Metabolic comorbidities in Cushing's syndrome

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Abstract

Cushing's syndrome (CS) patients have increased mortality primarily due to cardiovascular events induced by glucocorticoid (GC) excess-related severe metabolic changes. Glucose metabolism abnormalities are common in CS due to increased gluconeogenesis, disruption of insulin signalling with reduced glucose uptake and disposal of glucose and altered insulin secretion, consequent to the combination of GCs effects on liver, muscle, adipose tissue and pancreas. Dyslipidaemia is a frequent feature in CS as a result of GC-induced increased lipolysis, lipid mobilisation, liponeogenesis and adipogenesis. Protein metabolism is severely affected by GC excess via complex direct and indirect stimulation of protein breakdown and inhibition of protein synthesis, which can lead to muscle loss. CS patients show changes in body composition, with fat redistribution resulting in accumulation of central adipose tissue. Metabolic changes, altered adipokine release, GC-induced heart and vasculature abnormalities, hypertension and atherosclerosis contribute to the increased cardiovascular morbidity and mortality. In paediatric CS patients, the interplay between GC and the GH/IGF1 axis affects growth and body composition, while in adults it further contributes to the metabolic derangement. GC excess has a myriad of deleterious effects and here we attempt to summarise the metabolic comorbidities related to CS and their management in the perspective of reducing the cardiovascular risk and mortality overall.

Introduction

Cushing's syndrome (CS) is a severe disease due to the complications of glucocorticoid (GC) excess (Fig. 1). The increased morbidity and mortality in these patients is due to the cardiovascular, thrombotic, metabolic, infectious and musculoskeletal complications (1, 2, 3). To understand, treat and ideally prevent these complications is a major challenge to the treating physician and the multidisciplinary team looking after the patient. These issues

Invited Authors' profiles

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Dr Francesco Ferrau originally from Messina, Sicily, Italy, was a recent visiting PhD student at Barts and the London School of Medicine, where he studied the aetiology and pathogenesis of pituitary adenomas for his PhD.
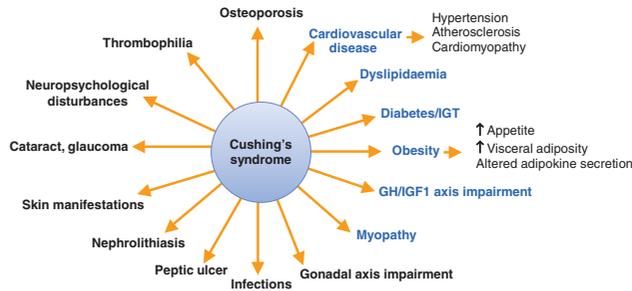
Figure 1

Cushing's syndrome comorbidities. CS is associated with numerous deleterious changes in various organ systems. The ones discussed in this review are marked with blue. IGT, impaired glucose tolerance.

are further complicated in patients with iatrogenic CS, where the underlying disorder often contributes to increased cardiovascular and musculoskeletal complications. This review focuses on the pathomechanisms and clinical consequences of the interrelated deleterious effects of GC excess on glucose, lipid and protein metabolism and on the changes observed in adipose tissue, muscle and cardiovascular system as well as on the growth hormone (GH)/insulin-like growth factor 1 (IGF1) axis. GC-excess related thrombotic complications, which further contribute to the CS-associated high mortality rate due to cardiovascular disease, will be specifically addressed in another review of this issue of the *European Journal of Endocrinology*.

GCs and glucose metabolism

Glucose metabolism alterations are common in CS: overt diabetes mellitus (DM) is diagnosed in 20–47% of the patients (4), while impaired glucose tolerance (IGT) is present in 21–64% of cases (4, 5). No significant gender differences have been reported (6). Diabetic CS patients have been found to be older than normoglycaemic or prediabetic CS patients (7). Nevertheless, reported prevalence could be underestimated because available data were largely based on the measurement of fasting blood glucose. Indeed, in a study of 48 patients with active Cushing's disease (CD), over two-thirds of those with diabetes had been diagnosed by an oral glucose tolerance test (OGTT), thus confirming the inaccuracy of the fasting blood glucose for assessing the prevalence of diabetes in CS (8). Therefore, an OGTT has been suggested in all patients with endogenous CS (9).

Increased DM prevalence has been reported in ectopic (74%) compared to pituitary (33%) and adrenal (34%) CS (10). This indirectly suggests that the degree of cortisol excess can be relevant for the development of altered glucose metabolism. No definitive data are available to link the duration of CS with glucose tolerance abnormalities. Alterations of glucose metabolism can persist after the remission and cure of CS (4). The prevalence of CS in diabetic populations has been studied and data suggest that routine screening of patients with DM for CS is not recommended (11). Several prospective screening studies of diabetic patients who lack specific clinical symptoms of hypercortisolism revealed a prevalence of CS that reaches 3.3% (12). More recently, only 0.6% of a large cohort of diabetic patients unselected for signs or symptoms suggestive of hypercortisolism have been found to be affected with CS. A case finding approach to identify CS is recommended in patients with diabetes and hypertension who are difficult to control despite complex treatment (13). The prevalence of abnormal glucose tolerance is controversial in patients with subclinical CS (sCS), depending on the criteria adopted to diagnose sCS and DM (14, 15). Data from some studies in which OGTT results are available suggest that there is no significant difference in the prevalence of IGT or DM in patients with sCS due to an adrenal adenoma compared to non-functioning adrenal lesions (14). On the other hand, a large study found an increased prevalence of DM, diagnosed according to the American Diabetes Association guidelines, in adrenal adenoma patients with sCS compared to those with non-functioning adrenal lesions (16). Similarly, a recent investigation carried out on 268 patients with adrenal incidentaloma demonstrated that HbA1c was significantly increased in sCS compared to subjects harbouring non-secreting lesions, suggesting an adverse metabolic profile in sCS patients, in line with other previous reports based on fasting glycaemia and insulin levels (17). Accordingly, other recent studies found an increased prevalence of glucose tolerance abnormalities among patients with sCS compared to patients with non-functioning adrenal adenomas (15). The prevalence of glucose metabolism abnormalities in patients with monolateral vs bilateral adrenal lesions is the same, even if sCS was more frequent among patients with bilateral disease (18). Insulin sensitivity parameters were reduced in both non-functioning adrenal adenoma or in sCS, compared to healthy controls. There was no difference in terms of insulin sensitivity between the two groups of adrenal lesion patients, but subjects with sCS had a significantly higher prevalence of IGT and a higher area under the curve

for glucose (19). These data and other studies demonstrate that patients with adrenal incidentaloma, regardless of the functional status, show insulin resistance (20, 21, 22, 23). Moreover, one study found a correlation between adrenal lesion size and insulin resistance (20). Therefore, we could turn the question around and ask whether insulin, a known growth factor, might have a potential role in the development and growth of the adrenal lesions (20). On the other hand, patients with non-functioning adrenal lesions could develop insulin resistance because of a subtle alteration of cortisol secretion dynamics not identifiable as autonomous sCS (22).

Mechanism ▶ Impairment of glucose metabolism in CS patients is due to a combination of several concurrent GC-related effects which directly and indirectly affect glucose homeostasis, mainly through the induction of gluconeogenesis and the disruption of insulin receptor signalling (Fig. 2). Patients with CS have an increased HOMA-index and a reduced insulin sensitivity index regardless of BMI, suggesting that hypercortisolism *per se* impacts on insulin sensitivity (8). GC excess increases glucose production in the liver by directly inducing the expression of key enzymes for gluconeogenesis (primarily by increasing the transcription of glucose-6-phosphatase); indirectly providing substrates for gluconeogenesis through the stimulation of lipolysis and proteolysis; antagonising the action of insulin; and enhancing the action of different insulin-antagonist hormones, especially glucagon (24).

GCs increase the expression of MAPK phosphatase 3 (MKP3), which in turn activates forkhead box O1 (FOXO1) promoting its translocation from cytosol to nucleus. Activated FOXO1 increases the transcription of the peroxisome proliferator-activated receptor gamma (PPARγ) co-activator 1 alpha (PGC1α) and also interacts with PGC1α to initiate the transcription of genes coding for gluconeogenic enzymes (phosphoenolpyruvate carboxy-kinase, glucose 6-phosphatase) (25). The mechanisms through which GCs alter hepatic gluconeogenesis and insulin sensitivity also involve crosstalk of the GC receptor (GR) with other signalling pathways. Indeed, an interplay has been found between GR and liver X receptors (LXRs), which are known to heterodimerise with the retinoid X receptor (RXR) to regulate cholesterol turnover and glucose metabolism. In a recent study, the LXRs/RXR complex reduced the transcriptional activity of GR, since LXR activation was able to reduce dexamethasone-stimulated elevation of circulating glucose in rats and suppress dexamethasone-induced mRNA expression of

hepatic glucose-6-phosphatase in rats, mice and human hepatoma cells (26). Moreover, the PPARα signalling pathway, which is required to suppress glucose-stimulated insulin secretion during fasting, has been found to be involved in the dexamethasone-mediated insulin resistance in the liver (27).

GCs also affect glucose metabolism through specific actions on skeletal muscle (Fig. 2). The detrimental effects of GC excess on glucose uptake and glycogen synthesis in the skeletal muscle is due to the interference with the post-receptor signalling pathway of the insulin receptor. Dexamethasone reduces the tyrosine-phosphorylation of insulin receptor substrate (IRS)-1, and consequently the activation of phosphatidylinositol 3 kinase (PI3K) and protein kinase B (PKB)/AKT, leading to impaired glucose transporter type 4 (GLUT4) migration to the cell surface. GCs also lower glycogen synthase kinase (GSK) phosphorylation and block the ability of insulin to dephosphorylate and activate glycogen synthase, leading to reduced glycogen synthesis (28).

GCs indirectly impair insulin signalling via effects on proteins and lipid metabolism. GC excess is associated with increased protein breakdown, leading to increased concentrations of amino acids. Amino acids interfere with the intracellular insulin pathway by inhibiting IRS tyrosine-phosphorylation and activation of PI3K (29). Hypercortisolism is also associated with increased lipolysis, causing free fatty acid (FFA) elevation, which affects insulin receptor signalling and, consequently, glucose uptake and disposal (24, 30). The increased intramyocellar fat and fatty acid metabolites content, consequent to the increased FFA influx into skeletal muscle coupled with decreased mitochondrial fat oxidation, lead to increased serine-phosphorylation of IRS1, via increased activity of serine kinases such as PKC. Serine-phosphorylation of IRS1 impairs its tyrosine phosphorylation by the insulin receptor and thus its activation, leading to the disruption of the insulin signalling cascade (30).

The typical visceral pattern of fat distribution in CS patients leads to increased leptin and reduced apelin levels, which have been associated with insulin resistance (24, 31). In mice, plasminogen activator inhibitor 1 (PAI1) expression was upregulated in white adipose tissue by corticosteroids, and transgenic PAI1 deficiency was able to counteract the negative effects of GCs on glucose metabolism, reducing insulin resistance (32).

Chronic hypercortisolism causes pancreatic β-cell dysfunction (Fig. 2). GC excess leads to impaired uptake and metabolism of glucose in β cells by reducing the expression levels of glucose transporter type 2 and
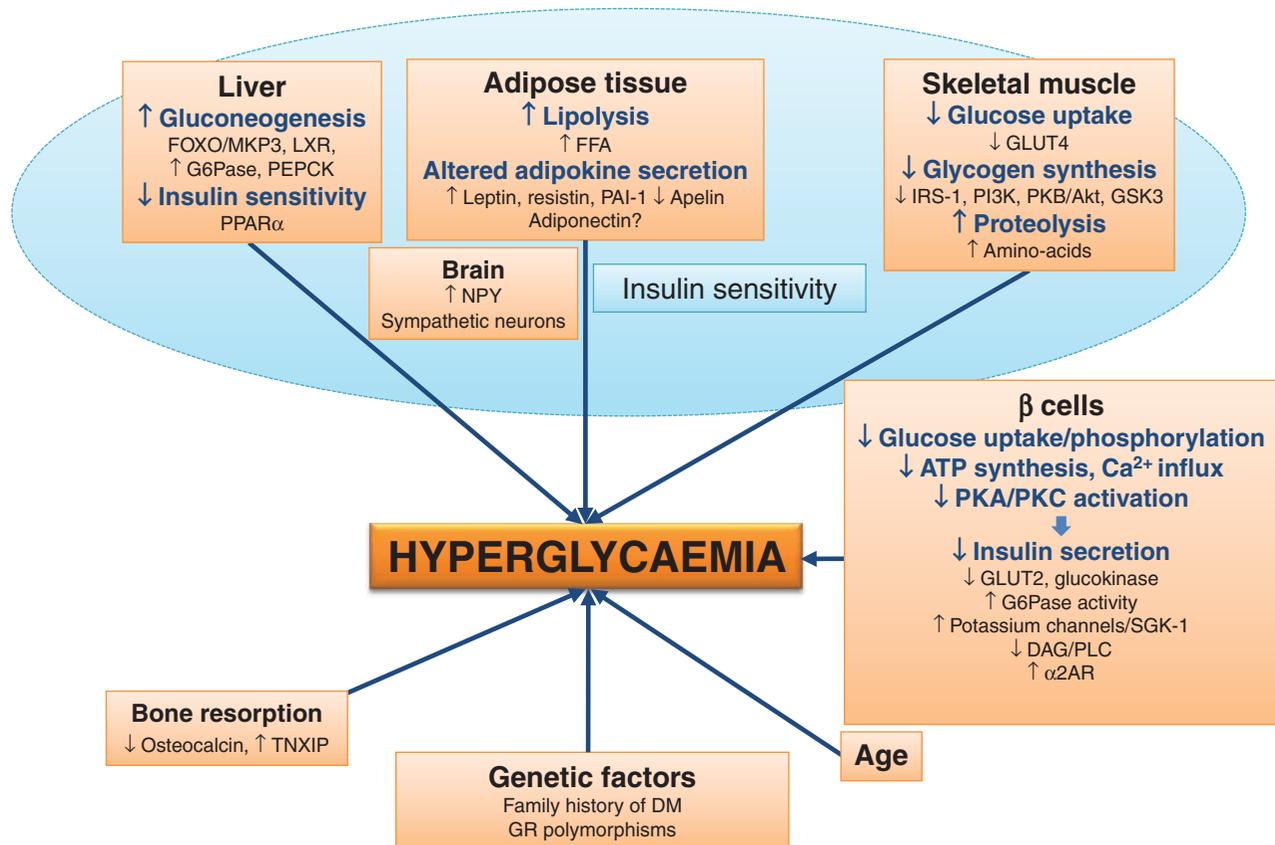
Figure 2

Hyperglycaemia in Cushing's syndrome. Hyperglycaemia in CS is due to several GC-related actions on the liver, skeletal muscle, adipose tissue and pancreas. GCs induce an increase of gluconeogenic enzymes, via upregulation of the forkhead transcription factor FOXO1 and mitogen activated kinase phosphatase 3 (MKP3) and via cross talk with other signalling pathways such as LXR. They reduce insulin sensitivity in the liver (with the contribution of PPARα signalling and GC-associated effects on hypothalamus) and skeletal muscle. The adipose tissue contributes to the insulin resistance through the deregulation of adipokine secretion and free fatty acid (FFA) release. GCs affect β-cell function interfering with glucose uptake and β-oxidation as well as with protein kinase A/protein kinase C (PKA/PKC) activation with a consequent reduction of

insulin secretion. Bone remodelling via reduced osteocalcin and increased thioredoxin-interacting protein 1 (TNXIP) can further contribute to the complex mechanism leading to glucose metabolism impairment. Age and genetic predisposing factors have been shown to be involved as well. G6Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase; PPARα, peroxisome proliferator-activated receptor α; PAI1, plasminogen activator inhibitor 1; NPY, neuropeptide Y; GLUT4, glucose transporter type 4; IRS1, insulin receptor substrate 1; PI3K, phosphatidylinositol 3 kinases; PKB, protein kinase B; GSK3, glycogen synthase kinase3; ATP, adenosine triphosphate; GLUT2, glucose transporter type 2; SGK1, serum/GC regulated kinase 1; DAG, dyacylglycerol; PLC, phospholipase C; α2AR, α2 adrenergic receptor; DM, diabetes mellitus; GR, GC receptor.

glucokinase leading to a decrease in ATP synthesis and calcium influx, resulting ultimately in reduced insulin secretion. Overexpression of the noradrenergic receptor, deregulation of ion channels and inhibition of the DAG-phospholipase C pathway have also been demonstrated to play a role in the GC-mediated alteration of insulin secretion (24). It was also recently reported that

short-term exposure to GCs reduced the insulinotropic effects of glucagon-like peptide 1 (GLP1) (33). While GCs acutely inhibit insulin secretion, chronically the GC-induced increased insulin resistance leads to hyperinsulinaemia (34). In rats, GCs impact on the mass and function of pancreatic α cells, causing hyperglucagonaemia (35).

In addition, some data suggest that hypothalamic arcuate nucleus GC signalling modulates hepatic insulin responsiveness via neuropeptide Y (NPY) and the sympathetic system (36).

The individual sensitivity to GCs can also contribute to the occurrence of glucose metabolism alterations: the beneficial GR polymorphism A3669G is associated with a reduced risk of GC-induced DM regardless of age, sex, BMI, family history of DM and duration of disease (37).

Recent data have also linked glucose homeostasis to bone remodelling in CS, in addition to the detrimental direct effect of GCs on bone. CS patients display low levels of osteocalcin which in turn has been associated with impaired glucose metabolism, insulin resistance and visceral fat (VAT) (38, 39). Thioredoxin interacting protein (TXNIP)-1 is upregulated in bone cells by GCs and might contribute to reduced osteocalcin levels. Endogenous CS patients have increased bone expression of TXNIP, which has been associated with high levels of glucose and insulin, increased insulin resistance and decreased insulin sensitivity. The direct link between altered TXNIP, osteocalcin and glucose abnormalities requires further study (40). The intricate interplay between genetic predisposing factors, aging, alteration of insulin sensitivity and β-cell function contributes to the development, progression and severity of the GC-induced abnormalities in glucose metabolism.

Management ▸ In endogenous CS, the first step in the management strategy is the treatment of the underlying cause, with the consequent normalisation of cortisol levels, which generally leads to an improvement of the glucose metabolism. The potential benefit of adrenal surgery on the metabolic profile of patients with adrenal adenomas and sCS, in comparison to a more conservative management, is controversial and still a matter of debate, with a preponderance of studies suggesting that patients' glucose profiles and consequently cardiovascular risk would be improved by adrenalectomy (41, 42, 43). However, definitive rapid correction of hypercortisolism in CS is not always easily achievable. The most recently introduced medical therapy for CD, pasireotide, is potentially diabetogenic. Furthermore, glucose metabolism abnormalities can persist after cure.

Insulin sensitizers, such as metformin and thiazolidinediones, are considered as first-line therapy. These can be combined with agents that increase postprandial insulin secretion such as sulphonylureas and meglitinides. The effect of metformin (and possibly thiazolidinediones) on fat tissue and other organ AMP-activated protein kinases

(AMPK) could be beneficial (44, 45). Pioglitazone may have a potential negative effect on heart and bone (46). Thiazolidinediones have been demonstrated to have a direct adrenocorticotropic hormone (ACTH)-lowering effect on corticotrophinomas in vitro, but no convincing results arose from clinical trials on patients with CD (47). GLP1 analogues and dipeptidyl peptidase four inhibitors could be helpful in the management of GC-induced diabetes, increasing glucose dependent insulin secretion and reducing glucagon secretion as well as having positive effects on β-cell mass and function, appetite, adipocyte modulation, fat distribution, hyperlipidaemia, heart and bone (48, 49). Incretin-based medications have been suggested for the management of pasireotide-induced hyperglycaemia, since somatostatin receptor inhibition leads to reduced secretion of GLP1, glucose-dependent insulinotropic polypeptide and insulin (50, 51). Conventional tailored schemes of treatment with insulin analogues can be required when oral hypoglycaemic agents are not effective (24).

GCs and obesity

GCs severely affect adipose tissue biology and endocrine function via several mechanisms (Fig. 3). Chronic hypercortisolism determines a redistribution of body fat deposition leading to increased abdominal adiposity, with the related metabolic consequences. Direct and indirect GC effects on the central nervous system that influence eating behaviour can contribute to the obese phenotype of CS patients.

GC effect on appetite ▸ Although excess GCs are generally thought to increase appetite, studies on animals, healthy controls or patients treated with GCs short or long term produced controversial results in terms of appetite and energy intake (52). While in healthy volunteers a modest appetite increase can be shown, in patients the underlying illness, type of steroid medication and duration of exposure can influence appetite, energy intake and body weight changes (52, 53). Furthermore, endogenous hypercortisolism can considerably differ from the iatrogenic GC excess in terms of effects on appetite and food intake. CD patients have an increased preference for dietary fat compared to weight-matched and normal-weight controls (54). High-dose GC treatment, especially if not associated to high-dose carbohydrate intake, does not necessarily lead to weight or abdominal fat gain in rodents, probably also because of the diabetes-related catabolic effects (55). In humans, the rapid development
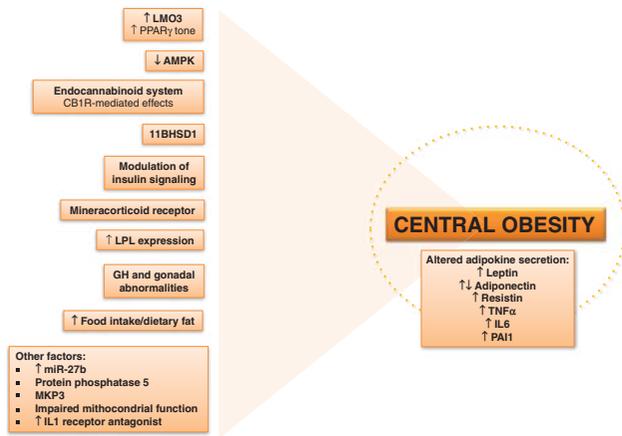
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Figure 3

Central obesity in Cushing's syndrome. Fat tissue redistribution in CS leads to central obesity and metabolic complications. Visceral obesity is associated to altered adipokine secretion further contributing to insulin resistance, inflammation and fat accumulation. LMO3, LIM domain only 3; PPARγ, peroxisome proliferator-activated receptor γ; CB1R, cannabinoid receptor 1; AMPK, AMP-activated protein kinase; 11BHSD1, 11β-hydroxy-steroid dehydrogenase enzyme; LPL, lipoprotein lipase; mIR27b, microRNA 27b; MKP3, MAP kinase phosphatase 3; TNFα, tumour necrosis factor-alpha; IL6, interleukin 6; PAI1, plasminogen activator inhibitor 1; IL1, interleukin 1.

of CS with very high cortisol levels, often due to ectopic ACTH-secreting tumours, can also lead to the lack of the otherwise typical weight gain in CS (56).

In animal models, chronic stress and the related increase in GCs lead to cravings for 'comfort food' (fat and carbohydrates) via a sequence of interconnected amygdala-limbic system-hypothalamic events (57). The resulting increased dopamine levels in the nucleus accumbens may then lead to reduced stress. Some data in humans also support this hypothesis (57). Weight gain would occur as a consequence of a stress-related shift to comfort food, with a redistribution of fat from periphery to the central compartment facilitated by GC in synergy with insulin (57).

Both *in vitro* (58) and *in vivo* (44) studies showed that GCs increase hypothalamic endocannabinoids. GCs and cannabinoids have been shown to increase hypothalamic AMPK activity resulting in increased appetite (59). Moreover, GCs have been shown to upregulate the gene expression of orexigenic peptides (NPY and agouti-related peptide) in rat arcuate nucleus also via AMPK (60). The GC effect on hypothalamic AMPK is lacking in cannabinoid

receptor 1-knockout (KO) animals, supporting the hypothesis that cannabinoids are involved in the hypothalamic AMPK and possibly the appetite effects of GCs (55).

GC effect on adipose tissue ▸ GCs are crucial for adipocyte differentiation (61) by inducing key adipogenic transcription factors (62) and they are known to regulate 20% of the adipose expressed genes (63). GCs sensitize pre-adipocytes to the action of insulin, leading to enhanced adipogenic action (64). GCs alter glucose metabolism and insulin sensitivity in adipocytes and they regulate lipid turnover by modulating the expression of genes involved in lipid storage and mobilization (65, 66). VAT is thought to be differentially responsive to GCs than subcutaneous fat (SAT). Indeed, GC binding to tissue homogenates and GR mRNA expression is higher in omental fat than in SAT (67, 68). Moreover, GCs were able to induce insulin resistance in human omental but not in subcutaneous adipocytes (69). In humans, GC excess results in increased abdominal VAT deposition and reduced peripheral subcu-taneous adipose depots. This can be only partially explained by the reduced lipolysis in central adipose tissue and the increased triglycerides uptake in abdominal adipocytes due to lipoprotein lipase overexpression (70, 71). Several different mechanisms can contribute to determining GC-mediated adipose tissue changes (Fig. 3).

11β-hydroxysteroid dehydrogenase type 1 activity ▸ Intra-cellular GC metabolism is regulated by the activity of two isoforms of the 11β-hydroxysteroid dehydrogenase enzyme, 11BHSD1 and 11BHSD2. 11BHSD1 promotes conversion of inactive cortisone to cortisol, whereas 11BHSD2 inactivates cortisol into cortisone. 11BHSD1 is widely expressed throughout the body, including the liver, visceral fat and SAT (72). Cortisol can reach a GR via several routes: direct binding of cortisol to GR; inacti-vation of circulating cortisol by 11BHSD2 in the kidney, then reactivation in peripheral tissues by 11BHSD1; and GR-induced local 11BHSD1 expression further amplifying intracellular cortisol availability (Fig. 4, panel A). The importance of excess or lack of locally produced GCs has been studied using global or tissue-specific overexpression or KO of 11BHSD1 (Fig. 4, panels B, C and D). GC treatment of global 11BHSD1-KO animals failed to cause CS. However, rodents with selective deletion of 11BHSD1 in the adipose tissue or in the liver were not protected from increased adiposity, decreased lean mass, glucose intolerance and hyperinsulinaemia or reduced grip strength following GC treatment. These findings would
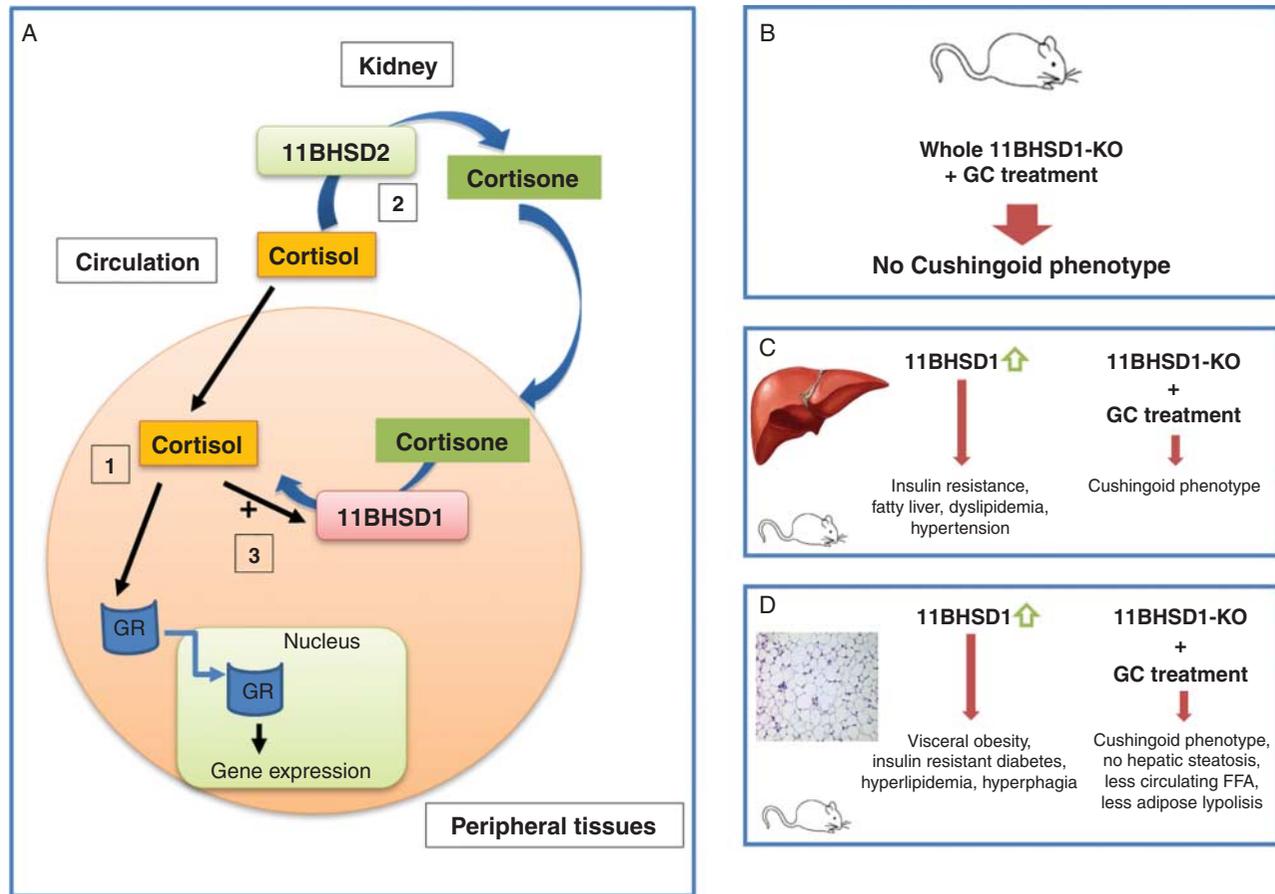
Figure 4

The 11β-hydroxysteroid dehydrogenases. Panel A: GC metabolism and tissue availability is regulated by the activity of two isoforms of the 11β-hydroxysteroid dehydrogenase enzyme 11BHSD1 and 11BHSD2. Cortisol can reach glucocorticoid receptor (GR) via several routes: [1] direct binding of cortisol to GR; [2] inactivation of circulating cortisol by 11BHSD2 in the kidney, then reactivation in peripheral tissues by 11BHSD1; and [3] GR-induced local 11BHSD1 expression further amplifying intracellular cortisol availability. Panels B, C, D: Clinical phenotype of mice with transgenic global (B) or tissue specific (C, liver; D, adipose tissue) deficiency of 11BHSD1 after GC treatment or overexpression (green arrow) of 11BHSD1. FFA, free fatty acids; KO, knockout.

suggest the importance of GC reactivation in tissues other than adipose and liver in the development of the CS phenotype. However, GC-treated adipose-specific 11BHSD1-KO mice were protected from lipid accumulation in the liver, increased circulating FFA and increased expression of lipolytic enzymes in fat tissue but not from increased expression of fatty acid transporters in the liver, suggesting that hepatic FFA uptake is not affected by 11BHSD1 expression in the adipose tissue. Interestingly, liver-specific 11BHSD1-KO did not protect the mice from adverse liver effects, suggesting the importance of 11BHSD1 expression in the adipose tissue in mediating negative metabolic effects on the liver (73). In another study, corticosterone treatment increased 11BHSD1 expression in adipose tissue of mice, and 11BHSD1 knockdown by shRNA attenuated GC-induced lipolysis and ameliorated insulin resistance in adipocytes (74). Adipose tissue-specific overexpression of 11BHSD1 leads to increased corticosterone content in fat tissue and associates to signs of the metabolic syndrome with visceral obesity, worsened by a high-fat diet, pronounced insulin-resistant diabetes, hyperlipidaemia and hyperphagia despite hyperleptinaemia (75). The importance of local GC action in the adipose tissue has also been shown in transgenic mice overexpressing 11BHSD2 exclusively in the adipose tissue, as these animals resist weight gain on

high-fat diet due to reduced fat mass accumulation (76). Similarly, adipose GR deficient mice have been shown to be protected from high-fat diet-induced obesity (77).

Liver-specific 11BHSD1 overexpression resulted in mild insulin resistance (without altered fat depot mass), fatty liver, dyslipidaemia and hypertension. These data would suggest that elevated hepatic expression of 11BHSD1 can be related to the pathogenesis of metabolic syndrome in the absence of obesity, like the metabolically obese but normal-weight individual (78). In humans, the key role of 11BHSD1 in determining Cushingoid features is demonstrated by a unique patient with CD who did not manifest the characteristic clinical signs, due to a functional defect of 11BHSD1 activity (79). Although 11BHSD1 mRNA is increased in the adipose tissue of obese individuals (80), this does not seem to apply to CS patients. 11BHSD1 mRNA and protein expression of omental adipose tissue is 13-fold higher in obese subjects compared with controls, but no difference was seen between CS patients and normal weight controls (81). The authors speculated that the lack of increase of 11BHSD1 expression in CS patients could be due to the downregulation of the enzyme as a result of long-term chronic cortisol overstimulation, representing a local defensive mechanism of the adipose tissue preventing 11BHSD1 overexpression, which in turn would be responsible for a further cortisol increase.

AMPK activity ▸ Several studies have found that GC tissue specifically influences AMPK activity (Fig. 5). In an animal model of CS, corticosterone treatment inhibited adipose tissue AMPK, which would support the accumulation of lipids in VAT (44). GC-induced adipose AMPK inhibition has also been confirmed in patients with CS who exhibited a 70% lower AMPK activity in VAT as compared with both non-functioning adrenal adenoma and control patients and was associated with increased mRNA expression of fatty acid synthase. AMPK activity showed a negative correlation to cortisol burden in these patients (45).

The endocannabinoid system ▸ The endocannabinoid system is also involved in the adverse metabolic features of chronic GC exposure. Scerif *et al*. (55) showed significant differences in GC-treated WT and cannabinoid receptor type 1 KO mice, and more recently, similar data showed the role of the cannabinoid system in GC-induced changes (82).

The mineralocorticoid receptor ▸ The possibility of GC-induced adipose tissue mineralocorticoid receptor (MR)

activation leading to pro-adipogenic effects has been raised. MR expression is increased in the adipose tissue of obese subjects (83) and has been shown to play a crucial role in adipocyte differentiation (84, 85). Furthermore, MR blockade was able to reduce the expression of pro-inflammatory and pro-thrombotic factors and to increase the expression of adiponectin in adipose tissue of obese diabetic mice (86) as well as attenuated adipocytes dysfunction and insulin resistance in obese mice (87). On the other hand, in primary cultures of human pre-adipocytes and adipocytes, GR expression is several 100-fold higher than MR. Silencing of GR, but not MR, blocked the pro-adipogenic actions of cortisol. In addition, GR silencing blocked the effects of cortisol on adipokines expression, while MR knockdown only increased leptin expression (88). Moreover, recently, transgenic mice overexpressing MR have been shown to be paradoxically protected from high-fat diet-induced obesity (89).

LIM domain only 3 ▸ LIM domain only 3 (LMO3), a pro-adipogenic factor that is more expressed in VAT than in SAT, could be the link between GCs and VAT changes. LMO3 has been recently demonstrated to be upregulated by GCs, to correlate with 11BHSD1 levels and to promote adipogenesis via increasing PPARγ tone in human VAT (90). As LMO3 is not expressed in rodent visceral tissue, this mechanism could also explain the well-known striking difference in GC-induced visceral adiposity in humans and mice.

Other mechanisms ▸ In mice, GCs have been recently shown to upregulate miR-27b, which inhibits the browning of white adipose tissue, a process which leads the white adipose tissue to express the uncoupling protein UCP1 (as brown adipose tissue) and to dissipate energy. MiR-27b inhibition improved GC-induced central fat accumulation (91). A recent animal model study suggests a role of protein phosphatase 5 (PP5), which is supposed to be a regulator of the transcriptional activity of GR, in adipose tissue distribution. Mice knocked in with a defective form of PP5 had less VAT surrounding gonads and kidneys and smaller adipocytes, probably via a disruption of the GC-related effects on pre-adipocyte differentiation (92). GCs, via FOXO1, increase the expression of MKP3. Mice lacking MKP3 are protected from some GC-related metabolic effects such as weight gain, increased adiposity, liver lipid accumulation and insulin resistance (93). In mice, an impairment of mitochondrial function has been related to increased epididymal adiposity after GC treatment (94). Recent *in vitro* data showed that
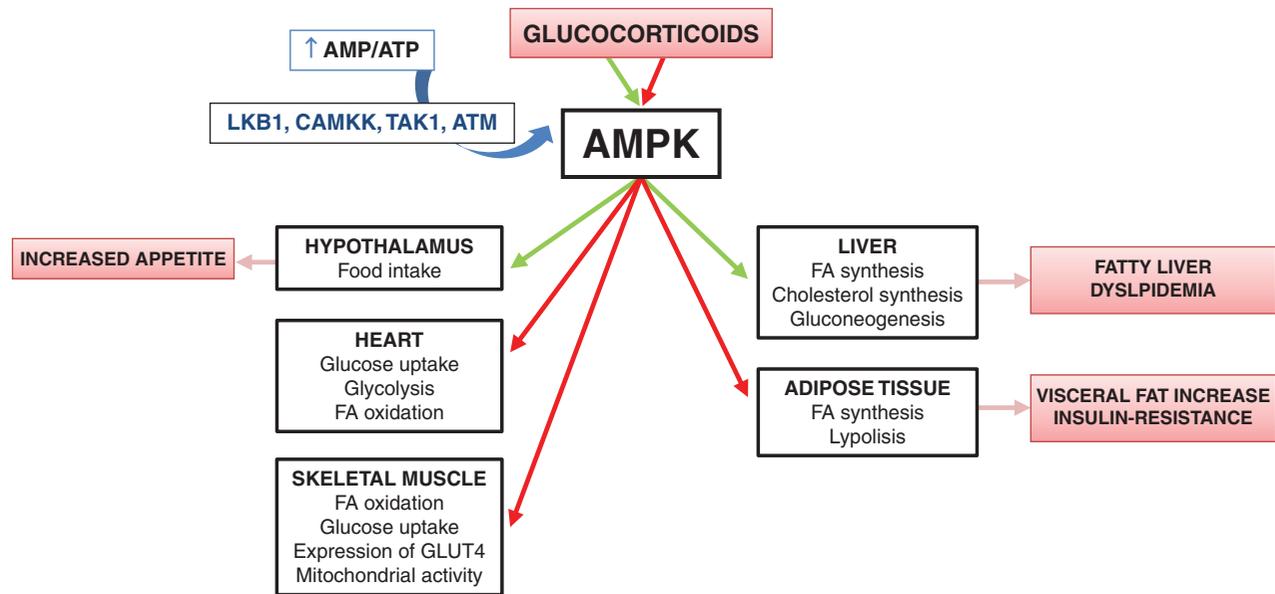
Figure 5

GCs and AMPK. AMP-activated protein kinase (AMPK), a cellular energy status sensor, is activated by high adenosine mono-phosphate (AMP)/ATP ratio through phosphorylation (LKB1, CAMKK, TAKI, ATM) and expressed in different tissues that exert several metabolic related actions (black frame boxes). GCs stimulate (green arrow) and inhibit (red arrow) AMPK expression/activity in hypothalamus, liver and adipose tissue contributing to increased appetite, visceral fat deposition, hepatic steatosis, dyslipidaemia and insulin resistance. GLUT4, glucose transporter type 4; FA, fatty acids; LKB1, liver kinase B1; CAMKK, calmodulin kinase kinase; TAK1, transforming growth factor-β-activated kinase; ATM, ataxia telangiectasia mutated.

dexamethasone induces proliferation, differentiation and basal metabolic activity of human brown adipocytes but inhibits their function under adrenergic stimulation (95).

The adipose tissue in CS patients ▸ Fat distribution shows an increase in VAT and VAT/SAT in CS, and without the normal male to female difference in VAT. Fat distribution correlated with age but not with glucose levels, circulating cortisol, ACTH or lipids (96). In one study, the total body fat of CS patients was found to be associated with increased levels of IL1 receptor antagonist (IL1RA), which decreased significantly after reduction of fat mass following cure. The authors suggested that IL1RA, which binds competitively to the IL1 type I receptor, abolishing the effects of the leptin-induced reduction in food intake, could mediate leptin resistance in CS, thus contributing to body composition change (97). In CS patients, VAT and abdominal SAT, measured by magnetic resonance imaging (MRI), have been found to be higher compared to controls, but no difference has been found in limb subcutaneous adipose tissue between patients and matched controls (98). The same authors also showed that CD patients after a mean of 20 months of remission had significantly reduced total, abdominal visceral and sub-cutaneous and bone marrow adipose tissue compared to active disease, although most of them were still over-weight or obese (99). Correction of hypercortisolism is generally associated with a reduction of VAT and SAT mass, although adverse metabolic profile and increased cardiovascular risk can persist after remission (1, 100, 101). Although CS patients experience loss of weight and fat in the short-medium term after remission (102), long-term follow-up demonstrated increased abdominal fat mass compared to age- and gender-matched controls (103). In another study, long-term cured CS patients had total fat mass and trunk fat mass comparable to patients with active disease and higher than gender-, age- and BMI-matched healthy controls (104). Similarly, studies on prolonged follow-up of CD children and adolescents reported persistence of increased total body fat, ratio of VAT to SAT and waist circumference (105, 106). The effects of exogenous hypercortisolism on body composition can be different from those seen in endogenous CS. A comparison of the body composition of patients cured from endogenous CS with rheumatoid arthritis patients treated with a low-dose of GCs showed that the increase in

total body fat and trunk fat is higher in endogenous than exogenous CS (107). Recently, it has been shown that patients with CS due to primary pigmented nodular adrenal disease have a typically milder obese phenotype than other forms of CS, even when their cortisol levels are comparable and their CS is not atypical or cyclical (108). The authors speculate that patients with CS due to perturbations of the cAMP/PKA pathway may be less obese because of increased PKA activity and resulting altered downstream regulation of cAMP-related lipogenic and lipolytic proteins (109). The influence of DHEA, the levels of which can be altered in CS, on fat distribution is not clear. Patients with adrenal adenoma and sCS were found to have increased VAT, measured by CT, similarly to patients with overt CS (110).

Ghrelin and adipokines in CS ▸ Patients with active CD have lower ghrelin levels compared to BMI-matched controls, which cannot be explained by increased insulin resistance, as they had similar indices of glucose metabolism. They also showed that ghrelin levels did not correlate with ACTH or cortisol in patients with active CD and did not parallel the reduction of cortisol that shortly follows surgery, suggesting no direct effect of cortisol levels on ghrelin. Cured patients showed an improvement in glucose and lipid homeostasis and increased ghrelin levels, suggesting an indirect effect of cortisol on ghrelin, mediated by the modulation of metabolic signals (111).

GCs, directly or indirectly via the metabolic changes, affect the endocrine activity of adipose tissue. The effects of GC excess on adiponectin levels are controversial. In an animal model of CS, plasma adiponectin levels were significantly higher than those of the WT littermates but mRNA and protein levels were significantly decreased in the adipose tissue. Bilateral adrenalectomy eliminated both their Cushing's phenotype and their increase in plasma adiponectin levels (112). In contrast, in humans, adiponectin levels did not differ between patients and BMI-matched controls (111). In another study, non-obese CS patients had lower adiponectin concentrations compared to non-obese controls, but this difference was not present when comparing obese CS patients and obese controls. This suggests that obesity is crucial when considering adiponectin levels in CS patients (113). Conflicting data have been reported about adiponectin levels before and after resolution of CS (104, 114, 115).

Leptin levels have been shown to be higher in patients with CS compared to normal weight or BMI-matched controls (115). Leptin does not seem to be acutely affected by the cortisol drop following neurosurgery but it has been found to be decreased in patients after long-term remission (116).

Resistin is significantly higher in female CS patients than in female control subjects and does not significantly change after cure, despite improvement of obesity and metabolic profile (116). TNFα levels are unchanged in CS (115). However, the soluble TNFα receptor 1 is increased in CS compared to BMI-matched controls before and after remission, suggesting a potential contribution of TNFα pathway to the persistent dysmetabolic features and increased cardiovascular risk observed after cure (104). The same applies to IL6, which is higher in active CS patients and remains increased after cure (104).

Liver steatosis in CS patients ▸ Liver-specific disruption of GR activity leads to reduced hepatic lipid content in animal models (117). Lack of 11BHSD1 decreases hepatic steatosis, but the extent of the contribution of 11BHSD1 expression and activity in the liver to the lipid accumulation is controversial (73, 118, 119). MKP3 deficiency has been shown to protect mice from GC-induced fatty liver (93).

Non-alcoholic fatty liver disease (NAFLD) is more frequent in people with obesity and diabetes and is considered the liver manifestation of metabolic syndrome. The combined effects of increased lipogenesis and reduced fatty acid oxidation in the liver, increased circulating FFA, VAT accumulation and dysregulation of adipokines can affect the development of hepatic steatosis in CS patients. In one CT-based study, hepatic steatosis was found in 20% of the CS patients with active disease. There was a significant negative correlation between both liver attenuation and liver/spleen ratio with total abdominal fat area, VAT area, the percentage of VAT and the visceral to SAT ratio (120).

Dyslipidemia in CS

GCs regulate both lipolytic and adipogenic processes. Different studies investigating the lipid profile of GC excess subjects showed dyslipidaemia in 37–71% of CS patients (1, 8, 100, 121). Hypercholesterolemia was found in 16–60% of the CS patients, with increased triglycerides in 7–36% of cases (1, 8, 100, 121). HDL levels can be reduced, but some authors did not report decreased HDL cholesterol or high total cholesterol/HDL and LDL/HDL ratios in active CS patients (8, 99). Improvements of dyslipidaemia after cure/remission occur, but an adverse lipid profile can persist in approximately 30% of the patients (1), probably due to GC-induced modifications on

adipose tissue. Patients with adrenal adenoma and sCS have been shown to have significantly increased cholesterol and triglyceride levels and decreased HDL cholesterol levels compared to controls, contributing to the increase in cardiovascular risk (121). However, in a subgroup of sCS patients no significant improvement was observed after adrenalectomy (122). Moreover, in another cohort of adrenal adenoma patients, the occurrence of dyslipidaemia was associated with the presence of impaired glucose metabolism rather than to the subtle cortisol hypersecretion *per se* (123). Patients undergoing long-term treatment with GCs causing typical Cushingoid fat deposition were demonstrated to have higher LDL and lower HDL cholesterol compared to GC-treated patients without the typical pattern of fat distribution (124). In contrast, another study addressing the effects of exogenous GC treatment showed no association between GC use and dyslipidaemia, and unusually, an increased HDL cholesterol level was found in patients treated with GCs who were above 60 years old (125).

Mechanism ▸ GCs increase the expression of adipose triglyceride lipase and hormone sensitive lipase (HSL), which are in charge of stored lipid breakdown in mature adipocytes, causing an increase in circulating FFA levels (126). GCs also stimulate lipolysis by increasing cAMP levels, with consequent activation of PKA, which can serin-phosphorylate HSL and perilipin. Perilipin facilitates HSL translocation and activity (65, 126, 127). GCs can also directly and indirectly affect cAMP levels and, consequently, lipolysis, by decreasing the expression of phosphodiesterase PDE3b, an enzyme involved in cAMP breakdown, and by impairing the insulin dependent activation of PDE3b, resulting in reduced cAMP degradation (65, 126). Lipolytic effects of GCs can also be related to the modulation of responsiveness to other hormones such as catecholamines and GH (70, 126).

While the lipolytic actions are associated with short-term exposure to GCs, the pro-adipogenic effects become prominent in chronic hypercortisolism. GCs increase the expression and activity of lipoprotein lipase, which is required to hydrolyse triacylglycerols circulating in the blood within the VLDL or chylomicrons, promoting uptake and storage in the adipose tissue (128, 129, 130). Therefore, in adipose tissue the two processes can occur at the same time. Mature adipocytes release FFA via the action of triglyceride lipase and HSL, while GC-induced differentiation of pre-adipocytes leads to adipocyte accumulation. In addition, via the increased circulating FFAs, ectopic storage of lipids in liver and skeletal muscle is increased (131). Increased liver lipogenesis then leads to increased secretion of VLDL, which becomes a further source of lipid storage in the adipose tissue (132).

Modifying factors, such as diet, can contribute to the balance of pro-adipogenic vs lipolytic effects. GCs increase dietary fat intake and induce neo-lipogenesis from substrates like glucose. GCs were also shown to inhibit AMPK activity in visceral adipose tissue leading to increased lipogenesis and fat storage. CS patients have 70% lower AMPK activity in visceral adipose tissue compared to control subjects with a consequent increase of fatty acid synthase expression (44, 45). In transgenic mice, the impact of selective adipose- or liver-specific 11BHSD1 overexpression on lipid levels has been discussed previously. The N363S GR polymorphism was reported to be associated with an enhanced sensitivity to GCs and with increased BMI and LDL cholesterol levels, as well as an increased risk of cardiovascular disease (133). GH deficiency combined with altered gonadal function can contribute to lipid abnormalities.

Management ▸ There are no guidelines specifically addressing how to treat dyslipidaemia in CS. However, in these patients, an aggressive management of lipid alterations is recommended due to the increased cardiovascular risk. Experimental treatment with 11BHSD1 inhibitors improved triglyceridaemia by reducing hepatic VLDL secretion and increased liver fatty acid oxidation. A 28-day treatment with INCB013739, a selective inhibitor of 11BHSD1, was shown to improve insulin sensitivity and lowered total and LDL cholesterol in patients with type 2 DM (134, 135).

The side effects of cortisol-lowering drugs on lipid levels should be considered: ketoconazole reduces total, intermediate density and LDL cholesterol and apoB levels by around 25% (136); mitotane increases total cholesterol by 68%, inducing an elevation of LDL cholesterol and apoB (137). In patients treated with ketoconazole, the use of pravastatin and rosuvastatin instead of other statins has been suggested to reduce the risk of myotoxicity, since these lipid-lowering drugs are not metabolised by CYP3A4 which is inhibited by the treatment with ketoconazole (138).

GCs and GH abnormalities

A normal GC milieu is needed for normal growth as GCs are important modulators of GH secretion and action and play an essential role in the differentiation as well as the function of somatotrophs (139). Both lack and excess of GCs lead to reduced height and lower GH levels.

Low GH levels of infants with congenital secondary hypoadrenalism can be restored with GC replacement (140, 141). GCs can also restore GH response to provocative stimuli in Addison's disease (142). While acute exposure to GCs stimulates the GH/IGF1 axis, chronic excess has a profound negative effect. Exercise-induced GC response is coupled with increased GH levels and acute GC administration is followed by an increase in GH (143, 144). Prolonged GC excess, however, is a well-known negative regulator of GH secretion. Short stature and delayed linear growth are typical features of paediatric CS, and slowed growth is common in children undergoing long-term/high-dose GC therapy. Spontaneous catch-up growth is unlikely even after successful treatment in paediatric CS (145). A significant proportion of paediatric CD patients cured by surgery alone or in combination with radiotherapy shows abnormalities of GH secretion after long-term cure, which are more common and persistent if other pituitary hormone deficits are present (146).

There is evidence supporting the negative impact of hypercortisolism on GH secretion in adult patients as well. In a small study, GH response to pyridostigmine/GHRH was found to be impaired in 14 CS patients. The GH/IGF1 axis recovered at 6 months after successful treatment in half of the patients, at 12 months in two and at 18 months in one patient. GH axis recovery was more common in patients in whom there was recovery of the hypothalamic-pituitary-adrenal (HPA) axis as well (147). Data from the KIMS database showed that GH deficient (GHD) patients with a previous history of CD had the same characteristics in terms of body composition and lipid levels but a higher prevalence of diabetes, hypertension, bone fractures and lower BMD, as well as a more impaired quality of life compared to GHD patients with other aetiologies (148). As GCs significantly impact on adiposity and BMI, which in turn can affect GH secretion, BMI alone can only partially explain GH impairment in CS patients (149).

In CD patients, GH impairment can also be the consequence of surgical treatment and/or radiotherapy. GHD is present in adult CD patients after long-term remission even if treated only with transsphenoidal neurosurgery. Male gender and the duration of disease were the most relevant predictors of GH/IGF1 axis disturbances after cure of CD (150).

Patients with adrenal adenomas and subclinical hypercortisolism had a reduced GH secretion reserve compared to patients with non-functioning adrenal adenomas after adjusting for age and BMI. GH secretion improved after recovery (151).

Mechanism ▶ The stimulation of GH secretion induced by an acute increase of GCs could be playing a part in physical or emotional stress-induced GH increase (139). One of the suggested mechanisms involves a steroid hormone-induced GTP binding protein (dexamethasone-induced Ras protein, dexras), which activates ERK1/2 and leads to phosphorylation of the E-twenty-six transcription factor family member ELK1. ELK1 has binding sites on the GH promoter (139). On the other hand, long-term GC excess can alter GH secretion dynamics via increasing somatostatinergic tone (152). Chronic hypercortisolism has multiple deleterious effects on growth, and some of them are uncoupled from GH/IGF1 secretion impairment. Indeed, GCs (i) impair gonadal function, mainly via inhibiting gonadotropin secretion and GnRH transcription, but also by interfering with testicular function and sex hormone-binding globulin synthesis in the liver; (ii) exert catabolic actions on bone and muscle; (iii) negatively affect calcium/vitamin D metabolism; and (iv) exert direct inhibitory effects on the growth plate (153, 154).

Management ▶ Early treatment with GH after cure of CS is an important consideration. Studying 13 paediatric patients with GHD following successful CS treatment, hGH therapy resulted in better height SDS (−1.3) and somewhat improved BMI (155), suggesting that early treatment of GHD is recommended to achieve optimal growth, while excess adiposity can be a persistent long-term complication (155). These results were in line with previous findings (156). Several studies compared adult CD and non-functioning pituitary adenoma (NFPA) patients treated with GH. Quality of life improved significantly with a 6-month GH treatment in cured CD patients (148). A 3-year follow-up study of GH-treated CD ($n=160$) and NFPA ($n=879$) patients found that the prevalence of metabolic syndrome, which was not significantly different at baseline, was significantly higher in CD than NFPA patients (23.4 vs 9.2%). The prevalence of diabetes, cardio- and cerebrovascular disease was higher in patients with a history of CD compared to NFPA patients, suggesting that GHD CD subjects are predisposed to adverse metabolic features and increased cardiovascular risk (157). Comparing the effect of GH treatment on lean body mass in cured CD and NFPA patients, NFPA patients showed significant improvement while CD patients did not, suggesting that GHD patients previously treated for CD could be resistant to the protein anabolic effect of GH even years after remission (158).

Heart and vasculature in CS

CS patients have increased cardiovascular morbidity and mortality due to several closely related factors such as hypertension, metabolic changes, coagulopathy and specific GC-related alterations of the heart and vasculature (Fig. 6).

Hypertension ▶ Hypertension has been reported in 55–85% of the CS patients and has been associated with disease duration. Hypertension has been shown to persist

after cure in 24–56% of the cases, especially when patients are older at diagnosis or have a longer history of hypercortisolism or a longer duration of untreated hypertension (4).

Several mechanisms have been suggested to play a role in causing hypertension in CS: mineralocorticoid activity of cortisol; activation of renin-angiotensin system; enhancement of cardiovascular reactivity to vasoconstrictors; increased β-adrenergic receptor sensitivity to catecholamines; suppression of the vasodilatory system; increased cardiac output, total peripheral resistance and
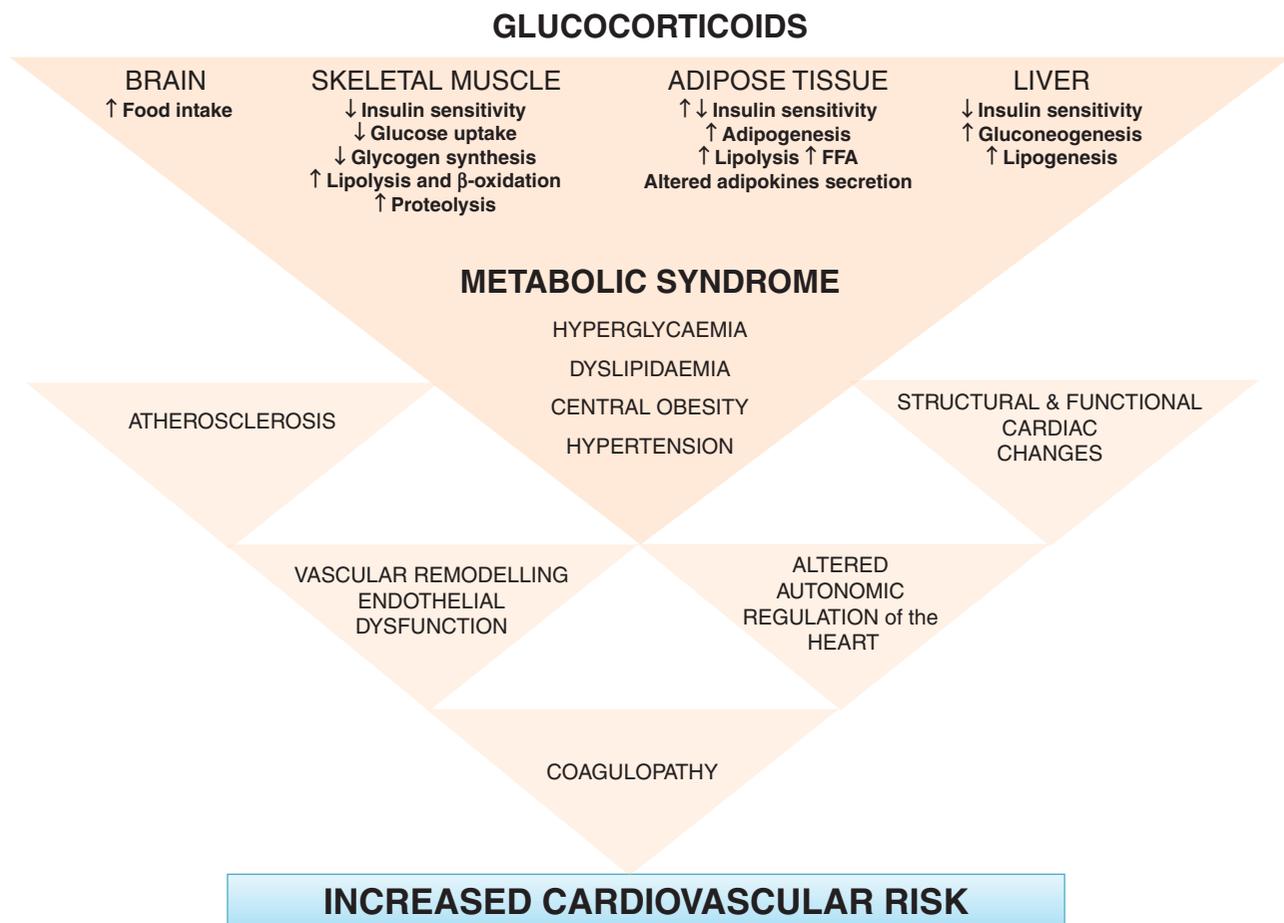
Figure 6

Cardiovascular risk in Cushing's syndrome. Metabolic syndrome is extremely common in CS. GCs induce hyperglycaemia by increasing hepatic gluconeogenesis and disrupting the insulin signalling in the liver, skeletal muscle and adipose tissue with a consequent decrease of peripheral use and disposal of glucose. Central fat accumulation is coupled with deregulation of adipokine secretion and facilitated by GC-related increased appetite, enhanced adipogenesis and altered lipid storage.

Hyperlipidaemia, a major cardiovascular risk factor, is strictly associated to the increased mobilization of free fatty acids (FFA) from the adipose tissue and increased hepatic lipogenesis. The CS patient with obesity, diabetes mellitus, dyslipidaemia, hypertension, procoagulant phenotype and severe structural and functional alterations of the heart and vessels is the prototype of a patient with increased cardiovascular risk.

renovascular resistance; insulin resistance; and sleep apnoea (159, 160).

GCs can induce hepatic synthesis of angiotensinogen, increase angiotensin II receptor type 1 concentration in the brain and peripheral tissue and enhance both angiotensin II-stimulated inositol phosphate-3 production in vascular smooth muscle cells and its central actions (159, 161). GC-induced increase of erythropoietin contributes to vasoconstriction and therefore hypertension (162). Endothelin has been found to be increased in CS patients (163). This increase could be related to the GC excess-induced endothelial damage and increased vascular permeability, causing endothelin hypersecretion in the circulation with the consequent permissive effect on the atherogenic and pressor action of endothelin. GCs can downregulate the plasma membrane sodium–calcium exchanger as well (164). CS patients have been shown to have a blunted vascular and renal response to pharmacological doses of atrial natriuretic peptide compared to normal controls and hypertensive subjects (165). Impaired production of vasodilators including prostaglandins, prostacyclins and compounds of the kallikrein–kinin system might also contribute to CS hypertension (160). Moreover, cortisol-induced hypertension is characterised by reduced activity of the nitric oxide pathway (166). GCs have been shown to downregulate nitric oxide synthase cofactor tetrahydrobiopterin (BH4) and GTP cyclohydrolase 1, the rate-limiting enzyme in the production of BH4, thus contributing to the reduced endothelium-dependent relaxation (167).

Vascular damage ▶ Vascular remodelling and increased vascular oxidative stress play a major role in CS-associated cardiovascular comorbidities. The cross-sectional media area of subcutaneous small resistance arteries was significantly greater in CS compared to hypertensive patients and controls, while the media to lumen ratio was greater in hypertensive and CS patients compared to controls (168). MR activation by GCs can mediate vascular damage, since mineralocorticoids have growth promoting and pro-fibrotic activities, which lead to remodelling and fibrosis of small vessels (169). CS patients with metabolic, cardiovascular and bone complications have increased oxidative stress markers, such as 15-F2t-IsoP and TBX2, compared to CS patients without complications or to control subjects. Vitamin E levels, a suggested marker of the antioxidant status, were significantly lower in CS patients compared to controls. These findings would suggest that excess GCs induce pro-oxidative processes which, in combination with the metabolic comorbidities, lead to a vicious loop of worsening oxidant-antioxidant balance and increased cardiovascular morbidity and mortality (170).

Patients with CS have been demonstrated to have increased carotid intima media thickness (IMT) (1, 100) and increased endothelial dysfunction, as shown by studies reporting impaired flow mediated vasodilatation (171, 172). Atherosclerotic plaques are more prevalent in CD patients than in the control population even after long-term remission and correlation with insulin resistance and central adiposity (1, 173). IMT correlated with waist-hip-ratio and fasting glucose, but no correlation was found with the cortisol levels (173).

Increased coronary calcifications and non-calcified coronary plaque volume, quantified by multidetector CT coronary angiogram scan, have been found in patients with active CS (3). Increased prevalence of coronary artery disease persists even after long-term remission of CS, since more coronary calcification and non-calcified plaques were found in cured CS patients than in age- and gender-matched controls (174). Coronary flow reserve is more commonly reduced in CS patients, compared to matched controls, and correlates negatively to urinary free cortisol (175).

Several studies found patients with adrenal adenomas and sCS to experience cardiovascular events more frequently than patients with non-functioning adrenal adenomas, and this increased prevalence of cardiovascular disease was found to be associated with subclinical hypercortisolism (16, 176, 177).

Inflammatory markers, such as soluble tumour necrosis factor receptor (sTNFR1), IL6, IL8, IL1b and glutathione peroxidase (GPx), which is involved in preventing cells from oxidative damage, have been found to be increased in CS patients. sTNFR1 was found to be the strongest predictor of IMT in CS along with GPx, IL1b and cortisol after a low-dose dexamethasone suppression test (178). In another cohort of patients with active or cured CS, sTNFR1 has been found to correlate with the Agatston score, being a predictor of coronary calcifications (179).

Endothelin, homocysteine, VEGF, osteoprotegerin and cell adhesion molecules (such as soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) have been found to be increased in CS patients with active disease, while taurine, a suggested protective factor, is decreased. These parameters all improved or normalized after successful therapy (180, 181, 182, 183).

Cardiac morphology ▶ Cardiac morphology changes have been associated with chronic hypercortisolism, and

specifically with the length of exposure to GCs, and functional modifications have been also reported.

Echocardiography in CS patients showed that up to 70% of the patients with active CS presented abnormal left ventricular (LV) mass parameters: 42% presented concentric hypertrophy and 23% concentric remodelling. Major indices of systolic and diastolic functions were reported to be normal. After remission of hypercortisolism, LV mass parameters improved considerably but did not normalize completely (184). In a previous paper, echocardiographic assessment of CD patients revealed LV hypertrophy and diastolic dysfunction as well as subclinical LV systolic dysfunction, which reversed after remission (185). Using cardiac MRI, subclinical systolic biventricular and left atrial (LA) systolic dysfunction associated with increased LV mass was found in CS patients compared to the controls. Remarkably, the study showed that the effective treatment of hypercortisolism improved the systolic performance of both ventricles and LA, reduced LV mass and LV wall thickness and led to the regression of the concentric LV remodelling pattern. The treatment-related decrease in LV mass was independently associated with changes in glucose metabolism and BMI. Moreover, on the basis of the absence of gadolinium enhancement of the myocardium, the study ruled out dense replacement myocardial fibrosis in uncomplicated CS (186). However, a previous study showed increased myocardial fibrosis, assessed by echocardiography, in CS patients compared to healthy controls and hypertensive patients (187). On ECG, changes in the QT interval have been found to be a specific feature of CD patients and to correlate with hypercortisolaemia regardless of other risk factors suggesting a cardiotoxic effect of cortisol excess *per se* (188). Altered autonomic cardiac regulation can contribute to the overall increased cardiovascular risk. Several studies found abnormalities in cardiovascular autonomic regulation in CS patients, as suggested by reduced heart rate variability (189, 190).

Patients with adrenal adenomas and sCS have been found to have increased LV mass compared to healthy controls and subjects harbouring non-functioning adrenal adenomas (191). Significant structural and functional modifications of the heart and vasculature can also be detected in paediatric CS patients (192).

Management ▸ In patients with endogenous CS, the identification and removal of the source of hypercortisolism lead to an improvement of hypertension in a significant proportion of patients (193, 194). The same seems to apply to patients with sCS in which

adrenalectomy has been shown to be more effective than medical treatment (195).

However, in order to prevent cardiovascular events, a tailored antihypertensive treatment is often required before surgery, or when the surgical approach is unsuccessful, or when the disease is cyclical, or in combination with cortisol-lowering medical treatment, or when hypertension persists despite the surgical or pharmacological control of hypercortisolism. Indeed, some medium/long-term follow-up studies of CS patients in remission showed a persistence of high blood pressure and a still increased risk of morbidity and mortality due to the increased cardiovascular risk (1, 100, 196). This risk is more consistent for cardio- and cerebrovascular events than for heart failure (196). While the length of exposure to cortisol excess has been clearly linked to the persistence of high blood pressure, the role of age at onset of hypercortisolism is not clear.

The management of hypertension in CS patients cannot leave out of consideration the mechanisms through which the GC excess determines the increased blood pressure and cardiovascular damage. Angiotensin-converting-enzyme inhibitors and angiotensin receptor blockers, with their cardioprotective effects, have been recently proposed as a first-line treatment to which calcium-antagonists, known to delay atherosclerosis and prevent stroke, and/or mineralocorticoid receptor antagonists can be added according to the severity of the condition and the presence of hypokalaemia (197). In the proposed treatment algorithm, α-blockers could be considered as the further next step. The use of thiazides and β-blockers should be limited to selected cases after the appropriate dose titration of the previously mentioned agents due to their potential metabolic side-effects (197).

With regard to the effects of cortisol-lowering drugs on hypertension, it has been shown that ketoconazole ameliorates blood pressure in more than 80% of the cases (198) and mitotane in 63% (199). Mifepristone was able to improve diastolic blood pressure and/or reduce the number of hypertensive drugs in 52% of the patients (200), but in other studies some patients experienced a worsening of hypertension and hypokalaemia (201). Metyrapone treatment can be also associated with increased blood pressure and hypokalaemia due to the rise in steroids with mineralocorticoid activity. Pasireotide, alone or in combination with cabergoline and ketoconazole, has been demonstrated to improve hypertension (202, 203). Retinoic acid has been demonstrated to be effective in reducing blood pressure as well (204). The new compound LCI699, which acts by inhibiting

aldosterone synthase and 11β-hydroxylase, can be beneficial in terms of blood pressure (205), but, similarly to metyrapone, the increased mineralocorticoids can lead to a worsening of hypertension.

Muscle in CS

Around 60% of the CS patients experience proximal muscle atrophy and weakness, which have been reported to be more frequent in men (6). CS patients have reduced lean mass, due to muscle loss of the limbs, compared to obese subjects with the same total fat mass (206). Respiratory muscle strength does not seem to be affected in CS (207). Muscle damage can persist both short- and long-term after cure. Indeed, the reduced muscle area in the arms showed no relevant improvement 6 months after successful treatment (102). MRI body composition assessment of CD patients 20 months after remission showed that total and limb skeletal muscle is actually reduced compared to active disease (99). The authors speculated that this could be due to the GC replacement therapy after cure since it inversely correlated with muscle mass. They also found that the difference in patients' muscle mass between active disease and remission disappeared when disregarding post-menopausal women, suggesting a role of the oestrogen deprivation as well (99). Furthermore, a long-term follow-up study demonstrated that patients with CS had reduced limb skeletal muscle mass, but similar total lean body mass, compared to age- and gender-matched controls (103).

Muscle fibre conduction is also altered in CS patients. Creatinine kinase, plasma myoglobin and muscle fibre conduction velocity were reduced in CS patients compared to healthy age-, sex- and BMI-matched controls, and creatinine kinase and muscle fibre conduction velocity correlated with disease duration (208). However, electromyography alterations typical of classical myopathy are rarely seen in the early stage of disease (209).

GC-induced changes in muscle are evident after a few days of GC administration. In healthy volunteers 8 mg dexamethasone administration for 7 days resulted in a decrease in the muscle fibre cross-sectional area, loss of myosin and a reduction in power (210). Short-term effects, more common in intensive care units, generally involve proximal and distal muscles. The type, dose and duration of steroid treatment determine the occurrence and severity of myopathy. Fluorinated steroids (such as dexamethasone or betamethasone) cause myopathy more frequently than non-fluorinated agents (such as prednisolone and hydrocortisone) (211). The reason why fluorinated agents are more frequently associated to myopathy is not clear, but it could be speculated that different GCs exert different genomic and non-genomic actions and impact on different signalling pathways, having different anti-inflammatory or anti-proliferative effects, with consequent different metabolic outcomes (212). Chronic GC excess generally has a more prominent effect on the proximal muscles (213). Interestingly, in aging subjects (without CS) muscle mass loss and decreased function were not associated to circulating or urinary cortisol, but muscle strength correlated with quadriceps 11BHSD1 mRNA expression, supporting the importance of local cortisol conversion and tissue-specific cortisol metabolism rather than overall circulating levels in determining negative effects of GCs (214).

Mechanism ▶ GC-induced muscle atrophy affects mainly fast-twitch or type II fibres with less or no impact observed in type I, the more oxidative type of fibres (211, 215). Histological features of GC-related myopathy are non-specific atrophy of type IIb muscle fibres, the absence of inflammatory infiltrate, variations in fibre size with centrally placed nuclei and, rarely, signs of muscle necrosis (215). GCs reduce skeletal muscle mass both by inhibiting protein synthesis and by increasing the rate of protein degradation (Fig. 7). GCs inhibit the transport of amino acids into the muscle and interfere with the stimulatory action of insulin, IGF1 and amino acids on AKT and mTOR and, consequently, on the phosphorylation of eIF4E-binding protein 1 and the ribosomal protein S6 kinase 1, which are known to play a crucial role in the protein synthesis machinery (211, 216). A recent study demonstrated that caveolin 1 (CAV1) is a critical regulator of muscle homeostasis through the modulation of the insulin-signalling pathway. GC response elements have been identified in the promoter of the CAV1 gene, and dexamethasone treatment reduced CAV1 protein and mRNA expression with a concomitant reduction in insulin receptor alpha (IRα) and IRS1 levels in C2C12 myotubes. In addition, CAV1 knockdown decreased the protein levels of IRα and IRS1, and overexpression of CAV1 prevented the dexamethasone-induced decrease in IRα and IRS1 proteins (217). GCs promote proteolytic degradation of myofibrillar and extracellular matrix proteins by inducing the expression of components of the ubiquitin and proteasome system; activating the lysosomal system, as suggested by the presence of markers of autophagy in the muscle of GC-treated animals, and stimulating the calcium-mediated proteolytic system (218). GC-induced FOXO and C/EBPβ trigger the expressions of genes involved in muscle protein
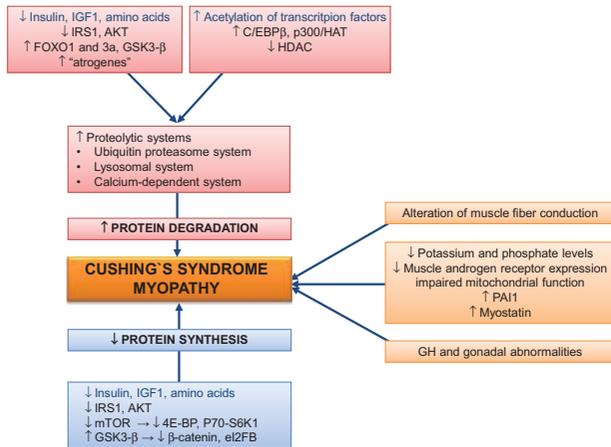
Figure 7

Cushing's syndrome myopathy. GCs induce muscle loss by impairing the anabolic action of insulin, IGF1 and amino acids. GCs increase protein degradation via the disruption of PI3K/AKT pathway and the stimulation of transcription factors (FOXO1 and 3a, GSK3-β) leading to the transcription of genes involved in muscle atrophy (atrogenes). GCs reduce protein synthesis via the inhibition of the transport of amino acids in the muscle and the inhibition of the stimulatory action of IGF1 and amino acids on mTOR with the consequent decreased activity of 4E-BP1 and S6K1. GSK3-β can also contribute to the reduced protein synthesis through decreased β-catenin and eIF-2B. GC-induced protein acetylation leads to increased proteolysis. The deleterious effect of GH and gonadal axis impairment contribute to the muscle disease as well. IRS1, insulin receptor substrate 1; FOXO1 and 3a, forkhead box protein O1 and 3a; GSK3-β, glycogen synthase kinase 3 beta; C/EBPβ, CCAAT/enhancer binding protein beta; HDAC, histone deacetylases; mTOR, mammalian target of rapamycin; P70-S6K1, P70S6 kinase1; 4E-BP, eIF4E-binding protein 1; PAI1, plasminogen activator inhibitor 1.

breakdown via the ubiquitin proteasome system and autophagy. A GC-induced increase of p300/HAT and a decrease of histone deacetylases expression and activity can lead to hyperacetylation and consequent activation of transcription factors involved in protein degradation pathways. Moreover, GC-related proteasome-dependent degradation of other factors, such as MyoD and myogenin that are involved in anabolic pathways, can further contribute to muscle loss. Stimulation of GSK3 by GCs may also be involved in the inhibition of protein synthesis through decreased β-catenin and eIF-2B and increased muscle proteolysis (211, 218). Moreover, GCs inhibit the production of IGF1 in muscle, which contributes to muscle development and integrity by increasing protein synthesis

and myogenesis while decreasing proteolysis and apoptosis (211, 219). GCs stimulate myostatin, an inhibitory growth factor that downregulates protein synthesis, proliferation and differentiation of muscle satellite cells, precursors of skeletal muscle cells (220, 221). GCs can indirectly affect muscle physiology through downregulating gonadal function and reducing the expression of the androgen receptor in skeletal muscle (219). Other factors have also been thought to be implicated in GC-associated myopathy, such as the GC effects on potassium and phosphate levels and mitochondrial function (209, 222). A recent study showed that mice lacking PAI1 are protected from GC-induced muscle atrophy (32).

In humans, however, the mechanism leading to muscle loss may not be exactly the same as in rodents, and the precise contribution of anti-anabolic and catabolic GC effects to myopathy needs to be clarified. In contrast with animal studies, GCs did not regulate the expression of proteolytic genes in CS patient muscle. No change was found in mRNA levels for cathepsin D (a lysosomal proteinase), m-calpain (a Ca^{2+}-activated proteinase), ubiquitin, 14-kDa ubiquitin-activating enzyme E2 and 20S proteasome subunits (which are critical components of the ubiquitin-proteasome proteolytic process) in the skeletal muscle of CS patients. The authors speculated that this could be due to the occurrence of adaptive regulatory mechanisms, consequent to chronic hypercortisolism, preventing sustained increased protein breakdown to avoid continuous rapid muscle wasting (223).

Intramuscular adipose tissue is linked to an adverse metabolic profile. In animal models, GCs induced an increase of intramuscular adipose tissue through the induction of pre-adipocyte differentiation mediated by inhibition of IL4 (224). In contrast, intramuscular adipose tissue is not increased in CS patients compared to BMI-matched controls (98).

Management ▸ The treatment of GC-associated myopathy should start with the correction of the underlying cause of endogenous hypercortisolism. In case of iatrogenic hypercortisolism, the discontinuation or dose titration of the steroid drug, or the replacement with a non-fluorinated agent (such as prednisolone), should be considered. Experimental treatments such as IGF1, branched-chain amino acids, creatinine, testosterone, nandrolone and DHEA and glutamine have been proposed (215). Only a few studies have demonstrated that aerobic and resistance exercises are effective in attenuating GC-induced muscle atrophy (211).

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Summary

The diagnosis and management of endogenous CS is one of the most challenging and could be one of the most rewarding tasks of an endocrine team. Exogenous GCs are hugely effective treatments for a number of severe diseases, while their use in a high percentage of cases inevitably will lead to iatrogenic CS. In this context, efforts should focus on synthetic GCs devoid of negative metabolic effects while retaining anti-inflammatory properties. The elucidation of the mechanisms involved in metabolic complications of excess GCs may lead to solutions for prevention and treatment and provides exciting challenges for both basic and clinical science. Many questions remain open: What is the molecular mechanism underlying GC-induced changes on the morphology and deteriorated function of the heart? What are the pathophysiological mechanisms involved in the persistence of increased morbidity and mortality in CS patients even after long-term remission? What is the molecular basis of the different actions of synthetic GCs? What is the exact relevance of GC action on other hormonal axis alterations and their contributions on metabolic changes. Comparative studies are required to examine the cardiometabolic consequences in exogenous vs endogenous hypercortisolism. The effectiveness of specific drug combinations to control hypertension and reduce cardiovascular risk in hypercortisolism needs to be further studied.

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