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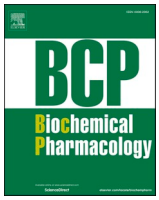

Review

Adipose tissue in cortisol excess: What Cushing's syndrome can teach us?

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ABSTRACT

Endogenous Cushing's syndrome (CS) is a rare condition due to prolonged exposure to elevated circulating cortisol levels that features its typical phenotype characterised by moon face, proximal myopathy, easy bruising, hirsutism in females and a centripetal distribution of body fat. Given the direct and indirect effects of hypercortisolism, CS is a severe disease burdened by increased cardio-metabolic morbidity and mortality in which visceral adiposity plays a leading role. Although not commonly found in clinical setting, endogenous CS is definitely underestimated leading to delayed diagnosis with consequent increased rate of complications and reduced likelihood of their reversal after disease control.

Most of all, CS is a unique model for systemic impairment induced by exogenous glucocorticoid therapy that is commonly prescribed for a number of chronic conditions in a relevant proportion of the worldwide population.

In this review we aim to summarise on one side, the mechanisms behind visceral adiposity and lipid metabolism impairment in CS during active disease and after remission and on the other explore the potential role of cortisol in promoting adipose tissue accumulation.

1. Introduction

1.1. Cushing's syndrome and adipose tissue

Cushing's Syndrome (CS) is a severe condition featured by a prolonged elevation in plasma cortisol levels, due to either exogenous steroid use or to excessive endogenous cortisol production. The most common cause of CS by far is the exogenous glucocorticoids (GCs) therapy from any administration route [1], mainly prescribed for chronic disorders such as rheumatological conditions, asthma and chronic obstructive pulmonary disease. Endogenous CS is instead a rare disease, with an estimated annual incidence ranging between 2 and 3 [2] to 8 cases per million people/year [3].

The most common cause of endogenous CS is Cushing's Disease (CD), due to adrenocorticotropic hormone (ACTH) hypersecretion by corticotroph pituitary adenoma [4]. Among ACTH-dependent causes, malignant non-pituitary corticotropin-secreting tumours are responsible for ectopic CS [5]. Endogenous CS may be also caused by autonomous adrenal overproduction of cortisol (due to either benign or malignant adrenal tumours, or by bilateral primary micro- and macronodular adrenocortical hyperplasia) accounting for around 20 % of all cases [6].

Although CS has some pathognomonic features such as purple striae, easy bruisability, thin skin and proximal myopathy that raise clinical suspicion for cortisol excess, other signs commonly present in CS like hirsutism, weight gain and visceral obesity are also frequent in highly prevalent conditions like polycystic ovarian syndrome and metabolic syndrome [7]. For this reason, CS diagnosis is challenging, especially when cortisol is only mildly increased.

CS is burdened by increased mortality due to its several comorbidities including cardiovascular, metabolic, immunological, psychiatric,

Abbreviations: 18F-fluorodeoxyglucose, 18FDG; 11 β-HSD, 11β-Hydroxysteroid dehydrogenase; acc, acetyl-CoA carboxylase; ACTH, corticotropin; AMPK, AMP-activated protein kinases; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; BMI, body mass index; cAMP, cyclic adenosine monophosphate; CD, Cushing's disease; CS, Cushing's syndrome; EAT, epicardial adipose tissue; FAS, fatty acid synthetase; FFAs, free fatty acids; G6Pase, glucose-6-phosphatase; GC, glucocorticoid; GR, glucocorticoid receptor; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; HSL, hormone-sensitive lipase; HPA, hypothalamic–pituitary–adrenal axis; KLF9, Krüppel -like factor 9; IL, interleukin; LDL, low-density lipoprotein; LMO3, LIM domain only 3; LPL, lipoprotein lipase; LV, left ventricular; MR, mineralocorticoid receptor; NAFLD, non-alcoholic fatty liver disease; PEPCK, phosphoenolpyruvate carboxykinase; PET-TC, positron emission tomography-computed tomography; PKA, protein kinase A; PGC1α, peroxisome proliferator-activated receptor γ co-activator 1 alpha; POMC, proopiomelanocortin; PPAR, peroxisome proliferator-activated receptor; TG, triglycerides; UCP1, uncoupling protein-1; VLDL, very-low density lipoprotein; WAT, white adipose tissue.

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and musculoskeletal disorders [8–10]. Regarding the metabolic ones, it has been observed that hypercortisolism leads to metabolic syndrome, characterised by central obesity, insulin resistance and glucose intolerance [11]. CS shares several features with metabolic syndrome including visceral adiposity that is one of its hallmarks, especially when associated with proximal limbs myopathy. These alterations are directly induced by chronic GCs overexposure that on one side promotes an increase in visceral adiposity and the accumulation of fat-depots in liver, skeletal muscle and pancreas while on the other, stimulates lipolysis in subcutaneous adipose tissue with release of free fatty acids (FFAs) into the circulation [10,12].

This review aims to summarise the multiple and complex effects of GCs on adipose tissue and lipid metabolism, their reversal after disease control and the effect of different compounds on visceral adiposity and lipid profile in CS, Fig. 1.

1.2. 11β-Hydroxysteroid dehydrogenase

Intracellular GC metabolism is regulated by two isoforms of the 11β-Hydroxysteroid dehydrogenase (HSD) enzyme; the type 1 (11β-HSD1) converts inactive cortisone into cortisol that binds the intracellular glucocorticoid receptor (GR). This isoform is localised in several tissues, including liver, adipose tissue, testis, ovary and lung [13]. The 11β-Hydroxysteroid dehydrogenase type 2 (11β-HSD2) instead operates the opposite conversion of cortisol to cortisone, preventing illicit occupation of mineralocorticoid receptor (MR) by cortisol in aldosterone target tissues, especially the kidneys [14].

To date, several studies suggest that increased 11β-HSD1 expression and activity are involved in the pathogenesis of obesity and insulin resistance [15–22]. Two studies have shown that cortisol increases 11β-HSD1 expression in omental adipose tissue [18–19]. Bujalska et al. analysed cultured omental and subcutaneous adipose stromal cells finding the exclusive expression of the 11β-HSD1 in these cells. Furthermore, its enzymatic activity was more efficient in the omental than in the subcutaneous fat and it was further stimulated by concomitant treatments with cortisol and insulin [18]. The authors speculated that this mechanism could lead to a constant exposure of GCs in omental fat, suggesting that visceral obesity might be a sort of "Cushing's syndrome of the omentum". Similarly, a higher expression of 11β-HSD1 gene correlated with waist circumference was found in another study on postmenopausal women [19]; as previously observed, 11β-HSD1 expression was inversely correlated with insulin sensitivity and its activity was enhanced when adipocytes were incubated with cortisol in vitro in a dose dependent fashion. A low expression of 11β-HSD2 mRNA was also found, but its detection was inversely correlated with central obesity and total body fat mass, suggesting an additional contribution of local increase in cortisol in visceral adiposity accumulation in obese individuals [19].

The importance of 11β-HSD1 activity has been confirmed in murine models with over- or downregulated 11β-HSD1 expression. Mice with transgenic overexpression of 11β-HSD1 in adipocytes have normal serum GC concentrations but elevated local GC levels in adipose tissue; this results in a higher tendency to develop visceral obesity and metabolic syndrome [23]. Conversely, 11β-HSD1 knockout mice seemed protected from the cardiovascular and metabolic side effects of GCs excess, including obesity. In fact, these mice presented a better lipid profile and did not display increased visceral adiposity [24–26]. A knockout model of adipose and liver specific 11β-HSD1 resulted in reduced lipolysis and circulating fatty acid excess. However, these mice presented other Cushingoid manifestations such as hypertension, increased adiposity and myopathy caused by GC excess that might be secondary to the reactivation of GCs by 11β-HSD1 at the target tissue level [27].

Furthermore, consequences of long-term 11β-HSD1 suppression in

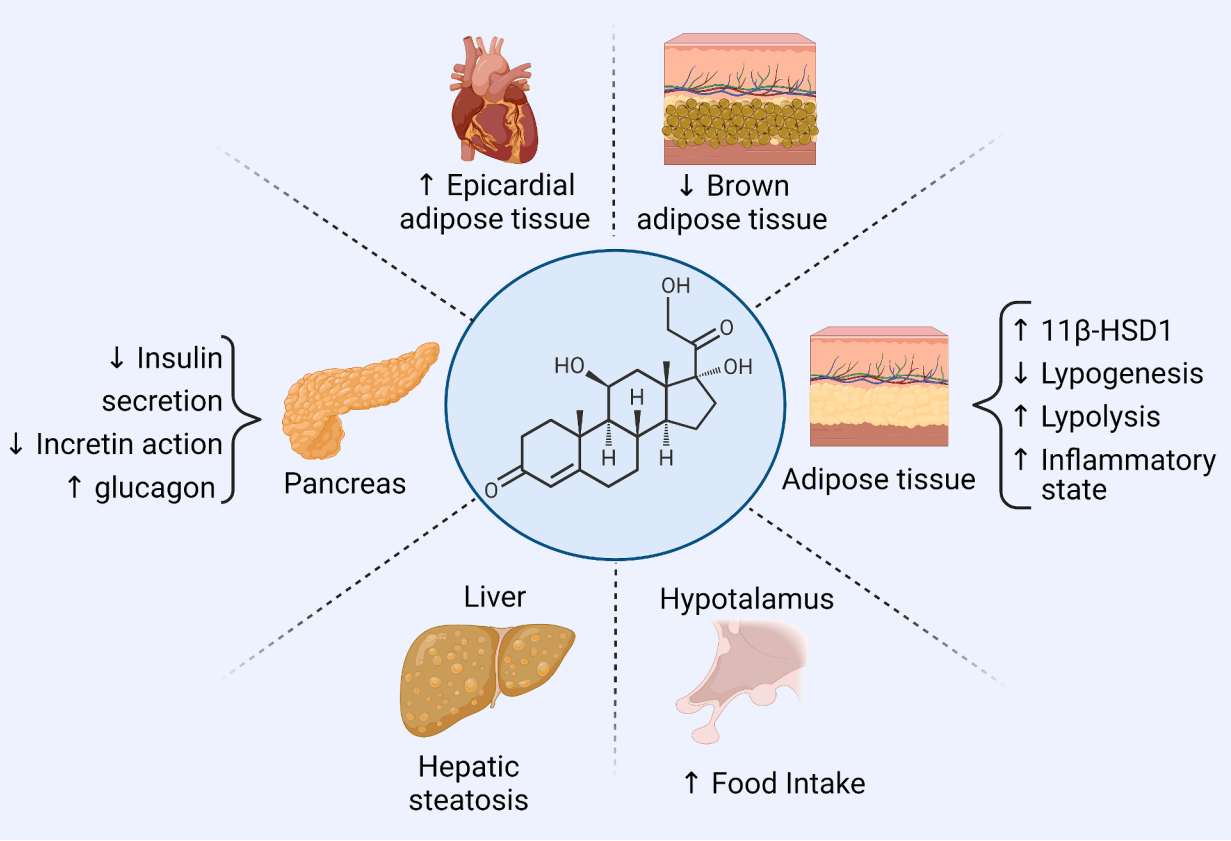

Fig. 1. Schematic representation of systemic cortisol actions.

humans are still unknown [28].

To date, several placebo-controlled trials have been conducted using different selective 11β-HSD1 inhibitors in the treatment of type 2 diabetes or in obese patients. In the study of Heise et al. on the use of two inhibitors, RO-151 and RO-838 with metformin in diabetic patients over 4 weeks, the results showed an improvement in parameters like body weight, HbA1c and insulin sensitivity. However, RO-151 caused an increase in ACTH plasma levels which can raise some concerns in the long run [29].

The increase in ACTH together with dehydroepiandrosterone was confirmed with other 11β-HSD1 inhibitor (i.e. INCB13739) tested on 302 patients with type 2 diabetes mellitus for 12 weeks. However, the levels of both hormones remained within the upper limit of normal and returned to baseline values after treatment discontinuation at follow-up visit. A reduction in body weight, total cholesterol, low-density lipoprotein (LDL), triglycerides (TGs), fasting plasma glucose, glycosylated haemoglobin (HbA1c) levels was observed in treated patients [30].

A similar trial with different doses of MK-0916 inhibitor was performed for 12 weeks in patients with diabetes mellitus and metabolic syndrome. Authors observed positive effects on body weight, blood pressure, HBA1c, but no significant changes in fasting glucose levels. The highest doses caused an elevation of androstenedione and dehydroepiandrosterone, which returned to normality 3 weeks after the end of the study, as previously observed [31]. Furthermore, in none of the studies that reported the elevation of adrenal androgens there was a worsening of hirsutism [30–32].

The MK-0916 and MK-0736 inhibitors were tested in 249 obese patients with hypertension; they induced a modest reduction in body weight and LDL levels after a 12-week trial. On the other hand, MK-0736 caused a decrease in high-density lipoprotein (HDL) levels [32]. A similar study evaluated the effectiveness of the reversible inhibitor of 11β-HSD1, AZD4017, in 31 obese women with idiopathic intracranial hypertension. An increase in HDL cholesterol with decrease of total cholesterol/HDL ratio and an increase in lean muscle mass were observed; however, body mass index (BMI) and body fat mass remained unchanged, as well as the level of TGs and HbA1c [33]. Based on these findings, selective 11β-HSD1 inhibitors could be considered a promising therapeutic option for the management of cortisol-related comorbidities, although none have been tested in the setting of CS.

1.3. 11β-Hydroxysteroid dehydrogenase 1 in CS

Data regarding 11β-HSD1 activity in the adipose tissue of CS patients are limited. As previously reported, 11β-HSD1 mRNA and protein expression in omental tissue were increased in obese patients compared to normal weight controls, but surprisingly this was not observed in CS patients [34]. Therefore, GCs do not directly control 11β-HSD1 activity in CS as there is no correlation between cortisol levels and the enzyme. It has been also speculated that this relative downregulation might represent a defence mechanism against hypercortisolism to prevent further increase in cortisol levels. Based on these findings, 11β-HSD1 inhibitors seem to have a marginal role in the visceral fat reduction in active CS.

However, 11β-HSD1 plays a key role in other Cushingoid manifestations as it has been demonstrated in CS who did not develop the classic Cushing's phenotype. It is indeed well recognized that the severity of clinical expression does not invariably correlate with the absolute levels of circulating cortisol. The first observation of this came from a case report described by Tomlinson et al. who described a patient diagnosed with CD but without any features or complications related to cortisol excess. Further investigations proved a defect in the enzyme activity with a concomitant increase in cortisol clearance rate [35]. A similar case of a patient affected by cortisol-producing adrenocortical adenoma was lately reported, and the same enzymatic deficiency was found [36].

2. Dyslipidaemia

Dyslipidaemia is probably the most neglected complication in CS as it has been only marginally evaluated. The scant studies available converge to point to a raised total and LDL cholesterol and TG concentrations, and reduced HDL cholesterol levels [37]. These alterations were equally present in pituitary and adrenal CS and tended to persist one year after curative surgery to some extent [38–39]. This was the case of patients with persistent obesity that maintained higher total and LDL cholesterol compared to sex- and age-matched, but not BMI-matched, controls as far as 5 years after remission [39].

Data on the effects of cortisol-lowering medications are scarce as well. Pasireotide, a somatostatin receptor multiligand used in the treatment of recurrent/persistent CD, proved to significantly reduce total and LDL-cholesterol irrespective of whether patients were taking lipid-modifying agents or not. Since this improvement was observed in both patients with normalised urinary free cortisol and uncontrolled patients, the effect was probably mostly related to the waist and BMI reduction obtained in the whole cohort [40]. Similar results were observed for the steroidogenesis inhibitors that induce an overall improvement of lipid profile together with metabolic and anthropometric features and hormone control, the only exception being represented by mitotane Table 1. [40–69]

3. Lipid synthesis

Although cortisol has lipolytic actions in isolated conditions, chronic hypercortisolism contributes to excess adiposity, altering food intake and influencing the storage of fatty acids [70]. CS patients have 30 % more fat mass, higher waist-to-hip and visceral-to-subcutaneous fat ratios than normal people. Alterations in visceral fat have been demonstrated also in mild autonomous cortisol secretion and in patients with non-functional adrenal tumours [71–73], pinpointing the importance of chronic exposure in the development of these metabolic changes.

In humans there are two types of adipocytes namely, brown adipose tissue (BAT) and white adipose tissue (WAT) [74], while the former oxidise fatty acids derived from TGs to generate heat due to the presence of the BAT-specific to uncoupling protein (UCP), the latter is involved in storage of energy as TGs; brown adipocytes oxidise fatty acids derived from TGs to generate heat due to the presence of the BAT-specific UCP1. Upon UCP1 activation, the energy generated by mitochondrial is released as heat instead of being used by ATP synthase [75].

In WAT, GCs have a dual effect since they increase lipolysis in subcutaneous adipose tissue, whereas stimulate the hypertrophy and differentiation of adipocytes in visceral adipose tissue [10,12]. In BAT, primarily found in infants and young children, chronic GCs exposure causes a reduction of UCP1 activation [76–77].

Although BAT has been basically considered non-existent and without physiologic relevance in adults [78], it was later observed that most important BAT areas are WAT depots that have gained BAT-like features, such as UCP1 expression [79] and regions of functionally active BAT can be identified by 18F-fluorodeoxyglucose positron emission tomography-computed tomography (18FDG PET-CT) [80].

WAT and BAT are both capable of undergoing a browning process characterised by a substantial upregulation of UCP1 expression. The resulting cells in WAT have been known as beige [81], brite [82], inducible [83] or recruitable [84] adipocytes.

Various treatments have been identified to induce browning, with cold exposure being the most pertinent and commonly employed in clinical studies. Previous reviews have extensively reviewed these agents [85–86].

The functional analysis of the browning process revealed contrasting results depending on the chosen experimental conditions. When using cold acclimation as a potent model for browning and initiating the process in mice housed at 21 °C, the evidence for increased UCP1 gene expression or UCP1 protein amount in classical BAT is weak. Conversely,

Table 1
Effects of cortisol-lowering medications on lipid and glucose profile, anthropometric features.

Drug	Effect on total Cholesterol/LDL	TGL	HDL	BMI/Waist	Fasting glucose levels/insulin need	HbA1c
Steroidogenesis inhibitors						
- [C]Levo/Ketoconazole	↓ [41–43]	=/↑ [43–44]	↓ [44]	↓ [43–47]	↓ [41,43–47]	↓ [44]
- Metyrapone	↓ [48]	NA	NA	↓ [48–49]	↓ [48–52]	↓ [48,50,52]
- Osilodrostat	↓ [53–55]	= [53]/↓ [54–55]	↓ [53–54]	↓ [53–56]	↓ [53–56]	↓ [53–56]
- Mitotane	↑ [41,57–60]	↑ [59–60] /= [41,58]	↑ [60][60]	↓ [60]	↓ [60]	NA
- Etomidate	NA	NA	NA	NA	↓ [60–61]	NA
Pituitary-directed drugs						
- Pasireotide	↓ [40,66]	= [40,66]	= [40,66]	↓ [40,66]	↑ [40,66]	↑ [40,66]
- Cabergoline	NA	NA	NA	↓ [68]	↓ [68–69]	↓ [69]
GR antagonists						
- Relacorilant	NA	NA	NA	NA	↓ [67]	NA
- Mifepristone	= [62–63]	= [62–63]	↓ [62–63]	↓ [62,64]	↓ [62,64–65]	↓

"↑": Increased level; "↓": reduced level; "=": no effect; NA: no data available,

brite/beige adipose depots showed significant increase in UCP1 expression in this condition, suggesting a preponderant role of functional browning of brite/beige tissues at cold temperature. However, when mice were housed at 30 °C (a thermoneutrality condition for mice), a substantial boost in UCP1 gene expression and UCP1 protein were observed in classical BAT depots, with practically no UCP1 gene expression in brite/beige tissues. This apparent paradox is related to the full differentiation status of BAT at 21 °C, which cannot be further activated by UCP expression. Under these conditions, increased thermogenesis can be achieved exclusively by stimulating cell proliferation, rather than enhancing cellular differentiation [87].

Studies performed in rodents showed that GCs have an inhibitory effect on BAT development and activity, most likely mediated via the GR. An increased BAT activity was found after adrenalectomy [88–89], while GC replacement normalised BAT activity [88,90].

Interestingly, one study on male mice model showed the opposite role of ACTH on activation of BAT and browning of WAT; thus in overt hypercortisolism there could be a balance between ACTH activation and BAT inhibitory effects of GCs on UCP1 transcription and function, that are possibly caused by GCs interfering with the adenylyl cyclase/ cyclic adenosine monophosphate (cAMP)/ protein kinase A (PKA) signalling pathway [91].

Indeed, in patients with CD with high ACTH levels, it was shown [92] that energy expenditure was not significantly decreased compared to matched healthy controls; so it might be possible that long-term exposure to hypercortisolism overcome the stimulating effect of ACTH at the fat tissue level, leading to a neutral effect on BAT activation in vivo.

In vitro studies using primary cultures of human BAT showed upregulation of UCP1, Cidea, and Ppargc1a mRNA levels in response to GCs [77,93]. However, the duration and dosage of GC treatment influenced the results, with varying effects on basal UCP1 gene expression. Adrenergically-induced UCP1 expression is consistently decreased by GC in both human and rodent adipocytes [93].

Direct investigations into the impact of GCs on BAT in humans are scant, primarily due to challenges in sample collection [94–95]. The commonly employed methods, measuring 18FDG uptake in BAT or supraclavicular skin temperature, are indirect and do not precisely assess thermogenesis. For instance, 18FDG PET-CT quantifies the glucose uptake, occurring even in the absence of UCP1 [94–95]. Despite these issues, current knowledge suggests that GCs exert a suppressive effect on BAT in humans [96].

Observational studies examining retroperitoneal fat revealed a lower prevalence of BAT-positive patients in groups with cortisol-producing adenomas and secondary hypercortisolism [96]. Additionally, there was a trend towards a negative correlation between urinary cortisol secretion and retroperitoneal UCP1 [96]. Retrospective studies demonstrated a decreased prevalence of BAT-positive individuals in patients receiving chronic GCs compared to controls [77]. Experimental studies on long-term effect of GC therapy showed a reduced 18FDG uptake in BAT and lower supraclavicular skin temperature in response to cooling (19 °C) in individuals pre-treated with oral prednisolone for 7 days [97]. Interestingly, the effects of GCs on human BAT seem to be time dependent. Short-term hydrocortisone infusion increased basal and isoprenaline-induced supraclavicular skin temperature [98]. Similarly, short-term (36 h) administration of prednisolone enhanced 18FDG uptake in BAT and leads to an increased supraclavicular skin temperature upon cold exposure [77].These data indicate that, in contrast to results reported for long-term hypercortisolism, short-term GC treatment may increase BAT thermogenesis in humans.

The impact of impaired BAT activity on the development of GC-induced obesity in humans remains to be further explored.

3.1. *Lipolysis*

Lipids are stored in adipose tissue as TGs. Lipases are enzymes which break down TGs, increasing plasmatic levels of fatty acids. The interaction between GCs and GR leads to an increase in transcription and activation of different lipases, such as adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and lipoprotein lipase (LPL) [70,99–100]. ATGL converts triacylglycerol to diacylglycerol by releasing one fatty acid. HSL could catalyse the same reaction to a lesser extent of ATGL, but it also converts diacylglycerol to monoacylglycerol. LPL instead hydrolyses triacylglycerol from chylomicrons and very low-density lipoproteins, which transport lipids from the gut to other tissues through the circulation system. The subsequent greater availability of FFAs is picked up in the visceral region. This mechanism is rather fast, since the increase in lipolysis starts few hours after GC exposure [99,101].

The efficiency of these lipases is also regulated by catecholamines that have a paramount lipolytic effect, especially in VAT [102].

Their binding to the β-adrenergic receptors activates the activity of adenylyl cyclase, leading to an elevation of cAMP levels, the target of which is the protein PKA. PKA phosphorylates HSL and promotes its translocation to the lipid droplets enhancing its lipolytic activity [103]. PKA also phosphorylates perilipin, which leads to an increase of HSL activity [104]. It is also supposed that the perilipin pathway could activate ATGL promoting its lipolytic activity [105–106].

The β-adrenergic signalling cascade is instead inhibited by phosphodiesterases, which break down cAMP reducing PKA activity.

GCs could interfere with the β-adrenergic pathway by altering cAMP levels [70,100,107], but the mechanisms behind this action remain unclear. Dexamethasone was found to increase cAMP levels in primary cultures of adipocytes and concomitantly enhance PKA activity and decrease phosphodiesterases expression [70]. As a result, an increased phosphorylation of HSL and perilipin was observed but without a greater translocation of HSL to the lipid droplets. Another study confirmed that GCs induced a rise in intracellular cAMP due to the decrease in protein kinase B and phosphodiesterases expression and activity [108]. However, other studies did not report cAMP elevation during in vitro incubation with GCs alone [101,109].

Lastly, a blunted growth hormone secretion is commonly found in CS, chronic GCs users, as well as in obese patients [110–111]. An impairment of the somatotropic axis could contribute to the increased visceral adiposity and decreased lean mass, since growth hormone has a physiological lipolytic role and protein anabolic effect [112–113].

3.2. Lipogenesis

As known, excess adiposity may be secondary to an increased synthesis and storage of lipids in pre-existing adipocytes (hypertrophy) or to the recruitment and differentiation of preadipocytes to mature adipocytes (hyperplasia) [16].

The latter mechanism, known as adipogenesis, is the most studied in hypercortisolism. Indeed, cortisol stimulates the differentiation of preadipocytes in a dose-related fashion [114], activating proadipogenic regulators such as peroxisome proliferator-activated receptor γ (PPARγ) and LIM domain only 3 (LMO3) [115–118]. LMO3 is highly expressed in visceral adipose tissue, where it is upregulated by GCs and correlates with 11β-HSD1 levels. LMO3 stimulates PPARγ activity enhancing visceral adipogenesis. Furthermore, preadipocyte differentiation could be enhanced by prostaglandin D2 synthase, activated by GCs binding to MR [117–118]. Cortisol is able to bind MR in CS due to the saturation of the enzymatic activity of 11β-HSD2 in the condition of cortisol excess. [119].

The increase in adipogenesis is supposed to lead to hyperplastic adipose tissue expansion; however, in CS patients, adipose tissue has hypertrophic adipocytes, which indicates that the dominant mechanism involved GC-stimulated synthesis and lipids storage [120].

Only a few studies investigated GCs' action on lipogenesis. In VAT, AMP-activated protein kinases (AMPK) activity is approximately reduced by 70 % in CS patients with respect to those with non-functioning adrenal adenoma and control subjects [121–122]. AMPK activity inhibits fatty acid synthesis and downregulates lipogenic enzyme gene transcription [123–124], thus the AMPK downregulation leads to an increase in lipid anabolic pathways.

Another study shows that corticosterone, together with insulin, stimulates lipid synthesis up to 66 % in cultured adipose tissue [125]. However, other studies delivered opposite results, showing a down-regulation of fatty acid synthase and acetyl CoA carboxylase in rats [126].

Lipid storage may also be increased through the re-esterification of FFAs into TGs. However, other studies did not support that hypothesis [127–128] as GCs seemed to reduce re-esterification within adipose tissue instead [127]. Similarly, the phosphoenolpyruvate carboxykinase, the enzyme involved in re-esterification, was reduced in adipose tissue during GCs treatment [128]. Cortisol action on lipid metabolism is schematically depicted in Fig. 2.

4. Insulin resistance and diabetes mellitus

The prevalence of DM in CS patients ranges between 20 and 45 %, although the actual prevalence is thought to be underestimated since the oral glucose tolerance test is not routinely performed in clinical practice especially in case of normal fasting glycaemia [129]. As a general rule, the extent of cortisol excess is directly related to with insulin resistance and DM development [130], although this has not been invariably confirmed. The insulin-resistance induced by GCs in adipose tissue could be explained by a reduction of glucose uptake through inhibition of the

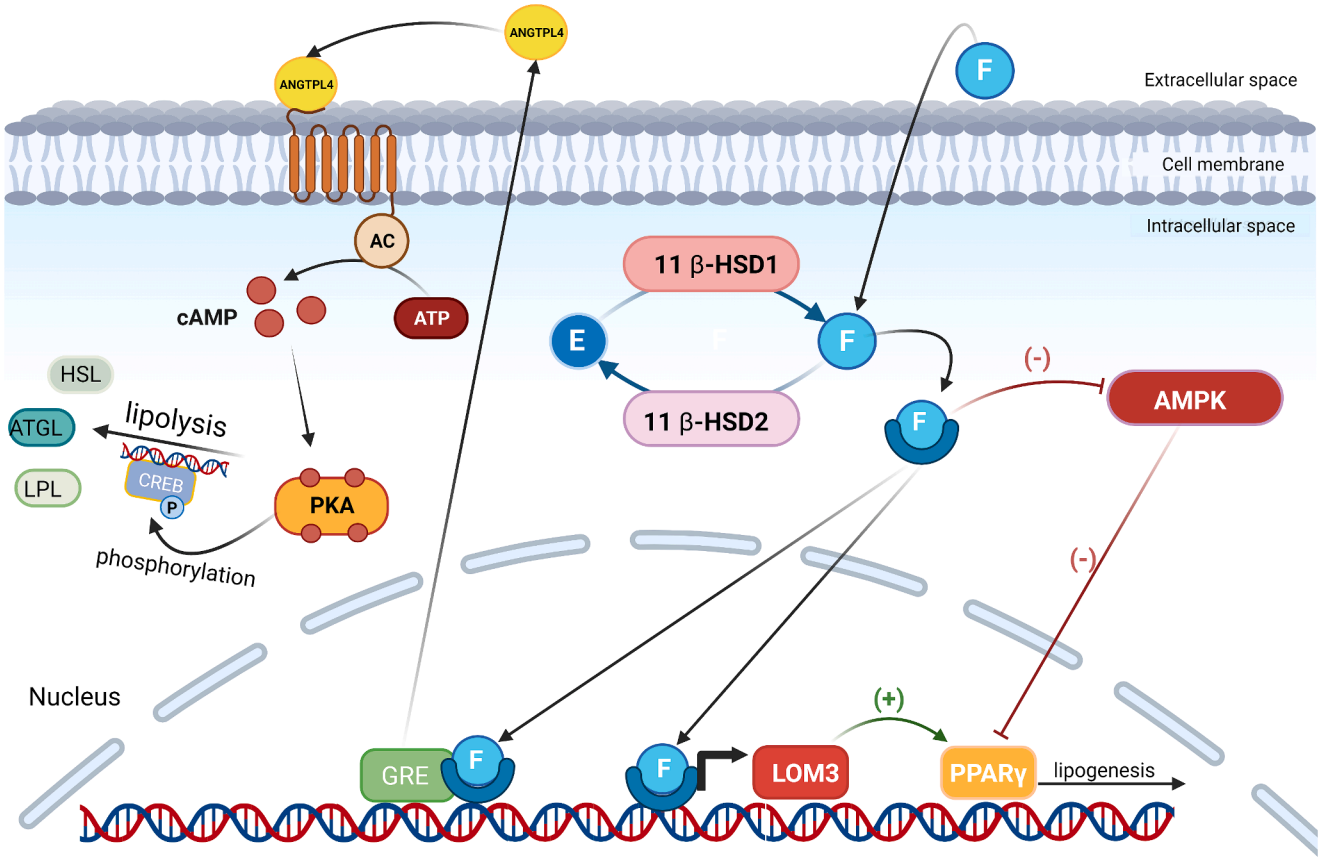

Fig. 2. Schematic representation of cortisol effects on lipid metabolism. F, cortisol; E, cortisone; GRE, glucocorticoid responsive element; AMPK, AMP-activated protein kinases; AC, adenylyl cyclase; PPARγ, peroxisome proliferator-activated receptor γ; cAMP, cyclic adenosine monophosphate; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; ATGL, adipose triglyceride lipase; PKA, protein kinase A; ANGTPL4, angiopoietin-like 4; 11 βHSD, 11β-Hydroxysteroid dehydrogenase. Figure made with Biorender.

translocation of glucose transporter type 4 on surface membrane [131]. Another possible contribution derives from a downregulation of insulin receptor substrate 1 expression. Moreover, it was observed a reduction of glucose uptake and a lower expression of insulin receptor substrate 1 on human omental fat cultures, but in line with the different action of GCs on adipose tissue-subtype, it was not observed in subcutaneous tissue [132]. GCs induce hepatic gluconeogenesis by increasing the transcription factor forkhead box protein O1 and upregulating of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), key enzymes involved in the process [133].

Moreover, GCs have a catabolic effect on muscle and the amino acids from protein degradation, along with the elevated FFAs, provide further substrates for hepatic gluconeogenesis [134–135]. In muscle cells GCs reduce the glucose uptake by reducing the translocation of the glucose transporter type 4 on the surface membrane [136]. Moreover, GCs showed to impair the phosphorylation of insulin signalling proteins in a murine model. In particular, a reduced phosphorylation of protein kinase B phosphorylation, that controls muscle glucose uptake, and of glycogen synthase kinase-3, which controls glycogen synthesis [137] have been reported. In liver cells GCs also stimulate de novo lipogenesis, enhancing the effect of insulin as described above [138–139].

The high circulating FFAs levels induced by GCs are preferably accumulated in visceral fat and in the liver inducing hepatic steatosis in the long run. Interestingly, Dalle et al showed that mice with a selective GR knock-out in adipose tissue not only determined an improvement in glucose tolerance and insulin sensitivity, but also a considerable reduction of TGs hepatic amount. These data strengthened the primary role of GCs in glucose and lipid metabolism control and the GR in the adipocytes as a potential therapeutic target for metabolic syndrome [140].

The diabetogenic effect of GCs is also due to their direct action on pancreatic cells. Most of the in vitro experiments on pancreatic ß-cells cultures proved a decreased insulin secretion upon GC exposure [141], whereas in vivo studies showed hyperinsulinemia and ß-cells hyperplasia [142]. It was postulated that this finding may be an adaptive response to counterbalance insulin resistance induced by GCs [143]. Thereby, the majority of CS patients present hyperinsulinemia. Cortisol also stimulates alpha pancreatic cells to produce glucagon, enhancing the hyperglycemic status [144].

It is noteworthy to stress that even a few days of oral administration of GCs leads to glucose impairment and insulin resistance also in healthy individuals [145–146]. Diabetes is present at CS diagnosis in almost 32 % of endogenous Cushing's syndrome patients, with a higher prevalence in the ectopic form of CS [147].

The diabetogenic effect of GCs is also due to a blunted "incretin effect". Incretin hormones are gut peptides that are secreted after meal intake and stimulate insulin secretion [148]. In healthy volunteers, dexamethasone treatment did not change fasting incretin hormones, but a reduced incretin effect was observed especially in people who developed glucose intolerance [149–150]. In CD patients with diabetes mellitus, a blunted glucose dependent insulinotropic polypeptide response to a mixed meal tolerance test was observed compared to the non-diabetic group [151]. Incretin mimetics, such as glucagon-like-peptide-1 receptor agonist, may improve the insulin sensitivity in CS patients, even in patients with Pasireotide-induced hyperglycemia [152].

5. Food intake

In normal conditions, the hypothalamic–pituitary–adrenal axis (HPA) activity is stimulated during prolonged fasting [153]. The subsequent elevation of GCs levels leads humans to choose high-caloric foods to assimilate energy substrates [154]. Acute supraphysiological exposure to GCs was shown to induce hyperphagia in animals, whereas results in humans are not always consistent [155–157].

In chronic hypercortisolism this process could cause an elevation in calories consumption in non-fasting period, along with increased circulation of FFAs contributing to adipose tissue accumulation. CS patients frequently reported increased subjective hunger and food consumption during active disease. It has been observed that CD patients usually pick high-fat products to eat compared to normal controls [158]. Interestingly, in CD both higher cortisol values and abdominal fat stores after remission were associated with lower post-meal satisfaction and fullness [159]. These findings pointed that once reached eucortisolemia, the HPA axis continues to regulate appetite, but exact mechanisms need to be elucidated. It should be mentioned that even fat stores may play a role in food intake, since hypothalamus is known to sense circulating fatty acids [160].

Two gastrointestinal hormones, ghrelin and peptide YY involved respectively in meal initiation [161] and termination (by enhancing satiety and decreasing food intake) [162–163], were not correlated with appetite and craving scores in CD patients. While peptide YY levels showed no changes after remission, fasting and post-prandial plasmatic ghrelin increased following cortisol normalisation. These findings were confirmed by other studies in which the expression of ghrelin and its receptor were increased in fasting conditions by GCs action [164–165]. However, it remains unclear whether this increase in ghrelin levels observed after remission is mainly due to decreased GC's concentration or to weight loss.

A possible role in food intake might be played also by the adipokine profile imbalance. Leptin is an adipocyte-derived protein which regulates food intake, body mass and takes part in lipolysis, proinflammatory responses and other functions [166–168]. Leptin concentrations tended to be higher in patients with CS compared to BMI-matched or normal weight controls [169]; furthermore, hypercortisolism might cause a reduction in leptin's sensitivity [157,170–171]. These alterations can favour the development of obesity, hyperphagia and metabolic disorders [157,172], all features present in CS.

GCs can also affect appetite by upregulating the gene expression of orexigenic peptides, like neuropeptide Y [173], and Agouti-related protein [174]. Neuropeptide Y increases food intake and the proportion of energy that is finally stored as adipose tissue. Other studies showed that GCs stimulate hypothalamic endocannabinoids with consequent increase in AMPK function, finally enhancing the sense of hunger [121,175–176].

ACTH is one the products of the enzymatic cleavage of the prohormone proopiomelanocortin (POMC); POMC-derived peptides are known to play a critical role in obesity through the melanocortin-receptor type 4; POMC deficiency or alterations in its processing are indeed associated with obesity [177]. POMC was found increased in ACTH-dependent CS, especially in ectopic forms [178] but the rate of efficient processing of the prohormone in such conditions and its impact on food intake have not been explored in cortisol excess states.

Interestingly, ectopic CS due to small-cell lung carcinoma showed a marked increase in Agouti-related protein that might contribute to the weight loss frequently found in these patients despite severe cortisol excess. These results were later confirmed in another study comparing ectopic CS of non-malignant origin with CD [179].

6. Adipose tissue's proinflammatory state

GCs are known for their anti-inflammatory actions and are usually used to suppress immune response. However, long-term hypercortisolism is characterised by a state of chronic low-grade inflammation. Indeed, several studies showed that active CS patients had increased plasmatic concentrations of proinflammatory cytokines such as interleukin (IL)-6, IL1-β and tumour necrosis factor-α [180–181]. Also obesity is a state of chronic low-grade inflammation with elevated levels of tumour necrosis factor-α and IL-1, driven by dysfunctional adipose tissue [182–183]. This inflammation is characterised by the increased presence of macrophages, with a predominance of the proinflammatory M1 macrophage phenotype over the M2 anti-inflammatory one

[184–185].

A preponderance of proinflammatory M1 macrophages, namely CD68 + and CD11c + macrophages, was also observed in CS patients [186]. as well as an increase in CD4 + T lymphocytes. All these markers were significantly increased compared to BMI-matched controls, confirming that the inflammatory state was not secondary to the mere weight excess. Moreover, macrophages could abolish the differentiation of preadipocytes, inducing a phenotypic switch into myofibroblast through the action of macrophage-derived factors [187–189]. This process causes an increase in pro-fibrotic markers in adipose tissue which has already been reported in patients with CS [190].

Furthermore, a reduction in the production of vimentin, an intermediate filament expressed in mesenchymal cells, which may indicate a compromised adipose tissue structure caused by GCs was observed in CD patients. These alterations can further contribute to the augmented levels of proinflammatory cytokines in CD patients [186].

GCs can bind both GR and MR; it should be recalled that in presence of low 11β-HSD2 activity, as it is the case of adipocytes, cortisol is the main ligand of MR since in physiological condition concentration of cortisol is 100- to 1000-fold higher than those of aldosterone and this effect is taken to the extreme in patients with hypercortisolism [191–192]. The activation of the MR in adipose tissue led to a release of proinflammatory cytokines from M1 macrophages [193]. This additional proinflammatory state was also observed in adipocytes where the GR was genetically deleted, suggesting that MR signalling induces proinflammatory state itself [194].

Lastly, a down-regulation of glucocorticoid-induced leucine zipper (an anti-inflammatory factor normally enhanced by GCs) and an activation of multiple proinflammatory pathways, as toll-like receptors, leucine-rich family 3 gene receptor and purinergic receptors were also described in obesity [195–197].

Overall, the adipose tissue inflammation may be a potential cause of the increased systemic inflammation found in CS, leading to insulin resistance and cardiovascular mortality. Future studies are needed to investigate the mechanisms through which GCs increase inflammation within adipose tissue and the effects of specific treatment in CS.

7. Hepatic steatosis

Hepatic steatosis is commonly found in CS and could lead to non-alcoholic fatty liver disease NAFLD [12,198] which in turn paves the way toward fibrosis and hepatic cirrhosis, both well known risk factors for hepatocarcinoma [199–200]. Although not systematically assessed, NAFLD seems to be highly prevalent in CS patients. Indeed, a CT-based study found hepatic steatosis in 20 % of active CS patients [198]; a negative correlation between both liver attenuation and liver/spleen attenuation ratio with abdominal fat area, visceral adipose tissue area and the ratio between visceral and subcutaneous adipose tissue confirmed a strong connection between visceral adiposity and liver hepatic steatosis [198]. Conversely, in a cohort of NAFLD patient the level of cortisol where slightly higher than the control groups [201].

The intricate regulation of hepatic lipid content involves various mechanisms such as food intake, glucose and lipid regulation, all under the direct or indirect influence of GCs. Cortisol exerts its effects by both upregulating several processes such as lipogenesis, gluconeogenesis, and increasing circulating FFAs and glucose levels, and concurrently inhibiting β-oxidation of fatty acids. Additionally, cortisol stimulates the secretion of very-low density lipoprotein (VLDL), contributing to a reduction in lipid deposition [12].

As previously described, hypercortisolism enhances food intake leading to the absorption of fatty acids by enterocytes, used to synthesise TGs that are released into the lymphatic system as chylomicrons. At this point, plasmatic LPL releases FFAs and glycerol from chylomicrons; FFAs are uptaken by muscle, adipose tissue, and liver where they are re-converted to TGs and stored in lipid droplets. Glycerol is instead picked up by the hepatocytes for gluconeogenesis as well as chylomicron remnants by the LDL-receptor [202].

Following a meal, glucose is absorbed by glucose transporter type 2 into the liver, where it is deposited as glycogen or metabolised [203], whereas amino acids can be converted to glucose by the liver to a lesser extent under fed conditions through gluconeogenesis. [204–205] Gluconeogenesis is enhanced in the presence of cortisol excess which accelerates the degradation of skeletal muscle proteins, leading to muscle atrophy. Furthermore, glucose obtained through this pathway could be converted to FFAs and stored as TGs, especially in visceral adipose tissue [206].

GCs further promote gluconeogenesis through the transcriptional activation of PEPCK and glucose-6-phosphatase (G6Pase) [207–209].

This stimulation is directly promoted by the binding of the GR complex to the glucocorticoid response unit located in the promoter region of these genes, and by the activity of nuclear receptor co-factor peroxisome proliferator-activated receptor-γ co-activator 1 alpha (PGC1α), a co-factor whose expression is also increased by GC [203,210]. Recently, GCs have been shown to stimulate the expression of Krüppel-like factor 9 (KLF9), a transcription factor that in turn stimulates the expression of PGC1α [211]. In a knock-out KLF9 mouse model, hyperglycemia induced by chronic dexamethasone treatment was significantly lower than in wild type mice confirming the role of GC/GR complex – KLF9 – PGC1α pathway in the activation of the hepatic gluconeogenic program [212]. The expression of PEPCK and G6Pase is promoted by catecholamines and glucagon, while insulin exerts inhibitory control over these enzymes [207–209]. As previously detailed, hypercortisolism induces hyperinsulinemia and insulin resistance, attenuating insulin-mediated glucose transport in tissues. Additionally, glucagon levels rise concomitantly [213–214] and the catecholamine-induced glycogenolysis is enhanced GCs [215]. The synergistic impact of these factors culminates in high blood glucose levels [203,214], a part of which is picked by the liver where it is converted into FFAs and stored as TGs within lipid vesicles.

TGs originate from the esterification of FFAs with glycerol or via de novo synthesis. Typically, FA esterification accounts for 60 % of TG synthesis. The contribution of de novo FFA synthesis is minimal during fasting periods but increases up to 20 % of the whole TG amount after food intake thanks to the higher acetyl-CoA availability [216–217]. GCs exert a regulatory control over several genes encoding enzymes involved in de novo FFA synthesis, including ACC and fatty acid synthase (FAS). Hyperglycemia, hyperinsulinemia, and GCs play integral roles in the de novo FFA synthesis pathway. In insulin resistance state, TG synthesis during fasting can be increased up to fivefold [218–220]. Transcriptional regulation of de novo FFA synthesis involves sterol regulatory element-binding protein-1c, stimulated by insulin, and carbohydrate response element-binding protein, stimulated instead by hyperglycemia [221–222]. As already described, GCs impaired both insulin and glucose plasma concentrations, activating sterol regulatory element-binding protein-1c and carbohydrate response element-binding protein, at the same time. This activation enhances the transcription of acetyl-CoA carboxylase (ACC) and FAS, along with the upregulation of glycolytic and lipogenic genes [223]. While some studies suggested a synergistic effect of GCs and insulin on ACC gene expression [224–226], others pointed to a pivotal role of insulin for GC-induced ACC stimulation [139,227–228]. recent study showed an activation of MAPK phosphatase 3 by GCs as an additional mechanism behind the higher hepatic lipid synthesis [138].

GCs induce a relatively modest elevation in plasmatic levels of FFAs. Specifically, GCs promote lipolysis in subcutaneous adipose tissue, leading to an increase in FFA levels. Simultaneously, hypercortisolism enhances the uptake of FFAs by the liver and other tissues such as the heart and skeletal muscle [30,229–231]. These concurrent processes constitute primary contributors to hepatic lipid deposition [216,232–235]. The uptake of FFAs is governed by a multitude of transporters [236–238]. A study conducted on rats demonstrated the upregulation of CD36, a FFA transporter, in the liver during GCs

treatment [239].

GCs exert inhibitory control over the transcriptional activity of peroxisome proliferator-activated receptor alpha (PPARα), leading to a reduction in the expression of mitochondrial acyl-CoA dehydrogenases. This mechanism results in the attenuation of FFA beta-oxidation [240–242]. However, the increase in glucagon levels might stimulate FFA oxidation [213,243] and consequent ketone formation [244]. Nevertheless, this latter stimulatory effect is counteracted by hyperinsulinemia, which suppresses this pathway [203,245], thus the overall impact of GCs on FFA beta-oxidation in the liver is its restraint. Interestingly, GCs also modulate some pathways that mitigate lipid deposition. GCs may lead to a reduction in the degradation of apolipoprotein B and in the expression and activity of triacylglycerol hydrolase, both integral to VLDL secretion [246–247]. Triacylglycerol hydrolase catalyses the hydrolysis of TGs in the hepatocytes and a fraction of the released FFAs is re-esterified to TGs [248]. A proportion of these TGs binds to ApoB100, culminating in the formation of VLDL particles. These particles are concentrated in the Golgi apparatus and subsequently secreted as VLDL [247,249–250]. The combined effects of increased TG synthesis and a modest rise in VLDL secretion attributable to excess GCs lead to a net TGs accumulation within the hepatic tissue [247,250].

8. Increased epicardial adipose tissue

The epicardial adipose tissue (EAT) is a fat depot of the heart in contact with myocardium and coronary arteries with multiple functions. EAT serves as a local energy source that releases FFAs into the bloodstream; however, it can also produce inflammatory adipocytokines that enhance in a paracrine way monocytes adhesion to endothelial cells [251]. Due to this latter action, the increase in EAT has been found to be an independent predictor of coronary artery disease, as it seems to be involved in early stages of atherogenesis [252].

The effects of hypercortisolism on EAT volume and left ventricular (LV) function were evaluated in patients with CS [253–254]. The presence of hypercortisolism itself was found to be and independent determinants of epicardial fat volume [254]; in fact, EAT volume was increased in active CS compared to controls, possibly contributing to the increased cardiovascular risk in this setting [253], and it remains higher despite remission, although a decrease was observed after treatment [254].

Similar effects occur in patients with long-term steroid treatment due to rheumatic disorders [255].

Furthermore, it was found that CS patients had higher LV mass index, concentric remodelling and impaired LV relaxation compared to control subjects [253]; these structural changes were independently correlated with EAT volume, suggesting a major role of EAT in CS-induced LV dysfunction.

As well known, EAT has local effects on the cardiac structure, volume and function in severely obese patients, but also in people without metabolic syndrome [256–258]; so it could be hypothesised that CS related cardiomyopathy is associated to adipocytokines release from EAT into the cardiomyocytes, promoting cardiac fibrosis and contractile dysfunction [259–260].

Notably, it was demonstrated that EAT was also composed by brown adipocytes, with a UCP-1 mediated thermogenic function and a putative cardio-protective role [261]; thus, it could be speculated that besides a quantitative change there is also a qualitative modification of the EAT in CS, with an increase of the white adipocytes and a relative decrease in brown adipose cells, as it occurs with aging.

The loss of BAT protective functions in the heart could be a major player in CS cardiomyopathy, but more studies are needed to better explain this relationship.

9. Cortisol action in obese patients

Considering all the above described effects of hypercortisolism on adipose tissue, many studies investigated the possibility of a reverse relationship between obesity and cortisol. It has been studied whether impaired cortisol levels are present in obesity and thus promote fat accumulation. To date, most of the studies have shown conflicting data [262], and recent *meta*-analyses were unable to prove a positive association between obesity and cortisol excess in epidemiological studies [263–264].

The 24 h urinary free cortisol is one of the widely used and accepted screening methods for the diagnosis of endogenous cortisol excess. However, results in literature are inconsistent, with some studies reporting hypercortisolism and others hypocortisolism in obese patients [265–269]. These heterogeneous results might depend on the fact that traditional screening methods for cortisol excess provide a snapshot of the day of the assay without giving a real estimation of the total tissues' exposure of cortisol for longer periods. For this reason, multiple samples are frequently required in case of suspected CS [270].

Recently, the measurement of hair cortisol has gained increasing interest since it is less influenced by daily fluctuation and can provide an excellent estimation of cortisol exposure in the previous months [271–272]. Recent studies have observed that hair cortisol levels are increased in obese people [273] and positively correlated to body weight [274] and waist-to-hip circumference [275]. The same trend was observed also for hair cortisol concentrations and obesity in children. [276].

Several studies have studied the alterations of the HPA axis in obese patients, as summed up by a systematic literature review [263]. The HPA axis follows a well-defined circadian rhythm of secretion, with cortisol peaking in the morning before awakening and slowly decreasing throughout the day, reaching the nadir at bedtime [277].

Some authors evaluated the cortisol awakening response by measuring salivary cortisol in the morning. As observed for other parameters, results were conflicting; some studies found a negative relationship between low morning cortisol levels and obesity [278–282], whereas others found an elevation of morning peaks [283–285] or no relationship at all [286–287].

An increased HPA activity to both physical and psychological acute stressors has been observed in people with abdominal obesity [269,279,288–290]. For instance, a higher ACTH and cortisol response to CRH was observed in women with abdominal obesity compared to controls [291]. Similarly, women with a greater waist-to-hip ratio displayed an enhanced cortisol response to ACTH stimulation [292].

The loss of the circadian rhythm of cortisol secretion is a typical hallmark of CS. Its elevation in non-CS patients was positively correlated with BMI or waist circumference, as observed in all studies [267,278,284,293] but one [281]. A community sample of 120 control patients, found elevated late-night salivary cortisol levels in patients with metabolic syndrome but the total endogenous daily cortisol secretion measured by the area under the curve of multiple cortisol sampling was not influenced by age or metabolic syndrome [294].

The maintenance of the normal HPA regulation has also been assessed through the low dose dexamethasone suppression test. A normal response consists of a decrease of serum cortisol to less than 50 nmol/L on the morning after dexamethasone administration [7]. However, results were not homogenous since testing protocols and dexamethasone doses differed among studies. In general, no correlation was observed between the loss of negative feedback and generalised obesity [269,293], whilst abdominal obesity seemed to be associated with a reduction of the feedback mechanism and increased post-dose cortisol levels [294,295]. Indeed, the waist-to-hip ratio seems to be linked to a less effective cortisol inhibition after dexamethasone, while these results were independent of the BMI. The possibility of low absorption and excessive drug distribution of dexamethasone has been questioned for obese patients, and alternative methods of performing the suppression test have been used proving the same results. This issue can be overcome by measuring serum cortisol and dexamethasone levels after suppression to confirm adequate drug bioavailability; a dexamethasone $>$ 4.5 nmol/

L improved accuracy and interestingly, its level was not correlated to either body weight or BMI [296].

10. CS remission and fat distribution

To date, few studies have investigated the effects of surgery or medical treatment on cortisol-related comorbidities, Table 2. It has been observed that despite complete remission, several patients do not completely revert cortisol-related comorbidities, in particular they maintain cardiovascular, bone and quality of life impairment that result in enduring elevated mortality rate [297–299].

One of the major determinant of this high risk condition is the persistent increase of typical hallmarks of the metabolic syndrome; Faggiano et al. showed that one year after remission BMI did not change and the waist to hip ratio despite its decline following cortisol normalisation remained significantly higher than age-matched controls [38].

Similarly, CS patients maintained abnormal body composition and impaired adipokines secretion even after long-term remission [181]. Lower levels of adiponectin, an adipokine with anti-atherogenic and anti-inflammatory activity, with concomitant elevation of soluble TNFα receptor 1 and IL-6 were found in CS patients in remission compared to controls. This impaired adipokine profile that correlates with visceral obesity, may contribute to the low-grade inflammation and cardiovascular risk observed in CS patients irrespective of the disease activity [300].

They also observed that long-term remission led to a gradual reduction of leptin levels. On the other hand, considering a shorter remission time, Cizza et al. found persistently elevated levels of leptin ten days after surgery for CD [301].

Another study [302] found that a 6-month remission decreased weight, waist circumference and nearly all fat depots, like VAT, pelvic bone marrow adipose tissue, subcutaneous adipose tissue as assessed by magnetic resonance imaging. This resulted in an improvement in fat distribution, with a reduction in visceral/total fat and visceral fat/skeletal muscle ratios, all indexes known to be associated with hepatic steatosis and metabolic syndrome [303,304].

Furthermore, Lonn et al. [305] observed that after normalisation of cortisol levels these patients present a reduction in total adipose tissue and a redistribution from viscera to legs. Regarding bone marrow adipose tissue, also Maurice et al. found a normalisation of its accumulation during remission [306] .

A study with a longer follow-up after remission reported a persistent increase in visceral fat and persistent difficulties in losing weight, with BMI exceeding 25 kg/m^2 in most of the cases [307].

Likewise, the EAT excess improved after biochemical remission, although it remained more represented than in control patients [253], whereas no changes were observed for the pericardial fat after cortisol control [254].

11. Conclusions

Several mechanisms are involved in the pathogenesis of visceral adiposity in CS, including cortisol direct action on adipocytes inducing adipogenesis, 11β-HSD1 overexpression and 11β-HSD2 saturation and increased intake high-energy food. However, the relative weight of each contributor to the clinical picture of CS is still to be determined and required further investigation. What is clear is the detrimental effect of central adiposity on cardiovascular health, since it is the key determinant of the sustained proinflammatory profile that has been observed even after long-term remission in CS patients. Despite the reversal of most of the cortisol-related complications after effective control of CS, BMI and especially waist circumference remained impaired exposing the patients to higher risk of cardiovascular events. Among treatments addressing adverse metabolic effects of cortisol at tissue level the 11β-HSD1 inhibitors provided promising data suggesting their future application to mitigate side effects of exogenous GCs therapy for chronic conditions and potentially as adjunctive treatment in the management of CS comorbidities. However, concerns have been raised about their

Table 2
Studies reporting the variations of anthropometric parameters, adipose tissue and adipokine profile in patients with Cushing's Syndrome.

First author	Year of publication	Study type	Remission time	Anthropometric parameters		Adipose tissue		Adipokine profile	
Lönn L	1994	Prospective observational	8 ± 2 months	Weight	↓	TAT VAT Legs adipose tissue	↓ ↓ ↑		
Cizza G	1997	Prospective observational	10 days	Weight BMI	= =			Leptin	=
Faggiano A	2003	Prospective observational	12 months	BMI WHR	= ↓				
Leong GM	2006	Prospective observational	3 to 7 years	BMI	=	VAT	=		
Barahona MJ	2009	Case-control	11 ± 6 years					Adiponectin sTNF-R1 IL-6	↓ ↑ ↑
Geer EB	2012	Prospective observational	6 months after GC replacement discontinuation mean 20.1 months (9 – 42 months)	Weight Waist circumference	↓ ↓	VAT SAT Pelvic BMAT VAT/SM VAT/TAT	↓ ↓ ↓ ↓ ↓	Leptin	↓
Maurice F	2018	Cross-sectional	43 ± 4 months	BMI	=	VAT dSCAT/ sSCAT BMAT	↓ ↓ ↓		
Maurice F	2018	Cross-sectional	> 2 years	BMI WHR	= =	EAT	↓		
Wolf P	2021	Prospective observational	8.1 – 10.9 months	BMI	=	EAT PAT	↓ =		

BMI = body mass index; WHR: waist-to-hip ratio; VAT = visceral adipose tissue; SAT = subcutaneous adipose tissue; BMAT = bone marrow adipose tissue; SM = skeletal muscle; TAT = total adipose tissue; dSCAT = deep subcutaneous adipose tissue; sSCAT = superficial subcutaneous adipose tissue; EAT = epicardial adipose tissue; PAT = pericardial adipose tissue.

safety in vivo in the long-run and further data are warranted. Targeting the cAMP-dependent pathway seems also an intriguing option but still to be explored.

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Alessandro Bavaresco: Writing – original draft, Investigation, Data curation. **Pierluigi Mazzeo:** Writing – original draft, Investigation, Data curation. **Martina Lazzara:** Writing – original draft, Investigation. **Mattia Barbot:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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