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PATIENTS WITH HYPERPROLACTINEMIA

Katarina Berinder

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CLINICAL AND EPIDEMIOLOGICAL  
PERSPECTIVES

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*To Fredrik, Victoria & Louise*



## ABSTRACT

Hyperprolactinemia (HPL) is the most common endocrine disorder of the hypothalamic-pituitary axis and prolactinomas the most frequent pituitary tumour. A majority of the patients are women in reproductive age seeking health care for menstrual disorders or infertility. Because a majority of the patients are relatively young at diagnosis and the medical treatment often life-long, it is essential to study the efficacy of treatment and long-term results in these patients. Data are lacking in women with HPL concerning parity and there is still a void of studies evaluating cancer risk and whether the metabolic state is altered in patients with elevated prolactin (PRL) levels. This thesis, which contains four studies addressing these issues, is aimed to improve our knowledge and the quality of clinical management in patients with HPL.

*Long-term outcome of treatment:* A total of 271 women with HPL were retrospectively evaluated. At diagnosis, 87% of the women presented with menstrual disturbances and 47% had galactorrhea. The outcome after up to 29 years of clinical follow-up (median 9.3 years) showed a good treatment result with restoration of menses and galactorrhea in 94% of the women. Medical therapy with dopamine (DA) agonists normalised PRL levels in 71% of the patients and 80% had a total or partial degree of tumour reduction. Fifty-three per cent (9 of 17) of the surgically treated patients and one third (three of nine) of the patients treated with radiotherapy exhibited long-term normalisation of PRL levels without medical treatment.

*Parity and pregnancy outcome:* In this matched cohort study (271 women with HPL and 1084 comparison subjects) a reduced parity was found in HPL patients, mainly because there were more nulliparous women and fewer women with more than two children. Parity was inversely associated with HPL status ( $P$  for trend = 0.0009). No increased risk of pregnancy or delivery complications was found. In addition, outcomes of the newborns did not differ between patients and controls.

*Cancer risk:* A small, though significant, increased risk of overall cancer was found in 969 patients with HPL (668 women and 301 men) as compared with matched comparison subjects (hazard ratio [HR] 1.31; 95% CI 1.02-1.68) which was mainly due to increased risk of upper gastrointestinal cancer in all patients and hematopoietic cancer in females. Risk of breast cancer did not differ between patients and controls. Furthermore, a reduced risk of prostate cancer by 60% was found in HPL men (HR 0.40; 95% CI 0.16-0.99).

*Metabolic assessment:* Evaluation of 14 consecutive patients with prolactinomas (eight women and six men) before and after normalisation of PRL levels by DA agonist therapy revealed that HPL men had an unfavourable metabolic profile at diagnosis. After therapy, a significant decrease of body weight, waist circumference and body fat% was found in the men. A positive correlation between PRL levels and low-density lipoprotein (LDL) cholesterol at diagnosis was seen and LDL cholesterol decreased after 2 months of DA agonist treatment. Furthermore, peripheral insulin sensitivity evaluated with a euglycemic hyperinsulinemic clamp tended to improve after therapy. This improvement was associated with a reduction in PRL levels.

In conclusion, the results of this thesis show that HPL patients can be effectively treated with DA agonists in the long-term perspective and emphasise its role as first-line therapy. Women treated for HPL have a reduced parity, but there are no increased risks during pregnancy or for their offspring. The small increased risk of cancer that was found in HPL patients and the possible negative effect of elevated PRL levels on metabolic state need to be further evaluated; however, it implies the need for an active treatment approach and close follow-up of patients with HPL.

*Key words:* prolactin, hyperprolactinemia, dopamine agonist treatment, parity, pregnancy outcome, cancer risk, lipids, insulin sensitivity



## LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-IV):

- I. **Berinder K**, Stackenäs I, Akre O, Lindén Hirschberg A, Hulting A-L.  
Hyperprolactinaemia in 271 women: up to three decades of clinical follow-up.  
*Clinical Endocrinology* 2005; 63: 450-455.
- II. **Berinder K**, Hulting A-L, Granath F, Lindén Hirschberg A, Akre O.  
Parity, pregnancy and neonatal outcomes in women treated for hyperprolactinaemia compared with a control group.  
*Clinical Endocrinology* 2007; 67: 393-397.
- III. **Berinder K**, Akre O, Granath F, Hulting A-L.  
Cancer risk in hyperprolactinemia patients – a population-based cohort study.  
*Submitted*.
- IV. **Berinder K**, Nyström T, Höybye C, Hall K, Hulting A-L.  
Insulin sensitivity and lipid profile in prolactinoma patients before and after normalization of prolactin by dopamine agonist therapy.  
*Pituitary* Dec 2010; E-pub ahead of print.



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## LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BIA	Bioelectrical impedance analysis
BMI	Body mass index
Brc	Bromocriptine
Brc-QR	Quick-release form of Brc
Cab	Cabergoline
CI	Confidence interval
CT	Computed tomography
D <sub>2</sub>	Type 2 dopamine (as in D <sub>2</sub> receptors)
DA	Dopamine
DM	Diabetes mellitus
FFA	Free fatty acid
FFM	Fat free mass
GH	Growth hormone
GI	Gastrointestinal
GnRH	Gonadotropin-releasing hormone
HbA <sub>1c</sub>	Haemoglobin A <sub>1c</sub>
HDL	High-density lipoprotein
HMWA	High molecular weight form of adiponectin
HOMA	Homeostasis model assessment
HPL	Hyperprolactinemia
HR	Hazard ratio
ICD	International classification of diseases
IGF-I	Insulin-like growth factor 1
IGFBP-1	IGF-binding protein-1
LDL	Low-density lipoprotein
LH	Luteinising hormone
MRI	Magnetic resonance imaging
NHL	Non-Hodgkin lymphoma
OR	Odds ratio
PIN	Personal identity number
PL	Placental lactogen
PRL	Prolactin
PRLR	Prolactin receptor
RIA	Radioimmunoassay
5-HT	Serotonin
SD	Standard deviation
SR	Stereotactic radiosurgery
TG	Triglyceride
TNF- $\alpha$	Tumour necrosis factor-alpha
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
WHR	Waist-hip ratio

# 1 INTRODUCTION

Hyperprolactinemia (HPL) is the most common endocrine disorder of the hypothalamic-pituitary axis and prolactinomas the most frequent pituitary tumour. A majority of the patients are women in reproductive age seeking health care for menstrual disorders or infertility. The prevalence is lower in men who typically present with symptoms that are due to hypogonadism and tumour expansion. Since a majority of the patients are relatively young at diagnosis and the treatment often life-long, it is crucial to follow the efficacy of treatment and long-term results in these patients. Data are lacking in women with HPL concerning parity and if there is an increased risk of pregnancy complications. Furthermore, there is still a void of studies evaluating cancer risk and metabolic effects of prolactin (PRL) in patients with HPL. This thesis focuses on different clinical and epidemiological outcomes in HPL patients, with the aim of improving our knowledge and the quality in clinical management of these patients.

## 1.1 PROLACTIN

### 1.1.1 History

PRL was discovered in 1928 as a pituitary factor that could induce milk secretion in rabbits [1]. A few years later similar observations were made in pigeons [2]. Riddle *et al.* named this factor prolactin because of its ability to stimulate milk production and they developed the pigeon crop assay that became the standard assay procedure for more than 30 years [2]. However, there were doubts if PRL existed in humans because growth hormone (GH) has PRL-like properties and early attempts to separate PRL and GH failed. Finally, in 1971, human PRL was isolated and a specific radioimmunoassay (RIA) was developed [3]. This finding opened a new era in the understanding of pituitary diseases and classification of PRL disorders in humans. In the early 1970s the findings that the dopamine (DA) agonist bromocriptine (Brc) (at that time called CB154) strongly inhibited PRL secretion and lactation in the postpartum period [4, 5] and its inhibition of PRL and reduction in tumour size in prolactinoma patients [6, 7] were important discoveries and the beginning of a change in treatment regimen of prolactinoma patients.

### 1.1.2 Structure

Human PRL is a polypeptide hormone composed of 199 amino acids (23 kDa). The tertiary structure of PRL with a four-helical bundle topology (Fig. 1) is similar to that of two other hormones, GH and placenta lactogen (PL), which all are members of the hematopoietic cytokine family [8]. It is thought that these three hormones evolved from a common ancestral gene by duplication [9, 10]. PRL circulates mainly in a monomeric form but there are variants of PRL because of posttranslational modifications, such as proteolytic cleavage, dimerisation, polymerisation, phosphorylation and glycosylation [11]. In general, these PRL variants have reduced biological activity. Large molecular isoforms (>150 kDa) are termed macroprolactin, which mainly are due to complexes of PRL and IgG [12]. Macroprolactin has markedly reduced bioactivity [13] and is considered to exhibit no systemic response *in vivo*. However, macroprolactin may be identified in different PRL immunoassays and may lead to apparent HPL [14].

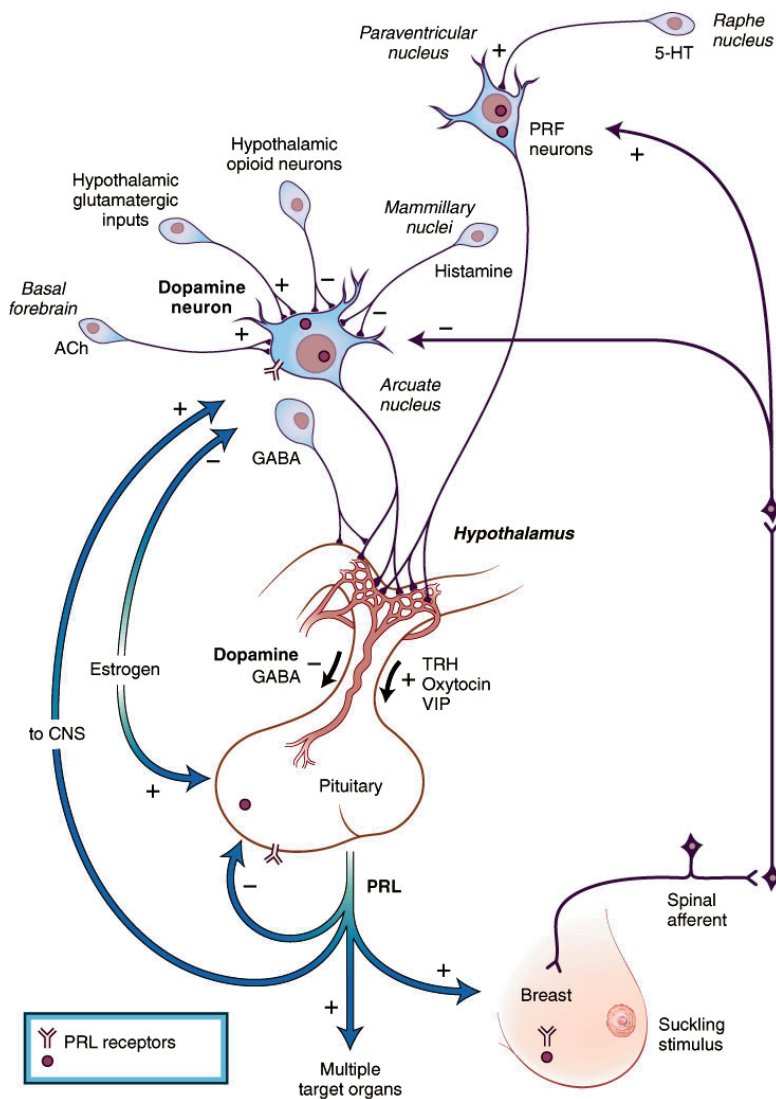


**Figure 1.** The tertiary structure of human PRL composed of a bundle of four antiparallel  $\alpha$ -helices. Modified figure from Keeler C et al., *J Mol Biol* 328, 1105-1121 (2003). This figure is reproduced with permission from the publisher, Copyright © 2003, Elsevier.

### 1.1.3 Regulation

Human PRL is synthesised in and secreted from lactotroph cells in the anterior pituitary and these cells comprises about 15-25% of functioning anterior pituitary cells [15]. The lactotrophs differ from the other pituitary cells, i.e. they have a high basal secretory activity and PRL secretion is mainly under a tonic inhibition by hypothalamic DA [11]. DA reaches the pituitary via the hypothalamic-pituitary portal system and inhibits PRL by binding to type 2 dopaminergic ( $D_2$ ) receptors on the lactotrophs, leading to a rapid suppression of PRL release from secretory vesicles, inhibition of PRL gene expression and lactotroph proliferation [16]. This mechanism is the rationale for the treatment of HPL patients with DA agonists. In addition, other potential inhibiting factors on PRL release are somatostatin and  $\gamma$ -aminobutyric acid (GABA). Furthermore, PRL exerts a negative feedback on its own release, by stimulating hypothalamic DA synthesis [16]. Although the control of PRL secretion is mainly inhibitory, there are several known PRL-releasing factors, including thyrotropin-releasing hormone (TRH), vasoactive intestinal polypeptide, oxytocin and endothelin [17]. The physiological relevance of these factors is questionable, however. Oestrogens stimulate lactotroph cell proliferation as well as PRL secretion [18]. Moreover, oestrogens activate secondary responses that may influence PRL gene transcription, i.e. inhibiting dopaminergic hypothalamic activity and upregulating TRH receptors [19]. Furthermore, PRL secretion is increased by different forms of stressors. A summary of the regulation of PRL secretion is presented in Figure 2.

Apart from the lactotrophs in the anterior pituitary, PRL is also produced by different extrapituitary sites e.g., mammary gland, placenta, uterus, prostate, brain and in the immune cells where it may function as an autocrine or paracrine factor [20]. The production of PRL in extrapituitary tissues is not regulated by DA.



**Figure 2.** Regulation of the hypothalamic-pituitary-prolactin (PRL) axis. The predominant effect of the hypothalamus is inhibitory, an effect mediated principally by dopamine secreted by the tuberohypophyseal dopaminergic neuron system. One or more prolactin-releasing factors (PRFs) probably mediate acute release of PRL as in suckling and stress. There are several candidate PRFs, including thyrotropin-releasing hormone (TRH), vasoactive intestinal polypeptide (VIP), and oxytocin. PRF neurons are activated by serotonin (5-HT). Oestrogen sensitises the pituitary to release PRL, which feeds back on the pituitary to regulate its own secretion (ultrashort-loop feedback) and also influences gonadotropin secretion by suppressing the release of luteinising hormone-releasing hormone (LHRH). Short-loop feedback is also mediated indirectly by prolactin receptor regulation of hypothalamic dopamine synthesis, secretion, and turnover. Published in Williams Textbook of Endocrinology, Chapter Neuroendocrinology. Reproduced with permission from the publisher. Elsevier illustration from [www.elsevierimages.com](http://www.elsevierimages.com). © Elsevier Inc. All rights reserved.

In humans, PRL is secreted episodically during the day with the highest concentrations occurring at sleep [15]. In addition, a sleep-independent endogenous circadian rhythm with a nocturnal rise has also been demonstrated, possibly driven by the nucleus suprachiasmaticus in the hypothalamus [21]. Women have higher PRL concentrations than men and PRL levels decrease with age [22].

#### **1.1.4 Action**

PRL is best known for its action on the mammary gland during pregnancy. This action includes stimulation of lobuloalveolar growth, milk synthesis and maintenance of milk production [11]. Lately, numerous biological functions have been attributed to PRL in humans and different animal species and more functions have been ascribed to PRL than all the other pituitary hormones together. These actions have been divided into reproduction, endocrinology and metabolism, growth and development, immunoregulation, brain and behaviour and water and electrolyte balance [23]. A knock-out model of the PRL-receptor (PRLR) gene in mice leads to major reproductive defects with sterility in female mice and an inability to lactate [23]. However, the full diversity of PRL effects in humans is not completely understood. There are genetic causes of hypopituitarism in humans, such as mutations of different pituitary-specific transcriptional factors (POU1F1, Prop1), which are associated with PRL deficiency [24]. However, these mutations also lead to other hormone deficiencies, including GH, thyroid stimulating hormone (TSH) and gonadotrophins. Furthermore, extrapituitary PRL is not affected. Isolated PRL deficiency has been described in women not able to lactate [25, 26]. Mutations of the PRL gene or PRLR gene has thus far not been described in humans. We therefore do not have a human clinical model to evaluate the consequences of absence of PRL.

PRL acts via a specific membrane receptor that is a single-pass transmembrane protein, a member of the cytokine receptor superfamily and closely related to the GH receptor [27]. The PRLR is activated by ligand-induced dimerisation of two receptor subunits, with signal transduction mainly via the Janus kinase/Stat pathway [23]. Activated Stat proteins translocate into the nucleus and bind to specific promoter elements on PRL-responsive genes.

## **1.2 HYPERPROLACTINEMIA**

### **1.2.1 Epidemiology**

The prevalence of HPL in the literature varies from 0.4% in an unselected normal population up to 5-17% in women with reproductive disorders [28, 29]. However, we have no figures of the prevalence or incidence in Sweden. Furthermore, it might be difficult to establish the precise prevalence of HPL because some patients might not have symptoms of elevated PRL levels, or have unspecific symptoms.

In a previous meta-analysis, the overall estimated prevalence of pituitary adenomas was 17% (14% in autopsy studies and 23% in imaging studies), where PRL-producing adenomas were the most common pituitary tumour (43%) [30]. However, the majority of pituitary tumours identified in autopsy studies are small (< 3 mm) and of unknown clinical significance [31]. In the meta-analysis, macroadenomas occurred at a rate of 1 per 600 persons [30]. Two population-based studies evaluating clinically significant pituitary adenomas revealed a

prevalence of prolactinomas of 44 and 62 per 100 000 inhabitants [32, 33] and prolactinomas accounted for 57% [32] and 66% [33] of all pituitary tumours. In addition, the incidence of pituitary adenomas were evaluated in northern Finland, where the incidence rate of prolactinomas was 2.2 per 100 000 person-years [34]. These figures demonstrate that prolactinomas are relatively common in the general population, where an increased knowledge of the clinical aspects of HPL for optimal diagnosis and follow-up is essential. Prolactinomas occur most frequently in females between 20-50 years of age. In the third decade of life, the female-to-male ratio is reported to be 6-14:1 [34, 35]; however, the frequency of prolactinomas is equal between sexes after the fifth decade of life [36]. Men are typically about 10 years older at diagnosis (mean age at diagnosis 42 years in males vs. 31 years in females) [32].

### 1.2.2 Causes

There are numerous conditions that may cause elevated PRL levels, which can be divided into three main categories: physiological, pathological and pharmacological (Table 1) [15]. When HPL is identified in a patient, it is important to identify physiological aetiologies, such as pregnancy, lactation and stress. It is also important to identify medications that alter DA

**Table 1.** Aetiology of hyperprolactinemia

Physiologic	Pathologic	Pharmacologic
Stress	<b>Hypothalamic disorders</b>	<i>Anticonvulsants</i>
Exercise	Tumours	Phenytoin
Lactation	Craniopharyngioma	<i>Antidepressants</i>
Pregnancy	Germinoma	Imipramine
Sleep	Meningeoma	Amitriptyline
	Metastases	Fluoxetine
	Infiltrative diseases	<i>Antihistamines H<sub>2</sub></i>
	Sarcoidosis	Cimetidine
	Tuberculosis	Ranitidine
	Langerhans-cell histiocytosis	<i>Antihypertensives</i>
	Suprasellar surgery	$\alpha$ -methyldopa
	Cranial irradiation	Labetolol
		Reserpine
	<b>Pituitary disorders</b>	Verapamil
	Prolactinoma	<i>Anti-emetics</i>
	Acromegaly	Domperidone
	Plurihormonal adenoma	Metoclopramide
	Macroadenoma (compressive)	<i>Neuroleptics</i>
	Lymphocytic hypophysitis	Chlorpromazine
	Empty sella syndrome	Fluphenazine
	Pituitary stalk section	Haloperidol
		Perphenazine
	<b>Other</b>	Risperidone
	Primary hypothyroidism	Thioridazine
	Chronic renal failure	<i>Oestrogens</i>
	Liver cirrhosis	<i>Opioids</i>
	Epileptic seizures	Morphine
	Chest wall lesions	Methadone
	Polycystic ovarian disease	Heroin
	Idiopathic	

Adapted from Williams textbook of Endocrinology (2007) [15].



tonus. When physiological and pharmacological causes to persistent HPL can be ruled out, a PRL-producing pituitary adenoma will be one likely explanation. However, it is important to distinguish a prolactinoma from a large pituitary tumour with secondary HPL and also to identify plurihormonal pituitary adenomas (Table 1). Elevated PRL levels without any visible pituitary adenoma and with other known causes of HPL excluded are considered as idiopathic HPL. This may reflect a prolactinoma too small to be detected by current radiological techniques or a hypothalamic regulatory dysfunction [37]. In this thesis we are focusing on patients with prolactinomas and idiopathic HPL.

### 1.2.3 Symptoms

The classic clinical presentations in women with HPL are menstrual disturbances, infertility and galactorrhea (Table 2). HPL women with regular menses may be infertile because of a short luteal phase [38]. In men, elevated PRL levels typically lead to hypogonadism, with a loss or decrease in libido, erectile dysfunction, impotence and oligospermia or azoospermia [36]. In HPL patients, elevated PRL levels cause hypogonadism by reducing luteinising hormone (LH) pulsatility [39], possibly via inhibition of the hypothalamic gonadotropin-releasing hormone (GnRH) [40]. Furthermore, high PRL levels are considered to inhibit ovarian and potentially testicular function directly [15]. Another possible mechanism of the hypogonadism seen in HPL patients with large tumours may be a direct tumour mass effect on the gonadotroph cells in the pituitary. Both women and men with HPL and hypogonadism have an increased risk of reduced bone mineral density, most notably in trabecular bone [41, 42]. Earlier studies suggested that PRL might have a direct effect on the bone. Nowadays, the general opinion is that gonadal dysfunction is the major cause of reduced bone mass seen in

**Table 2.** Signs and symptoms of hyperprolactinemia

Associated with hyperprolactinemia
<i>Women</i>
Oligomenorrhea
Primary or secondary amenorrhea
Infertility
<i>Men</i>
Decreased libido
Impotence, erectile dysfunction
Oligospermia, azoospermia
Gynecomastia
Galactorrhea
Anxiety
Depressive symptoms
Osteopenia
Associated with tumour mass
Headache
Visual impairment
Pituitary insufficiency
Cranial nerve palsies

HPL patients, reviewed in [43]. In a small study of HPL women with normal menstrual function, no difference in bone mineral content was found compared with controls [44]. Men often have macroprolactinomas at diagnosis [45], and consequently, frequently present with symptoms that are due to mass effects of the pituitary tumour (e.g., headache, visual field defects or pituitary insufficiency). The reason for the preponderance of large tumours in men is not fully understood. One explanation might be that men more often are diagnosed at a later stage; however, it may also reflect a gender difference in the pathogenesis and growth of the pituitary tumour. Delgrange *et al.* demonstrated that the predominance of large prolactinomas in men was not due to a longer delay in diagnosis, but rather to the greater proliferative potential of the tumours, as indicated by a higher index of Ki-67 [46] and confirmed by other studies [47, 48]. No correlation was found in patients with macroprolactinomas between tumour size and age at diagnosis or duration of symptoms [48]. On the other hand, these data need to be interpreted with caution because of the insidious nature of the symptoms and signs of HPL in males, which makes it difficult to establish the onset of disease.

#### 1.2.4 Diagnosis

To establish the diagnosis of HPL, a single measurement of serum PRL level above the upper normal limit is, in general, sufficient to confirm the diagnosis of HPL [49]. However, since PRL is secreted episodically during the day and there are different stressors that can increase PRL levels (such as pain during venepuncture and heavy exercise); slightly elevated PRL levels should be confirmed in additional sampling. A careful history, including medications, physical examination, screening blood chemistries, thyroid-function tests and pregnancy test will exclude almost all causes of HPL other than hypothalamic-pituitary disease [37]. Consequently, the next step in the investigation is a radiological examination of the hypothalamic-pituitary region, preferably a magnetic resonance imaging (MRI) with gadolinium enhancement [50]. Generally, there is a close association between the size of the prolactinoma and PRL levels [51], a macroprolactinoma being more likely if PRL levels exceed 250 µg/l [37]. Secondary causes of HPL are most typically associated with PRL levels less than 100 µg/l. The “hook effect” must be considered in large pituitary adenomas with mildly elevated PRL levels when using some immunoradiometric assays in that a false low PRL level may wrongly exclude the diagnosis of a prolactinoma. The hook effect is seen when an excess of antigen saturates the recognition sites on both the unlabeled and labeled antibodies and thus impairs the antigen-antibody formation, resulting in low antigen determination [52]. However, this can be overlooked with an appropriate dilution of the sample. On the contrary, macroprolactinemia must be considered in a patient with elevated PRL levels with no symptoms related to HPL. It is important to be aware of which PRL assay is used and its ability and accuracy to measure monomeric PRL, since this varies greatly between different assays [53]. The golden standard to detect the presence of macroprolactin is gel filtration chromatography, but the polyethylene glycol precipitation method is reproducible and easy to perform and is the method of choice for most laboratories [14]. A recent study by Wallace *et al.* followed 51 patients with macroprolactinemia and normal monomeric PRL levels for a median period of 10 years [54]. The study demonstrated no symptomatic progression or increased monomeric PRL levels in any of the patients. The authors concluded that macroprolactinemia is benign and, DA agonist treatment, prolonged follow-up or imaging investigations are not necessary.

## 1.2.5 Treatment

The primary goal of treatment in HPL patients is to normalise PRL levels and thereby restore symptoms such as hypogonadism, infertility and galactorrhea, as well as to reduce tumour size, especially in patients with large pituitary tumours. As suggested by Klibanski, indications for treatment in prolactinoma patients include infertility, oligomenorrhea or amenorrhea, bothersome galactorrhea, gynecomastia, testosterone deficiency, macroadenoma and enlarging microadenoma [55]. According to treatment recommendations from the Endocrine Society, there is no indication to treat asymptomatic patients with microprolactinomas [49]. In fact, small studies of patients with untreated microprolactinomas who were followed on an average of 4 to 6 years have shown that the risk of tumour growth is low (0-15%) and that these patients actually may have clinical and radiographic improvement [56-58]. Nevertheless, in addition to the increased risk of osteopenia in HPL patients with gonadal dysfunction, knowledge of long-term risks associated with persistent elevated PRL levels is limited, and consequently, there is a need for studies evaluating different clinical outcomes in these patients.

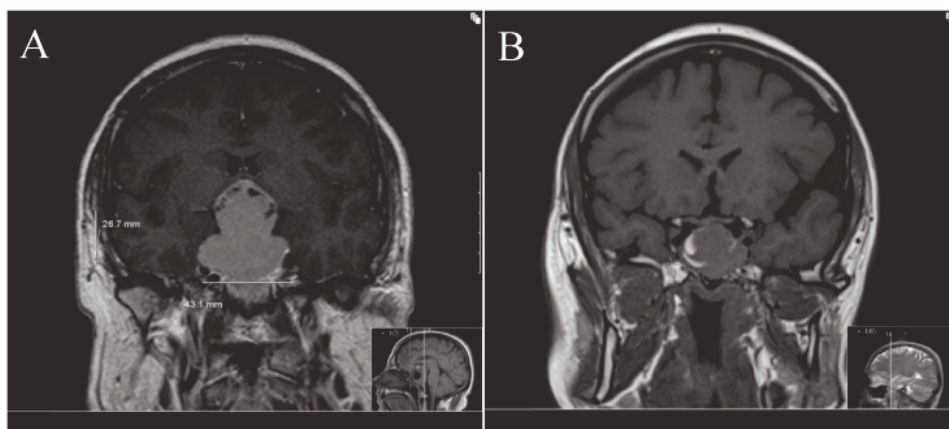
### 1.2.5.1 Medical treatment

Today, DA agonists are widely accepted as the first line treatment in patients with HPL [49, 59, 60]. In Sweden, there are three DA agonists available for treatment of HPL disorders: Brc, cabergoline (Cab) and quinagolide. The first DA agonist used in clinical practice to treat HPL was Brc, which was introduced more than 35 years ago. Brc is an ergot-derived DA agonist, with D<sub>2</sub> receptor agonistic and D<sub>1</sub> receptor antagonistic properties. It is also known to act on serotonin (5-HT) receptors [61]. The half-life of Brc is relatively short, about 3 h, with a duration of action of 8-12 h and divided daily doses are often necessary [62]. The therapeutic dose of Brc generally varies between 2.5-15 mg per day. Cab is an ergot-derived DA agonist with high affinity for D<sub>2</sub> receptors and low affinity for D<sub>1</sub> receptors. Cab also has agonistic effects on 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors [61]. The half-life of Cab is long (63-109 h) [63] and it can therefore be administered once or twice weekly in patients with HPL. In 12 healthy volunteers, the maximal PRL inhibition effect of Cab disappeared after 12-20 days (at a single dose between 0.5 and 1.5 mg) [64]. Typically, Cab therapy is started with a low dose (0.25-0.5 mg weekly); for maintenance, the usual range is 0.25-2.0 mg per week. Quinagolide is a non-ergot oral DA agonist with specific D<sub>2</sub> receptor affinity with little or no effect on D<sub>1</sub> receptors [65]. It has a longer half-life than Brc (24-h duration of action) and can be administered once daily [66, 67]. The therapeutic dose of quinagolide varies normally between 75 and 300 µg per day. Quinagolide is available in Sweden and in several European countries but not in the USA or Japan.

### *Outcome of medical therapy*

Brc is effective in normalising PRL levels, restoring gonadal function and reducing tumour size in a majority of HPL patients [68-70]. Twenty-six studies evaluating Brc effect on tumour shrinkage in 271 patients with macroprolactinomas were reviewed by Bevan *et al.* [71]. The authors demonstrated that 79% of the macroprolactinoma patients had at least 25% tumour shrinkage and 89% of the tumours shrank to some degree. In Figure 3, an example of the efficacy of Brc treatment on tumour shrinkage, in a male patient with a macroprolactinoma, is presented. Several studies in patients with prolactinomas have shown that Cab is more effective than Brc in normalising PRL levels and reducing tumour size [72, 73]. Moreover, treatment with Cab has proven to be effective in prolactinoma patients resistant to other DA agonists [74]. In a review by Kars *et al.* Cab normalised PRL levels in

75-90% of patients with micro- or macroprolactinomas, in addition, an average decrease in tumour volume of 72-92% was reported [75]. Finally, prolactinoma patients resistant to Brc might respond to quinagolide. In 28 women resistant to Brc (doses 15-30 mg per day) quinagolide normalised PRL levels in 39% and resumed normal gonadal function in 75% [76]. In summary, Cab has been proven to be the most effective DA agonist in treating HPL patients to date.



**Figure 3.** MRI images of a male patient with a macroprolactinoma, at diagnosis (A) and after one year (B) of Brc treatment. This patient participated in Study IV. The pictures are presented with the patient's kind permission.

#### *Side effects of DA agonists*

The most common side effects of all DA agonists are nausea, vomiting, headache and dizziness. These side effects can be minimised by starting the treatment with a low dose at bedtime and thereafter gradually increasing the dose [37]. Other side effects might include constipation, dry mouth, dyspepsia, orthostatic hypotension, nasal congestion, psychiatric symptoms, digital vasospasm and, in some rare cases, pulmonary or retroperitoneal fibrosis. Numerous studies have demonstrated that Cab has fewer side effects than Brc. In a comparative study of Brc and Cab in women with HPL: 12% of the patients treated with Brc vs. only 3% in the Cab-treated patients had to discontinue treatment because of side effects [72]. Furthermore, quinagolide has been reported to have less side effects than Brc [77, 78]. However, side effects are very individual and in the case of non-severe adverse side effects, switching to another DA agonist is recommended.

During the past years, concerns have been raised about the long-term safety of Cab in the context of potential cardiac valve effects. Two studies of Parkinson patients published in 2007 showed that treatment with Cab was associated with an increased risk of cardiac valve regurgitation, particularly doses higher than 3 mg per day and duration of treatment more than 6 months [79, 80]. The significance of these findings in patients receiving DA agonists for HPL is not established. There are some important differences between the use of DA

agonists in Parkinson patients and patients treated for HPL, in that the doses of DA agonists typically are much higher in Parkinson patients on the contrary, HPL patients are in general treated with DA agonists for a longer time. A recent meta-analysis of the current literature regarding the risk of cardiac valvulopathy in HPL patients treated with Cab is reassuring in the sense that eight of nine observational studies did not demonstrate any increased risk of clinically significant valvular regurgitation in HPL patients [81]. Only one study, by Colao *et al.*, found an increased prevalence of clinically significant tricuspid regurgitation in prolactinoma patients (54%) treated with Cab compared to controls (18%) [82]. However, there was an unexpected high prevalence of tricuspid regurgitation also in the control group. The mechanism by which DA agonists may cause valvular fibrosis is thought to be mediated via the stimulation of 5-HT<sub>2B</sub> receptors, expressed in the heart valves. This action leads to mitogenesis and proliferation of fibroblasts and, in turn, causes valvular regurgitation [83, 84]. Cab has high affinity for 5-HT<sub>2B</sub> receptors, whereas Brc has a weaker affinity and is thought to be a partial agonist [61].

#### 1.2.5.2 Surgery

Because of the efficacy of medical treatment, only a minority of patients with prolactinomas require surgery. Indications for transsphenoidal surgery include intolerance or resistance to DA agonists, persistent visual field defects despite medical therapy and pituitary apoplexy with neurological signs in macroadenomas [50]. Surgical outcomes in 50 published series, summarised by Gillam *et al.*, demonstrate that 75% of microprolactinomas and 34% of macroprolactinomas achieved initial surgical remission [59]. However, the recurrence rate is relatively high, yielding a long-term cure rate of 61% in microprolactinomas and 26% in macroprolactinomas. This difference can be explained to some extent by the sometimes infiltrative growth of macroprolactinomas.

#### 1.2.5.3 Radiation therapy

Another treatment option for prolactinoma patients not responding to medical treatment, surgery, or both is radiation therapy. In a summary of 250 patients conventional fractionated radiotherapy has been reported to normalise PRL levels in 34% [59]. A disadvantage of conventional radiotherapy is the latency of hormonal reduction and the risk of developing pituitary insufficiency [85]. Another mode of radiotherapy is gamma knife stereotactic radiosurgery (SR). Gamma knife SR has been reported to normalise PRL levels in 18-47% of prolactinoma patients and the risk of pituitary insufficiency was 14-26% in patients followed for at least 4 years [86-88]. In a study of 35 patients with prolactinomas receiving SR, normoprolactinemia was achieved in 37%; however, the onset of action was slow with a median time of hormonal normalisation of 96 months [87]. Thus, one drawback with radiation therapy is time to remission, often making medical treatment necessary during this period.

### 1.3 PREGNANCY

#### 1.3.1 Pituitary gland during pregnancy

The normal pituitary gland enlarges gradually throughout pregnancy, with a total increase of 120-136% at the end of the third trimester [89, 90]. During pregnancy, the height does not normally exceed 10 mm, but directly postpartum a size up to 11.6 mm has been demonstrated

[91]. Pituitary enlargement is due to an oestrogen-stimulated increase in the number of lactotrophs [92]. Conversely, gonadotrophic cells show a substantial reduction in hormone content. PRL concentrations increase gradually during pregnancy with a mean level of 250 µg/l at the end of the third trimester [93]. In contrast, Divers *et al.* found that the progressive rise in maternal PRL levels was not seen in a group of 54 microprolactinoma patients, without Brc therapy, followed during pregnancy [94]. However, in another study of women with prolactinomas, the normal increase of PRL concentrations during pregnancy was found [95]. After delivery, PRL levels drop but increase in response to suckling.

### 1.3.2 Pregnancy in hyperprolactinemia patients

Because of the stimulatory effect of oestrogen on the lactotrophs, concerns regarding growth of prolactinomas during pregnancy have been raised. Molitch made a summary of 19 studies (363 women with microadenomas, 84 women with macroadenomas) showing that the risk for symptomatic tumour enlargement during pregnancy was only 1.4% in microadenomas but 26% in macroadenomas [96]. Current treatment practice is to discontinue DA agonist therapy in patients with microprolactinomas once pregnancy is diagnosed and to follow the patients with controls of visual fields. On the other hand, in macroprolactinomas an individual treatment strategy has to be made and close monitoring of symptoms of tumour enlargement throughout pregnancy is mandatory [50].

Interestingly, pregnancy may be beneficial in women with HPL and prolactinomas in that PRL levels in many patients decrease after pregnancy. It has been shown that PRL levels normalised postpartum in 17-29% of the women with HPL [97-99]. However, the underlying mechanism for this positive effect of pregnancy on prolactinomas is not known. Different speculations have been raised e.g., changes in the vascularisation of the adenoma that are due to a stimulatory effect of oestrogens may result in necrosis or microinfarction of the pituitary adenoma [93, 97]. Moreover, pregnancy may accelerate a spontaneous improvement in HPL women, especially in non-tumoral HPL [99]. This finding is in line with a study evaluating PRL levels before and after first pregnancy in non-HPL women, demonstrating a decrease in PRL levels after pregnancy as well as significantly lower PRL levels in parous women compared with nulliparous controls [100].

#### 1.3.2.1 DA agonist treatment and pregnancy

The safety of taking Brc during early pregnancy is well documented, i.e. no deleterious effects on foetal or neonatal development have been reported [101-104]. Follow-up studies of children exposed to Brc in early pregnancy have shown no adverse effects on childhood development [104, 105]. Less data on foetal outcome in women on continuous Brc treatment throughout pregnancy are available, although no teratogenic effects have been reported [95, 106]. During the past decade, there is increasing knowledge of using Cab to induce pregnancy, where no increased risk for miscarriage or foetal malformations has been seen [107-109]. A recent study by Stalldecker *et al.* evaluated 103 pregnancies induced by Cab. The authors also reviewed the literature of studies assessing the safety of Cab in early pregnancy [110]. In summary, in 763 published pregnancies with Cab during pregnancy no increased risk of spontaneous abortions, preterm delivery or neonatal malformations was found. However, because of the potential risk of cardiac valvulopathy associated with Cab treatment, the use of Cab during pregnancy must be carefully considered. Safety data on quinagolide in early pregnancy are limited but there are no reports of any detrimental effects

on pregnancy or foetal development [76, 111]. To summarise, the treatment of choice when inducing pregnancy in HPL women should be Brc based on the long experience with this drug. However, although there are a number of studies focusing on drug teratogenicity, we lack data on parity in HPL women.

## **1.4 PROLACTIN AND CANCER**

PRL is an extremely versatile hormone with numerous separate biological effects across vertebrates [20]. For more than three decades, PRL has been suspected of being involved in tumour growth and there are emerging data that PRL has a role in tumour proliferation in various cancer forms [112]. Furthermore, both PRL and PRLR are expressed in different human cancer cell lines, supporting its action as a autocrine/paracrine growth factor [113]. However, it is difficult to establish the relative contribution of locally produced PRL and PRL of pituitary origin to the growth of tumours. Studies of cancer risk in HPL patients are scarce [114] and we lack data on the long-term risks in HPL patients. Furthermore, due to current treatment guidelines, some patients with asymptomatic HPL may be left untreated if fertility is not an issue [49, 50]. It is therefore essential to evaluate cancer risks in patients with HPL.

### **1.4.1 Breast cancer**

Breast cancer is the most common cancer in Swedish women, representing 29% of all female cancer cases [115]. Each year just over 7 000 women with breast cancer are diagnosed. Several established risk factors are linked to breast cancer, such as age, family history, ethnicity, early menarche, late menopause, nulliparity, oral contraceptives, hormonal replacement therapy and obesity [116].

In experimental studies substantial information supports a role of PRL in breast cancer aetiology. More specifically, PRL enhances cell proliferation [117, 118], inhibits apoptosis [119] and increases cell motility [120]. In addition, transgenic mice overexpressing PRL develop mammary carcinomas [121]. However, because of species differences, caution should be used when extrapolating results from animal studies to humans. In humans an association between circulating PRL levels and breast cancer prognosis has been observed [122], but clinical trials using DA agonists as an additional treatment in breast cancer patients were ineffective, reviewed in [123]. Some epidemiological studies suggest that there is an association between PRL levels and risk of breast cancer [124, 125]. In one large prospective case-control study of postmenopausal women PRL was associated with a modestly increased risk of breast cancer (relative risk 1.34, 95% confidence interval [CI] 1.02-1.76) in the highest vs. lowest quartiles of PRL levels [124]. However, when interpreting the results, it is important to consider that this study evaluated the association between breast cancer risk and PRL levels within the normal range. There are some case reports of breast cancer in both women and men with prolactinomas [126-128], raising the question of whether patients with very high PRL levels have an increased risk of breast cancer. So far, no association between breast cancer risk and HPL patients has been shown [114, 129].

### 1.4.2 Prostate cancer

The most common malignancy in Swedish men are prostate cancer and around 9000 men are diagnosed each year, representing on third of all male cancers [115]. The causes of prostate cancer remain poorly understood and there are only three well-established risk factors: age, ethnicity and family history [130].

Unlike mammary tissue, the experimental evidence of a proliferative effect of PRL on prostate tumorigenesis is less established. It is well-recognised, however, that PRL has a trophic effect on prostate cells and is essential for the normal growth and development of the prostate [131]. The human prostate produces PRL, expresses PRLR and PRL is proposed to act as a direct growth and proliferation factor on the prostate [132]. However, the role of PRL in human prostate cancer is not clear. In one prospective case-control study PRL levels were measured in 144 men with prostate cancer and 289 controls [133]. Consistent with a previous study [134], no correlation was seen between PRL levels and prostate cancer. Colao *et al.* studied 20 men with prolactinomas and 20 controls and demonstrated that PRL excess was associated with decreased prostate size, which, in turn, was associated with reduced testosterone levels [135]. After 24 months of treatment with Cab testosterone levels and prostate volume were normalised. To date, only one small study (31 male patients) has evaluated prostate cancer risk in prolactinoma patients, where no cases of prostate cancer was found [114].

### 1.4.3 Other cancer forms

Elevated PRL levels have been associated with other tumour types and it has been suggested that locally produced PRL could play a role in tumour development in gynaecological [136], tongue [137], colon [138] and hematopoietic cancer [139, 140]. There is a bi-directional connection between the neuroendocrine and the immune system and PRL is considered to be important for the maturation and functional maintenance of the immune system [23]. PRLRs are expressed by virtually all subtypes of immune cells, whereas PRL is produced mainly by lymphocytes [141]. Actions of PRL on the immune system were first proposed based on animal studies in which pituitary insufficiency was induced by surgical ablation of the gland [142] or treatment with Brc [143], leading to a suppressive effect on the bone marrow and a decreased immune response, respectively. In contrast, elevated PRL levels have been observed in various autoimmune diseases in humans, such as, systemic lupus erythematosus and rheumatoid arthritis [144]. Furthermore, elevated PRL levels have been associated with hematopoietic malignancies. Human cell lines of B-lymphoblastoid cells [145] and Non-Hodgkin lymphoma (NHL) cells [146] are known to produce PRL. Elevated serum PRL levels have been observed in patients with acute myeloid leukaemia [140] and in advanced multiple myeloma [139]. However, the role of PRL in hematopoietic malignancies is not clarified; nor is it clear whether there is a link between PRL excess in humans and hematopoietic cancers.



## 1.5 PROLACTIN AND METABOLISM

For some years, PRL has attracted attention as a metabolic hormone. Increasing evidence in animal studies suggests that PRL is important in the regulation of carbohydrate and lipid metabolism in different target organs (i.e. pancreatic islets and adipose tissue) [147]. Throughout lactation, PRL has an essential role to supply the mammary gland with nutrients for milk synthesis, with a shift in lipid production from adipose tissue towards mammary gland through action on different key enzymes in lipid metabolism [148]. Previously, the overall opinion was that PRL affected adipose tissue via an indirect mechanism in humans; however, lately it is established that PRL acts directly on human adipose tissue via functional PRLRs [149]. During pregnancy, adaptive changes occur in the pancreatic  $\beta$ -cells to compensate for the increased need of maternal insulin. In rodents, lactogenic hormones (PRL and PL) stimulate growth of pancreatic  $\beta$ -cells and insulin production, in addition, the threshold for glucose-stimulated insulin secretion is lowered, reviewed in [147]. In support of these findings it has been demonstrated that PRLR-deficient mice have reduced islet  $\beta$ -cell mass, insulin content and insulin secretion [150], all of which underline the role of lactogenic hormones in pancreatic development and function. However, the effect of PRL and PL on pancreatic function is not fully understood in humans.

### 1.5.1 Hyperprolactinemia and weight

PRL promotes fat storage in different animal species. Elevated PRL levels stimulate food intake and are associated with increased body weight in rats [151-153], with weight loss after suppression of PRL by Brc treatment [152]. In addition, sustained HPL in humans has been linked to a high prevalence of obesity, especially in men and patients with macroprolactinomas [154-156]. Furthermore, a history of recent weight gain was more frequent among patients with prolactinomas than among patients with clinically inactive pituitary tumours, despite equally frequent hypogonadism in both groups and no difference in the tumoral mass effect on the central hypothalamus [155]. A normalisation of PRL levels by treatment with DA agonists may lead to weight loss [154, 155, 157] but findings are often contradictory [158, 159]. At present, the linkage between HPL and weight gain is unclear. However, reduced central dopaminergic tone as a result of sustained HPL [154] or increased hypothalamic pressure has been proposed as possible mechanisms. In addition, drugs that block  $D_2$  receptors promote a gain in body weight [160, 161]. Baptista *et al.* reported that PRL levels correlated positively with body mass index (BMI) in a subset of men treated with DA antagonists [162]. However, it is not clear if elevated PRL levels, induced by DA antagonists, *per se* have a role in the weight gain seen in these patients. Today, there is no strong evidence that PRL is an important factor in human obesity.

### 1.5.2 Hyperprolactinemia and lipid profile

Dyslipidemia is regarded as one of the major risk factors for cardiovascular disease. The role of low-density lipoprotein (LDL) cholesterol in the development and progression of atherosclerosis is well established and that LDL-lowering therapy reduces the risk for coronary heart disease [163]. The effect of PRL on lipid metabolism in humans is not well established. HPL has been associated with hypercholesterolemia and hypertriglyceridemia in women with prolactinomas [164]. In a recent study total and LDL cholesterol were higher in 22 patients with prolactinomas than in 20 age- and gender-matched controls [165]. In other studies, however, no differences in lipoprotein profiles have been reported [159, 166]. Fahy *et*

*al.* found no difference in lipid profile in HPL patients at diagnosis when compared with controls though a significant reduction in total and LDL cholesterol was seen after treatment with Brc [167]. One explanation for the alterations in lipoprotein profile observed in some women with HPL could be linked to oestrogen deficiency. Hypoestrogenism is associated with increased total and LDL cholesterol and reduction in high-density lipoprotein (HDL) cholesterol and oral oestrogen reverses these abnormalities [168]. PRLRs are highly expressed in the liver, which is central to metabolic homeostasis [147], but there is very limited work on the effect of PRL in this tissue. In conclusion, the effect of PRL on lipid profile, if any, is scanty and incomplete.

### 1.5.3 Hyperprolactinemia and glucose homeostasis

Insulin is one of the key regulators of carbohydrate and fat metabolism. Insulin resistance is a condition in which regular insulin levels fail to exert the normal effects of insulin action in peripheral tissue. Human pregnancy is a physiological state of insulin resistance. The mediators of insulin resistance seen in late human pregnancy are not fully elucidated but are considered, at least partially, to be related to alterations in cortisol and reproductive hormones, such as PL, progesterone and PRL [169]. Furthermore, the cytokine tumour necrosis factor-alpha (TNF- $\alpha$ ) has been associated with insulin resistance during late pregnancy in humans [170], possibly via impaired insulin signalling [171].

In the mid-1970s, it was demonstrated that patients with HPL had reduced glucose tolerance and hyperinsulinemia following a glucose load [172, 173]. Consequently, Landgraf *et al.* suggested a diabetogenic effect of PRL [173]. Since then, various clinical studies have indicated that HPL is associated with reduced glucose tolerance, elevated basal and glucose stimulated insulin levels and insulin resistance [174-176]. Increased insulin resistance using the homeostasis model assessment (HOMA) index is described in patients with hyperprolactinemia [159, 177]. Furthermore, that the insulin sensitivity index (ISI composite) is reduced in the hyperprolactinemic state, improving after normalisation of PRL by DA agonist therapy [166]. A recent study demonstrated that when evaluated by a euglycemic hyperinsulinemic clamp, 16 prolactinoma patients were more insulin resistant than controls [178]. The reason for the increased insulin resistance seen in HPL patients has not been clarified. In a rat adipocyte culture system Ryan *et al.* found that PRL, PL and progesterone, reduced maximal glucose transport and the authors suggested that the insulin resistance of pregnancy may be related to a post-binding defect in insulin action [179]. Moreover, insulin binding to monocytes and erythrocytes was decreased in HPL patients indicating a down-regulation of insulin receptors [175], which may decrease the effect of insulin *in vivo*. Foss *et al.* found that insulin levels were significantly higher after glucose ingestion in patients with elevated PRL levels compared with controls, and that the suppression of serum free fatty acid (FFA) levels were smaller [176], suggesting an impaired antilipolytic effect of insulin. Impairment of glucose tolerance may in part be due to the inhibitory effect of FFA on peripheral glucose uptake. Taken together, a potential reduced effect of insulin and down-regulation of insulin receptors might contribute to the insulin resistance found in HPL patients.

#### 1.5.3.1 Prolactin and adiponectin

Adipose tissue acts both as an energy storage depot and as an endocrine organ that secretes various cytokines and hormones, collectively called adipocytokines. The role of

adipocytokines in insulin resistance and their possible inflammatory effects has been extensively studied in recent years [180]. Adiponectin, a peptide hormone produced by the adipose tissue, has an important role in energy homeostasis through the regulation of glucose and fatty acid metabolism in peripheral tissues [181]. Adiponectin is the most abundant adipocytokine in the human circulation, with higher levels in women than men and the levels are inversely associated with BMI, fasting insulin and glucose levels [182]. Adiponectin is positively correlated with insulin sensitivity and hypoadeponectinemia is associated with obesity and development of diabetes mellitus (DM) type 2 [183]. Nilsson *et al.* demonstrated that PRL inhibits adiponectin secretion from human adipose tissue *in vitro* [184], resulting in the speculation that this suppression may be one factor leading to the increased insulin resistance seen in HPL patients. So far, the role of adiponectin secretion in patients with HPL has not yet been evaluated.

#### 1.5.4 Dopamine agonist treatment and metabolism

In seasonally obese rodents Brc has been shown to decrease body weight and improve glucose tolerance [185, 186]. These beneficial metabolic effects are associated with a reduction in elevated norepinephrine and 5-HT activities in the hypothalamus, with possible suppression of hepatic glucose output and adipose lipolysis [185], indicating that DA agonists may act through the neuroendocrine system to improve peripheral energy metabolism, at least in animal studies.

In non-HPL humans administration of a quick-release form of Brc (Brc-QR) has been associated with beneficial metabolic effects [187, 188]. A mean reduction in haemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) of approximately 0.6% was demonstrated in patients with DM type 2 treated with Brc-QR as monotherapy or in combination with sulphonylurea [187]. In addition, a reduction in FFA, triglycerides (TGs) and a minor decrease in total cholesterol was observed. Kamath *et al.* showed a reduction in mean daylong plasma glucose, TG, FFA and total cholesterol in 13 obese non-diabetic women after 8 weeks of treatment with Brc-QR, but neither circulating plasma insulin concentrations nor the ability to mediate glucose disposal changed [188]. In both these studies [187, 188] the improvement in metabolic variables was accompanied with a significant reduction in PRL levels within the normal range. The authors of the latter study raised the question of whether the findings were due to the decrease in PRL concentration, to an unknown mediator associated with the change in dopaminergic activity or to a direct effect of Brc-QR [188]. Conversely, in another study, treatment with Brc (6-8 months) in patients with DM type 2 did not have any positive effects on body weight, body fat per cent, lipid values or insulin resistance, evaluated with HOMA index [189]. However, there are those advocating the use of Brc-QR as a new treatment modality for type 2 diabetes [190].

## 2 AIMS

The overall aim of this thesis was to increase our knowledge of the consequences of elevated PRL levels in humans and the outcome of therapy in HPL patients in order to improve the clinical management of these patients.

Specific aims were:

1. To evaluate long-term therapeutic results in women with HPL, focusing on medical treatment
2. To study parity in women with HPL
3. To investigate if patients with HPL have an increased risk of pregnancy complications or adverse pregnancy outcome
4. To study the risk of cancer in HPL patients
5. To investigate the metabolic state in patients with HPL before and after medical therapy

## 3 SUBJECTS AND METHODS

### 3.1 STUDY SUBJECTS

**General remarks on the classification of HPL patients (Papers I, II, III [Hospital cohort] and IV):** To establish the diagnosis of HPL a PRL level above the upper normal limit had to be found. Pregnancy, lactation and patients with secondary causes of HPL (e.g. primary hypothyroidism, chronic renal and hepatic failure, medication that may cause HPL, plurihormonal pituitary tumours or tumours affecting the pituitary stalk) were excluded. At least one radiological examination of the sella region had been performed in all patients. The pituitary adenoma was regarded as a microadenoma if maximal tumour diameter was <10 mm and as a macroadenoma if  $\geq 10$  mm [191]. If the patient had elevated PRL levels without a visible pituitary tumour and secondary causes of HPL were excluded, idiopathic HPL was considered (Paper I). An overview of the patients participating in the four different studies in this thesis is presented in Table 3.

**Table 3.** Patients and comparison subjects included in this thesis, n (%).

	Patients	Females / Males	HPL	Micro- adenoma	Macro- adenoma	Comparison subjects
Study I	271*	271 / 0	74 (29)	160 (63)	21 (8)	-
Study II	271*	271 / 0	74 (29)	160 (63)	21 (8)	1084
Study III	969	668 / 301	na	na	na	9618
Study IV	14	8 / 6	0	8 (57)	6 (43)	-

HPL=idiopathic hyperprolactinemia; na=non-applicable. \*16 patients had no initial radiological examination available.

**Papers I, II:** The study population consisted of 271 prevalent women with HPL at the Karolinska University hospital (161 patients from the Department of Endocrinology, Metabolism and Diabetology and 110 patients from the Department of Obstetrics and Gynaecology). The patients had their HPL diagnosis between 1974 and 2001 and were identified using the clinics own patient registers. Informed written consent was obtained from all patients. Sixteen patients declined to participate in the study.

In Paper II we used the Register of Population to identify four comparison subjects for each HPL patient, matched by sex, birth year and county of residence.

**Paper III:** The HPL patient cohort consisted of two parts. First, a hospital cohort of women and men with a diagnosis of prolactinoma between 1974 and 2005 at the Karolinska University hospital; incident patients from 1993 onwards (n=236) and prevalent and incident patients diagnosed between 1974 and 1992 (n=148). Second, via the Swedish National Patient Register, we identified all patients hospitalised for HPL from 1987 to 1995 using the

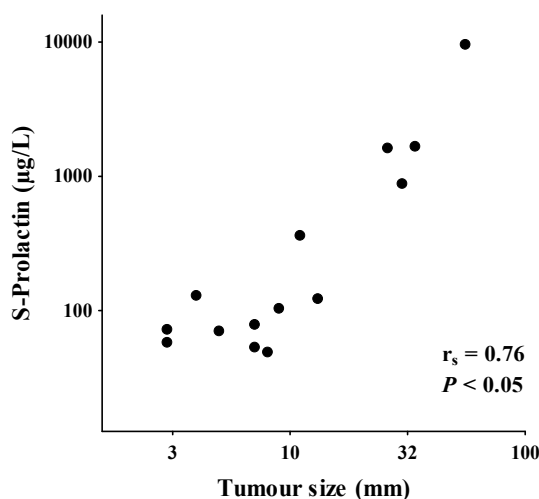
ninth revision of the International Classification of Diseases (ICD) (ICD-9: code 253.1). In total 682 individuals were identified in the register. We excluded 66 patients who were included in the hospital cohort and 31 patients with a concomitant diagnosis of acromegaly, leaving 585 patients. For each patient in the whole HPL cohort (n=969), we identified 10 comparison subjects matched by sex, birth year and county of residence via the Register of Population. The comparison subjects were alive on January 1, in the year of diagnosis of the corresponding case. Seventy-two comparison subjects were unidentifiable or died between the date of selection and start of follow-up and were therefore omitted from the study, leaving 9618 comparison subjects for the analysis.

**Paper IV:** Fifteen consecutive patients with a newly diagnosed PRL-producing pituitary adenoma from the out-patient clinic at the Department of Endocrinology, Metabolism and Diabetology, Karolinska University hospital were included. One woman was excluded because of pregnancy. Thus, the final sample consisted of 14 patients (eight women and six men). Inclusion criteria were elevated PRL levels found on at least two occasions and a MRI of the hypothalamic-pituitary region that confirmed a pituitary adenoma. Additional exclusion criteria to the above-mentioned ones were patients on medication for hyperlipidemia and DM. Baseline characteristics of the study participants are presented in Table 4. Mean age at diagnosis was 39.7 ( $\pm 13.7$ ) years. Male patients had higher median PRL levels than females (1260 [123-9600]  $\mu\text{g/l}$  vs. 72 [49-131]  $\mu\text{g/l}$ ,  $P = 0.004$ ) and larger tumours. In agreement with previous findings [51] a positive correlation between PRL levels and tumour size at diagnosis was found (Fig. 4).

**Table 4.** Characteristics of 14 consecutive patients with prolactinomas at diagnosis, treatment modality and participants in the clamp (Paper IV)

Gender (F/M)	Age (Years)	PRL at diagnosis ( $\mu\text{g/l}$ )	Tumour size (mm)	Gonadal Status	Treatment	Clamp
F	32	131	4	Ameno	Pravidel	
F	71	105	9	RT	Pravidel	
F	32	58	3	HC	Pravidel	Yes
F	18	80	7	Ameno	Dostinex	Yes
F	29	54	7	HC	Pravidel	Yes
F	34	73	3	Oligo	Pravidel	
F	47	71	5	HC	Pravidel	
F	26	49	8	HC	Pravidel	Yes
M	39	891	30	RT	Pravidel	
M	52	123	13	RT	Pravidel	
M	43	9600	56	Hypogonadal	Pravidel	
M	33	1630	26	Hypogonadal	Pravidel	Yes
M	44	361	11	Hypogonadal	Pravidel	Yes
M	56	1693	34	Hypogonadal	Pravidel	

F=female, M=male, PRL=prolactin, Clamp= euglycemic hyperinsulinemic clamp, Amenorrhoea=secondary amenorrhoea, Oligo=oligomenorrhoea, HC=hormonal contraceptive, RT=replacement therapy with dermal oestrogen (one woman) and testosterone (two men), Pravidel®=Bromocriptine, Dostinex®=Cabergoline.



**Figure 4.** The association between pre-treatment PRL levels and maximal tumour diameter (log scale) at diagnosis in 14 patients with prolactinomas (Paper IV). Spearman rank correlation test.

## 3.2 REGISTRIES

Sweden has high-quality population-based registries that provide a unique opportunity to conduct nationwide epidemiological studies [192]. All Swedish residents alive from 1947 onwards have been assigned a 10-digit personal identity number (PIN), a unique personal identifier referred to in all medical records and official registers [193]. Through the use of the PIN, it is possible to unambiguously link information between different databases.

### 3.2.1 The Swedish Registry of Population (Papers II, III)

The Register of Population kept by Statistics Sweden contains the official Swedish population data, including name, PIN, current address, marital status, dates of death and migration. Such population data have been available since 1960. The registry is based on reports from the Swedish tax authority and includes information on household income from the income tax return form. The registry was used to identify comparison subjects matched by birth year, gender and county of residence (Papers II, III).

### 3.2.2 The Swedish Medical Birth Registry (Paper II)

The Medical Birth Register was founded in 1973 and includes demographic data and prospectively collected information on reproductive and medical history as well as complications that occur during pregnancy, delivery and the neonatal period on practically all deliveries in Sweden [192]. Antenatal, obstetric and neonatal data are recorded in a standardised manner starting with the first antenatal visit and continues until the mother and child are discharged from hospital after delivery. From the registry, we collected information

on parity, maternal, pregnancy and neonatal characteristics of the patients and comparison subjects (Paper II).

### **3.2.3 The Swedish National Patient Registry (Paper III)**

In 1964, the Swedish National Board of Health and Welfare started collecting data on hospital discharge diagnoses in the National Patient Registry and since 1987 the register has complete national coverage. Each record in the registry corresponds to one hospital admission and contains patient data, hospital identification, administrative data and medical information including main and secondary discharge diagnosis and major interventions. The healthcare system in Sweden is almost exclusively financed by taxes and in-hospital medical service is organised by the community. Since the patients in a majority of the cases are obliged to use the hospital in their county of residence, the National Patient Registry is considered population-based and attributed to the county where the patient lives. Since 2001, the register also contains outpatient visits at specialised caregivers. The registry was used to identify all patients admitted to hospital for HPL from 1987 to 1995 (Paper III).

### **3.2.4 The Swedish Cancer Registry (Paper III)**

The Swedish Cancer Registry was established in 1958 and covers the whole population. Approximately 50 000 malignant cases of cancer are registered every year. It is mandatory for every health care provider to report all newly diagnosed malignant neoplasms, diagnosed at clinical, morphological or other laboratory examinations and those diagnosed at autopsy. Information on the site and histopathological features of the tumours is recorded. Reports are sent to one of six regional oncology centres for coding and quality checks. The Cancer registry is estimated to be more than 95% complete [194]. We used the registry to collect information on all cancer diagnoses and the date of these diagnoses for HPL patients and comparison subjects (Paper III).

## **3.3 STUDY DESIGN**

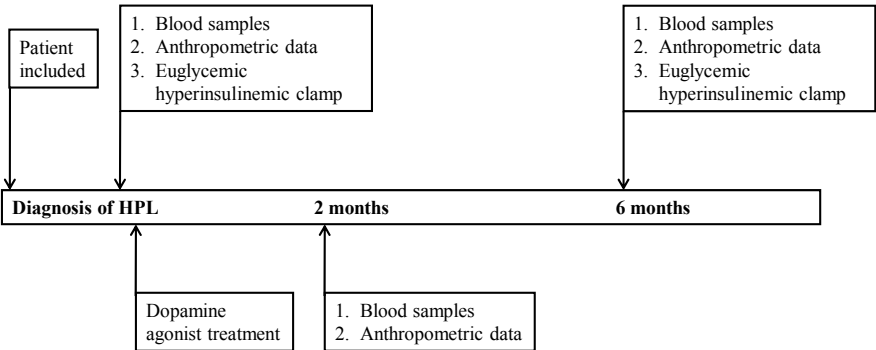
**Paper I:** This study was a retrospective chart review. Patient records were reviewed in retrospect regarding clinical data, radiological findings, treatment strategy, histopathological diagnosis and outcome after long-term follow-up. The follow-up started at the time of HPL diagnosis in the patient file and continued until the last recorded visit at the clinic or December 31, 2002. The patient cohort was analysed as a whole but also divided into three groups according to radiological findings at diagnosis: no visible tumour, microadenoma or macroadenoma. Treatment result was focused on medical therapy, but outcome after surgery and radiation therapy were also evaluated.

**Paper II:** In this matched cohort study we retrieved information of all births among patients and comparison subjects since 1974 in the Swedish Medical Birth Registry, together with information of the antenatal period, delivery and neonatal period. We analysed pregnancy data as a whole but also made restrictions to the first pregnancy to account for the possible dependence in multiparae between repeated pregnancies. Some of the variables analysed in our study were not accessible from the start of the birth register, i.e. induction of labour and smoking and we therefore restricted these data to the period of full coverage. Furthermore, when analysing parity, we made a restriction to women born after January 1, 1953 to possibly ensure full coverage of the mother's fertile period in the Birth registry.



**Paper III:** In this matched cohort study the exposed cohort was the HPL patients and the unexposed cohort the matched individuals from the general population. By linking data to the Swedish Cancer Registry, we ascertained all malignant tumours in patients and comparison subjects. At the time of linkage, cancer register data were available through 2007. The follow-up was started at the date of HPL diagnosis (cases in the National Patient Register), the year of prolactinoma diagnosis (incident cases Hospital cohort) or January 1, 1993 (prevalent cases Hospital cohort). Follow-up continued until death, cancer diagnosis or December 31, 2007, whichever occurred first. When we calculated risk for all cancers, the diagnosis of first cancer or death was used as the individual endpoint of follow-up. When analysing specified cancer forms, the follow-up ended at diagnosis of that specific cancer or death.

**Paper IV:** This study was an observational case serie of consecutive prolactinoma patients attending our out-patient clinic. Anthropometric data and laboratory measurements were studied at baseline and after 2 and 6 months of DA agonist therapy. Blood samples were obtained in the morning after an overnight fast for measurements of hormones (PRL, TSH, fT4, testosterone, GH, insulin-like growth factor I [IGF-I], IGF-binding protein-1 [IGFBP-1]), metabolic variables (glucose, HbA1c, insulin, C-peptide, lipid profile, leptin, adiponectin) and inflammatory markers (hsCRP, TNF- $\alpha$ ). Peripheral insulin sensitivity was determined using the euglycemic hyperinsulinemic clamp technique at baseline and after 6 months of treatment. An overview of the study protocol is presented in Figure 5. The treatment with DA agonists started with a low daily dose, and according to the PRL response, the dose was gradually increased. Thirteen patients were treated with Brc (mean dose 5.7 [ $\pm$ 3.9] mg per day [range 1.25-15 mg]) and one patient was treated with Cab (0.5 mg per week) because of side effects of Brc. To normalise PRL levels the male patients required significantly higher doses of Brc than the females (8.5 [ $\pm$ 4.1] mg vs. 3.2 [ $\pm$ 1.2] mg,  $P$  = 0.007).



**Figure 5.** Schematic view of the study protocol (Paper IV)

## 3.4 METHODS

### 3.4.1 Anthropometric evaluation (Paper IV)

Physical examination included measurements of height, weight, waist and hip. BMI was calculated as weight divided by the square of height ( $\text{kg/m}^2$ ). Waist and hip circumference were measured to calculate waist-hip ratio (WHR). Total body fat (kg), body fat per cent (%) and fat free mass (FFM) (kg) were determined by bioelectrical impedance (BIA) using a body composition analyser (Tanita, TBF-300). The measurements were controlled for sex, age and weight. BIA is a safe and non-invasive technique for assessing human body composition and measures the resistance of body tissues to a small electrical signal. The principle of BIA is that an electric current passes through the body at differential rates depending on body composition and thus can be used to estimate body fat and FFM [195].

### 3.4.2 Insulin sensitivity (Paper IV)

Insulin sensitivity was assessed using the euglycemic hyperinsulinemic clamp technique according to De Fronzo *et al.* [196], which is a widely used experimental procedure for the determination of insulin sensitivity and considered as the “golden standard” method [197]. In brief, intravenous catheters were inserted into the right arm for substrate (insulin/glucose) infusion. A superficial dorsal hand vein was cannulated in retrograde fashion with a 21-gauge butterfly needle and kept patent by a slow saline infusion. The hand was kept warm by an electric device for intermittent sampling of arterialised venous blood. Insulin (Actrapid, NovoNordisk A/S, Copenhagen, Denmark) was infused at a 10-min priming infusion followed by a constant infusion of  $40 \text{ mU/m}^2/\text{min}$  for 110 min. Furthermore, 20% dextrose (Fresenius Kabi, Stockholm, Sweden) was infused and the rate of dextrose infusion was adjusted to achieve a blood glucose level of  $5.0 \text{ mmol/L}$  based on arterialised samples withdrawn every 5th min from an ipsilateral dorsal hand vein (heated air box set at  $55^\circ\text{C}$ , University of Nottingham, U.K.). When a steady state is attained the exogenous glucose infusion rate equals the amount of glucose disposed in body tissues. Whole-body insulin sensitivity (M-value) was calculated from the amount of glucose infused during the last 30 min of the clamp divided by body weight (kg) and period (min) and expressed as  $\text{mg/kg/min}$ . The amount of infused glucose equals whole-body glucose disposal when the endogenous glucose production is suppressed. The standard version of the euglycemic clamp (insulin  $40 \text{ mU/m}^2/\text{min}$ ) will turn off endogenous glucose output in the vast majority of patients and thus gives an estimate of peripheral insulin sensitivity [197]. A drawback, however, is that the clamp yields an estimate of insulin sensitivity to only one level of hyperinsulinemia, and it could be argued that the conditions created with the clamp are non-physiological.

Insulin sensitivity was also assessed using HOMA and the insulin resistance index (HOMA-IR) was calculated (fasting glucose [ $\text{mmol/l}$ ] \* fasting insulin [ $\mu\text{U/ml}$ ]/22.5) [198]. To convert insulin from SI units to conventional units values were divided by 6.945. A HOMA-IR  $\geq 2.77$  has been suggested to indicate insulin resistance [199].

### 3.4.3 Biochemical assays

Because PRL concentrations demonstrate diurnal variation, blood samples are normally performed in the morning, ideally in a non-stressful setting. In the retrospective follow-up study (Paper I) we were not able to control for these parameters.

During the study period 1974-2002 (Paper I), serum concentrations of PRL were measured by four commercial assays. RIAs from 1974 to 1992: Biodata Serono and Farnos Diagnostica and immunofluorimetric assays from 1992 to 2002: DELFIA and Modified DELFIA. However, all these assays had the normal range of PRL between 3-19 µg/L in women and we could therefore compare the different PRL levels in the study. In Paper IV, serum PRL was measured using commercial chemiluminescence immunoassay (Beckman Coulter Unicel, DXI). The normal range was 3-27 µg/L (women <50 years), 3-20 µg/L (women ≥ 50 years) and 3-13 µg/L (men). Because of the specificity of this PRL method, sera were not screened for macroprolactin.

In-house RIAs were used for IGF-I and IGFBP-1 with individual serum samples from the different investigations in the same assay. IGF-I was measured after ethanol extraction and cryoprecipitation and using des (1-3) IGF-I as a ligand [200]. The standard deviation (SD) scores of IGF-I were calculated from the regression of healthy adult subjects [201]. The RIA for IGFBP-1 was performed according to Póvoa *et al.* [202]. The geometrical means of IGFBP-1 in 595 non-diabetic middle-aged women and men are 41 (95% CI 39-44) and 19 (95% CI 18-20) µg/L, respectively [203]. Plasma total cholesterol, HDL cholesterol and TG were measured by automated colorimetric methods (UniCel DXC800, Beckman Coulter, Fullerton, CA, USA). LDL cholesterol was calculated according to the formula suggested by Friedewald *et al.* [204]. The upper normal limit for total cholesterol in a reference population was 6.1 mmol/L (<31 years), 6.9 mmol/L (<51 years) and 7.8 mmol/L (≥51 years); the corresponding values for LDL cholesterol were 4.3, 4.7 and 5.3 mmol/L, respectively. Commercial kits were used for the following factors: S-insulin and S-C-peptide by electrochemiluminescence immunoassays (Roche Diagnostics GmbH, Mannheim), S-total adiponectin and S-leptin by RIA kit (Millipore Corporation, Linco Research, Inc., USA), S-TNF-α by chemiluminescent immunometric assay (Immulite, Siemens, Gwynedd, Great Britain) and P-hsCRP by an automated immunoturbidimetric assay (Beckman Coulter, Fullerton, CA, USA) with a lower detection limit of 0.2 mg/L. HbA1c was measured by cation exchange chromatography (MonoS column) with HPLC (Bio-Rad). S-TSH, S-free T4, S-testosterone, S-GH and P-glucose were all measured using routine assays.

### 3.4.4 Statistical analysis

Results are presented as mean (± SD) if normally distributed data, otherwise as median and range (min-max). Comparisons between two independent groups were performed using the unpaired t-test when data were normally distributed (Papers II, IV) otherwise, the Mann-Whitney rank sum test was used (Papers I, IV). For the comparison between three or more groups in non-normally distributed data, the Kruskal-Wallis test was performed (Paper I). Wilcoxon signed rank test was used for the analysis of continuous variables with non-normally distribution in two dependent groups (Papers I, IV). For comparison between repeated measurements, repeated measure analysis of variance (ANOVA) was used followed by Tukey *post hoc* test. In non-normally distributed data Friedman ANOVA was used (Paper IV). In Paper IV, correlation analyses were performed using linear regression, with log-transformed data when required. In non-normally distributed data Spearman Rank order correlation test was used ( $r_s$ ). The  $\chi^2$ -test was used for comparison between categorical variables (Paper I). In Paper II, Conditional logistic regression was used when comparing dichotomous outcomes between patients and controls and presented as odds ratios (ORs) with 95% CIs. Quantitative outcomes were analysed by ANOVA and presented as mean differences between patients and controls with 95% CIs. Pregnancy-related outcomes for

patients and controls were compared according to the matched design for the first pregnancy. The generalised estimating equation method was used when analysing repeated pregnancies, analysing the data unmatched and adjusting for covariates (maternal age, smoking and household income) and repeated pregnancies. The generalised estimating equation method is valuable to use when observations within the same group can be expected to be correlated, such as repeated pregnancies in the same woman. In paper III, Cox proportional hazard regression was used to analyse the relative risk of cancer in the exposed vs. unexposed cohort and presented as hazard ratios (HRs) with 95% CIs. A two-sided  $P$ -value  $< 0.05$  was considered statistically significant. Statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc.) (Paper I), SAS statistical software (SAS Institute, Inc., Cary, NC, USA) (Papers II, III) and Statistica, Statsoft version 9.0 (Tulsa, OK, USA) (Paper IV).

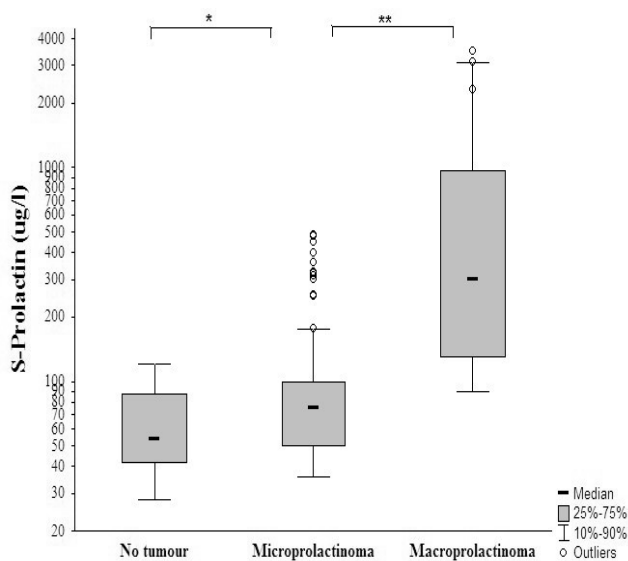
### **3.4.5 Ethical approval**

The studies were approved by the Ethics Committee of the Karolinska University Hospital (Papers I, II) and the Stockholm Regional Ethical Review Board (Papers III, IV).

## 4 RESULTS

### 4.1 CLINICAL FOLLOW-UP OF HPL PATIENTS (PAPER I)

The mean age at diagnosis was 31 ( $\pm$  9.5) years in the 271 women with HPL. Menstrual disturbances were present in 87% of women in reproductive age and 47% had galactorrhea. PRL levels were significantly higher in women with menstrual disturbances than in women who reported normal menstrual function (78 [25-3500] vs. 45 [27-250]  $\mu\text{g/l}$ ,  $P < 0.001$ ). Furthermore, PRL levels were higher in patients with amenorrhea vs. oligomenorrhea (84 [25-3500] vs. 63 [25-500]  $\mu\text{g/l}$ ,  $P = 0.007$ ). No difference in PRL levels was found between patients with or without galactorrhea (77 [25-1360] vs. 70 [25-3500]  $\mu\text{g/l}$ ,  $P = 0.578$ ). Eight patients (4.5%) had visual field defects at diagnosis. At radiological examination, 63% ( $n=160$ ) of the patients had microprolactinomas, 8% ( $n=21$ ) had macroprolactinomas and 29% ( $n=74$ ) had no visible tumour. Median PRL levels were 72 (25-3500)  $\mu\text{g/l}$  at diagnosis and differed between the three groups according to radiological findings (Fig. 6).



**Figure 6.** Serum PRL levels (logarithmic scale) according to radiological findings at diagnosis (no tumour [ $n=74$ ], microprolactinomas [ $n=160$ ] and macroprolactinomas [ $n=21$ ]) in women with HPL. \*  $P = 0.005$  (no tumour vs. microprolactinoma) \*\*  $P = 0.0001$  (microprolactinoma vs. macroprolactinoma)

A majority of the patients that received DA agonists had been treated with Brc; 71% had only received Brc and 95% had received Brc at any time. Mean dose of Brc was 4.8 ( $\pm 3.9$ ) mg per day; the mean dose of quinagolide was 95.6 ( $\pm 66.3$ )  $\mu$ g per day and Cab 0.5 ( $\pm 0.2$ ) mg per week. Thirty-seven per cent of the patients reported side effects (nausea, vomiting, fatigue, postural hypotension, dizziness or headache) of Brc and 38% of quinagolide. Side effects of Cab could not be evaluated because of insufficient data.

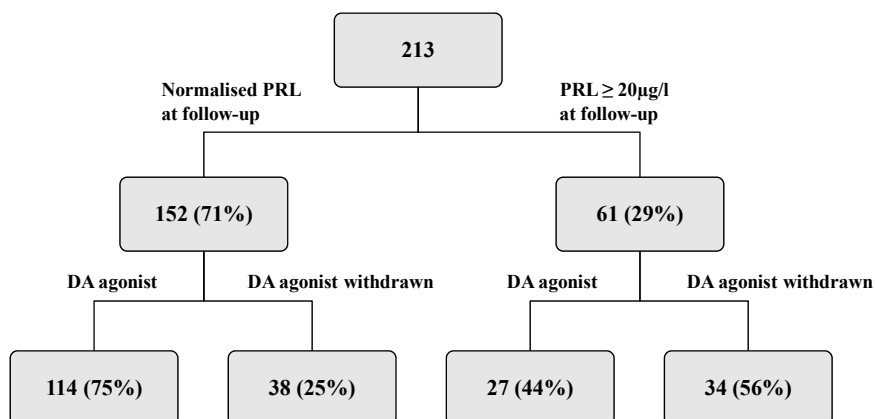
The median period of observation in all patients was 9.3 (0.5-29) years. At follow-up, normalised menstrual function had been obtained in 94% and resolution of galactorrhea in 94%. Furthermore, a significant decrease in PRL levels from 72 (25-3500)  $\mu$ g/l to 14 (0-89)  $\mu$ g/l in the whole patient cohort ( $P < 0.001$ ) and 72% of the patients had a normal PRL level. The treatment results did not differ according to radiological findings at diagnosis or if the patients were divided into groups based on observation time (Table 5).

**Table 5.** PRL levels at clinical follow-up in women with HPL in relation to radiological findings at diagnosis and duration of disease

	PRL ( $\mu$ g/l) Median (range)	P - value
All patients	14 (0-89)	0.370*
Microadenomas	13 (0-85)	
Macroadenomas	16 (0-54)	
Idiopathic HPL	14 (2-89)	
Duration of disease (months)		0.483*
6-59 ( $n=46$ )	9.0 (1-78)	
60-119 ( $n=74$ )	14 (2-45)	
120-179 ( $n=39$ )	17 (2-85)	
180-239 ( $n=30$ )	12 (0-89)	
240-299 ( $n=45$ )	14 (1-69)	
$\geq 300$ ( $n=9$ )	11 (0-52)	

\*Based on Kruskal-Wallis test between groups.

In the solely medically treated patients 152 of 213 patients (71%) had a normalised PRL level at follow-up; of these patients 38 (25%) had stopped medical treatment (Fig. 7). Altogether, 38 of 213 patients (18%) had a normal PRL level without medical therapy at follow-up in all patients. The effect of DA agonists on tumour size was analysed in 83 medically treated patients with a micro- or macroadenoma at diagnosis and who had a computed tomography (CT) or MRI scan both at diagnosis and follow-up. In total, 66 of 83 patients (80%) showed complete disappearance or partial reduction in tumour mass. Three patients had an increase in tumour size; all three had a microadenoma and tumour progression was due to non-compliance in two patients and side effects of Brc in one patient.



**Figure 7.** Outcome of medical treatment in 213 women with HPL at follow-up. Data are presented as number of patients (%). Data were not available in 24 patients and 3 patients were excluded from the analysis because of an observation time of less than 6 months.

Totally, 17 patients were operated on (as primary treatment in 3 patients and because of side effects or lack of response to medical therapy in 14 patients). The patients were followed after surgery for a median time of 11 (1.7-26) years. Initial cure was achieved in 10 patients (59%) and 1 patient relapsed after 34 months, giving a cure rate of 53% in both micro- and macroadenomas (56% in microadenomas and 50% in macroadenomas). Two of the surgically treated patients also received radiation therapy after surgery (external radiation and SR, respectively), none of these two patients were cured (normal PRL levels without medical treatment) at follow-up. However, one patient (surgery and external radiation therapy) had normalised PRL levels with Brc treatment at follow-up. The other patient (surgery and SR) had nearly normalised PRL levels (PRL 29 µg/l) without medical therapy, at follow-up. Five patients developed pituitary insufficiency after surgery (one of these patients had also received external radiation therapy). In total nine patients received radiation therapy, two patients received external radiation therapy and seven patients SR. In the seven patients treated with gamma knife SR, three had normalised PRL level at follow-up without concomitant medical treatment. These three patients received SR because of intolerance to DA agonists.

A benign course was seen in the seven women without treatment. One patient had a microprolactinoma at diagnosis; in the remaining patients no visible pituitary adenoma was found. Six patients had menstrual disturbances at diagnosis, which normalised spontaneously in four patients and no information was available in two patients. Median PRL levels decreased from 36 (25-70) µg/l to 12 (6-15) µg/l.

## 4.2 PARITY AND PREGNANCY OUTCOME IN HPL PATIENTS (PAPER II)

During follow-up, 162 deliveries by 76 women were found in the patient cohort after diagnosis of HPL and 1220 deliveries by 519 women in the control group. The mean age at first live birth in the patients was 29.0 ( $\pm 4.4$ ) years and in controls 27.2 ( $\pm 4.8$ ) years, ( $P = 0.0002$ ). HPL patients had reduced parity, which was mainly due to more nulliparous women and less women with more than two children (Table 6). Parity was inversely related to HPL status ( $P$  for trend = 0.0009). The OR of having three or more children was 0.31 (95% CI 0.16-0.60) in the patient cohort.

**Table 6.** Comparison of parity in HPL patients and controls, restricted to subjects born after January 1, 1953. Odds ratios (ORs) for the association between HPL and parity are presented.

	<b>Patients</b> (n=174) <i>n (%)</i>	<b>Controls</b> (n=696) <i>n (%)</i>	<b>OR</b> (95% CI) <sup>†</sup>
Nullipara	75 (43)	244 (35)	1
1-para	33 (19)	117 (17)	0.86 (0.53-1.39)
2-para	54 (31)	223 (32)	0.70 (0.45-1.09)
$\geq 3$ -para	12 (7)	112 (16)	0.31 (0.16-0.60)

<sup>†</sup>  $P$  for trend = 0.0009

No significant difference was found in pregnancy or neonatal characteristics between the HPL cohort and comparison subjects in the first child as well as in all pregnancies regarding the following variables: previous miscarriages, weeks of gestation, birth weight, Apgar score (5 min), gender, induction of labour, vacuum extraction, forceps delivery, caesarean delivery or postpartum stay for the mother. These data were adjusted for factors that we know can interfere with pregnancy outcomes, i.e. maternal age, smoking, household income (as a measure of socioeconomic status) and parity (in all pregnancies). Duration of neonatal care in all pregnancies was 0.9 days shorter in the HPL cohort compared with controls (95% CI -1.7 to -0.1). Congenital malformations of the newborns were found in 1.2% ( $n=2$ ) in the HPL cohort and in 3.0% ( $n=37$ ) in the controls. The two malformations found in the offspring of HPL patients were choanal atresia and dislocation of the hip.

## 4.3 CANCER RISK IN HPL PATIENTS (PAPER III)

The aim of this study was to assess the risk of cancer in a large cohort of women and men with a diagnosis of HPL. In total, 969 HPL patients (668 women and 301 men) were followed for an average of 13.2 ( $\pm 5.3$ ) years and 9618 comparison subjects for 13.9 ( $\pm 4.9$ ) years, yielding 12 822 and 134 099 accumulated person-years of follow-up, respectively. Mean age at first recorded diagnosis of HPL was 40.8 ( $\pm 16.4$ ) years. Before the diagnosis of HPL, 40 cases of cancer were observed in the patients and 191 cases in the comparison subjects.



In Table 7, the relative risks of cancer associated with HPL in the whole patient cohort are presented. During follow-up, 73 malignant tumours were identified in the patients and 660 tumours in the comparison subjects, which yielded an increased overall cancer risk in HPL patients (HR 1.31; 95% CI 1.02-1.68). The excess risk was mainly attributed to an increased tumour incidence in females ( $P$  for homogeneity between sexes = 0.020). Twelve patients with breast cancer were identified during the follow-up in female patients (HR 1.09; 95% CI, 0.60-1.99) and none in male patients. A significantly reduced risk of prostate cancer was observed among HPL patients (HR 0.40; 95% CI 0.16-0.99). Furthermore, increased risks of upper gastrointestinal (GI) cancer (HR 3.69; 95% CI 1.70-8.03) in both males and females and haematopoietic cancer in females (HR 3.51; 95% CI 1.06-11.6) were found. The risk of cancer in the hospital cohort and register cohort was also analysed separately, where the overall cancer risk remained significantly increased only in the register cohort.

**Table 7.** Hazard ratios (HRs<sup>1</sup>) for the relative risks of cancer associated with hyperprolactinemia (HPL) in Sweden.

Cancer site (ICD-7)	Observed cancers <sup>2</sup>		HR (95% CI)
	HPL patients (n=969)	Comparison subjects (n=9618)	
All cancer (140-209)			
All	73	660	<b>1.31</b> (1.02-1.68)
Female	45	317	<b>1.72</b> (1.25-2.36)
Male	28	343	0.94 (0.63-1.40)
Upper GI (140-151)			
All	9	27	<b>3.69</b> (1.70-8.03)
Female	4	8	<b>5.34</b> (1.56-18.3)
Male	5	19	<b>2.95</b> (1.07-8.14)
Lower GI (153,154)			
All	12	89	1.67 (0.89-3.12)
Female	6	35	2.36 (0.96-5.81)
Male	6	54	1.28 (0.53-3.06)
Lung (161-163)			
All	3	64	0.49 (0.15-1.56)
Female	2	28	0.81 (0.19-3.44)
Male	1	36	0.27 (0.04-1.97)
Breast (170)			
Female	12	118	1.09 (0.60-1.99)
Male	0	1	
Cervix (171)	3	9	3.51 (0.93-13.3)
Corpus uteri (172)	4	26	2.25 (0.76-6.70)
Ovarial (175)	2	18	1.22 (0.28-5.35)
Prostate (177)	5	136	<b>0.40</b> (0.16-0.99)
Hematopoietic (200-209)			
All	5	42	1.81 (0.68-4.81)
Female	4	16	<b>3.51</b> (1.06-11.6)
Male	1	26	0.64 (0.08-4.85)

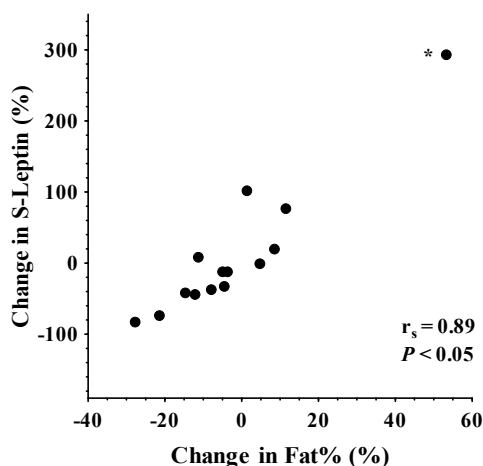
<sup>1</sup>Bold type: 95% CI does not include 1.00

<sup>2</sup>Based on 12822 person-years in the patients and 134099 person-years in the comparison subjects  
ICD=International Classification of Diseases; CI=confidence interval; GI=gastrointestinal

#### 4.4 METABOLIC EVALUATION IN HPL PATIENTS (PAPER IV)

In this observational study of 14 consecutive patients with newly diagnosed prolactinomas we compared different anthropometric and metabolic variables before and after treatment with DA agonists. At diagnosis, none of the women were obese ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ), whereas three of the six men were obese. Elevated WHR ( $\geq 0.85$  in women and  $\geq 0.90$  in men) was found in one of seven females (1 missing) and in five of six males.

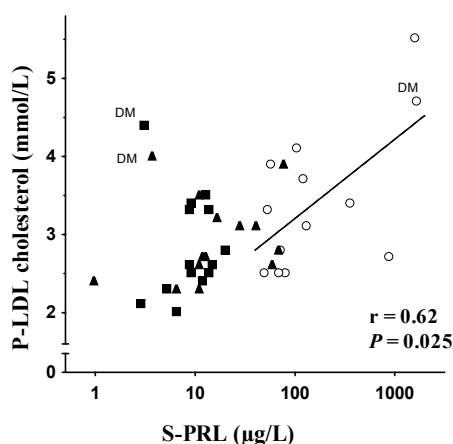
In men a significant decrease in body weight (95.6 [80.7-110.1] to 83.4 [77.8-99.1] kg,  $P = 0.046$ ), BMI (27.6 [23.4-32.9] to 25.4 [22.8-29.6]  $\text{kg/m}^2$ ,  $P = 0.046$ ), waist circumference (101 [91-113] to 95 [89-102] cm,  $P = 0.028$ ) and body fat% (21.9 [17.6-31.9] to 18.3 [15.6-28.0] %,  $P = 0.028$ ) was observed after 6 months of treatment. In contrast, no changes in anthropometric variables were seen in women. Per cent reduction in weight was positively correlated to per cent reduction in PRL levels in the whole patient group ( $r_s = 0.73$ ,  $P < 0.05$ ). No correlation was found between per cent change in weight and Brc dose at 6 months ( $r_s = -0.37$ ,  $P = \text{NS}$ ). Leptin values decreased significantly after 6 months of DA agonist therapy in men (6.1 [2.1-19.6] to 2.8 [1.4-4.3]  $\mu\text{g/l}$ ,  $P = 0.046$ ), which was probably due to the close positive correlation between per cent change in leptin and body fat% ( $r_s = 0.89$ ,  $P < 0.05$ ,  $n = 14$ ) (Fig. 8).



**Figure 8.** Correlation between per cent change in S-Leptin and Fat% after 6 months of DA agonist treatment when compared with diagnosis in 14 prolactinoma patients. If the outlier (\*) is excluded;  $r = 0.80$ ,  $P < 0.001$ .

### Lipid profile

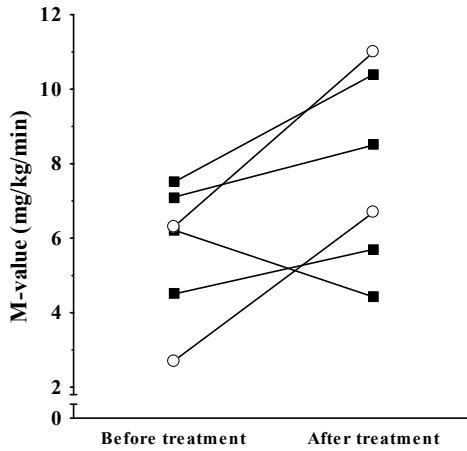
Lipid values were within the reference range at diagnosis in a majority of our patients. The relation between LDL cholesterol and serum PRL levels are presented in Figure 9. A positive association between LDL cholesterol and PRL levels at diagnosis was found ( $r = 0.62$ ,  $P = 0.025$ ). After 2 months of DA agonist treatment, a significant reduction in total cholesterol ( $5.4 [\pm 1.0]$  vs.  $4.8 [\pm 0.7]$  mmol/l,  $P = 0.002$ ) and LDL cholesterol ( $3.4 [\pm 0.9]$  vs.  $2.9 [\pm 0.6]$  mmol/l,  $P = 0.003$ ) was found when compared with diagnosis. There was no correlation between LDL cholesterol and dose of Brc at 2 or 6 months ( $r = 0.02$ ,  $P = 0.958$ ;  $r = 0.04$ ,  $P = 0.901$ , respectively) or between per cent reduction in LDL cholesterol and Brc dose ( $r = -0.18$ ,  $P = 0.586$ ). In addition, no association was observed between reduction in LDL cholesterol and weight loss ( $r = 0.41$ ,  $P = 0.157$ ) or change in fat% ( $r = 0.33$ ,  $P = 0.274$ ). HDL cholesterol or TGs did not change after treatment.



**Figure 9.** The relation between P-LDL cholesterol and S-PRL in prolactinoma patients at diagnosis (O) and after 2 (▲) and 6 (■) months of DA agonist therapy. The regression line for the correlation between P-LDL cholesterol and S-PRL at diagnosis (O) is presented (n=13). The patient with diabetes at diagnosis is marked DM.

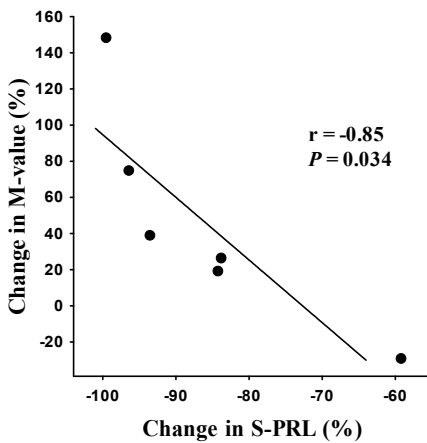
### Glucose, insulin and insulin sensitivity

One male patient had repeated elevated fasting glucose levels at diagnosis and was therefore considered to have DM type 2. The other patients had normal fasting glucose values. Using the HOMA-IR and the value of 2.77 as a threshold for insulin resistance [199], one woman and two men had values indicating increased insulin resistance at diagnosis. Fasting glucose, HbA<sub>1c</sub>, insulin, C-peptide levels and HOMA-IR did not change after normalisation of PRL levels at 6 months. Insulin sensitivity evaluated by the euglycemic clamp technique in six patients before and after 6 months of DA agonist therapy tended to improve: M-value at baseline  $5.7 (\pm 1.8)$  and at 6 months  $7.8 (\pm 2.6)$  mg/kg/min ( $P = 0.083$ ) (Fig. 10). No clear consensus has been established regarding the cut-off levels of normal insulin sensitivity according to euglycemic clamp. However, an M-value  $< 6$  mg/kg/min has been considered as an insulin-resistant state [205].



**Figure 10.** M-value in four women (*filled square*) and two men (*open circle*) with HPL at diagnosis and at normalised PRL levels after 6 months of DA agonist treatment ( $P = 0.083$ ).

A significant correlation between per cent improvement in insulin sensitivity and per cent decrease in PRL levels ( $r = -0.85$ ,  $P = 0.034$ ) was observed (Fig. 11). In the five patients treated with Brc who performed the clamp the association between per cent improvement in insulin sensitivity and reduction in PRL levels remained, but there was also an association to Brc dose ( $r = 0.92$ ,  $P = 0.029$ ), which in turn, was associated with reduction in PRL levels ( $r = -0.90$ ,  $P = 0.035$ ).



**Figure 11.** Correlation between per cent change in M-value and in S-PRL after 6 months of DA agonist treatment when compared with diagnosis in the six prolactinoma patients who performed the clamp.

We found no association between total adiponectin and PRL levels at diagnosis ( $r = -0.43$ ,  $P = 0.123$ ), no change in serum adiponectin concentrations before and after PRL normalisation (6.3 [3.8-14.0] mg/l vs. 6.2 [3.4-12.0] mg/l,  $P = 0.250$ ) and no association between per cent change in M-value and per cent change in adiponectin levels ( $r_s = 0.37$ ,  $P = \text{ns}$ ). Actually, two male patients with very high PRL levels at diagnosis (9600 and 1630  $\mu\text{g/l}$ ) had the highest adiponectin levels (6.4 and 6.5 mg/l, respectively) among men. S-IGFBP-1 levels did not change between the hyperprolactinemic and normalised state. Females had higher S-IGFBP-1 levels than males (48 [13-97]  $\mu\text{g/l}$  vs. 15 [8-39]  $\mu\text{g/l}$ ,  $P = 0.001$ ), which is in accordance with previous findings [203].

## 5 DISCUSSION

### 5.1 METHODOLOGICAL CONSIDERATIONS

#### *Considerations of the patients and study design in Papers I, II*

The patients who were classified as idiopathic HPL might be a somewhat heterogeneous group. Idiopathic HPL may reflect a microprolactinoma that is too small to detect with available radiological imaging techniques or possibly an altered DA tonus [37]. In our group of patients with non-tumoral HPL, many had their diagnosis in the early 1970s or 1980s and almost one third had an X-ray examination of the sella region or encephalogram. Consequently, it was difficult to detect small adenomas. In addition, even with early CT or MRI scans, pituitary adenomas smaller than 4-5 mm were not consistently detected. Conversely, in recent years we have become aware of macroprolactin. We cannot exclude the possibility that there are patients with macroprolactinemia in the group with no visible tumour because further analysis was not performed to distinguish between the different forms of PRL. On the other hand, a majority of our patients had clinical symptoms associated with HPL and normalised their PRL levels after treatment, which is not the case with macroprolactinemia. In an attempt to overcome this potential problem, the patients were analysed as a whole cohort but also divided into three groups based on radiological findings at diagnosis. However, we did not find any major differences in treatment results between these groups.

The ideal study design in Paper I, would have been a structured prospective follow-up study. However, a retrospective chart review with a clear research question is a valuable method allowing for long-term follow-up, but the validity is hampered by the retrospective assessment of the study variables. The major limitations include incomplete and missing documentation, lack of structure and uniformity in following patients and problems in controlling for bias and confounders [206].

#### *Consideration of the patients and study design in Paper III*

Some of the patients in Paper III were identified via the use of the Swedish Patient Registry. When using register data to identify patients with a specific diagnosis, there is a risk of misclassification if the diagnosis is not confirmed via the patient file. To increase the specificity of the prolactinoma diagnosis, we excluded patients with a diagnosis of acromegaly. This is because an average of one third of acromegalic patients have a concomitant increase in PRL levels [207] and acromegaly has been associated with an increased cancer risk [208].

During the study period, we could not identify patients with HPL who were handled only as out-patients. We assumed that a majority of the patients diagnosed between 1987 and 1995 were hospitalised at least once during the investigation of HPL because of current practice at that time. If not, this might have led to the inclusion of patients with a more severe disease and potentially other co-morbidities or exposures that might increase cancer risk and thus overestimate the risk of cancer in our study. Apart from the matching variables, we were not able to adjust for other factors associated with risk of cancer, such as smoking, alcohol consumption and BMI. In the context of our findings, the increased risk of upper GI cancer (head and neck cancer in particular) could theoretically be confounded by smoking. However, we have no indication of an increased smoking prevalence among PRL patients, which would

have been required for bias toward increased risks. An indirect sign of smoking in our cohort was the tendency of reduced lung cancer risk in patients, indicating no excess smoking. If anything the contrary is true. Moreover, as always in observational settings, unknown confounders could not be controlled for. Finally, despite our HPL cohort being one of the largest to date, statistical power was limited and negative results must be interpreted with particular caution.

#### ***Consideration of the patients and study design in Paper IV***

In general, recruitment of consecutive patients with newly diagnosed prolactinomas should be unbiased. However, inclusion of patients from a specialised out-patient clinic might lead to enrolment of patients with more severe symptoms and larger tumours. This probably explains why we have a higher proportion of male patients than that found in the literature [35]. Our study sample was heterogeneous regarding the presence of hypogonadism at diagnosis and one could stress stricter inclusion criteria in the study design. Ideally, to be able to overlook the effect of gonadal hormones on metabolic parameters, all patients should have been on stable hormone substitution. However, such an approach would oppose current treatment recommendations for prolactinomas [50] and result in a delay of treatment start.

The patients in the present study were their own controls. One could argue that lack of a control group could preclude the possibility of defining the effects on metabolism of PRL vs. other factors. However, previous studies have demonstrated that treatment with Brc-QR in non-HPL patients with DM type 2 [187] or in obese women [188] results in improved glycemic control and lipid profile, with a simultaneously significant decrease in PRL levels within the normal range. Thus, a control group treated with Brc would probably not have been able to clarify whether the improvement in metabolic parameters was due to a reduction in PRL levels or to effects mediated by DA agonists. Furthermore, treatment of healthy controls with Brc would not have been feasible from an ethical point of view. A proper control group could have been DA agonist naïve patients with prolactinomas and to evaluate metabolic variables before and after normalisation of PRL levels by surgery, preferably microprolactinomas in order to ascertain a high surgical success rate. However, the first-line treatment for prolactinomas is medical therapy [59]. Finally, it cannot be excluded that other factors (e.g., diet and exercise) changed during the study period, although the patients were urged to keep a stable lifestyle (except for the male patient with DM).

## **5.2 INTERPRETATIONS AND IMPLICATIONS**

### **5.2.1 Clinical presentation (Papers I, IV)**

In Paper I, a majority of the 271 women with HPL presented with menstrual disturbances, microadenomas and modestly elevated PRL levels (median PRL 72 µg/l). The few female patients in Paper IV were similar to the female patients in Paper I: all had microprolactinomas and the median PRL level at diagnosis was 72 µg/l. In contrast, all men in Paper IV had macroprolactinomas and highly elevated PRL levels (median PRL 1260 µg/l). The predominance of macroprolactinomas in our male patients is in accordance with previous studies [45, 48, 209]. The reason for the high proportion of large tumours in men is still not fully understood, but could be related to a greater proliferative potential of the tumours in men [46-48, 210].

We found a difference in PRL levels between female patients reporting normal menstrual function, oligomenorrhea or amenorrhea, with increasing PRL levels associated with the severity of menstrual impairment (Paper I). In patients with elevated PRL levels, an impaired LH pulsatility has been observed [39, 211], possibly because PRL inhibits GnRH release [40]. The reason for the more severe menstrual disturbances in women with highly elevated PRL levels may be a more pronounced inhibition of GnRH neurons with higher PRL levels. Furthermore, all our male patients had hypogonadism symptoms (e.g., decreased libido and impotence) prior to diagnosis. Of importance is that two of six male patients had testosterone substitution already at referral to our department, which was initiated before the prolactinoma was diagnosed. This observation points to the importance of evaluating PRL levels in men with hypogonadism.

The presence of galactorrhea should lead to control of PRL levels to confirm or deny the diagnosis of HPL. However, galactorrhea is not a mandatory finding in HPL patients and its prevalence is known to vary substantially: 30-89% in females [57, 212, 213] and 19-36% in males [69, 209]. About half of our female patients presented with galactorrhea (Papers I and IV) whereas none of the male patients had galactorrhea (Paper IV). No association between PRL levels and galactorrhea was found; nor did we find any significant differences in the presence of galactorrhea between multiparous and nulliparous women (Paper I). The reason for the diverse prevalence of galactorrhea reported in the literature might reflect whether registration was based on anamnesis or on breast examination. Moreover, individual susceptibility could play a potential role. Finally, not only PRL is necessary for galactopoiesis, i.e. oestrogens, progesterone, cortisol and several other hormones and growth factors are involved [11].

### **5.2.2 Outcome of treatment (Paper I)**

In the early 1970s, measurement of human PRL in serum [3] became available in clinical practice and soon afterwards Brc was introduced to treat HPL patients. Many patients with HPL are treated life-long with DA agonists, but when initiating this study the literature contained sporadic reports on long-term follow-up. We therefore conducted this retrospective study with the longest reported follow-up in the literature so far. We found a good result of treatment in this cohort of 271 women with HPL, with restoration of menstrual function in 94% of the women and resolution of galactorrhea in 94%. In the solely medically treated patients, 71% had normal PRL levels at follow-up and 80% had partial reduction or complete disappearance of the pituitary tumour, confirming previous studies evaluating treatment efficacy [68, 69, 72]. The results in our study were similar in patients with non-tumoral HPL, microprolactinomas and macroprolactinomas. Furthermore, treatment results were not related to duration of follow-up, suggesting a prompt and lasting effect of DA agonists in a majority of the patients with HPL. In 2009, Kars *et al.* evaluated 72 patients with macroprolactinomas showing that 65% of the patients had normalised PRL levels by DA agonist treatment after a mean observation time of 10.2 ( $\pm 6.1$ ) years [214]. In one third of the patients additional surgery was necessary due to intolerance or resistance to DA agonists [214].

Nearly all of our patients were treated with Brc because only Brc was available when most patients started DA agonist therapy and in accordance with our treatment practice. We did not find any unexpected adverse side effects of the different DA agonists. However, the potential association between cardiac valvulopathy and Cab [79, 80] was not a known concern at that time and consequently echocardiography was not performed on a regular basis.



One potential problem in this study was that the patients were identified retrospectively without complete information about dropouts. However, patients who dropped out from follow-up are probably those with less complicated disease and our finding of a favourable outcome of treatment in this cohort, may still be an underestimation. On the other hand, the number of patients who dropped out of clinical follow-up because of death or cure is probably limited since the age at diagnosis is low and, according to our treatment guidelines, we continue follow-up in a majority of the HPL patients.

An important issue regarding the treatment of HPL patients concerns when to withdraw medical therapy. One of the first studies evaluating the results after discontinuation of Brc treatment demonstrated that PRL remained normal in only 2 of 30 patients (7%) [68]. In the present study, 18% (38 of 213) of the solely medically treated patients had a normal PRL level at follow-up after DA agonist withdrawal. This figure is consistent with a recent meta-analysis of 19 studies in which 21% had persisting normoprolactinemia after discontinuation of DA agonist treatment [215]. Dekkers *et al.* found that the probability of treatment success was highest when Cab was used for at least 2 years [215]. Furthermore, there are data indicating that elevated PRL levels may resolve after pregnancy [97-99] and menopause [216]. However, because of limited information in the patient files, we could not evaluate different predictive factors that would indicate a successful withdrawal of medical therapy.

In the surgically treated patients, a long-term cure rate of 53% was observed, which is in accordance with previous studies evaluating surgical success in both micro- and macroprolactinomas [51, 217]. Gamma knife SR normalised PRL levels in three of seven patients and in the remaining patients PRL levels decreased. Based on our experience and other studies [87, 88], we consider SR an important adjunctive therapy in patients intolerant or resistant to DA agonists. However, further studies are warranted to evaluate efficacy as well as complications.

### 5.2.3 Parity and pregnancy outcome (Paper II)

HPL women were about 2 years older than controls at their first term-pregnancy. Furthermore, women treated for HPL had a reduced parity in that more women in the HPL cohort were nulliparous and fewer women had more than two children. The probability of having more than two children was reduced by 69% in the HPL cohort. The explanation for these findings might be that many women with HPL are diagnosed during an investigation of infertility and treatment for HPL is needed before they can get pregnant [37]. Thus, these patients might be delayed in their childbearing and therefore have a shorter fertility period and are possibly less likely to have many children. However, if these patients are recruited because of being involuntarily childless, this may lead to a selection of women wishing to become pregnant. Such selection would bias the study to overestimate parity among the patients and thus strengthen our finding of a reduced parity associated with HPL. In a summary by Weil a pregnancy rate of about 80% could be achieved after Brc treatment in women with HPL wishing to become pregnant [103]. However, this is the first study evaluating overall parity in a large group of women treated for HPL compared with controls.

Because of the register-based design we could not retrieve information about dose or duration of Brc treatment when inducing pregnancy. However, the therapeutic safety of Brc in early pregnancy has been well-documented in previous studies [101-104]. Concerns have been raised whether the relative hypoprolactinemia induced by continuous Brc therapy could be harmful for the luteo-placental function. To our knowledge, no such effects have been

reported, however [95, 103]. In addition, human decidua secrete PRL independent of DA agonists and normal levels of PRL are present in amniotic fluid during Brc treatment [20]. In the current study, we have information about two patients who were treated with Brc (1.25-5 mg per day) throughout five pregnancies because of macroprolactinomas and a potential risk for tumour enlargement. In accordance with previous reports [95, 106], all five pregnancies were uneventful and had a favourable outcome, i.e. the mothers delivered healthy babies.

Characteristics during pregnancy or delivery did not differ between patients and comparison subjects in our study. Interestingly, there were no significant differences between patients and controls in the odds of induction of labour, vacuum extraction, forceps delivery or caesarean delivery, which indicates that the complex process of parturition is not disturbed in HPL women. Furthermore, characteristics of the newborn did not differ, indicating no excess risk in the offspring of HPL women. The female-to-male ratio in our data was non-significantly in favour of more female babies: 53% female offspring in the patients compared with 48% in the controls. However, the adjusted OR of having a female baby was 1.2 (95% CI 0.8-1.7). In contrast, Raymond *et al.* found a slight predominance of male babies (55%) in children born to mothers treated with Brc during a part (n=41) or throughout (n=23) their pregnancy [105]. We used the length of the in-patient period for the newborns as an indirect marker of a complicated delivery or increased morbidity in the newborn. However, in these data, we did not find any such association. In contrast, we found that the adjusted difference in days of neonatal care in the offspring of HPL women was 0.9 days shorter than for the offspring in the controls. The reason for the shorter period of care is not known, but the HPL patients were older than the controls at their first delivery and there has been a change in praxis regarding how long mothers and children stay at hospital after delivery. Since the 1970s, the number of days that mothers stay at hospital after vaginal delivery has decreased from a mean duration of 6 days to 2 days [218].

No increased risk of congenital malformations of the live newborns in the patient cohort was found when compared with the comparison group. However, when interpreting these results, it is important to consider that this study was underpowered to detect differences in rare outcomes. If the birth register is used to study malformations, only congenital malformations known at discharge from the neonatal unit will be identified and therefore these figures may be underestimated in both patients and controls.

#### **5.2.4 Cancer risk (Paper III)**

From experimental models, there is accumulating evidence that PRL might play a role in tumorigenesis in a variety of human cancers [112]. Data on cancer risk in HPL patients, however, are sparse and imprecise. In Paper III, an increased overall cancer risk by 30% was seen in HPL patients when compared with matched individuals in the general population. This finding is in contrast to the only available study of overall cancer risk in prolactinoma patients, where no increased risk of malignancy was demonstrated [114]. The reason for this discrepancy is not clear, but the latter study was based on a much more limited number of patients (n=98) and a shorter follow-up (mean 3.6 years), resulting in reduced power to detect rare outcomes such as malignant tumours.

A major concern regarding PRL and cancer has been the potential growth-promoting effect of PRL on breast and prostate cancer. In Paper II, we found that HPL women were older at first-term birth and had a reduced parity compared with controls, which are known risk factors for

breast cancer [219]. In contrast, physiological states of elevated PRL levels (e.g., multiple pregnancies and prolonged breast feeding) are associated with reduced breast cancer risk. In the present study, we found no increased risk of breast cancer in HPL women. However, because of the relatively low number of breast cancer cases, we are not able to preclude an increased risk. Yet, our results are strengthened by a recent study showing no increased breast cancer risk in 1342 women treated for HPL [129].

Animal studies have indicated that PRL regulates prostate growth and proliferation and thus theoretically may affect carcinogenesis in the prostate [220, 221]. To date, only one study has examined the effects of HPL on human prostate in men with prolactinomas, demonstrating a reduced prostate size compared with controls which probably are due to the reduced testosterone levels found in the prolactinoma patients [135]. In our study, a reduced risk of prostate cancer by 60% was observed in HPL men, which is the first study to indicate such an association.

For several reasons, care is required when interpreting our results regarding breast and prostate cancer. First, oestrogens are a known risk factor for breast cancer [116] and androgens are thought to be instrumental in the development of prostate cancer [130]. A potential increased risk of breast and prostate cancer associated with elevated PRL levels may be counter-balanced by the frequently observed hypogonadism in HPL patients with lower exposure to oestrogens and androgens. On the other hand, our finding of a reduced risk of prostate cancer in HPL men might, in addition to chance variation, be related to low androgen exposure. Second, the treatment of choice in patients with HPL is DA agonists, which are highly effective in normalising PRL levels [69, 72, 77]. In the hospital cohort, 88% of the patients had a normal PRL level after therapy with DA agonists. We have no information about PRL levels in the register cohort, but one might assume that a majority had normal PRL levels during follow-up. In many patients, however, there is a delay in diagnosis and the patients might therefore have been exposed to elevated PRL levels for several years [46, 48]. Third, regarding men with HPL, androgen status is generally monitored and if testosterone levels do not normalise, androgen replacement therapy might be introduced. In such cases, prostate-specific antigen levels may be followed regularly, potentially resulting in increased detection of early prostate cancer [222]. Such vigilance would, however, bias the estimates towards a higher incidence of prostate cancer in the patient cohort and thus cannot be used to explain our finding of a reduced risk.

The overall increased risk of cancer in HPL patients in our study was mainly related to the augmented cancer risk in women. The reason for this gender difference is not known. However, prostate cancer, the most common cancer form in Swedish men [115], was significantly reduced in our HPL men and this might have a substantial impact on the total number of cancer cases in the male patients.

An almost four-fold increased risk of upper GI cancer was seen in the whole patient cohort as well as in women and men analysed separately. These tumours were mainly head and neck cancer, in contrast to the comparison subjects where a majority had cancer in the oesophagus or stomach. This finding is novel and unexpected for a non-pre-specified cancer form and should thus be interpreted with extra caution. Furthermore, the finding is based on few cases and the CIs are wide. However, elevated PRL levels have been observed in patients with cancer of the tongue, where PRL was an independent prognostic risk factor of survival [137]. Thus, it could be speculated that PRL might act as a growth-promoting factor on tumour

cells. An increased risk of hematopoietic cancers was observed in women. We cannot rule out that our observation might be due to chance because of the inconsistency between men and women and because of the low precision with wide CIs. Nevertheless, the finding is intriguing in terms of PRL's potential role in stimulating lymphocytic proliferation [223], its anti-apoptotic effects on lymphoid cells [224] and its association with acute myeloid leukaemia [140] and multiple myeloma [139].

A tendency of an increased risk of cervical cancer was noted in our HPL cohort (HR 3.51 [95% CI 0.93-13.3]). The role of PRL in cancer of the cervix remains elusive and no previous study has addressed the issue of whether prolactinoma patients have an increased risk of cervical cancer. Elevated serum PRL levels have been observed in patients with cervical carcinoma [225] but there are contradictory findings [226]. In addition, treatment with Brc in non-HPL women with advanced cancer of the cervix has not been successful [227]. The relevance of PRL and cancer risk in patients receiving anti-psychotics is unclear. There are reports of an increased breast cancer risk in DA antagonist-treated patients [228]; however, the findings are contradictory and inconclusive [229] and the relationship to PRL levels is unknown. In addition, patients receiving anti-psychotics may have other co-morbidities such as overweight, smoking and low physical activity that may predispose such patients to cancer. Furthermore, little is known about the potential influence of DA agonist treatment on cancer risk. However, in a recent meta-analysis, evaluating cancer risk in patients with Parkinson's disease, who often are treated with very high doses of DA agonists, a decreased risk of both smoking-related and non-smoking related cancer was found [230].

### **5.2.5 Metabolic evaluation (Paper IV)**

Although HPL is the most common hypothalamic-pituitary disorder the metabolic consequences of elevated PRL levels in humans and the effect of medical treatment remain obscure.

#### **Anthropometric variables**

In agreement with others, we found that obesity was mostly seen in our male prolactinoma patients [154, 156]. These obese men had an unfavourable metabolic profile at diagnosis, i.e. elevated WHR ( $>0.90$ ), increased waist circumference ( $>102$  cm), elevated TG levels, low levels of HDL cholesterol (in two men) and insulin resistance. All these parameters are components of the metabolic syndrome, which is a well-known risk factor for cardiovascular disease [231]. However, it is not known whether HPL patients have increased cardiovascular morbidity. Furthermore, it is not yet established why male prolactinoma patients tend to increase more in weight than women, but large pituitary tumours and highly elevated PRL levels may affect the hypothalamus and lead to hypothalamic DA depletion with possible effects on appetite and satiety [232]. Nonetheless, in one study, male prolactinoma patients were more obese than patients with clinically inactive tumours despite equal frequency of hypogonadism and tumour size, suggesting an effect on body weight of PRL *per se* [155]. After 6 months of treatment, a significant decrease in body weight, BMI, waist circumference and per cent body fat was observed in our male patients. No changes in anthropometric values were seen in the women.

#### **Lipid profile**

Dyslipidemia in patients with elevated PRL levels is another inconclusive area of research. For instance, there are studies indicating an association between HPL and

hypercholesterolemia [164, 165] but inconsistent and contradictory findings have also been reported [158, 159]. In our study, we did not find any major elevations of blood lipids at diagnosis. However, a positive association was observed between LDL cholesterol and serum PRL levels at diagnosis and furthermore, a reduction in total and LDL cholesterol levels was found after 2 months of DA agonist therapy. A possible explanation for the reduction in LDL cholesterol might be reversal of hypogonadism [168]. Yet, a majority of our female patients did not change their gonadal status because they were on hormonal contraceptives. Further, the improvement in testosterone levels in the four males without replacement therapy was not evident until evaluation at 6 months. Dietary changes and weight reduction could also contribute to the reduction in LDL cholesterol, although in this study the improvement in LDL cholesterol was seen in females despite unchanged weight and was apparent in males before weight loss. It therefore seems unlikely that normalisation of gonadal status or weight reduction is the only explanation for the improvement in LDL cholesterol in this study. Thus, one might speculate that PRL has an effect on LDL cholesterol homeostasis in humans.

### **Insulin sensitivity**

Several studies have shown that HPL patients have reduced glucose tolerance and increased insulin resistance [159, 173-175, 177, 178]. Only in one study the reference method for evaluating insulin sensitivity (i.e. the euglycemic hyperinsulinemic clamp) has been used in HPL patients, demonstrating that prolactinoma patients (n=16) were more insulin resistant than age- and BMI-matched controls and that PRL levels negatively correlated with the M-value [178]. In the present study, a tendency of improvement in peripheral insulin sensitivity was observed in the prolactinoma patients after treatment. The lack of a significant improvement in insulin sensitivity might be due to the limited number of patients (n=6) participating in the clamp study, and consequently, the power in this part of the study was reduced. However, five of six patients showed considerable improvement in the M-value. This finding corresponds to a recent study of 22 prolactinoma patients in which HOMA-IR improved after 6 months of therapy with DA agonists [233]. We found an association between per cent improvement in the M-value and per cent reduction in PRL levels, as well as an association between improvement in the M-value and Brc dose, which, in turn, was associated with a decrease in PRL levels. Thus, with our study design, it was not possible to differentiate the parallel effects of a reduction in PRL levels and Brc treatment in mediating improved insulin sensitivity.

Brc-QR has been shown to decrease glucose levels and improving both lipid profile and insulin sensitivity in obese persons and patients with DM type 2 [187, 188, 234]. The proposed rationale for using Brc-QR, in patients with DM type 2, is to provide a short acting pulse of DA agonist activity to centres in the brain that regulate peripheral metabolism [234]. However, it is not known whether treatment with more long-acting DA agonists, as in HPL patients, has the same positive effects on metabolic variables. In the 1980s, two studies evaluated insulin sensitivity and glucose tolerance, respectively, in HPL patients before and after transsphenoidal surgery [164, 235]. These studies demonstrated that after normalisation of PRL levels by surgery alone; insulin sensitivity (evaluated by insulin tolerance test) improved [235] and that oral glucose tolerance improved and the plasma insulin response decreased [164]. These findings would strengthen the hypothesis that PRL is one possible mediator of the insulin resistance found in HPL patients.

IGFBP-1 is mainly produced by the liver and considered a short-term regulator of IGF-I bioactivity. Insulin is the main inhibitor of hepatic production of IGFBP-1 and thereby

increases free IGF-I levels [236]. Previous studies have shown that serum IGFBP-1 concentrations are positively associated with insulin sensitivity [237] and that low levels are related to increased risk of cardiovascular disease [238]. IGFBP-1 levels in our study did not change after treatment, indicating no major alterations in hepatic insulin sensitivity [239].

Adiponectin has an important role in energy homeostasis. Its concentration is lower in obese persons and in patients with DM type 2 and is positively correlated to insulin sensitivity [183]. PRL inhibits adiponectin secretion from human adipose tissue *in vitro* [184]. We found no association between total adiponectin and PRL levels at diagnosis and adiponectin levels did not change after normalisation of PRL levels. Furthermore, no association between improvement in the M-value and change in adiponectin levels was observed. Thus, in this study, which is the first to evaluate adiponectin levels in HPL patients, we could not confirm the hypothesis that elevated PRL concentrations suppress adiponectin levels and thereby being one factor that leads to the increased insulin resistance observed in HPL patients [240]. Consistent with previous results, we observed a sex difference in adiponectin levels [241], where women exhibited higher levels than men. So far, it is not clearly established which adiponectin form is the most clinically relevant, but the high molecular weight form of adiponectin (HMWA) has been regarded as the best marker [181]. In contrast, in a recent study total adiponectin, HMWA and the ratio between HMWA and total adiponectin had similar utility for the identification of insulin resistance and metabolic syndrome [242]. It is unclear whether analysis of HMWA in our study would have been beneficial and giving additional information.

## 6 GENERAL REMARKS AND FUTURE PERSPECTIVES

The overall aim of this thesis was to evaluate the consequences of elevated PRL levels on different clinical outcomes to improve our knowledge and quality in the clinical management of HPL patients. Our studies have demonstrated that, in the long run, HPL patients have good treatment results. However, despite the efficacy of medical treatment, approximately 10-15% of the patients have a total or partial resistance to DA agonist therapy [243]. These patients are a challenge to treat, especially those with large tumours who cannot be cured by surgery. Future studies are needed to evaluate new treatment modalities. Several PRLR antagonists are under development, these compounds were initially developed as an additive treatment in human breast and prostate cancer [112]. However, PRLR antagonists might be a valuable option in the future in DA agonist resistant prolactinomas to counteract the undesirable effects of elevated PRL levels. Furthermore, we lack data on long-term effects and risk for late complications in prolactinoma patients treated with gamma knife SR. We have an opportunity to evaluate the long-term outcome of SR in prolactinoma patients in that treatment of pituitary adenomas with gamma knife SR was first developed at the Karolinska hospital by Lars Leksell in the late 1960s. Another problem in the clinical management of HPL patients is when to discontinue medical treatment. We lack randomised controlled studies evaluating different strategies of DA agonist withdrawal in patients treated for HPL. Having such information would help to improve the selection of patients for discontinuation of medical therapy.

Based mainly on experimental and animal studies, concerns have been raised that HPL patients might have an increased risk of breast and possibly prostate cancer. In our study, however, we did not find any increased risk of breast cancer in HPL women, confirming the results in a previous study [129]. Moreover, we are the first to report a reduced risk of prostate cancer in HPL men. On the other hand, our finding that HPL patients have a small increased overall cancer risk underscores the need for further studies evaluating the potential effect of PRL as a growth-promoting factor in human cancers. To overcome the problem with low precision in our study, due to the relatively few number of cancer cases, might be to conduct a study with a larger sample size or by pooling results in a future meta-analysis. It is now 10 years since the National Patient Register started to collect data on all out-patient visits in specialised care. It could therefore be a valuable option to use this register, to be able to increase the number of patients with HPL, in a future study re-evaluating cancer risk in these patients.

After normalisation of PRL by DA agonist therapy, beneficial metabolic effects were detected in both women and men with prolactinomas. Further studies are warranted to clarify the role of PRL in lipid and glucose metabolism in humans. In addition, our finding of an unfavourable metabolic profile, especially in male patients with HPL, together with other studies demonstrating that HPL patients have impaired endothelial function [166] and a potential hypercoagulable state [165], raises the question of whether these patients have an increased risk of cardiovascular disease and mortality. We will use our patient cohort in Paper III to evaluate co-morbidity in HPL patients with special emphasis on cardiovascular disease. In our opinion, the metabolic profile might be one factor to consider in asymptomatic patients with non-tumoral HPL or microprolactinomas who, according to current clinical practice guidelines, may be left without therapy. Future studies will decide whether all patients with HPL need to be actively treated, regardless of cause and magnitude of the increase in PRL levels.

## 7 CONCLUSIONS

- Long-term follow-up of HPL patients confirms the effectiveness of DA agonist treatment in correcting hypogonadism, normalising PRL levels and reducing tumour size, emphasising its role as a first-line therapy in patients with HPL.
- Women with HPL are older at first-term pregnancy and have reduced parity compared with controls, due to more nulliparous women and less women with more than two children. The odds of having more than two children are reduced by almost 70%.
- We found no evidence of an increased risk of pregnancy or delivery complications or any negative effect on the offspring of women with HPL.
- A small, though significant, increased overall cancer risk was found in patients with HPL, which was mainly due to an increased risk of upper GI cancer in all patients and hematopoietic cancer in women.
- No increased risk of breast cancer was found in women with HPL and male patients had a reduced risk of prostate cancer.
- A positive association between LDL cholesterol and PRL levels was observed in prolactinoma patients and after normalisation of PRL levels by DA agonist therapy, total and LDL cholesterol levels decreased. Improvement in insulin sensitivity correlated to a decrease in PRL levels.



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