Adipokines and Cardiovascular Risk in Cushing’s Syndrome

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\textbf{Introduction}

Endogenous Cushing’s syndrome (CS) is a consequence of chronic exposure to high circulating levels of glucocorticoids. Cortisol is an end product of the hypothalamic-pituitary-adrenal (HPA) axis, and is produced by the adrenal cortex in response to pituitary adrenocorticotropic hormone (ACTH). ACTH in turn is regulated by hypothalamic secretion of corticotropin-releasing hormone (CRH) and vasopressin. CRH, vasopressin and ACTH secretion is controlled by cortisol levels through a negative feedback mechanism at the level of the hypothalamus and pituitary. In healthy individuals, cortisol is secreted in a circadian rhythm; levels peak in the morning and then decrease during the day to a nadir around midnight. Endogenous CS is characterized by loss of this circadian rhythm; levels peak in the morning and then decrease during the day to a nadir around midnight. Endogenous CS is characterized by loss of this circadian rhythm, together with impaired physiologic feedback within the HPA axis. CS is usually caused by excessive ACTH release from a pituitary corticotrope adenoma (Cushing’s disease, CD) and less frequently by ectopic ACTH or (very rarely) CRH production. CS can also be ACTH-independent when caused by increased cortisol secretion by adrenocortical tumors or hyperplasia [1].

Endogenous hypercortisolism is associated with increased cardiovascular risk as well as a number of comor-
vidities, and in untreated patients, results in a 4 times higher mortality rate than expected in the normal population [2].

Chronic glucocorticoid excess in CS is commonly associated with a cluster of cardiovascular risk factors that strongly impact morbidity and mortality, including visceral obesity with insulin resistance, impaired glucose tolerance, atherosclerosis, systemic hypertension, dyslipidemia and hypercoagulability [3].

An increase in visceral fat is a phenotypic feature of CS. Importantly, our understanding of the mechanisms whereby increased central adiposity leads to metabolic alterations and cardiovascular morbidity has been largely based on the demonstration that adipose tissue secretes a number of cytokines and bioactive compounds, the so-called ‘adipokines’. Adipokine, which include a variety of pro-inflammatory peptides, are involved in many physiological or pathological processes, including inflammation, endothelial damage, atherosclerosis, impaired insulin signaling, hypertension and bone remodeling. Adipokine dysregulation is a strong determinant of the ‘low-grade’ inflammatory state of obesity, promoting a cascade of metabolic aberrations leading to cardiovascular complications [4]. In CS, increased visceral adiposity [5, 6] is associated with altered production of adipokines, which contributes to the pathogenesis of the comorbidities observed in this condition. This review will highlight recent insights into the relationship between adipokines and cortisol, with particular regard to cardiovascular risk in CS. Because we will only focus on those adipokines that have been studied in CS, it is possible that other molecules that have been significantly associated with cardiovascular risk in other models of disease will not be discussed comprehensively in this review.

**Cushing’s Syndrome and Fat Distribution**

Conflicting data have been reported regarding fat distribution in patients with CD compared to BMI-matched controls. For example, one study reported that overweight women with CD had higher percent trunk fat (as assessed by DXA) than BMI-matched controls [7], whereas another reported that total and trunk fat in obese women with CD were comparable to BMI-matched controls [5]. Although total and trunk fat in obese CD females did not differ from BMI-matched controls in the latter study, a strong correlation was observed in CD patients between absolute trunk fat and intra-abdominal visceral area as assessed by CT. Indeed, the abdominal visceral to subcutaneous fat ratio (V/S) was significantly greater in CD women than in either obese or normal-weight controls [5]. Interestingly, the increase in the V/S ratio does not differ between female and male patients with CS, suggesting that hypercortisolism may override gender-related differences in fat distribution [6].

It is important to emphasize that concomitant growth hormone deficiency (GHD) in states of cortisol excess (CS-GHD), both functional or based on tumor-related somatotrope dysfunction, is a common feature of active [8, 9] as well as cured CD [10]. Magiakou et al. [8] found a marked reduction of both spontaneous and stimulated GH secretion in children with CS, which persisted a year after surgical remission of hypercortisolism. In a small study on 16 adult patients with CS, GHD was reported in the entire group prior to surgery, with 69% of patients having severe GHD [9]. Pecori Giraldi et al. [10] showed that 65% of CS patients assessed within a median of 3.3 years of follow-up after surgical resolution of hypercortisolism had impaired GH secretion. Of note, the duration of hypercortisolism predicted postsurgical GHD, suggesting a direct, detrimental effect of glucocorticoid excess on GH secretion [10]. This effect may be related to alterations of either GHRH [11] or somatostatin release [12] by high levels of glucocorticoids, or a direct inhibition of pituitary somatotropes by glucocorticoids [13].

CS-GHD contributes to the metabolic alterations typically observed in this pathological condition, even many years after resolution of hypercortisolism [14], although the degree of dysfunction varies between CS and GHD. In a small study that compared body composition in patients with either CS or GHD to normal controls, percent trunk fat was highest in CS and lowest in normal controls, with patients with GHD having trunk fat intermediate between patients with CS and controls, although the difference between groups was not statistically significant [15].

Patients with CS-GHD have an increased prevalence of diabetes mellitus, hypertension and cardiovascular diseases as compared with GHD patients with previous nonfunctioning pituitary adenomas [16, 17]. Of note, Johansson et al. [18] demonstrated that among cured CD patients with concomitant GHD, there was a trend towards reduction in body fat during 2 years of GH replacement. At the end of the treatment period, within-group reduction in body fat approached significance. Höybye et al. [17] showed a significant improvement of lipid profile in CS-GHD patients after 3 years of GH replacement.
**Leptin**

*Leptin and Cardiovascular Risk*

Leptin, the ob gene product, is an anorexigenic hormone mainly secreted by adipocytes in proportion to body fat (specifically subcutaneous fat) content. Levels are typically elevated in obesity, which is considered a leptin-resistant state [19]. Hyperleptinemia in obese individuals has been widely recognized as an independent cardiovascular risk factor associated with hyperinsulinemia and insulin resistance [20]. Compelling evidence also suggests a pathogenetic role of high leptin in atherosclerosis and endothelial dysfunction [20]. Leptin can induce release of reactive oxygen species and monocyte recruitment, promoting atherogenesis in bovine endothelial cells [21]. O’Rourke et al. [22] showed that in the presence of elevated glucose levels, leptin stimulated in vitro macrophage cholesterol ester synthesis, thus contributing to foam cell formation. A positive relationship between plasma leptin and coronary artery calcifications is demonstrated to directly inhibit ACTH-mediated, but not basal cortisol secretion from human adrenal cells in vitro in a dose-dependent manner [28]. This inhibition peaked after 24 h of exposure to human physiological leptin concentrations, suggesting a potential long-term effect of this adipokine on adrenal steroidogenesis [28]. In contrast, a dose-dependent enhancement of 11β-hydroxysteroid dehydrogenase type 1 activity (11β-HSD1), which converts inactive cortisone to cortisol, was seen in primary cultures of ob/ob mouse hepatocytes after addition of leptin, suggesting that this adipokine may in fact increase intra-tissue cortisol levels [29].

In vivo Studies: Animal Models

Similar to in vitro data, in vivo animal data regarding the effect of leptin on CRH and ACTH production are conflicting, and may again relate to differences in species and/or experimental design. Studies suggest a differential effect of acute versus chronic leptin administration on the HPA axis. Leptin acutely enhances hypothalamic CRH biosynthesis in rats, whereas its chronic administration exerts an inhibitory effect in mice [30]. A stimulatory effect of leptin on CRH expression was observed in the paraventricular nucleus (PVN) of rats 2–6 h after either intracerebroventricular (icv) or intraperitoneal administration of the peptide, consistent with its anorexigenic action [31, 32]. However, leptin infusion for 7 days was found to downregulate CRH expression in the mouse PVN and to prevent the compensatory rise of CRH in adrenalectomized mice [33]. A shorter period of subcutaneous leptin infusion did not have any effect on CRH expression in either mice or rats [30].

Interestingly, the effect of leptin on CRH secretion in rodents appears to be influenced by circulating glucocorticoids. CRH concentrations in the PVN rose by about 50% within 1–3 h of the icv injection of leptin in adrenalectomized rats, but this was not observed in animals with intact adrenals [34]. At the level of the pituitary, another study demonstrated that prolonged systemic administration of leptin increased ACTH concentrations in both adrenalectomized female rats and intact animals [30]. In contrast to these studies, chronic administration of leptin reduced POMC expression in the anterior pituitary of obese mice [35]. In rats, contrasting effects of leptin administration on steroid hormone secretion have been documented, with some studies showing a marked stimulation, and others describing a lowering effect of leptin on plasma corticosterone levels, depending on the route and duration of administration [30].

Complete leptin deficiency in the ob/ob mice is associated with decreased omental 11β-HSD1 activity and hypercorticosteronemia, which is reversed by chronic leptin

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replacement [36], suggesting that leptin may facilitate tissue cortisol synthesis. In contrast, mice selectively overexpressing omental 11β-HSD1 present with typical features of the metabolic syndrome, including visceral obesity and hyperleptinemia. Although a direct relationship between cortisol and leptin cannot be inferred from this study, the data suggest that increased intracellular concentrations of glucocorticoids in the omentum may contribute to increased leptin secretion in central obesity [37].

Human Studies

In vivo studies in humans overall support an inhibitory effect of leptin on the HPA axis with an inverse relationship reported between leptin pulsatility and that of ACTH and cortisol in healthy males [38]. Koutkia et al. [39] reported a synchronicity between leptin and cortisol pulse dynamics in healthy men, with changes in leptin preceding a reciprocal change in cortisol during an overnight fast.

Interestingly, studies also suggest that exogenous cortisol may impact leptin secretion. A 48-hour exposure to dexamethasone (1 μmol/l) was reported to enhance leptin release from omental adipose tissue in vitro from women (but not men) [40]. In humans, glucocorticoid effects on leptin levels may be dose-, body weight-, gender- and duration-dependent. Administration of pharmacological doses of dexamethasone to normal subjects has been shown to cause either an acute, sustained, dose-dependent rise in blood leptin concentrations [41–45], or no significant effect [46–48]. For example, acute dexamethasone administration (10 mg over 4 days) increased leptin concentrations in obese subjects, which was associated with baseline leptin and BMI regardless of gender, age, or insulin sensitivity [43]. Putignano et al. [48] found a significant increase in plasma leptin after lower doses of dexamethasone (1 mg, 0.035 mg/kg, 0.007 mg/kg, 0.015 mg/kg) only in obese women, but not in men or in normal-weight subjects of either gender.

In humans, there are gender differences in leptin levels. Circulating levels of leptin, normalized to total fat mass, are 2- to 3-fold higher in premenopausal women compared with men [49]. Leptin is secreted in large amounts by subcutaneous fat, and higher leptin levels in women are also consistent with greater subcutaneous fat in women compared to men [50]. In addition, sexual dimorphism in leptin levels is mediated by sex steroids. Testosterone is inversely related to leptin and inhibits leptin secretion, in contrast to a slight stimulatory effect exerted by estradiol [51].

Soluble Leptin Receptor

The soluble leptin receptor (sOB-R) derives from the proteolytic cleavage of leptin membrane receptors. Binding of the sOB-R to leptin determines levels of free leptin in serum [52]. Chan et al. [53] reported that leptin and sOB-R were inversely related, with the former being able to reciprocally regulate its binding protein in several acute conditions. Interestingly, these authors found a positive correlation between cortisol and sOB-R, which was also reported in anorexia nervosa [53, 54]. Finally, in a study examining secretory patterns of sOB-R and cortisol in healthy men, Gavrila et al. [55] found that profiles of both hormones were strongly related, such that the peak for both was reached at approximately the same time in the morning, and the nadir of sOB-R occurred about 2 h after that of cortisol, suggesting a possible interplay between these hormones.

Leptin and Cushing’s Syndrome

Several reports have documented elevated leptin concentrations in CS patients compared with normal-weight and BMI-matched controls [56–58] (table 1). Masuzaki et al. [57] showed that circulating leptin levels in CS patients (BMI range: 16.6–30.3) were similar to those in obese controls (BMI range: 27.8–56) but significantly higher than in healthy normal-weight subjects (BMI range: 16–23). Importantly, when leptin levels were evaluated in relation to the percentage of body fat measured by DEXA, they were significantly elevated in CS as compared with the entire control group. Consistent with these studies, Weise et al. [59] found a positive correlation between BMI and leptin in patients with active CS. However, no study thus far has evaluated the relative contribution of specific fat depots, namely visceral versus subcutaneous fat, to leptin levels in CS. This distinction is crucial, because subcutaneous fat is known to produce 2–3 times more leptin than omental fat [50]. CD, however, is characterized by a higher proportion of visceral compared to subcutaneous fat, with the latter being comparable in CD obese women and BMI-matched controls [5]. Of note, Widjaja et al. [60] reported higher leptin levels in CS males as compared with BMI-matched controls, likely consequent to greater trunk fat in CS, whereas no difference was observed in women with CS compared with BMI-matched controls, likely because of a similar proportion of subcutaneous fat in both groups.

A study of leptin pulsatility in 7 women with CD revealed a more than 2-fold elevation in daily leptin production rates as compared with age-, gender- and BMI-matched controls, which was driven by higher pulse am-
plitude rather than frequency [61]. Of note, approximate entropy of leptin release and cross-approximate entropy of cortisol and leptin were superimposable to those measured in controls. These findings argue in favor of an unexpected synchrony between leptin and cortisol in CD, with a preservation of normal responsiveness of fat cells to cortisol, despite its excessive and dysregulated production in that disorder. This evidence supports a possible direct influence of hypercortisolemia on hyperleptinemia in CD, although the same group did not find any correlation between leptin and cortisol secretion rates [61]. Similarly, Weise et al. [59] demonstrated maintenance of a normal diurnal leptin profile in 18 CS patients but did not observe any correlation between leptin and cortisol at any time point. This apparent dissociation between cortisol and leptin in CS was confirmed by the finding that CRH administration before and 10 days after surgical resolution of hypercortisolism in 12 CD patients did not impact leptin concentrations in spite of the significant differences in cortisol and ACTH response between the pre- and postoperative phases [59]. The maintenance of hyperleptinemia despite a fall in cortisol levels after transphenoidal surgery (TSS) was confirmed by other investigators [58] and suggests that factors other than cortisol hypersecretion may play a role in leptin overproduction in CD, such as a persistence of fat distribution abnormalities. In contrast to acute normalization of cortisol, which does not cause reductions in leptin levels, short-term fasting for a 36-hour period is associated with a reduction in levels of leptin and insulin in both CS and obese patients, although not to the extent seen in healthy controls [62].

Although leptin levels do not change acutely after successful TSS for CD, levels do decrease chronically, likely due to decreased BMI, fat mass, and insulin levels with persistent cortisol normalization. Widjaja et al. [60] reported a significant decrease in leptin levels about 2.5 years after TSS in 9 CS patients as cortisol, insulin, and BMI decreased into the normal range.

Kresk et al. [63] observed a significant decrease in the ratio of leptin to sOB-R, a marker of leptin bioavailability, 9 months after curative TSS in CD patients, similar to findings in obese patients following bariatric surgery [64]. This is likely a consequence of reductions in body weight, fat mass and insulin levels with cortisol normalization. Indeed, in the report by Widjaja et al. [60], insulin was the only predictor of leptin levels in active CS. This is in agreement with several studies showing a direct relationship between leptin and insulin in humans [65, 66], with the latter being an independent predictor of decreased leptin levels in obese subjects after significant weight loss [67].

In another study of patients with CS, a significant postoperative decrease in leptin levels after an average of 31 months was associated with reductions in cortisol and trunk fat, and a concomitant decrease in interleukin (IL)-1Ra, the molecule thought to antagonize leptin signaling in the hypothalamus [68]. This finding indicates that similar to obesity, CS may be a state of leptin resistance, which at least in part is mediated by an elevation of IL-1Ra levels during the active phase of the disease [68].

Concomitant GH deficiency may also play a role in the hyperleptinemia associated with active CS, given that GH directly inhibits ob gene expression in visceral fat, as shown in obese rats [69]. Indeed, in GHD patients without Cushing’s, leptin levels were significantly higher than in BMI-matched controls, but decreased to comparable levels after a year of GH replacement in parallel with a reduction of body fat [70]. These studies, however, do not conclusively determine whether elevations in leptin in states of GHD and cortisol excess are a direct consequence of low GH and high cortisol, or whether hyperleptinemia is consequent to coincident fat accumulation.

Overall, although the pathogenesis of hyperleptinemia in CS is not clear, it is interesting to speculate that the increase in leptin is a compensatory mechanism to antagonize glucocorticoid excess such as through inhibition of CRH release. Additionally, hyperleptinemia may represent an attempt to reduce the stimulatory effect of cortisol on food intake through leptin-mediated inhibition of the powerful orexigenic peptide neuropeptide Y and stimulation of the anorexigenic POMC and cocaine- and amphetamine-regulated transcript (CART) neurons [71].

In conclusion, most studies showed elevated leptin levels in active CS. Decreases in leptin secretion after correction of hypercortisolism appear to be dependent on the evaluation time (i.e. short-term vs. long-term after remission) and changes in body fat. Hyperleptinemia in CS may be a compensatory mechanism which is made ineffective by concomitant glucocorticoid excess and, possibly, by an obesity-like resistance to its action.

Adiponectin

Adiponectin and Cardiovascular Risk

Adiponectin is the protein product of the apM1 gene, which is mainly produced by visceral fat and circulates in blood in different forms of varying molecular weight [72, 73]. Low levels of adiponectin are a common feature of...
obesity and are associated with insulin resistance [74]. In normal subjects, acute insulin infusion during a hyperglycemic hyperinsulinemic clamp caused a significant decrease in high-molecular-weight adiponectin, but not in total or low-molecular-weight adiponectin [75]. The ratio of high-molecular-weight/total adiponectin is a stronger predictor of insulin resistance and metabolic syndrome than total adiponectin levels [76] and has been shown to be significantly lower in diabetic as compared with nondiabetic subjects [75].

The insulin-sensitizing action of adiponectin is mediated through mitogen-activated protein kinase in muscle and liver with consequent increase in free fatty acid oxidation and reduced hepatic glucose production [74]. In addition, adiponectin has important anti-atherogenic and anti-inflammatory activity. It inhibits adhesion of monocytes to endothelial cells and transformation of macrophages to foam cells in vitro [77]. Moreover, adiponectin increases nitric oxide production in human aortic endothelial cells, and low levels of the adipokine are associated with impaired endothelium-dependent vasodilatation in patients with diabetes as well as in healthy subjects [78]. Adiponectin is inversely related to CRP and the cytokines tumor necrosis factor-α (TNF-α) and IL-6; the latter two are powerful inhibitors of adiponectin expression and secretion in cultured human adipose cells. These data suggest that the role of adiponectin in cardiovascular risk may be partly mediated by interplay with these pro-inflammatory molecules [79].

In a large cohort of men without cardiovascular disease, subjects having total adiponectin levels in the highest quintile had a significantly decreased risk of myocardial infarction compared with those in the lowest after 6 years of follow-up [80].

**Adiponectin and the HPA Axis**

In vitro and Animal Studies

An association between adiponectin and the HPA axis has been reported in various in vitro and in vivo animal models, and will be summarized here. Incubation of murine 3T3-L1 adipocytes with dexamethasone reduces expression of the adiponectin gene, an effect that is completely reversed following dexamethasone withdrawal for 24 h [79]. Similarly, Makimura et al. [81] reported that in ob/ob mice, which are characterized by low adiponectin levels and insulin resistance, adrenalectomy normalized expression of adiponectin and improved insulin resistance. These data suggest that glucocorticoids are inhibitory to adiponectin secretion and support the hypothesis that restoration of adiponectin levels may mediate the improvement in insulin sensitivity observed in adrenalectomized ob/ob mice. Transgenic overexpression of 11β-HSD1 in murine adipose tissue led to elevated intra-adipose corticosterone concentrations associated with low adiponectin expression, visceral obesity and insulin resistance [82]. Similarly, adipocyte-specific inactivation of glucocorticoid action in a transgenic murine model over-expressing 11β-HSD type 2 (11β-HSD2) led to a favorable metabolic profile, including reduction of food intake, increased expression of adiponectin, and a decrease in central fat accumulation and insulin resistance [83], further confirming an inverse relationship between glucocorticoids and adiponectin.

**Human Studies**

The few human studies that have been published thus far examining the impact of short-term glucocorticoid administration on adiponectin levels in humans are small in size and contradictory. Two studies reported no effect of glucocorticoid administration on adiponectin levels despite a concomitant impairment of insulin sensitivity in either normal [84] or obese [85] subjects. In contrast, another study reported an increase in circulating adiponectin levels in healthy male athletes who were administered 60 mg of prednisolone daily for a week [86], while Fallo et al. [87] demonstrated a significant decrease in adiponectin 30 and 60 min after a 25-mg injection of hydrocortisone in 5 healthy volunteers compared with placebo. Although differences in study populations and variations in study protocols including dose and duration of glucocorticoid administration may account for some of these discrepancies, it is likely that a longer exposure to elevated glucocorticoid levels may be necessary to detect changes in adiponectin concentrations associated with significant metabolic alterations.

Interestingly, a 24-hour profile of circulating adiponectin and cortisol in 6 young men indicated substantial differences in secretory patterns of the two hormones, despite a similar timing of peak hormone secretion. In particular, adiponectin, but not cortisol, plateaued during the day and then reached its nadir 2 h after cortisol at night [55].

**Adiponectin and Cushing’s Syndrome**

A few small studies have reported adiponectin levels in patients with CS (table 1). These studies were limited by small size and heterogeneous characteristics of the samples, as well as by the fact that only total adiponectin was measured. As previously mentioned, adiponectin circulates in a variety of molecular forms and the pre-
dominant molecular forms in CS have not been reported. In particular, it could be important to measure high-molecular-weight adiponectin, which, as noted, has been shown to significantly predict insulin resistance and metabolic syndrome in patients with diabetes [76]. This may yield results that differ from those reported thus far in this condition. This would be particularly true if associations of cortisol and adiponectin were driven by associated changes in insulin resistance, as is likely.

In one study, Fallo et al. [87] reported no difference in adiponectin levels between 11 obese patients with CD and BMI-matched controls, suggesting that increased body fat rather than hypercortisolism may be the main determinant of adiponectin in obese CS. However, this study also reported no difference in adiponectin levels in obese versus nonobese CD, and lower adiponectin levels in nonobese CD compared with nonobese controls, suggesting that hypercortisolemia does impact adiponectin levels directly, independently of body weight. The well-known inverse relationship between insulin sensitivity and adiponectin was confirmed in both obese and nonobese CD [87]. However, a limitation of this study was that female and male patients were analyzed together, despite the well-known sexual dimorphism in adiponectin regulation, with men having lower levels than women subsequent to inhibitory effects of testosterone on adiponectin release [88].

Another study of 14 women with CD failed to show any difference in adiponectin levels during the active phase of the disease compared with BMI-matched controls, possibly because of comparable insulin sensitivity in the two groups [89]. In addition, adiponectin levels remained unchanged 10 months after successful TSS compared with baseline values, despite a significant decrease of BMI, insulin resistance and cortisol, arguing against the regulation of adiponectin by endogenous glucocorticoids in CD [89]. These observations were confirmed by Kresk et al. [63].

Although Barahona et al. [90] reported that adiponectin levels were significantly lower in active CS as well as in cured CS after 11 ± 6 years as compared with healthy controls, this difference was no longer significant when patients were stratified based on their estrogen status.

As previously mentioned, GHD may coexist in patients with CD, and this may be a confounder in some of the published studies, although data regarding adiponec-

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**Table 1. Adipokine patterns in CD**

<table>
<thead>
<tr>
<th>Adipokines</th>
<th>Pattern in active CS patients vs. BMI-matched controls</th>
<th>Pattern in CS after correction of hypercortisolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>Increased [56–58, 62]</td>
<td>Unchanged [58]</td>
</tr>
<tr>
<td></td>
<td>Increased only in men [60]</td>
<td>Decreased [60, 63, 68]</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Decreased in non-obese; no difference in obese CD vs. non-obese [87]</td>
<td>Unchanged [63, 89, 90]</td>
</tr>
<tr>
<td>Resistin</td>
<td>Increased in females [63]</td>
<td>Unchanged [63]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Unchanged [68, 129, 130]</td>
<td>Increased in hypoadrenal patients [129]</td>
</tr>
<tr>
<td></td>
<td>Increased sTNF-R1 [90]</td>
<td>Increased sTNF-R1 vs. BMI-matched controls [90]</td>
</tr>
<tr>
<td>IL-6</td>
<td>Unchanged [129, 130]</td>
<td>Increased in hypoadrenal patients [129]</td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>Increased expression of Ang II receptor 1A [166]</td>
<td>Not known</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Increased [172]</td>
<td>Decreased vs. controls [173]</td>
</tr>
<tr>
<td></td>
<td>Increased although not significantly [173]</td>
<td>9 months</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>Decreased [89]</td>
<td>Increased [89, 191]</td>
</tr>
<tr>
<td></td>
<td>Increased; similar to controls with lower BMI [192]</td>
<td>3–24 months</td>
</tr>
</tbody>
</table>

*sTNF-R1 = Soluble TNF-α receptor.*
Resistin levels in GHD are also somewhat conflicting. Some reports showed no difference in total adiponectin concentrations between GHD patients at baseline and healthy controls [44, 91], and others described significantly lower levels in patients with GHD [92]. Giavoli et al. [92] also observed a significant elevation of this adipokine in GHD patients compared with baseline values after a year of treatment with GH, possibly related to favorable changes in body composition. However, this finding was not confirmed by others [44, 91].

In conclusion, future larger studies including isoform measurements are needed in order to conclusively assess whether a potential dysregulation in adiponectin production may contribute to the high metabolic and cardiovascular risk profile in CS patients.

Resistin

Resistin and Cardiovascular Risk

Resistin is a dimeric protein produced by macrophages and adipocytes especially those in visceral fat, and its name derives from its role in inducing insulin resistance in mice [93]. It regulates insulin sensitivity in skeletal muscle and liver, and its expression in human macrophages is reduced after incubation with rosiglitazone [94].

Resistin is elevated in obesity and is positively associated with insulin resistance and the degree of glucose intolerance in both humans and animal models [95]. Resistin is considered a marker of inflammation and a contributor to atherosclerosis. In vitro studies report that resistin dose dependently induces proliferation of smooth muscle cells [96], and rh-resistin enhances the release of endothelin-1, vascular cell adhesion molecule (VCAM), and intercellular adhesion molecule-1 (ICAM-1) from human endothelial cells [97]. Resistin predicts coronary artery calcification in the metabolic syndrome [98] and was independently associated with arterial stiffness in the Baltimore Longitudinal Study of Aging [99]. Recent evidence from the Framingham Offspring Study suggests that elevated levels of resistin are inversely related to left ventricular fractional shortening, a marker of left ventricular systolic function [100]. Of note, resistin was an independent predictor of heart failure in the same cohort [101]. Moreover, resistin has been demonstrated to be independently associated with increased risk of myocardial infarction and ischemic stroke in a large cohort of middle-aged subjects, and in the Women's Health Initiative Observational Study [102, 103].

Resistin and the HPA Axis

In vitro and Animal Studies

In vitro and animal studies have shown a positive relationship between glucocorticoids and resistin. Incubation of 3T3-L1 adipose cells with dexamethasone enhanced expression of the resistin gene, suggesting that glucocorticoid-induced insulin resistance may be at least partly mediated by resistin [104]. However, another study using the same cell line could not confirm these results [105]. In prepubertal mice, resistin gene expression increased following injection of dexamethasone into the pituitary and decreased following adrenalectomy [106]. Notably, resistin gene expression was found in rat adrenal glands [107].

Resistin and Cushing’s Syndrome

In the only study published thus far evaluating resistin levels in CS, resistin was significantly higher in 10 CS female patients compared with age-, sex-, and BMI-matched controls (table 1), and was positively associated with BMI, but not with urinary free cortisol or insulin [63]. Nine months after surgery, there was no significant decrease in resistin concentrations, despite a significant reduction in total body fat and trunk fat [63]. Interestingly, Ermetici et al. [108] reported significantly higher resistin levels in patients with adrenal incidentalomas as compared with controls.

The in vitro action of GH on resistin levels is controversial, and its incubation with 3T3-L1 adipocytes caused either inhibition or no effect on resistin expression [105]. Similar to recovered CS patients [63], a year of GH replacement in GHD patients did not significantly change resistin levels [109].

In summary, effects of resistin in hypercortisolemic states and/or associated GH deficiency remain to be determined.

Tumor Necrosis-Factor-α

TNF-α and Cardiovascular Risk

TNF-α is a pro-inflammatory cytokine with important regulatory effects on lipid metabolism, adipocyte function and insulin signaling [110]. Its expression has been shown to correlate with percent body fat and insulin resistance in humans [111]. Moreover, TNF-α produced by periarteriolar fat in obese rats impairs endothelium-dependent vasodilatation through inhibition of insulin-mediated release of nitric oxide [112]. Thus, TNF-α may be an important link between insulin resistance and vas-
cular disease. In addition, TNF-α has been reported to upregulate release of ICAM-1 and VCAM-1 on the surface of endothelial cells, which facilitate leukocyte adhesion to vessel walls [113]. These data confirm a prominent role of this adipokine in the pathogenesis of atherosclerosis and endothelial damage. Indeed, elevated levels of TNF-α predicted cardiovascular events in a cohort of 2,225 healthy elderly subjects during an average follow-up of 3.6 years [114]. Levels of TNF-α at the 95th percentile of the control distribution were associated with a 3-fold increase in recurrent acute ischemia in a case-control study including 544 patients [115].

**TNF-α and the HPA Axis**

In vitro and Animal Studies

Cross-talk between TNF-α and glucocorticoids is of paramount importance in modulating the immune response. TNF-α may also affect the HPA axis; it causes an elevation of glucocorticoid concentrations in acute inflammation [116]. TNF-α not only activates the HPA axis and enhances glucocorticoid production but also modifies tissue sensitivity to the anti-inflammatory effects of glucocorticoids [117].

Incubation of explanted rat hypothalami with graded concentrations of TNF-α showed a dose-dependent increase in CRH secretion, which was prevented by the pre-administration of glucocorticoids. Of note, an increase in ACTH secretion was only observed after exposure to the highest doses of the cytokine [118]. In contrast, incubation of normal rat anterior pituitary cells with TNF-α inhibited CRH-stimulated ACTH release in a dose-dependent manner, without affecting basal secretion [119].

Finally, exposure of adult human adrenal cells to TNF-α enhanced basal and ACTH-stimulated cortisol release [120]. Because TNF-α expression has been demonstrated in human adrenal glands [120], one may hypothesize that TNF-α impacts cortisol secretion in adrenal tissue through a paracrine mechanism.

TNF-α administration potently enhanced transcriptional activity of 11β-HSD1 causing an increase in intracellular concentrations of cortisol [37]. Interestingly, co-administration of dexamethasone and insulin further potentiated the effect of TNF-α on 11β-HSD1 [37]. Thus, in conditions characterized by upregulation of 11β-HSD1, such as obesity, the concentration and action of cortisol were locally amplified by several factors that include TNF-α and other adipokines, triggering a ‘vicious circle’ or a ‘fast-forward’ feedback whereby adipose tissue and liver are continuously subject to the deleterious effects of glucocorticoid excess [121].

Studies in vitro assessing regulatory effects of glucocorticoids on TNF-α secretion in adipocytes have reported conflicting results, likely as a consequence of different experimental models. Sewter et al. [122] did not show any significant change in TNF-α release from either human adipose tissue or isolated adipocytes after 20 h of incubation with cortisol. In contrast, chronic treatment with dexamethasone for 15 days inhibited TNF-α expression in immortalized human pre-adipocytes in line with the immunosuppressive action of glucocorticoids [123]. Consistent with these data, glucocorticoids have been shown to inhibit TNF-α production in several animal models [124], while adrenalectomy causes elevated TNF-α levels during a septic insult in rats [125].

**Human Studies**

Similar to in vitro findings, studies examining the relationship between glucocorticoids and TNF-α in humans have reported conflicting results. One in vivo study showed that short-term dexamethasone administration in healthy men did not change TNF-α levels, despite concomitant occurrence of some dexamethasone-related metabolic perturbations [126]. In contrast, another study in healthy volunteers reported a significant suppression of TNF-α production following administration of either pharmacological or physiological doses of hydrocortisone (80 or 20 mg, respectively) [127]. Similarly, TNF-α release was suppressed after a stress-induced rise in glucocorticoid levels and a decline in TNF-α levels was observed concomitantly with circadian, physiological variations in cortisol, confirming the existence of a strict relationship between the two molecules [127].

Interestingly, in obese adolescent girls, high cortisol levels were an independent and direct predictor of TNF-α receptor 2, consistent with obesity being a pro-inflammatory state sustained by a relative and chronic excess of cortisol [128].

**TNF-α and Cushing’s Syndrome**

Three observational studies have reported that circulating TNF-α levels are within the normal range in CS patients [68, 129, 130] (table 1), and do not correlate with cortisol levels [68]. Another study assessing TNF-α concentrations in 7 CD patients reported that basal TNF-α levels were not significantly higher in the inferior petrosal sinus ipsilateral to the ACTH-secreting adenoma than in the contralateral sinus and peripheral blood [131]. Of note, administration of CRH induced a significant rise in ipsilateral TNF-α levels as compared with the other two sites, confirming in vitro evidence of an acute stimula-
tory effect of CRH on TNF-α secretion [131]. Indeed, incubation of cells from ACTH-secreting adenomas with CRH led to a significant increase in TNF-α levels in the medium as compared with pre-exposure concentrations [131]. Barahona et al. [90] reported that levels of the soluble TNF-α receptor 1 were significantly higher in patients with active CS than in controls.

These in vivo data intriguingly suggest that the physiological interplay between cortisol and TNF-α is altered in the presence of cortisol excess and that levels of the cytokine are not as low as expected. It has been speculated that TNF-α levels, although in the normal range, are inappropriately high in CS in the face of chronic hypercortisolism, contributing to the persistence of a low-grade inflammatory state. This phenomenon may be a consequence of a reduced sensitivity of TNF-α to the inhibitory action of cortisol and/or a concomitant enhancement of inflammation by other factors, such as visceral fat, other adipokines and GHD, which antagonize direct glucocorticoid effects [132]. This may partly explain the lack of correlation with cortisol levels observed in some studies [68, 130].

Of note, TNF-α levels were shown to increase early after TSS in patients with postoperative hypocortisolism [129]. Slightly lower TNF-α levels, though still higher than in the preoperative phase, were observed 10 days after TSS in patients on replacement doses of dexamethasone [129]. These findings support the hypothesis that short-term decreases in cortisol induce metabolic and adipokine changes, which, in turn, restore physiological regulation of TNF-α secretion. Yet, a state of low-grade inflammatory activation may persist after cure of CS. Barahona et al. [90] reported that soluble TNF-α receptor 1 was significantly higher both in patients with active CS and in patients who had been surgically cured of hypercortisolism for an average of 11 years compared with normal controls suggesting that TNF-α signaling may be persistently altered in CS patients even after long-term cure. This may maintain a ‘low-grade inflammation state’ in cured CS patients, leading to a persistently elevated cardiovascular risk profile [90]. Another possible explanation for persistent abnormalities may be glucocorticoid replacement, particularly if the dose is supraphysiologic. In the Barahona study [90], nearly one-third of patients were taking glucocorticoid replacement.

Of note, a recent paper evaluating adipokine levels in 20 patients with adrenal incidentaloma (of whom only 3 had subclinical CS) found higher TNF-α concentrations in these patients as compared with age-, sex-, and BMI-matched controls, suggesting that adrenal tissue may autonomously produce TNF-α [108].

GH overexpression in rats and mice is associated with reduced TNF-α levels [110]. However, data are conflicting and TNF-α has recently been shown to be higher in patients with acromegaly as compared with normal controls [135]. A study in humans with GHD showed a 220% increase in concentrations of this adipokine and a subsequent significant reduction after starting GH replacement [134]. The impact of associated GHD in some patients with CS on TNF-α levels is unclear.

In conclusion, the physiological relationship between glucocorticoids and TNF-α may be blunted in CS, thus contributing to a state of chronic inflammation, which may persist long-term after remission of glucocorticoid excess.

### Interleukin-6

**IL-6 and Cardiovascular Risk**

IL-6 is a cytokine which circulates as a glycosylated protein and has a wide range of actions including promotion of coagulation and immune/inflammatory reaction [135]. IL-6 is secreted by several cell types, including fibroblasts, endothelial cells and adipocytes, and plasma levels are significantly upregulated in human obesity and insulin resistance [136]. IL-6 administration directly altered insulin signaling in murine hepatocytes and 3T3-L1 adipocytes, and affected insulin-induced glycogenesis in liver cells [137]. A direct relationship has been observed between IL-6 in fatty tissue and insulin resistance in human obesity [138], and IL-6 has also been proven to be an important link between inflammation and atherosclerosis [139]. Indeed, IL-6 is induced by TNF-α in vascular tissue and has been shown to regulate CRP production in smooth muscle cells, which negatively affects expression of adhesion molecules, and endothelial function [139]. Elevated levels of IL-6 were associated with a doubling of the risk of cardiovascular and all-cause mortality in a cohort of 1,293 healthy, elderly subjects followed prospectively for a mean of 4.6 years [140], and had an important prognostic value in patients with unstable angina [141].

**IL-6 and the HPA Axis**

In vitro and Animal Studies

Similarly to other cytokines, IL-6 directly stimulates the HPA response to stress and inflammation. Incubation of rat hypothalami with IL-6 induced a rapid and dose-dependent release of CRH, and this effect was also observed in vivo after icv injection of IL-6 [142]. However, in vitro studies evaluating the effects of IL-6 on
ACTH synthesis and secretion have demonstrated contradictory findings, with a direct stimulatory effect on corticotrope cells [142], yet inhibitory effects on CRH-mediated ACTH secretion in rat anterior pituitary cell cultures [132]. Interestingly, activation of the immune response in mice lacking both CRH and IL-6 led to the release of significantly lower corticosterone levels than those measured in animals deficient in CRH or IL-6 alone, suggesting that IL-6 is an important CRH-independent stimulator of the HPA axis [143].

Indeed, IL-6 is able to directly and dose dependently induce the release of corticosteroids by animal and human cells in vitro and in vivo, and similar to TNF-α is considered an intra-adrenal factor regulating adrenal steroidogenesis [119]. Of note, it has been demonstrated that IL-6 and ACTH may act synergistically to enhance the production of corticosterone in rat adrenal cells [144]. Thus, it is possible that stress-induced increases in IL-6 contribute to the maintenance of homeostasis through the concomitant rise in glucocorticoid secretion.

Incubation of corticotrope adenoma cells with dexamethasone significantly inhibited intratumoral IL-6 release [145]. Adrenalectomized rats have elevated IL-6 levels, which significantly decrease after administration of corticosterone [146]. Although the dose-dependent inhibition of IL-6 by glucocorticoids has been documented in many studies in animal and human cell cultures, including adipocytes [147], some studies suggest that suppression is partial, likely from concomitant effects of other factors, such as catecholamines [132].

Human Studies

Human studies have suggested that the interaction between glucocorticoids and IL-6 is modulated by a complex network of signals, including TNF-α.

Administration of 80 mg of hydrocortisone to healthy volunteers suppressed IL-6 release, whereas stress-induced levels of glucocorticoids had no effect [127]. This observation, along with the finding that circadian variations of cortisol were not associated with decreased IL-6 secretion [127], seems to confirm that the relationship between glucocorticoids and IL-6 in humans is at least in part mediated by other factors, including TNF-α and/or catecholamines. Interestingly, Papanicolau et al. [148] showed that a high-intensity exercise test in healthy men caused a peak of catecholamines which was positively associated with a concurrent peak of IL-6. Pretreatment with dexamethasone or hydrocortisone attenuated, but did not block, the IL-6 peak.

It is well known that the stimulatory action of IL-6 on the HPA axis represents the basis for the immunomodulatory treatment of many illnesses including tumors. Daily administration of IL-6 to patients with cancer causes a significant rise in ACTH and cortisol levels over 7 days [149].

**IL-6 and Cushing’s Syndrome**

Data regarding IL-6 levels in CS are contradictory (table 1). One study reported that IL-6 levels are not suppressed in patients with active CS [129] and do not differ significantly from age-, sex- and BMI-matched controls [130]. However, Papanicolau et al. [129] showed that in patients with active CS, IL-6 may be more responsive to the tonic inhibitory action of glucocorticoids than other cytokines; IL-6 levels rose dramatically in the immediate postoperative period, when patients became hypoadrenal. This increase was more consistent than that observed for other adipokines, and was partially reversed by subsequent glucocorticoid replacement [129]. These data suggest the existence of a tonic negative feedback loop between endogenous cortisol and IL-6. However, the data are by no means conclusive, and another study [90] showed that IL-6 concentrations were significantly higher in women with active CS than in gender-, age-, and BMI-matched controls, and remained as elevated in cured patients even after a mean of 11 years of cure [90].

In 20 patients with cortical adenomas, of whom only 5 had been diagnosed with subclinical hypercortisolism, IL-6 levels were elevated compared with controls and were inversely related to urinary free cortisol concentrations [108]. In addition, Kushlinskii et al. [150] found that circulating IL-6 levels in patients with adrenal tumors were significantly higher than those in healthy controls, and were highest in patients with adrenocortical tumors. These data suggest possible autonomous production of this adipokine by adrenal tissue.

As previously mentioned, patients with CD often have GHD and this may affect IL-6 production. Although in vitro studies showed that GH administration is able to induce IL-6 expression in 3T3-L1 adipocytes, in vivo observations have documented an inverse relationship between GH and IL-6. In particular, Serri et al. [134] found a 340% increase in IL-6 in GHD patients as compared with controls, and a decrease in IL-6 following GH replacement.

In conclusion, inappropriately high levels of IL-6 in Cushing’s (which may or may not be related to GHD) represent another important pathogenic component of the inflammatory-related vascular and metabolic complications associated with chronic glucocorticoid excess.
Angiotensinogen

Angiotensinogen and Cardiovascular Risk

Angiotensinogen (AT), the precursor of the vasoactive peptide angiotensin II, is predominantly produced by the liver, followed by adipose tissue. AT is higher in obese than lean subjects, and is positively associated with blood pressure [151]. Indeed, AT mRNA expression in adipose tissue, and specifically in omental fat, is upregulated in obese patients [152]. In addition, AT overexpression in murine adipose tissue leads to adipocyte hypertrophy, an increase in fat mass, increased plasma AT levels, hyperinsulinemia, hyperleptinemia, and hypertension, whereas the opposite metabolic pattern is observed in the AT knock-out model [153]. AT re-expression in AT-null mice, which are lean and hypertensive, restores normal blood pressure [154].

Angiotensinogen and the HPA Axis

Dexamethasone administration induces AT gene expression and secretion in cultured mature mouse adipose cells and explants of rat adipose tissue ex vivo [155]. In addition, an upregulation of AT has been described in transgenic mice overexpressing adipose 11β-HSD-1 [156]. It would be intriguing to speculate that glucocorticoids may stimulate AT secretion, which, in turn, may act as either a vasoconstrictor or trophic factor for adipose tissue through its activation products (e.g. angiotensin II). Indeed, angiotensin II promotes differentiation of preadipocytes to adipocytes through release of prostacyclin [157] and cortisol has been shown to increase angiotensin II type 1 receptor gene expression in human adipocytes in a time- and dose-dependent manner [158]. However, data published thus far do not indicate a direct, causal relationship between AT and cortisol in human models [159]. Data regarding acute effects of AT or angiotensin infusion on HPA axis function are limited, with some studies describing no effect [160, 161], while others report a decrease in ACTH or cortisol secretion [162, 163] in humans. Of note, a recent paper by Sanchez-Lemus et al. [164] showed that angiotensin II receptor 1A (AT1A) blockade increased basal circulating levels of corticosterone in rats.

Angiotensinogen and Cushing’s Syndrome

Whether AT may play a role in any of the clinical features of chronic glucocorticoid excess is unclear because the scarce data published in CS so far are inconclusive. Of interest, one study showed that hypertension in CS is largely mediated by angiotensin II [165]. Shibata et al. [166] found an increase in the expression of AT1A in both mononuclear leukocytes and platelets of 7 patients with unilateral adrenal cortical adenomas causing CS compared with controls, which was reversed after adrenalectomy [166]. AT1A gene expression in these adrenal tumors was decreased compared with that from adrenal glands of control subjects (table 1).

Plasminogen Activating Inhibitor-1

Plasminogen Activating Inhibitor-1 and Cardiovascular Risk

Plasminogen activating inhibitor (PAI-1), which is produced by liver and adipose tissue, inhibits the activity of tissue-type plasminogen activator, an anticlotting factor, thereby favoring thrombus formation over ruptured atherosclerotic plaques. PAI-1 expression is elevated in visceral obesity, insulin resistance and hypertriglyceridemia, and its levels appear to predict risk for future development of both type 2 diabetes and cardiovascular disease [167, 168].

PAI-1 and the HPA Axis

Dexamethasone has been observed to induce a time- and dose-dependent expression and release of PAI-1 from subcutaneous and especially omental fat in vitro [169]. Interestingly, this stimulatory effect of glucocorticoids on PAI-1 production by adipose tissue appeared to be inhibited by coincubation with an inhibitor of 11β-HSD-1 [170]. Indeed, in situ hybridization revealed increased PAI-1 mRNA expression in visceral adipose tissue of obese subjects, which was positively related to the expression of 11β-HSD-1 mRNA, suggesting that local conversion of cortisone to cortisol may contribute to the observed elevation of PAI-1 levels in obesity [170]. Similarly, a 3-hour intravenous infusion of hydrocortisone enhanced PAI-1 circulating concentrations in both obese and normal women, and the percent increase between 180 and 240 min was significantly higher in obese subjects compared with controls [171].

PAI-1 and Cushing’s Syndrome

CS has been shown to be associated with increased levels of PAI-1 compared with healthy controls, which may relate to the hypercoagulable state frequently observed in patients with this condition [172, 173] (table 1). Of note, PAI-1 positively correlates with BMI in active disease. Levels are comparable to controls after a median of 36 months from surgical remission of hypercortisolism [173].
Similarly, GHD patients have increased levels of PAI-1, and this pattern correlates with BMI and waist-to-hip ratio [174, 175]. Indeed, GH was shown to enhance PAI-1 expression and secretion from adipocytes in vitro, although it has also been hypothesized that GH may indirectly downregulate PAI-1 production through its modulatory effect on the nitric oxide release from endothelial cells [175]. GHD may represent another cause of high PAI-1 levels in CS.

**Ghrelin**

**Ghrelin and Cardiovascular Risk**

Ghrelin is a 28-amino-acid acylated peptide mainly secreted by the stomach and represents the principal endogenous ligand for growth hormone secretagogue receptor (GHS-R) type 1a, whose expression is observed in the hypothalamo-pituitary region [176]. Ghrelin is also synthesized by the hypothalamus, pituitary, pancreas, kidney, heart, thyroid and Leydig cells. Although ghrelin does not meet the definition of an adipokine, it is involved in the regulation of glucose metabolism and lipogenesis both directly and through interactions with adipokines and is important to consider. Increasing evidence indicates that ghrelin, in addition to its GH secretagogue and orexigenic effects, also impacts diverse processes including ACTH and PRL secretion, glucose and lipid metabolism, gastric motility and acid secretion, cardiac function, sleep, and reproduction. In addition, ghrelin shows antiproliferative effects both in vivo and in vitro [176]. Ghrelin is present in the blood in two forms: the desacylated and the acylated form. The latter, which circulates at 2.5-fold lower concentrations than the unacylated, binds to the GHS receptors and mediates most of the endocrine actions of the molecule [177].

Ghrelin is known to stimulate the differentiation of pre-adipocytes into adipocytes and antagonize lipolysis [176]. Ghrelin levels are inversely correlated with BMI and insulin resistance, and food has an inhibitory effect on ghrelin, which is attenuated in obesity [176].

This orexigenic peptide also exerts anti-inflammatory and cardioprotective actions, partly mediated through its inhibitory actions on TNF-α, IL-1β, and IL-6 [178]. Ghrelin has been shown to inhibit mononuclear cell binding and nuclear factor-κB activation in human endothelial cells in vitro and in a rat model in vivo [179]. In addition, it has been found to directly increase endothelial nitric oxide synthase expression in humans with metabolic syndrome, improving endothelial dysfunction [180].

Moreover, ghrelin infusion increases cardiac output and reduces systemic vascular resistance in healthy humans [181, 182].

**Ghrelin and the HPA Axis**

In vitro and Animal Studies

Ghrelin acutely induces ACTH secretion, an effect that decreases following chronic administration of ghrelin [183]. The ghrelin-induced ACTH release is mediated by central mechanisms involving CRH and neuropeptide Y [176]. Ghrelin released from the rat hypothalamus stimulates CRH production [184]. In addition, the ability of GH secretagogues (GHS) to stimulate ACTH and cortisol requires an intact hypothalamic-pituitary axis as this effect is mediated by CRH rather than a direct effect of GHS on pituitary corticotropes [185]. In normal subjects, the extent of ACTH response to GHS does not differ from that following CRH administration and is sensitive to negative feedback inhibition by cortisol [186].

However, some direct stimulatory action of ghrelin on the pituitary cannot be ruled out, as GHS receptors (GHS-R) have been found on normal pituitary cells as well as corticotrope adenomas as well as normal pituitary cells [187], and a direct stimulatory effect of ghrelin on ACTH release in such tumors has been demonstrated in vitro [188]. Of note, even ectopic ACTH-secreting tumors show an exaggerated response to GHS, arguing against the use of ghrelin in differentiating between pituitary and extrapituitary causes of ACTH-dependent hypercortisolism [176].

Importantly, both the ghrelin receptor (GHS-R1a) and ghrelin mRNA have been detected in normal adrenal glands as well as in cortisol-secreting adenomas, suggesting that ghrelin may have an additional pathophysiological role in the regulation of the HPA axis [189].

Glucocorticoids may in turn affect ghrelin secretion. Although short-term dexamethasone treatment in rats did not cause a significant change in pituitary ghrelin expression, 1 week of treatment led to a significant decrease in ghrelin expression compared with controls [190]. Similarly, daily administration of 30 mg of prednisolone for 5 days to 8 healthy males induced a significant decrease in ghrelin levels [191]. It is possible that some of these changes in ghrelin levels are mediated by changes in insulin.

**Ghrelin and Cushing’s Syndrome**

Data available on ghrelin in CS do not conclusively define its relative contribution to the metabolic and cardiovascular features associated with chronic hypercorti-
Ghrelin levels have been reported as both increased and decreased in CS [89, 191, 192]. A potential limitation to the studies published so far is that only total ghrelin was measured and the acylated form of the molecule is responsible for the neuroendocrine actions of ghrelin, including its regulation of the HPA axis. Importantly, desacylated ghrelin appears to have an opposite effect on glycometabolic status compared with the acylated form, and has been reported to induce the growth of adrenocortical tumors [177].

In patients with CD, the ACTH response to ghrelin is exaggerated and even higher than that observed following CRH administration in the same subjects [176]. Otto et al. [191] reported that a single fasting plasma ghrelin measurement in 5 CS patients was significantly higher 24 months after successful surgical correction of hypercortisolism compared with values before intervention. Of note, this increase in ghrelin levels was inversely related to the significant postsurgical decrease in BMI, suggesting that the change in ghrelin may be mediated by the progressive weight loss induced by remission of CS, and the associated reduction in insulin levels (table 1). Similar findings were reported by Libè et al. [89], who noted that higher fasting ghrelin levels in 14 CD patients 10 months after surgical resolution of hypercortisolism compared with baseline were associated with a significant reduction in BMI and insulin levels over this period. Because insulin is known to inhibit ghrelin secretion [193], it is possible that insulin resistance associated with CS is responsible for lower ghrelin levels during the active stage of the disease. However, Libè et al. [89] could not find any correlation between presurgical ghrelin and insulin levels.

In a study by Giordano et al. [192], ghrelin levels measured every 15 min for 3 h in 8 CD patients were comparable to those found in normal controls with significantly lower BMI, despite significantly higher insulin and glucose levels in CD. Although the well-known inhibitory effect of glucose and insulin on ghrelin levels appears to be blunted in CD, acute administration of ghrelin to CD patients causes the expected physiological increase in glucose, suggesting that in the presence of chronic hypercortisolism, some metabolic actions of ghrelin are preserved. Of interest, no correlation was found between ghrelin and cortisol levels either before [89, 192] or a few days after successful surgery [89]. However, the sample size was small in these studies.

Published data do not show an association between GH and ghrelin levels in CS, although ghrelin is a GHS and CS is often associated with GHD [194, 195]. Interestingly, a recent study found that ghrelin suppression during insulin-induced hypoglycemia was similar in patients with GHD and normal subjects, and total ghrelin levels were not associated with either GH or cortisol levels [196]. In contrast, another study reported lower levels of total ghrelin in GHD patients at baseline compared with controls, followed by a subsequent increase in ghrelin levels after a year of GH treatment, which was associated with a significant decrease in body fat as compared with controls [92].

In conclusion, low ghrelin levels may contribute to increased cardiovascular risk in CD similar to that observed in obesity, but future studies are needed to further clarify this point, particularly by differentiating the interactions of acylated versus desacylated ghrelin with the HPA axis.

Conclusions

Adipokines are at the center of a complex network of signals that regulate metabolism and cardiovascular function. Imbalance of adipokine production is associated with increased cardiovascular risk in several conditions characterized by central fat accumulation, including CS. Indeed, elevation of leptin and resistin levels as well as the release of pro-inflammatory cytokines, such as TNF-α and IL-6, may lead to the high cardiovascular morbidity observed during the active phase of CS. Likewise, persistent impairment of adipokine secretion may contribute to the increased long-term cardiovascular risk in patients cured of CS described in some earlier reports. Future studies are needed to fully elucidate the interactions between clinical or subclinical increases in cortisol production and dysregulated adipokine secretion in CS, providing new insights into the pathogenesis of complications associated with this potentially lethal disease.

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