are similar to those in the underlying general population, although differences may exist in terms of the magnitude of the effect and of the cancer risk fraction attributable to hormone levels.

About 13% of the included NYUWHS subjects did not provide information about race, about 75% indicated they were non-Hispanic white and 12% indicated they were Black, Hispanic or other ethnicity. Restricting the analyses only to women who indicated they were non-Hispanic white did not change the direction and
strength of the observed associations.

Selection, recall and inverse-causation bias

One advantage of the nested case-control study design applied is that cancer case and control subjects originate from the same, well-defined source population, thereby minimizing the risk of control selection biases. Control subjects were selected at random among all cohort members who were alive, free of cancer, who had not had a bilateral ovariectomy or hysterectomy (for the ovarian and endometrial study respectively) up to the index date (date of cancer diagnosis for the case subject), and who matched the case on menopausal status at enrolment, age (±6 months) and date of recruitment (±3 months).

The menopausal status of subjects with equivocal data was confirmed by an FSH measurement. The exact cut-off points used for subject classification by levels of a hormone of interest could vary according to the laboratory kits used, populations, sample matrix and laboratory where hormone measurements are performed. The manufacturer of the FSH IRMA assay (Diagnostic System Laboratories, Webster, Texas, USA) has indicated FSH levels up to 10.26 IU/L for premenopausal women and 13.50 IU/L as lowest value for postmenopausal women. The concordance was examined between classifications made on the basis of FSH levels with different cut-off points and classifications made on the basis of self-reported data about menopausal status and, additionally, according to age distribution (women < 42 and > 55 years) separately for each cohort, using FSH data from more than 300 women from the 3 cohorts. On the basis of this analysis, women were classified as postmenopausal if their FSH measurement was > 12.75 IU/L. Additionally, all analyses in postmenopausal women were repeated excluding subjects that had an FSH value below 30 IU/L (a more conservative cut-off point). The results of these restricted analyses yielded very similar estimates of risk, suggesting that misclassification of menopausal status has not influenced our main findings.

For several case-control sets from the C-peptide, IGF-I and IGFBPs components of the ovarian and endometrial studies (6 and 4 sets, respectively), the matching for menopausal status did not hold. However, levels of IGF-I, IGFBPs and C-peptide change as a function of age and are not influenced by transition of women to menopause (after adjustment for age) and exclusion of the sets, for which the menopausal matching did not hold, did not influence the RR estimates of hormone-cancer associations. Only case-control sets for which the matching for menopausal status was respected were retained for the sex-steroids components of the ovarian and endometrial cancer studies.

The probability of recall bias in cohort studies with prospectively collected data is low. In our studies, about half of the NSHDS subjects did not have prospectively collected data about reproductive history and this information was collected by mailing questionnaires and/or telephone interviews to all study subjects after the index date (date of cancer diagnosis for each case-control matched set). For 13 of the ovarian cancer cases, who were deceased, only data from the medical records were available. For the endometrial study, a special effort was made also in the NYUWHS to collect and up-date information about hormone use up to the index date. Only baseline information was available for ORDET participants. To account for these differences in the collection of...
reproductive data, we always reported the crude estimates of risk and adjusted for variables for which we were confident in the quality of the data, such as pregnancies and OC use (concordance of more than 88%). In addition, mean hormone levels did not vary substantially across strata of potential confounders in our data and there is no indication from published cross-sectional studies that past use of exogenous hormones and pregnancies influence significantly endogenous sex steroids, C-peptide, IGF-I or IGFBPs levels. Similarly to results from studies on breast cancer and as were the expectations, the association of estrogens with endometrial cancer risk appeared stronger in the group of women who had never used HRT than in past users of HRT. In conclusion, although not optimal, adjustment for reproductive history variables, which could act as potential confounders, was adequate in our study.

The prospective design of the studies minimises the “inverse causation” bias, which may occur if the metabolic factors studied as potential determinants of cancer risk are in fact themselves subject to changes induced by the presence of a tumor, or by the consequences of the tumor diagnosis (e.g., stress, anticancer treatment). This is of particular relevance for studies on ovarian cancer, as a tumor growing in a key endocrine organ is likely to cause major alterations in the synthesis and circulating levels of sex steroids and other hormones. To reduce inverse causation bias, cases were selected if they were diagnosed at least one year after blood donation for the ovarian cancer study and at least six months after blood donation for the endometrial cancer study. In addition, analyses, restricted to matched case-control sets in which the case subject was diagnosed at 2 or more years after blood donation were also run.

Some limitations of the questionnaire data
A limitation of the available questionnaire data is that only self-reported data about diabetes diagnosis were available. The prevalence of diabetes in the control population was 5% at median age of at recruitment of 54.7 years for the ovarian study and 57.3 years for the endometrial cancer study. In comparison, the estimated prevalence of diabetes in 1995 and 2000 in adults aged 20 and over has been reported to be above 7.4% in USA, 9.3% in Sweden and 7.5% in Italy, indicating that the data on diabetes prevalence in the studies could have not been complete. However, other surveys have shown lower prevalence (5-6%) of diagnosed diabetes in American women over age 20. Additionally, there was no indication if the diabetes diagnosis was of type I or type II diabetes. Insulin-dependent diabetes (type I) usually develops at an earlier age and is characterised with reduced pancreatic secretion of insulin, while non-insulin dependent diabetes (type II) usually develops after age 35 and is generally associated with a prolonged period of insulin resistance and hyperinsulinemia, even if the increased insulin levels remain too low to maintain the normal glucose homeostasis. Only a few women reported diabetes diagnosis, but they had substantially higher C-peptide levels than non-diabetic women, suggesting that they had preserved endogenous insulin production. Hence, the majority of these study subjects probably had a non-insulin dependent (type II) diabetes mellitus. The initial hypothesis was that C-peptide concentrations will be directly related to risk of both ovarian and endometrial cancers and, consequently,
type II diabetes could act as a positive
confounder. Such a confounding effect of
previous diabetes diagnosis is of less
concern for the ovarian study, as generally,
diabetes has not been related to increased
risk of ovarian cancer and is less likely to
act as a classical confounder (it is not
related to disease). Additionally, pre-diag-
nostic C-peptide levels were not associated
with risk of ovarian cancer, thus failing to
adjust for diabetes diagnosis has not
resulted in an inflated or a spurious direct
association of C-peptide levels with risk of
ovarian cancer. On the other hand, diabetes
is an established risk factor for endometrial
cancer and the strength of the association
of C-peptide with risk was slightly
weakened, but not abolished in analyses
restricted to women without diabetes diag-
nosis. Thus, it is probable that some
residual confounding remained in our
model of C-peptide and endometrial
cancer. However, the association of C-
peptide with endometrial cancer was very
strong and adjustments for other variables
that can mediate the association of diabetes
with endometrial cancer, such as BMI,
SHBG and estrogens (in postmenopausal
women) did not abolish its significance.

Another potential limitation of these
studies is that there was no information
about family history of ovarian cancer and
if the study subjects were BRCA-1 or-2
mutation carriers, which prevented exami-
nation of the associations between endo-
genous hormones and ovarian cancer
according to the genetic background of the
study subjects. However, family history of
breast cancer in first degree relatives was
not significantly related to risk in these
studies.

Laboratory measurements
Hormone measurements in samples taken
about a year and a half apart, which were
available for part of the NYUWHS study
subjects, showed satisfactory to high intra-
class correlations, for both peptide and sex-
steroid hormones (in postmenopausal
women), indicating that the studied
hormones are fairly stable over time in a
given individual and that a single measure-
ment can be used to characterise an
individual’s average level.

All laboratory analyses (with the ex-
ception of 43% of subjects included in the
endometrial cancer and sex-steroids study)
were conducted on stored samples from
each cohort, at a single central laboratory,
the Hormone Laboratory at IARC, Lyon,
France. Samples from case subjects and
their matched control subjects were always
analyzed within the same batch, assay kit
and on the same day to minimise variations
or bias due to assay type or batch.
Laboratory personnel were unable to
distinguish between case and control
samples. To control the quality of the
hormone measurements, samples from a
pool of quality control plasma and 3
standard sera were inserted randomly in
each batch.

Mean hormone levels differed some-
what between the three cohorts, but such
variations were not confined to a single
cohort or a hormone measurement. Several
factors could have contributed to such
differences. Serum samples were available
for the NYUWHS subjects, while heparin-
nised plasma samples were available for
the NSHDS and the ORDET cohort
participants. It has been shown that mean
IGF-I and IGFBP concentration may differ
in serum, heparin or EDTA plasma samp-
les from the same subjects, but the corre-
lation between measurements in serum and
plasma are very high for IGF-I (r=0.95)
and good for IGFBPs 389. Variations in
blood collection, processing or storage conditions among the three cohort studies could have also contributed to the observed variation of mean hormone levels across cohorts. To account for these differences in mean hormone levels in the statistical analyses, hormone quantiles were defined on the basis of cohort study and laboratory method (see below). Additionally, relative risk estimates obtained by applying study-wide cut-off points were calculated and usually were of similar magnitude as the cohort-specific estimates. Thus it is reasonable to assume that despite differences in mean levels of IGF-I and IGFBPs the relative ranking of subjects by IGF-I and IGFBP concentrations was preserved.

For the endometrial cancer and sex-steroids study, mean hormone levels varied according to the type of hormonal assay applied – indirect methods (including a purification step) or direct (no-extraction) methods. A validation study conducted at the Hormonal Laboratory at IARC (where all estradiol, estrone, androstenedione and testosterone measurements by direct kits for this study were performed) showed high correlations between measurements performed by an indirect (reference) and direct methods with preservation of the relative ranking of the subjects. Additionally, a recent study by Dorgan et al. (2002) concluded that although absolute concentrations may differ, measurements by mass spectrometry and RIA (after an extraction step) yield similar estimates of between subject differences in serum concentrations of most sex-steroid hormones measured for these studies. Furthermore, a large pooled study of breast cancer did not show any important differences in risk estimates calculated from hormone measurements obtained by direct or indirect methods. For the current studies the direct assay kits for the hormonal analyses were carefully selected on the basis of: 1. high correlation with the hormone values obtained by the reference indirect method; 2. good performance of the direct kits in studies of hormone reproducibility over time; and 3. mean absolute hormone concentrations measured.

In conclusion, we believe that differences in hormone levels according to the laboratory methods applied and blood sampling procedures in the parent cohort studies should not have influenced substantially the associations of sex steroid hormones with cancer risk observed in our study.

Phase of menstrual cycle at blood donation

In premenopausal women, sex-steroid hormones (especially estrogens and progesterone) vary throughout the menstrual cycle. To minimise such variation, in the ORDET cohort, blood from premenopausal women was drawn during the luteal phase of the menstrual cycle (between 20th and 24th day after last menstruation). In NYUWHs, premenopausal women returned information on the date of the menstrual cycle that immediately followed blood donation, and for the studies on ovarian and endometrial cancers, NYUWHs premenopausal case and control subjects were matched for phase of menstrual cycle at blood donation. No information about phase of menstrual cycle was available for NSHDS participants. However, we believe our results were not influenced by the lack of matching for phase of menstrual cycle in the NSHDS premenopausal participants, as estrogens were measured only in samples from postmenopausal women and there is little or negligible variation throughout the menstrual cycle of androgens SHBG, IGF-I and IGFBPs.
Fasting status at blood donation

A limitation of this study is that about half of the blood samples were collected from non-fasting women. Fasting status influences blood concentrations of C-peptide, IGFBP-1 and, less so, IGFBP-2\(^7,238\), as also observed in our data (Tables 12). To reduce possible attenuation of the association of C-peptide and IGFBP-1 or -2 with cancer risk related to variations in insulin levels in non-fasting subjects, adjustments were made for fasting status in all statistical models tested. The associations of C-peptide with cancer risk were similar when cohort and fasting specific, or only fasting-specific cutoff points were applied. Additionally, unconditional logistic regression models (adjusted for all matching variables) showed very similar ORs in the groups of fasting and non-fasting women at blood donation, for both the ovarian and endometrial studies.

Statistical power

Both ovarian and endometrial cancer are relatively rare (e.g. in comparison with breast cancer) and combining the data of the three cohorts gave us the possibility of investigating prospectively these two gynecological cancers. Currently, these are by far the largest prospective studies on endogenous hormones in relation to ovarian and endometrial cancers.

One-to-one pair matching provides the most cost-effective design when cases and controls are equally ‘scarce’. However, if controls are more readily obtained than cases, two, three or even more controls matched to each case can be selected\(^394\). Generally, there is little justification to increase the case-control ratio beyond 1:4 because the gain in statistical power with each additional control beyond this point is small. Power calculations indicated that selection of more than 2 controls per case would have no significant contribution to the power of the studies. However, because of the limited number of cases available, the studies lacked sufficient statistical power to address associations in subgroups of menopausal status, histology or even detect relatively ‘weak’ associations, conferring less than 2 – 2.5-fold increase in relative risk for some of the analyses. Future studies with adequate statistical power are necessary to address hormone-cancer relationships in premenopausal women and according to histological subtype of the tumors.

METHODOLOGICAL CONSIDERATIONS (PAPER VI)

The strength of the cross-sectional study is that it included large numbers of both pre- and postmenopausal women with measurements of circulating androgens, SHBG, IGF-I and IGFBP-3 and, for a proportion of the postmenopausal women, estrone and estradiol. A limitation of this study is that it was based on hormonal measurements performed on blood specimens obtained at a single point in time. Thus, although the sex-steroid and peptide hormones of interest have been reported to have a good to high reproducibility,\(^380,381,395\) it is likely that the observed associations had been somewhat attenuated as a result of physiological within-subject fluctuations in hormonal concentrations at any given point in time and variability related to laboratory error. An additional concern is the relatively narrow age range of women classified as premenopausal. Most (82%) of these subjects were aged 40 or older, which implies that a number of them were effectively peri-menopausal at the time of blood sampling.

In the cross-sectional analyses anthropo-
pometrical data were used, which were either self-reported (in the NYUWHS cohort and 34% of the NSHDS) or measured (ORDET study and for 66% of the NSHDS participants). To account for the different quality of the data all main analyses were repeated in subgroups of subjects with measured or self-reported anthropometry. The pattern of the associations between hormone levels and BMI did not differ in these subgroup analyses.

**DISCUSSION: OVARIAN CANCER**

The nested case-control study on ovarian cancer is the second study conducted to date that investigated the relationship between prediagnostic circulating hormone concentrations and risk of ovarian cancer. At variance with the findings of Helzlsouer et al. (1995) no evident case-control difference in mean sex-steroid hormone levels was observed in the whole study population, or separately within sub-groups defined by menopausal status at recruitment. In the premenopausal group, however, there was an evident, but not statistically significant increase in the risk of ovarian cancer with increasing concentrations of androstenedione.

Three sets of results of this study, along with those of Helzlsouer et al. (1995) support the possibility that ovarian cancer risk could be related to the intra-ovarian environment during a woman’s reproductive years rather than to the secretion of reproductive hormones into the general circulation:

- **a. DHEAS in circulation is a reliable marker of adrenal sex-steroid activity, as this hormone is secreted nearly exclusively by the adrenal gland** and only a small percentage is generated from the peripheral conversion of DHEA. Blood DHEAS levels do not exhibit evident circadian or menstrual cycle variations, owing to long half-life and high concentration. The lack of association of DHEAS and ovarian cancer in this study, as well as in Helzlsouer et al. (1995) suggests that the adrenal contribution to circulating sex-steroids levels is not a major determinant of ovarian cancer risk.

- **b. SHBG is the major binding protein of testosterone and estradiol in the circulation and is an important determinant of their free and biologically active fraction. Increased concentrations of this protein were shown to be protective for hormone-related cancers, including breast, endometrium, and prostate. In the present study, increasing SHBG concentrations were either unrelated to, or slightly increased risk of ovarian cancer, thus arguing against a major role for circulating free testosterone and estradiol fractions.**

The inverse association between BMI and ovarian cancer risk in the present study may explain the unexpected increase in risk in the top SHBG quartile for postmenopausal women. Many studies have reported an inverse association of BMI with circulating SHBG and in this study the inverse correlation was stronger among postmenopausal than in premenopausal women.

- **c. Before menopause, androstenedione is the sex steroid hormone the ovary secretes most abundantly, whereas testosterone, which has a stronger androgenic activity, is secreted in about 10 times lower quantities. In contrast, after menopause, androstenedione is produced almost exclusively by the adrenals. Hence, the increase in risk with increasing androstenedione levels observed in premenopausal, but not in postmenopausal women, may be the expression of elevated...**
intra-ovarian concentration of this hormone during the premenopausal years. Similarly, in the study of Helzlsouer et al. (1995), differences in mean androstenedione levels between cases and controls reached statistical significance in the pre-, but not in postmenopausal women.

Further insights about the role of ovarian versus circulating hormone levels come from the observation that during the 4-5th month of fetal development, there is a parallel increase in the steroidogenic activity of ovarian interstitial cells and in the proliferative processes of OSE, which undergoes diffuse multilayered proliferation. These proliferative processes seem to end when the tunica albuginea is formed and separates the OSE cells from the hormonally active stroma. In contrast, in the testis, there is an early separation of the surface epithelium from the underlying sex cords by a much denser tunica albuginea, a difference that may account for the divergence in the growth patterns of the surface epithelium of the ovary and testis during fetal life and in the types of malignancies that develop during adult life (about 95% of testicular tumors are of germ-cell origin and the remaining part are mostly sex cord-stromal tumors).

The lack of association of ovarian cancer risk with circulating estrone levels in postmenopausal women is in line with the findings of Helzlsouer et al. (1995) and also with the relatively weak adverse effects of BMI and use of HRT reported in several epidemiological studies. In contrast, a full term pregnancy, is associated with about 100-fold increase in maternal circulating estrogen concentrations, but confers protection against ovarian cancer. However the effect of high estrogens during pregnancy could be counterbalanced by the concomitant increase in progesterone and SHBG concentrations and in addition, after the first 4 weeks of pregnancy, nearly all estrogens are synthesised not by the ovaries, but in the trophoblasts, i.e., the placenta.

The potential importance of paracrine and intracrine hormonal exposures has been discussed in relation to other malignancies, including those of the breast and prostate. Such mechanisms may be of particular relevance in the pathogenesis of ovarian cancer since normal OSE is avascular, hence the exposure to sex steroids or other hormones occurs primarily via paracrine and autocrine mechanisms and because OSE cells lining the inclusion cyst (believed to be the possible origin of ovarian malignancies) are in close proximity with the mitogen-producing components of the ovary. In fact, a hypothesis that postulates a major etiological role of ovarian synthesis of androgens and estrogens and decreased progesterone concentrations is in agreement with the two classical hypothesis for ovarian cancer pathogenesis – the incessant ovulation (as the steroidogenic activity of the ovaries is closely associated with their ovulatory activity) and the gonadotropin hypothesis (as postulated by Cramer and Welch, which proposes also indirect involvement of gonadotropins through stimulation of steroidogenesis).

In the ovarian cancer study, we also investigated the association of pre-diagnostic concentrations of circulating IGF-I, IGFBP-1, -2 and -3 and risk of ovarian cancer. No association of IGF-I or any of the IGFBPs with ovarian cancer risk was observed in the study population as a whole, or in women with cancer diagnoses after age 55. A strong direct association of IGF-I levels with ovarian cancer risk was observed among women who were
younger than age 55 at cancer diagnosis. The observed increase in risk exclusively among women with relatively early diagnosis could be due to the greater importance of the mitogenic and anti-apoptotic effects of IGF-I during ovulation related tissue remodeling of the surface epithelium. It could also reflect an interaction between elevated IGF-I and the ovarian steroidogenic and/or ovulatory activity before menopause. Alternatively, it is possible that mean IGF-I levels decline more rapidly with age among women whose levels were initially high and who were at increased ovarian cancer risk due to direct effects of IGF-I irrespective of any interactions with ovarian activity. Similar to our observation of an effect of IGF-I on ovarian cancer risk only at a comparatively early age, other studies have shown an association of IGF-I with breast density and breast cancer risk exclusively among premenopausal women and women with a relatively early age of cancer diagnosis. However, IGF-I alone cannot account for the overall pattern of the ovarian cancer occurrence and therefore confirmation from other prospective studies will be essential to elucidate its role.

Levels of the studied IGFBPs were not related to risk. In analyses restricted to women with cancer diagnosis before age 55, there was a weak tendency of increase in risk with increasing IGFBP-3 concentrations. Although this finding was not entirely anticipated, it coincides with other studies that showed an increase in cancer risk with elevated concentrations of IGFBP-3. The concomitant increase in ovarian cancer with levels of both IGF-I and IGFBP-3 may be a reflection of their common regulation by growth hormone. However, the confidence intervals of IGFBP-3—ovarian cancer models always included unity, and the point estimates for the effect of IGFBP-3 were significantly reduced after adjustment for levels of IGF-I, while the association of IGF-I with ovarian cancer remained significant after adjustments for levels of IGFBP-3, suggesting a leading role of elevated IGF-I concentrations.

In contrast to the results for IGFBP-3, there was a weak tendency for a protective effect of elevated circulating IGFBP-1 and -2 among women diagnosed at an early age. These, at first glance opposite effects of IGFBP-3 and of IGFBP-1 and 2 on ovarian cancer risk, could be explained by their differential regulation and metabolism. As discussed, the major stimulus for the synthesis of both IGF-I and IGFBP-3 is GH, but GH down-regulates the synthesis of IGFBP-1 and -2. As a result, circulating IGF-I levels are directly correlated with IGFBP-3 levels and inversely correlated with levels of IGFBP-1 and -2 (as also observed in our data). As IGFBP-1 and -2 form smaller binary complexes with IGF-I, which can cross the endothelial membranes, these binding proteins have been postulated to be major modulators of free, biologically active IGF-I that can reach target tissues, as also indicated in several cross-sectional studies.

The relationship of pre-diagnostic levels of C-peptide with risk of ovarian cancer was also examined. C-peptide concentrations were not associated with risk of ovarian cancer in the whole study population or in subgroups of pre- or post-menopausal women. Additionally, similar lack of association was observed when cohort and fasting specific, or only fasting-specific cutoff points were used to define hormone exposure categories, arguing...
against a major effect of C-peptide levels on risk of developing ovarian cancer.

Increased body weight and BMI are the most widespread hyperinsulinemic conditions and BMI has been related to a modest increase in ovarian cancer risk \(^{101,102}\), although in our data we observed a tendency for a protective effect of higher BMI \(^{110}\). Adjustment of the C-peptide-ovarian cancer models for BMI categories slightly increased the risk estimates, while adjustment of BMI-ovarian cancer models for C-peptide levels resulted in virtually no change in the point estimates. These observations suggest that the protective effect of obesity in our data was not mediated by an increase in insulin levels.

The interpretation of the epidemiological data on ovarian cancer occurrence in relation to hormonal hypotheses is complicated by a number of factors:

First, the synthetic pathways of most of the hormones suggested as being etiologically important are closely linked and, consequently, there is a high degree of correlation between their circulating levels. For example, gonadotropins provide the key stimulus for ovarian androgen and estrogen synthesis; ovarian androgen excess is usually associated with chronic anovulation and reduced synthesis of progesterone and, after menopause, circulating androgens are the precursor hormones for the synthesis of estrogens. Thus, there is an overlap, or complementarity, between the gonadotropin, ovarian androgen, estrogen and progesterone hypotheses. Likewise, there can be an overlap with the insulin and IGF-I hypotheses, since both insulin and IGF-I can enhance androgen synthesis.

Second, most of the epidemiological evidence currently available provides only indirect support for any particular hypothesis (Table 1), since many of the established or possible risk factors can be interpreted as being in favour of more than one hormonal hypothesis. Perhaps this is most striking for the reduction in risk among OC users, an observation that is consistent with hypotheses that implicate gonadotropins, androgens, progesterone, estrogens and even IGF-I (the oral intake of estrogens reduces circulating IGF-I levels \(^{414,415}\)). Another example is the possible increase in ovarian cancer risk among women with PCOS (although not strongly established), which could support the gonadotropin, androgen, progesterone or insulin hypotheses. Thus, as most of the available epidemiological evidence is indirect and consistent with several hormonal hypotheses, it is difficult to single out one hormone (or group of hormones) as a major determinant of risk.

Third, all epidemiological studies on ovarian cancer are undoubtedly hampered by the infrequency of the disease and only a very few studies could account for the diverse histological subtypes with reasonable statistical power. However, differences in the effect of established risk factors have been reported and age-specific incidence rates do vary according to histological subtype: the incidence of endometrioid, clear cell and mucinous tumors plateau after menopause, while the incidence of serous tumors continue to rise until a much greater age (Figure 2). The distinct mutational spectra that characterise specific subtypes of epithelial ovarian cancer also suggest involvement of diverse pathways (and possibly different hormonal factors) in malignant transformation \(^{37,416}\). Finally, the role of intra-ovarian versus circulating levels of sex-steroids and other hormones needs to be elucidated.
DISCUSSION: ENDOMETRIAL CANCER

The nested case-control study on sex-steroids and endometrial cancer in post-menopausal women confirmed previous observations in the NUYWHS of a direct association of prediagnostic circulating estrogen levels and an inverse association of SHBG concentration with endometrial cancer risk. The associations persisted in analyses restricted to cases diagnosed two or more years after blood donation. Similar results were reported also by the two largest retrospective case-control studies of post-diagnostic endogenous sex-steroid hormone levels and endometrial cancer.

Overall, concentrations of circulating androgens were directly related to endometrial cancer, although less strongly than were circulating estrogen levels. Adjustment of androstenedione and testosterone models for estradiol or estrone levels resulted in a decrease in the regression coefficients and loss of statistical significance, while adjustment of the estrogen models for androgen levels only slightly decreased the estimates and the associations remained significant. These observations suggest that estrogens are a major determinant of endometrial cancer risk, while circulating androgens contribute to endometrial cancer development mainly as precursor hormones for the synthesis of estrogens.

However, some independent effect of circulating androgens on tumor growth cannot be ruled out, particularly in the years close to diagnosis. When the analyses were restricted to cases diagnosed two or more years after blood donation the associations of androgens with endometrial cancer risk became weaker and there was a significant interaction between androgens and lag-time between blood donation and cancer diagnosis, indicating a stronger association of androgens with endometrial cancer risk in the two years prior to cancer diagnosis. Based on the results of a large case-control study, showing a strong positive association of androstenedione with endometrial cancer that persisted after adjustment for estrone, Potischman et al. (1996) proposed that early in the neoplastic process, abnormal endometrial cells may acquire the ability to produce estrogens locally from the plasma pool of androgens and thus gain a growth advantage that is independent of circulating estrogen levels. This hypothesis is supported by in vitro studies that showed an increased aromatase activity of endometrial neoplastic cells, but not of normal cells. If indeed, such increased capacity for aromatization is a particular characteristic of endometrial neoplastic cells, then the association of androgens with risk would be expected to be stronger in case-control studies, which are based on hormone measurements in samples collected after endometrial cancer diagnosis. In prospective studies, the associations would be expected to be stronger in the years immediately prior to diagnosis and to weaken with elapsed time between blood donation and cancer diagnosis, as observed in our data.

Prediagnostic concentrations of the exclusively adrenal androgen DHEAS were also directly and strongly associated with risk and this association persisted after adjustment for estrogens. DHEAS can be involved in endometrial cancer pathogenesis through several mechanisms. DHEAS is the most abundant, albeit weak, androgen and is the major source of circulating DHEA. It has been proposed that DHEA not only exerts androgenic but
also estrogenic effects in postmenopausal women due to the binding to vacant estrogen receptors of either DHEA itself or its metabolite 5-androstene-3β, 17β-diol. It is possible that the persistence of the association of DHEAS with endometrial cancer risk after adjustment for levels of estrogens could be due to this additional indirect estrogenic effect of DHEAS. Additionally, DHEAS has a long half-life in the circulation and can be used as a marker of adrenal sex-steroid synthetic activity, which may be of particular importance after menopause and reduction of the ovarian sex steroid synthesis.

In the IGF-component of the endometrial cancer study, prediagnostic blood concentrations of IGF-I, IGFBP-2 and -3 were not related to risk. Prediagnostic levels of IGFBP-1 were inversely associated with risk, but after adjustment for confounders, the association remained statistically significant only in the group of postmenopausal women.

The lack of association of IGF-I concentrations with risk is unlikely to be due to low measurement reproducibility of circulating IGF-I as several studies (including data from the ovarian and endometrial case-control studies) have shown a good to high reproducibility of blood IGF-I measurements over time. A similar lack of association of IGF-I with endometrial cancer has been observed in two case-control studies, one from Sweden and one from Greece.

Several studies have shown that women with BMI ≥ 25 have lower total IGF-I levels in comparison with women with BMI between 20 and 25 kg/m², while levels of IGFBP-3 are relatively stable across BMI categories or may even increase with BMI. The prevalence of overweight and obesity in this study was substantially higher in the case than in the control subjects (58% vs. 39%, respectively) and it is plausible to assume that the observed tendency for inverse associations of IGF-I levels and of IGF-I/IGFBP-3 molar ratio and of the direct association of IGFBP-3 with endometrial cancer in the crude models were a reflection of the confounding effect of obesity. Additionally, unlike most tissues where the key stimulus for the synthesis of IGF-I and IGFBP-3 is growth hormone, the major determinant of IGF-I levels in endometrium is estradiol and circulating IGF-I levels may not be a good marker of the local expression and action of IGF-I in the endometrium.

Before menopause, endometrial synthesis of IGFBP-1 is the result of the opposing effects of insulin (which inhibits synthesis) and progesterone (which stimulates synthesis) [Figure 4]. In contrast, after cessation of the cyclic ovulatory activity, insulin remains the main determinant of endometrial and other tissue IGFBP-1 synthesis. Thus, in postmenopausal women circulating levels of IGFBP-1 are expected to be a good reflection of the endometrial tissue levels of this binding protein. The observed inverse association between IGFBP-1 levels and endometrial cancer, which in postmenopausal women persisted after adjustments for BMI and other confounders lends further support for a possible protective effect of elevated levels of IGFBP-1 for endometrial cancer.

In the study we also observed a strong direct association of prediagnostic C-peptide concentrations (as markers of pancreatic insulin secretion) with endometrial cancer risk. As discussed (Figure 4), possible mechanisms that may link elevated insulin concentrations to increased
risk of endometrial cancer include its mitogenic and anti-apoptotic activity, the down-regulation of SHBG and IGFBP-1 and its role as a contributing cause of ovarian androgen excess, which in premenopausal women may cause chronic anovulation and progesterone deficiency.

One of the major causes of insulin resistance and hyperinsulinemia is excess weight and high BMI has been strongly associated with risk of developing endometrial cancer. In our analyses, the strength of the association between C-peptide and endometrial cancer risk was lowered by adjustment for BMI, but C-peptide concentrations remained strongly associated with cancer risk, indicating that hyperinsulinemia may be a risk factor for endometrial cancer that is independent of obesity. Conversely, BMI remained directly associated with endometrial cancer risk after adjustment for levels of C-peptide, probably due to the direct effect of obesity on sex-steroid hormone concentrations (e.g., in postmenopausal women adipose tissue is the major site where estrogens are synthesised).

Two large case-control studies have reported on the association of insulin with endometrial cancer. Weiderpass et al. (2003) observed a similar direct association of insulin with risk in analyses restricted to women who had never used HRT (120 cancer patients and 187 controls) although after adjustment for confounders, the association was no longer significant. Among women who had ever used HRT (140 cancer patients and 109 controls), however, insulin was inversely related to endometrial cancer. In the study of Troisi et al. (1997) (165 cases and 180 controls), risk was increased among women with elevated serum C-peptide, but the association was abolished after adjustment for BMI.

The major differences between the 2 case-control studies and the current one is that we measured C-peptide concentrations in prospectively collected samples from non-fasting women, while for the study of Weiderpass et al. (2003) and Troisi et al. (1997), serum samples, obtained after endometrial cancer diagnosis of the cases and from fasting women were collected. As discussed, the prospective design of our study minimizes “inverse causation” bias and to account for the measurement in non-fasting samples, we ran unconditional logistic regression models in sub-groups of fasting and non-fasting women at blood donation. The ORs for the association of C-peptide with endometrial cancer was similar in the two sub-groups and there was no indication for effect modification by fasting status.

Further, in the study of Troisi et al. (1997), only women with no previous diabetes diagnosis were included, while we did not exclude women with diabetes from our study. Restriction of our analyses to women without previous diabetes diagnosis weakened the association between C-peptide levels and endometrial cancer risk, but did not abolish it and women from the fifth quintile of C-peptide had more than 3-fold higher risk than women from the lowest quintile of C-peptide. However, only self-reported information about diagnosis of diabetes was available and it is possible that the real prevalence of diabetes in our population was higher than estimated from the questionnaire data.

In the study of Weiderpass et al. (2003) current users of exogenous hormones were included, while in our study only women who did not use exogenous hormones at blood donation were eligible. In the study...
of Weiderpass et al. (2003) control subjects who were either current or former users of HRT had significantly higher mean (and median) levels of insulin than never users (p < 0.0000), while in our study levels of C-peptide were not significantly different between former users and never users of HRT in the whole study population, or in analyses limited to control subjects or to cohort study. Furthermore, mean C-peptide concentrations of women who were ever users of HRT were always lower than those of women who never used HRT and there was no heterogeneity of RRs in subgroups of ever or never users of HRT. Studies that have investigated the effect of HRT on insulin concentration, sensitivity and glucose disposal have been largely contradictory, some of the differences probably due to the type of HRT used (estrogen versus estrogen plus progesterone), route of estrogen administration (oral versus dermal) and study population (obese versus lean, diabetic versus healthy women) 432-437. Further, larger prospective studies with high quality data about diabetes diagnosis are necessary to establish the effect of circulating insulin on risk of endometrial cancer and to investigate possible effect modification due to current or former use of different types of HRT.

**DISCUSSION: CROSS-SECTIONAL STUDY**

**Effect of BMI on hormone levels**

Well-established metabolic effects of obesity on circulating endogenous hormones include the progressive reduction in SHBG with increasing BMI in both pre- and postmenopausal women and the direct association with estrogens in postmenopausal women 7,8,12. Our data are consistent with these observations. A proposed mechanism for the reduction in SHBG with increasing BMI is related to the concomitant rise in insulin levels, since insulin has been shown to inhibit the hepatic synthesis of SHBG 7,12. In postmenopausal women, estrogens are produced from the conversion of precursor androgens or other estrogens, mainly in the adipose tissue, and their production is not regulated by feed-back mechanisms 12,438. As a consequence, after menopause, estrogen concentrations are directly related to the amount of adipose tissue. The increase in mean free estradiol (non-SHBG-bound) concentrations across BMI categories observed in our data was greater than the increase in total estradiol concentrations (69% vs. 29%), which is consistent with the dual effect of obesity toward increasing estrogen production and decreasing SHBG in circulation.

In pre- and postmenopausal women, increased BMI, waist-hip ratio or abdominal obesity have been associated with either no change 162,439-442 or with an increase 7,443-448 in total testosterone concentrations. Most of the studies in premenopausal women have shown an increase in free testosterone with increasing body weight 345,439,444,449, but very few such studies have been reported in postmenopausal women 441,442. Many previous studies did not find an association of obesity with levels of androstenedione 162,345,444,449-451 or DHEAS 345,439,442,444,447,448,450 in either pre- or postmenopausal women.

In our data, no significant correlations between BMI and androgens were observed in premenopausal women, although there was a non-significant 25% increase in free testosterone in women with BMI > 27 when compared to those with BMI < 21.5. In postmenopausal women, mean
levels of all androgens increased across BMI categories, but the increase in the top BMI category in comparison with the lowest was greatest for levels of free and total testosterone (153% and 65%, respectively). The increase in DHEAS -- an androgen synthesized exclusively by the adrenal glands -- was much less pronounced and not significant. Thus, these results suggest that in postmenopausal women, increased BMI may lead to an enhanced ovarian synthesis of androgens.

It has been proposed that the relationship between BMI and androgens is mediated by obesity–related changes in insulin and bioavailable IGF-I. *In vitro* studies have shown that both insulin and IGF-I can stimulate ovarian androgen synthesis. However, such 'gonadotropic' effect of insulin and IGF-I may be less evident before menopause at the background of high circulating sex-steroid hormone levels and when their synthesis is under the tight control of gonadotropins and controlled by powerful feed-back mechanisms. Additionally, some studies have suggested that the adipose tissue, with its 17β-hydroxysteroid dehydrogenase activity may also be an important site of peripheral testosterone production.

As observed in other cross-sectional studies, only very weak monotonous correlations of BMI with IGF-I or IGFBP-3 were observed. In the postmenopausal group, there was a clear non-linear association of IGF-I with obesity, with the highest IGF-I levels in women with BMI between 24 and 25 kg/m². Similar findings have been reported previously. The non-linear relationship of BMI with IGF-I may be the expression of obesity-related changes in the synthesis of insulin, GH, GH-receptor, IGFBP-1 and IGFBP-2. In lean individuals, or after a prolonged fasting, the low endogenous insulin production is associated with a decreased GH receptor levels, resulting in reduction in IGF-I synthesis in response to GH stimulation and a decrease in circulating IGF-I levels. In obese subjects, elevated insulin levels increase the free fraction of IGF-I (through decreasing IGFBP-1 and IGFBP-2) with a consequent negative feed-back on the secretion of GH from the pituitary and a reduction in total circulating IGF-I.

In contrast to the results in postmenopausal women, mean IGF-I concentrations did not differ across BMI categories in the premenopausal group. At least partially, the lack of association in premenopausal women might be due to the narrower range of BMI and the small number of subjects with BMI ≥ 30 (n=9). However, differences in the responsiveness of IGF-I to GH stimulation and in the proportion of lean and fat body mass, could also contribute to the different pattern of the association of BMI with IGF-I in pre- and postmenopausal women.

Several studies have reported an increase in IGFBP-3 with obesity, but in most of the studies, including the current one, there was little variation in IGFBP-3 levels across BMI categories.

### Interrelationship between sex-steroid hormones, SHBG, IGF-I and IGFBP-3

Sex-steroids and IGF-I-related hormones undergo parallel changes throughout life. There is a dramatic increase during puberty and then a substantial decrease with age. Still, the precise mechanisms of the links and the possible interactions between these hormonal systems remain largely unknown.

It has been proposed that endogenous sex-steroid hormones stimulate GH and
IGF-I synthesis, as supported by observations of minimal or no pubertal growth spurt in patients with hypogonadism, and that patients with both true precocious puberty and GH deficiency can exhibit a growth spurt indistinguishable from that of children with true precocious puberty and normal GH secretion. Additionally, both oral and trans-dermal exogenous androgens or androgenic progestins cause elevations in circulating IGF-I. The effect of exogenous estrogens depends on the route of administration. Oral formulations decrease IGF-I, most likely because of a first-pass effect on the liver of pharmacological doses of estrogens, while trans-dermal applications of estrogens do not seem to influence circulating IGF-I. Although not uniform, studies in premenopausal women have shown some degree of correlation between IGF-I and circulating estrogens and androgens, although no substantial variation of circulating IGF-I throughout the menstrual cycle.

Alternatively, IGF-I has been shown to influence the synthesis of sex-steroid hormones. Patients with isolated GH deficiency or with Laron syndrome (GH resistance from a defect in the GH-receptor), have delayed appearance of pubertal signs and a slow, protracted puberty, even though ultimately reaching full sexual development. In Laron-syndrome patients treated with high concentrations of exogenous IGF-I, levels of sex-steroid hormones have been shown to increase and some female patients developed hyperadrogenism with oligo/amenorrhea and acne. There is also in vitro evidence that IGF-I enhances the LH-dependent ovarian androgen production and that IGF-I might affect gonadotropin production at the pituitary level.

In this study, IGF-I concentrations were directly associated with those of all androgens, but the correlations were statistically significant only in postmenopausal women and for androstenedione in premenopausal women. Similar observations were reported by Helle et al (2002), but not in two other large cross-sectional studies in postmenopausal women. IGFBP-3 correlated only weakly with all androgens studied in both pre- and postmenopausal women, as also observed by others. Although the cross-sectional nature of this study does not allow conclusions to be drawn on the causal relationship between the sex-steroid and the IGF-I-axes, it does provide evidence for a direct association of IGF-I with circulating androgens in post- and possibly in premenopausal women.

As observed in most previous studies, IGF-I and IGFBP-3 were not related to estrone and total estradiol concentrations in postmenopausal women. However, Janssen et al. (1998) observed a direct correlation between free estradiol and IGF-I or IGFBP-3 levels, while in our data, weak inverse correlations were found. The observations of several epidemiological studies, taken together with the lack of substantial variation in IGF-I levels during trans-dermal estrogen replacement therapy, argue for a weak effect of circulating estrogens on plasma IGF-I concentrations in postmenopausal women.

Our findings of inverse associations of IGF-I or IGFBP-3 levels with SHBG in postmenopausal women concur with the results of other cross-sectional studies. It has been proposed that the underlying mechanism is the inhibition of hepatic SHBG synthesis by IGF-I, as shown in vitro. An interesting ob-
ervation is that the inverse association of IGFBP-3 with SHBG was stronger than that of IGF-I with SHBG in our data (also after adjustment for BMI) and in some other studies. No association of either IGF-I or IGFBP-3 with SHBG was observed in premenopausal women. However, currently, there is no evidence suggesting that the effect of IGF-I on the liver synthesis of SHBG differs according to menopausal status.

**IMPLICATIONS AND FUTURE RESEARCH**

A number of epidemiological risk factors for ovarian cancer have been clearly identified, but there is still relatively little understanding of the mechanisms through which they influence ovarian cancer pathogenesis. Although there is substantial indirect evidence that hormonal factors may play a role in ovarian cancer etiology, there is little direct evidence to support any specific hypothesis. Elevated ovarian androgen and estrogen synthesis and decreased progesterone concentrations are likely to be involved in ovarian cancer pathogenesis. The results from prospective cohort studies on circulating hormone levels with sufficiently large numbers of cases to allow analyses according to histological subtypes may provide more direct insights into the role of endogenous hormones and growth factors. A challenging new area of research will be to study the effect of differences in hormonal exposures during pregnancy on subsequent risk of epithelial ovarian cancer. The role of other endocrine factors – such as IGF-II, hepatocyte growth factor and inhibins, as well as the involvent of inflammatory processes and retrograde menstrual flow through the Fallopian tubes are also of interest.

Substantial experimental and epidemiological evidence point to an etiological role of elevated estrogen concentration for development of endometrial cancer in postmenopausal women, but the role of elevated androgen concentrations in the pathogenesis of the disease remains to be elucidated. Further epidemiological investigations with larger numbers of subjects and studies on specimens obtained from normal and neoplastic endometrium would be necessary to establish the role of elevated androgen concentrations in endometrial cancer development and progression.

Future prospective studies with adequate control for previous diabetes diagnosis and fasting status are necessary to confirm the findings for increased endometrial cancer in women with elevated C-peptide concentrations. Decrease in the prevalence of chronic hyperinsulinemia, through changes in lifestyle or medication, is expected to prevent endometrial cancer.

Better understanding of the hormonal pathways that influence risk of developing gynaecological and other malignancies would help choose optimal strategies for exogenous hormone supplementation and can give indications for the development of hormonal treatment or diagnostic tests.

The results of the cross-sectional analysis provide further evidence that increase in BMI influences circulating levels of sex steroids and IGF-related hormones in healthy women. Alteration of endogenous hormone metabolism may form a physiological and causal link between excess body weight and cancer risk as well as with cardiovascular disease, type II diabetes, and other chronic diseases. The prevalence of obesity is increasing rapidly world-wide, not only in economically developed countries, but also in developing
In Europe, about 50% of men and 35% of women are currently estimated to be overweight or obese, as are women in the Middle East and Latin America. The high prevalence of obesity is mostly explained by a lifestyle characterized by over-consumption of energy combined with low levels of physical activity. Avoidance of weight gain should become one of the mainstays of chronic disease prevention in modern societies. Governmental and non-governmental organizations should develop health policies and initiatives to promote a healthy diet, rich in fresh fruit, vegetables and cereals and low in saturated fats and rapidly digestible carbohydrates, and to encourage a physically active life. Although it is never too late to benefit from a healthier lifestyle, a special effort should be made to prevent overweight and obesity from early life, based on development of life-long healthy eating and physical activity patterns.
CONCLUSIONS

On the basis of the results from the nested case-control study on endogenous hormone levels and ovarian cancer it is concluded that:

- The study results do not support a major role for circulating testosterone, androstenedione, DHEAS, estrone (in postmenopausal women) and SHBG in ovarian carcinogenesis.

- The data suggest that elevated circulating androstenedione levels measured before menopause may be related to a subsequent increase in ovarian cancer risk and that such an association is one of the peripheral expressions of complex alterations of the intra-ovarian hormonal environment.

- Elevated IGF-I levels may be implicated in the development of ovarian cancer diagnosed before age 55. These results add ovarian cancer to the group of common tumors in the economically developed countries for which the IGF-I system is believed to play an important etiological role.

- Prediagnostic levels of IGFBP-1, -2 and –3 are not related to risk of ovarian cancer, although our results are compatible with a protective effect of IGFBP-1 and –2 in women who developed ovarian cancer before age 55.

On the basis of the results from the nested case-control study on endogenous hormone levels and endometrial cancer it is concluded that:

- In postmenopausal women, there is a strong direct association of prediagnostic estradiol and estrone concentrations and an inverse association of SHBG levels with endometrial cancer risk.

- The direct association of endometrial cancer risk with androstenedione and testosterone concentrations in postmenopausal women seems to be due primarily to their role as precursor hormones of estrogen synthesis, although they may have some independent effect, especially during endometrial tumor progression.

- Elevated concentrations of DHEAS are associated with an increased risk of endometrial cancer in postmenopausal women. This association may be mediated through DHEAS conversion to more active sex-steroids. Elevated prediagnostic DHEAS levels in endometrial cancer patients may also reflect increased sex-steroids synthesis by the adrenal glands.

- Chronic hyperinsulinemia, as reflected in the increased concentrations of circulating C-peptide, is associated with an increased risk of endometrial cancer in pre- and postmenopausal women. This association is independent of obesity.
• Increased circulating IGFBP-1 concentrations are associated with lower risk of endometrial cancer in postmenopausal women.

• Circulating concentrations of IGF-I, IGFBP-2 and IGFBP-3 do not appear to be related to endometrial cancer risk in both pre- and postmenopausal women.

On the basis of the cross-sectional analyses on circulating androstenedione, testosterone, DHEAS, SHBG, estradiol, estrone, IGF-I and IGFBP-3 it is concluded that:

• Increase in BMI influences circulating concentrations of sex steroid hormones and SHBG in both pre- and postmenopausal women.

• The effect of BMI appears to be stronger after menopause, when the powerful feed-back mechanisms that control estrogen and androgen synthesis before menopause are no longer functional.

• A clear non-linear relationship between BMI and circulating IGF-I was observed in postmenopausal women.

• There is an inverse association of IGF-I and IGFBP-3 concentrations with SHBG.

• In postmenopausal women, circulating IGF-I concentrations are directly correlated with total and free androgen concentrations.
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