Chapter 1

General Introduction and Outline of the Thesis

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The involvement of hormones secreted by the adrenal cortex in intermediary metabolism was first established in 1908, when the French investigators Bierry and Mallozziel discovered that adrenalectomized dogs developed lower fasting glucose levels [1]. In 1910, hypoglycemia was first described in association with Addison’s disease [2]. In the following decades, the critical role of the adrenal cortex in intermediary metabolism and energy homeostasis was further characterized [3]. A major advance was made in 1936 with the simultaneous isolation of the inactive form of the adrenal hormone cortisol, so called cortisone, by the Polish-born, Swiss chemist Tadeusz Reichstein [4] and the American scientist Edward Calvin Kendall [5]. This breakthrough enabled further experiments into the various physiological roles of adrenal cortex hormones. Not only were the metabolic effects of cortisone further explored, cortisone was also shown to reduce stress responses and traumatic shock in rats [6].

The amount of cortisone that could be isolated from bovine adrenal glands, however, was small, and the need to produce adrenocortico steroids through synthetic methods became soon apparent. This process was fuelled by the US entry into the Second World War, when rumors circulated that Luftwaffe pilots were taking adrenal extracts to increase their resistance to oxygen deprivation at high altitudes. Although this rumor was never confirmed, it induced an all-out quest for large-scale synthesis of active adrenal hormone, which was given even higher priority than the development of penicillin and anti-malarials [7,8].

In 1946, Lewis Hastings Sarett of Merck Research Laboratories succeeded in synthetically producing cortisone from desoxycholic acid [9]. By the summer of 1948, sufficient material was produced to initiate the first studies in humans. In clinical trials conducted in the Mayo clinic by the rheumatologist Philip Showalter Hench, a collaborator of Kendall, cortisone treatment for the first time improved the symptoms of Addison’s disease. In addition, Hench tested cortisone in patients with rheumatoid arthritis, driven by observations that joint complaints were reduced during pregnancy and jaundice, conditions in which sex steroids and bile acids are increased, substances that were found closely related to the novel discovered steroid cortisone. Indeed, cortisone treatment induced a spectacular reduction in joint tenderness and swelling in chronic rheumatoid arthritis patients [10,11]. In the following years, the use of adrenal cortex hormones formulations was successfully introduced in the treatment of an increasing number of diseases due to their potent anti-inflammatory and immunosuppressive effects. Tadeusz Reichstein, Philip Showalter Hench and Edward Calvin Kendall shared the Nobel Prize for Physiology or Medicine in 1950 ‘for research on the structure and biological effects of adrenal cortex hormones’. In the same year, cortisone was officially launched as a pharmacological agent.
Figure 1. Overview of the therapeutic and adverse effects induced by glucocorticoid treatment. (See page 366 for full color figure)

However, with its increasing use, it became quickly apparent that high-dose glucocorticoid (GC) treatment induced several undesired effects, ranging from salt and water retention to increased gastric acidity and psychosis. To this end, in the 1950-1960s, newer synthetic GC compounds were developed, including prednisolone and dexamethasone, which were designed to induce fewer side effects. Prednisolone, which is characterized by a 1,2 double bond in the A-ring, was first produced in 1955 by Schering Corporation and was introduced into the market a decade later under the brand name Meticortelone. When this structure additionally undergoes 9α-fluorination, 1-dehydrogenation and 16α-methylation, dexamethasone is yielded and this compound was introduced for clinical use a year later. Although these newer formulation significantly reduced side effects as compared to cortisone, many important side effects of GC treatment remain present today (Figure 1). Of these adverse effects, unfavorable changes in cardiometabolic parameters are prominent and include the development of glucose intolerance, diabetes, visceral adiposity, dyslipidemia, skeletal muscle atrophy and hypertension [12]. The mechanisms underlying these so called diabetogenic effects of GCs regarding glucose, lipid and protein metabolism were studied extensively in the 1960-1970s
and were mainly attributed to GC-induced insulin resistance [13-21]. After this period of intensive research on the metabolic effects of GCs, research slowed down in this area for about two decades (Figure 2).

Figure 2. The number of new publications on Pubmed with search terms “glucocorticoids” and “diabetes” shown per 5-year intervals. After 1975, the amount of research performed on the topic declined somewhat, but in recent years, the subject has received full attention.

In the last 15 years, however, research into the diabetogenic effects of GCs has been revived, mainly due to two different reasons. First, it became clear that deregulated (tissue) GC metabolism may play a role in ‘idiopathic’ obesity and metabolic syndrome, encouraged by the observation that several features of these conditions strikingly resemble the phenotype of chronic GC excess. It was postulated by Björntorp that low-grade chronic stress, characterized by (slightly) increased GC activity, may contribute to visceral obesity, the metabolic syndrome and cardiovascular disease [22]. In addition, increased tissue cortisol levels, generated from the inactive metabolite cortisone by enhanced activity of the enzyme 11-beta hydroxysteroid dehydrogenase (HSD) type 1, were demonstrated in adipose tissue derived from rodent models of obesity and from obese humans [23]. This makes the hormone 11-beta HSD type 1 an attractive therapeutic candidate for the treatment of obesity and its metabolic consequences. As such, a battery of pharmaceutical companies is currently developing compounds that may reduce tissue cortisol levels by targeting 11-beta HSD type 1. The amount of research done on this topic has skyrocketed during the last decade (Figure 3).

Second, improved understanding of the mode of action of GCs on the molecular and cellular level has led to the development of so-called dissociated glucocorticoid receptor (GR) agonists (Figure 4). In 1985, it was discovered that GCs exert their actions through
binding to the intracellular GR, following which this complex migrates to the nucleus, where it regulates target gene expression [24,25]. Additionally, it became clear that the anti-inflammatory actions and dysmetabolic effects of GCs may be the result of different genomic actions, i.e. transrepression and transactivation, respectively. This suggests that these effects may potentially be separated, which forms the basis of these novel GR agonists [25-32]. Several compounds with a dissociated profile are currently under development by different pharmaceutical companies and have shown promising results in disease and side effect animal models, where they reduced inflammation without altering glucose levels [33].

**Figure 3.** The number of new publications on Pubmed with search term "11-beta hydroxysteroid dehydrogenase" shown per decade. The number of publications on this topic has skyrocketed in the past 2 decades.

**Figure 4.** The number of new publications on Pubmed with search term "selective glucocorticoid receptor agonists" shown per decade. In the last decade, information on the development of these novel compounds has found its way to the public domain.
The work done in this thesis should be seen in light of the development of these dissociated GR agonists and was conducted within the framework of a relatively novel organization. In 2005, the fifth Dutch Technological Top Institute was founded by the name of Top Institute Pharma, in which the Dutch government, several pharmaceutical companies and all Dutch universities were brought together with the goal to develop so called priority medicines. In our Top Institute Pharma Project consortium (T1.106), we collaborated with NV Organon (more recently incorporated first by Schering-Plough and later by Merck Sharp & Dohme), who are currently developing GR agonists with a dissociated profile.

The clinical development of these novel compounds, however, faces important difficulties. The mechanisms by which prednisolone induces metabolic changes remain largely unknown. In addition, due to the differences in the type of GC administered, treatment duration, mode of administration and chosen dose, several contrasting reports exist in the literature. Also, since many studies were conducted more than 4 decades ago, it may be difficult to compare recent studies with the results obtained from those early studies due to different research methods and techniques that are currently being employed. Thus, in order to be able to further develop the dissociated GR agonists, it is highly important to first obtain detailed information regarding the metabolic side effects induced by prednisolone treatment and to select biomarkers that reflect these metabolic side effects in a dose-dependent manner. Only after these indicators of efficacy and safety have become available, it will be possible to design compounds with an improved therapeutic index compared to the currently-available classic GR agonists.

In the present thesis, of which the PANTHEON (Prednisolone peripherAl effects on glucose meTabolism, metabolic Hormones, insulin sEnsitivity and secretiON)-trials form the backbone, we aimed to assess the peripheral metabolic effects of both low-and high-dose GC treatment in healthy volunteers and patients with rheumatoid arthritis, focusing specifically on the effects of prednisolone on islet-cell function and insulin sensitivity of various metabolic pathways.

In chapter 2 of this thesis, an overview of the knowledge regarding the diabetogenic side effects of GC treatment is presented that was available at the time when our studies were initiated. Part II describes the effects of GC treatment on pancreatic islet-cell function. In chapter 3 mechanisms underlying the deleterious effects of GCs on beta-cell function are addressed in INS-1E cells in vitro, in particular the involvement of endoplasmic reticulum stress. In chapter 4, the effects of both acute and more prolonged high-dose GC treatment on beta-cell function are described, using mathematical modeling analysis of glucose, insulin and C-peptide concentrations obtained from standardized meal challenge tests. In chapter 5, we
further explored the effects of both low- and high-dose GC treatment on both alpha- and beta-cell function in healthy volunteers using both intravenous hyperglycemic clamp studies and meal challenge tests. In chapter 6, in order to further delineate the role of GR signaling in beta-cell function, we investigated the effects of single nucleotide polymorphisms (SNPs) in the GR gene on measures of beta-cell function in a cohort of 449 subjects. In chapter 7, we explored the potential of the novel class of glucose-lowering agents the glucagon-like peptide (GLP)-1 receptor agonists to prevent the dysmetabolic effects induced by GC treatment in healthy subjects. Part III of the thesis focuses on GC-induced insulin resistance and the mechanisms that may contribute to this well-known but only partly understood side effect of GC treatment. In chapter 8, the effects of both low- and high-dose GC treatment on several aspects of intermediary metabolism is addressed. Possible mediators of GC-induced insulin resistance in skeletal muscle, including impaired sphingolipid metabolism and mitochondrial dysfunction, are investigated in chapter 9. In chapter 10 the possible role of microvascular dysfunction induced by GC treatment was addressed and in chapter 11 we investigated the effects of GC treatment on adipose tissue function and adipokine secretion. In chapter 12, the role of the novel endocrine factor angiopoietin-like protein 4 in GC-induced metabolic alterations was studied, by combining an in vivo (randomized controlled trial in healthy individuals) and in vitro (cell culture) approach. In part IV of this thesis, using an oral glucose tolerance test to assess both insulin secretory response and surrogate markers of insulin resistance, the diabetogenic effects of GC treatment were investigated in two different rheumatoid arthritis populations, allowing to study the interaction with systemic inflammation. In chapter 13, the acute effects of high-dose prednisolone treatment on glucose tolerance, insulin sensitivity and insulin secretion in patients with early and active rheumatoid arthritis are described, whereas in chapter 14, the effects of chronic GC treatment on glucose tolerance, insulin sensitivity and insulin secretion are assessed in patients with long-standing rheumatoid arthritis.

In the general discussion (part V, chapter 15), an overview will be presented of the pathophysiology of glucose intolerance induced by GC treatment. Additionally, implications will be discussed for the development of dissociated GR agonists. Finally, since these compounds are still under development, a proposition will be made how GC-induced diabetes may best be treated in clinical practice.
REFERENCES


23. Morton NM, Seckel JR. 11beta-hydroxysteroid dehydrogenase type 1 and obesity. Front Horm Res. 2008; 36:146-64


