Hypothalamic–pituitary–adrenal axis activation and glucocorticoid-responsive gene expression in skeletal muscle and liver of Apc mice

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Abstract

Background Cancer patients at advanced stages experience a severe depletion of skeletal muscle compartment together with a decrease in muscle function, known as cancer cachexia. Cachexia contributes to reducing quality of life, treatment efficiency, and lifespan of cancer patients. However, the systemic nature of the syndrome is poorly documented. Here, we hypothesize that glucocorticoids would be important systemic mediators of cancer cachexia.

Methods To explore the role of glucocorticoids during cancer cachexia, biomolecular analyses were performed on several tissues (adrenal glands, blood, hypothalamus, liver, and skeletal muscle) collected from ApcMin/− male mice, a mouse model of intestine and colon cancer, aged of 13 and 23 weeks, and compared with wild type age-matched C57BL/6J littermates.

Results Twenty-three-week-old Apc mice recapitulated important features of cancer cachexia including body weight loss (−16%, P < 0.0001), muscle atrophy (gastrocnemius muscle: −53%, P < 0.0001), and weakness (−50% in tibialis anterior muscle force, P < 0.0001), increased expression of atrogens (7-fold increase in MuRF1 transcript level, P < 0.0001) and down-regulation of Akt–mTOR pathway (3.3-fold increase in 4EBP1 protein content, P < 0.0001), together with a marked transcriptional rewiring of hepatic metabolism toward an increased expression of gluconeogenic genes (Pcx: +90%, Pck1: +85%), and decreased expression of glycolytic (Slc2a2: −40%, Gk: −30%, Pklr: −60%), ketogenic (Hmgcs2: −55%, Bdh1: −80%), lipolytic/fatty oxidation (Lipe: −50%, Mgl: −60%, Cpt2: −60%, Hadh: −30%), and lipogenic (Acyl: −30%, Acacb: −70%, Fasn: −45%) genes. The hypothalamic pituitary–adrenal axis was activated, as evidenced by the increase in the transcript levels of genes encoding corticotropin-releasing hormone in the hypothalamus (2-fold increase, P < 0.01), adrenocorticotropic hormone receptor (3.4-fold increase, P < 0.001), and steroid biosynthesis enzymes (Cyp21a1, P < 0.0001, and Cyp11b1, P < 0.01) in the adrenal glands, as well as by the increase in corticosterone level in the serum (+73%, P < 0.05), skeletal muscle (+17%, P < 0.001), and liver (+24%, P < 0.05) of cachectic 23-week-old Apc mice. A comparative transcriptional analysis with dexamethasone-treated C57BL/6J mice indicated that the activation of the hypothalamic–pituitary–adrenal axis in 23-week-old ApcMin/− mice was significantly associated with the transcription of glucocorticoid-responsive genes in skeletal muscle (P < 0.05) and liver (P < 0.001). The transcriptional regulation of glucocorticoid-responsive genes was also observed in the gastrocnemius muscle of Lewis lung carcinoma tumour-bearing mice and in KPC mice (tibialis anterior muscle and liver).

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Introduction

Cancer cachexia is a devastating hypo-anabolic and catabolic syndrome characterized by a progressive intentional loss of body mass, essentially due to a severe depletion of skeletal muscle compartment, that cannot be reversed by conventional nutritional supports. The prevalence of cancer cachexia is quite variable, affecting 15% of prostate or breast cancer patients to 50% of colorectal cancer patients, and even 70% of pancreatic cancer patients. It has been estimated that 15.8 subjects per 10 000 of the total general population in European Union suffered from cancer cachexia in 2013, while it affected approximately 16.5 individuals per 10 000 people in the USA in 2014. Skeletal muscle depletion has very strong adverse effects for cancer patient health care as it increases the risk of surgical complications, and chemotherapy toxicity, ultimately reducing cancer patient’s quality of life and contributing to mortality.

Along with skeletal muscle depletion and functional related complications, an often-overlooked aspect of cancer cachexia is its systemic nature. Although skeletal muscle plays a central role in the syndrome, cancer cachexia also involves multiple tissues and organs with important metabolic functions, including brain, adipose tissue, and liver. To provide new insights into the mechanisms involved in cancer cachexia and to understand as a whole the diversity and complexity this syndrome, we looked for circulating molecular determinants of cancer cachexia. Our attention turned out to glucocorticoids (GC), a class of steroid hormones that play important metabolic functions in multiple organs and tissues. GC are synthesized and secreted by the adrenal cortex under the control of the hypothalamic-pituitary-adrenal (HPA) axis. GC bind to GC receptor, which then binds to GC response elements in the promoters of target genes to activate or inhibit their transcription. In skeletal muscle, GC administration has long been known to decrease protein synthesis and increase protein degradation, an effect that is mediated by a transcriptional program that decreases the expression of genes promoting protein synthesis and increases those promoting protein degradation. Accordingly, skeletal muscle mass loss is abrogated in LLC-tumour bearing mice with targeted deletion of GC receptor in skeletal muscle. Another target metabolic tissue of GC is the liver. Molecular studies have indicated that GC stimulated the transcription of genes encoding gluconeogenic enzymes to increase glucose production. According to clinical studies show that hepatic glucose metabolism is markedly impaired in cancer patients as exemplified by decreased hepatic glycogen store and heightened hepatic gluconeogenesis from lactate, glycerol or alanine. Finally, an increase in circulating GC level has also been reported in cancer patients as well as in animal models of cancer. However, it remains largely unsolved whether the HPA axis is activated during cancer cachexia to coordinate GC-dependent transcriptional responses in skeletal muscle and liver.

In the present study, we show that HPA axis activation is associated with an increase in GC concentration in the serum, skeletal muscle, and liver of 23-week-old Apc mice, a mouse model of intestine and colon cancer. This response was associated with the transcription of GC-responsive genes involved in skeletal muscle proteostasis and liver metabolism.

Materials and methods

Animals

Animal experiments were conducted in accordance with the European Community guidelines and with the authorization of the Ministère français de l’Enseignement Supérieur, de la Recherche, et de l’Innovation. Mice were co-housed with one to three other mice in filter-top cages with bedding and nest building enrichment in a temperature-controlled (20–21°C) with a 12-h light/dark cycle. Animals had free access to food and water.

Apc male mice were purchased from the Jackson Laboratory. Mice showing signs of distress (sores, weight loss greater than 20%, rectal bleeding) were removed from the study. For dexamethasone treatment, C57BL/6J male mice (87.4 ± 0.7 days) were randomly allocated to three groups: vehicle-treated (PBS), dexamethasone-treated (3 mg/kg daily i.p. injection of a water-soluble form of dexamethasone for 5 days) or vehicle-treated (PBS) groups. KPC female and male mice were obtained by breeding Ptf1a-p48-Cre+ mice. Age-matched and sex-matched control mice were [WT], Ptf1a-p48-Cre+ mice. Age-matched and sex-matched control mice were [WT], Ptf1a-p48-Cre+.
mice were euthanized by cervical dislocation. Euthanized before the onset of one of these endpoints. All corresponding wild-type (WT) or control littermates became available, allocated by genotype and paired with corresponding wild-type (WT) or control littermates. Tissues from 13- and 23-week-old wild type and Apc mice, dexamethasone-treated mice, and KPC mice were removed on anaesthetized mice (90 mg/kg ketamine and 10 mg/kg xylazine) between 10 am and 12 am. Due the heterogeneity of the disease progression, KPC and their control littermates were euthanized between 3 and 6 months of age. KPC mice were categorized as severe (SEV) upon reaching at least one of the following endpoints, body weight loss (based on the kinetic analysis of body weight), adipose tissue loss, abdominal distension, and/or lethargy. Moderate (MOD KPC) were euthanized before the onset of one of these endpoints. All mice were euthanized by cervical dislocation.

**Anaesthesia and tissue removal**

Tissues from 13- and 23-week-old wild type and Apc mice, dexamethasone-treated mice, and KPC mice were removed on anaesthetized mice (90 mg/kg ketamine and 10 mg/kg xylazine) between 10 am and 12 am. Due the heterogeneity of the disease progression, KPC and their control littermates were euthanized between 3 and 6 months of age. KPC mice were categorized as severe (SEV) upon reaching at least one of the following endpoints, body weight loss (based on the kinetic analysis of body weight), adipose tissue loss, abdominal distension, and/or lethargy. Moderate (MOD KPC) were euthanized before the onset of one of these endpoints. All mice were euthanized by cervical dislocation.

**Blood analyses and serum assays**

Haematocrit, glycaemia, and lactatemia were measured during tissue collection using a Mini Compur M1100 microspin, FreeStyle Optium Neo H glucometer (Abbott Diabetes Care), and Accutrend® Plus lactate analyser (Roche Diagnostics GmbH), respectively. Sera were prepared from blood samples removed from the tail vein under isoflurane (3%) anaesthesia between 10 am and 12 am in the fed state, and used for the measurement of corticosterone (MyBiosource) and β-hydroxybutyrate (APExBIO) concentrations.

**Analysis of pancreatic lesions in KPC mice, image acquisition, and analysis**

Pancreatic tissue was fixed in 4% formalin (Electron Microscopy Science); 4 μm slides of paraffin-embedded pancreatic tumours tissue were deparaffinized and rehydrated, stained with Mayer haematoxylin and eosin, dehydrated and mounted. Images were acquired with a Zeiss Imager M2 AX10 microscope and processed using Zeiss Zen. Pancreatic neoplasm lesions were assessed and classified according to the Baltimore consensus.

**Immunofluorescence analysis of myofibre cross-sectional area, image acquisition, and analysis**

Gastrocnemius muscle sections (10 μm) were prepared (Leica CM1950 cryostat microtome) and then immuno-stained (mouse anti-laminin, Sigma-Aldrich) using an Alexa Fluor 488 goat anti-mouse secondary antibody (Thermo Fisher Scientific). Slides were mounted with antifade mounting medium with DAPI (Vector Laboratories). Images were acquired (Zeiss Axio Imager 2 Microscope) and used for the quantification of muscle cross-sectional area (ImageJ).

**In situ force measurement**

The distal tendon of the tibialis anterior muscle of anaesthetized (90 mg/kg ketamine and 10 mg/kg xylazine) 23-week-old WT and Apc mice was attached to the force-position transducer apparatus, while mice knee was anchored and the sciatic nerve was stimulated. After determination of optimum muscle length (L₀), maximum rate of force development to peak twitch and maximum rate of relaxation to the twitch were analysed (Aurora Scientific). Maximum absolute isometric tetanic force (P₀) was determined as the maximal force recorded during a stimulation of 0.5 s at 100 Hz. Maximum specific isometric tetanic force (sP₀) was calculated as the maximum absolute isometric tetanic force divided by tibialis anterior muscle weight. Muscle fatigue was determined by recording force production during a 120 s stimulation protocol at 50 Hz.

**Isolation of RNA and reverse transcription-quantitative polymerase chain reaction analysis**

RNA was extracted (NucleoSpin RNA Set for NucleoZOL, Macherey-Nagel) from adrenal glands, hypothalamus, liver, and quadriceps muscle in Apc mice, gastrocnemius muscle, liver, and hypothalamus in dexamethasone-treated mice, and liver and tibialis anterior muscle in KPC mice; 200 ng of RNA were reverse transcribed (Reverse Transcriptase Core kit, Eurogentec). qPCR was carried out on 2 μL of cDNA (Takyon ~ No Rox SYBR MasterMix dTTP Blue, Eurogentec) in a total reaction volume of 10 μL. Primer sequences are provided in Supporting information, Table S1. Fluorescence intensity was recorded using a CFX96 Real-Time PCR detection system (Bio-Rad). Data were analysed using the ΔΔCT method of analysis. Reference genes (Ppia and Hprt for quadriceps muscle, Actb and Hprt for liver and adrenal glands, Actb and Tuba1 for hypothalamus) were used for normalization.

**Protein extraction and immunoblotting, data acquisition, and analysis**

Powdered quadriceps muscle and liver were homogenized (1:20 w:v) in a RIPA buffer (Cell Signalling Technology) supplemented with protease and phosphatase inhibitors (Roche). Samples were centrifuged (10 min, 12 000 g, 4°C).
Protein concentration of supernatants was determined (DC protein assay, Bio-Rad); 30 μg of protein in Laemmli buffer (Bio-Rad) were subjected to 8–16% gel electrophoresis (Bio-Rad) and transferred onto nitrocellulose membrane (Trans-Blot Turbo Transfer System, Bio-Rad). Membranes were then blocked with a Tris Buffered Saline-0.1% Tween 20 solution containing 5% non-fat dried milk for 2 h at room temperature and incubated overnight at 4°C with primary antibody against rpS6ser235/236 (1:1000), DDIT4 (1:1000), 4EBP1 (1:1000), PGClA (1:1000), CRTC2ser171 (1:1000), PKC1 (1:500), CREBser133 (1:1000) and CREB (1:1000), followed by probing with HRP-conjugated secondary antibody (Agilent, 1:2000). Membranes were incubated with ECL (Bio-Rad) and imaged (ChemiDoc MP Imaging System, Bio-Rad). The protein band intensity was determined using Image Lab™ 6.0. Stain-free technology (Bio-Rad) was used for protein normalization.

**Skeletal muscle and liver corticosterone content**

A solid-phase enzyme-linked immunosorbent assay (MyBiosource, San Diego, USA) was used for the measurement of corticosterone content in the *quadriceps* muscle and liver protein extracts of Apc mice.

**Statistical analysis**

Data are expressed as means ± SEM. Statistical analyses were performed using GraphPad Prism 9.0. Grubb’s test was performed to identify outliers. Data were tested for normal distribution using Shapiro–Wilk test. Mann–Whitney or two-tailed unpaired t-test with Welch’s correction if necessary, one-way ANOVA followed by Tukey’s multiple comparison test, Kruskal–Wallis test followed by Dunn’s multiple comparison test, and two-way ANOVA followed by Sidak’s multiple comparison test were used. Pearson correlation coefficient and log-rank (Mantel–Cox) test were also used. Gene expression overlapping significance, odds ratio, and Jaccard index were calculated with Fisher’s exact test using GeneOverlap package (http://shenlab-sinai.github.io/shenlab-sinai/) in R (version 3.6.3). Statistical significance was considered when \( P < 0.05 \).

**Results**

**Apc mice recapitulate the main features of cancer cachexia**

To investigate the mechanisms driving cancer cachexia, we used the Apc mouse, a mouse model of intestinal and colon cancer that harbours a heterozygous mutation in the adenomatous polyposis coli (APC) gene.28 Mutations in the APC gene activate the β-catenin-Tcf signalling and are responsible for familial adenomatous polyposis, an inherited cancer predisposition syndrome.30 The APC gene is mutated in more than half of colorectal cancer patients30 and the risk of colorectal cancer by the age of 40 is almost 100% in patients.30 Here, Apc mice experienced a progressive loss in body mass that began 15 weeks after birth, reaching −16% of their peak body mass at the age of 23 weeks (Figure 1A). Apc mice fed normally (Figure S1A; Data S1) and had similar tibia length (Figure S1B; Data S1) than WT age-matched littermates, indicating that the lower body mass of Apc mice was not due to a slower growth. Apc mice developed polyposis (Figure 1B), as well as anaemia (Figure 1C). The median survival of Apc mice was 26 weeks (Figure 1D).

Marked reductions in adipose tissue store (Figure S1C; Data S1) and skeletal muscle compartment were also observed. Cachexia in Apc mice differently affected skeletal muscles with mixed contractile and metabolic phenotypes, such as *quadriceps* (−24% at 13 weeks and −52% at 23 weeks) and *gastrocnemius* (−20% at 13 weeks and −53% at 23 weeks) muscles (Figure 1E), and those with a predominance of fast-twitch glycolytic fibres such as *extensor digitorum longus* (−24% at 13 weeks and −52% at 23 weeks) and *tibialis anterior* (−19% at 13 weeks and −49% at 23 weeks) muscles (Figure S1D; Data S1). *Soleus* muscle, a slow-twitch oxidative postural muscle, which has not developed atrophy yet at 13 weeks, became atrophic at 23 weeks (Figure S1D; Data S1). The reduction in *gastrocnemius* muscle mass was associated with a progressive decrease in muscle fibre cross-sectional area (Figure 1F). Finally, skeletal muscle depletion was also correlated with body mass loss (Figure S1E; Data S1).

Functionally, the absolute maximal isometric tetanic force of *tibialis anterior* muscle (\( P_0 \)) was significantly reduced in 23-week-old Apc mice compared with WT mice (Figure 1G). Specific muscle force (\( sP_0 \)) was not different (Figures 1G and S1F, Data S1), indicating that the loss in skeletal muscle mass strongly contributed to the loss in force. The maximum rates of force development and relaxation were also reduced during a single twitch response (Figure S1G; Data S1). Finally, muscle fatigue was also increased as shown by the faster and greater decrease in muscle force during a fatigue protocol (Figure 1H).

Molecularly, the mRNA levels of Trim63 (MuRF1) and Fbxo32 (MAFbx) encoding E3-ubiquitin ligases involved in ubiquitin-proteasome dependent proteolysis41 were increased in the *quadriceps* muscle of 23-week-old Apc mice compared with WT mice (Figure 1I). This was associated with an increase in the protein content of the translational repressors, DDIT442 and 4EBP143 (Figure 1J). The fast migrating hypo-phosphorylated active form of 4EBP1 was also increased (Figure 1J). Finally, the active phosphorylated form of ribosomal protein S6 was decreased (Figure 1J). Together, these data support the existence of an unbalance between

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Figure 1  Apc mice recapitulate the main features of cancer cachexia. (A) Relative changes in body mass in WT (n = 9) and Apc (n = 24 at 5 weeks) mice from 5 to 23 weeks after birth. (B) Polyp number in small intestine and colon of WT and Apc mice at 13 (WT n = 11; Apc n = 10) and 23 (WT n = 10; Apc n = 6) weeks. (C) Haematocrit in WT and Apc mice at 13 (WT n = 11; Apc n = 10) and 23 (WT n = 10; Apc n = 8) weeks. (D) Kaplan–Meier survival curve of WT (n = 31) and Apc (n = 31) mice indicates that half-life survival of Apc mice was 26 weeks. (E) Quadriceps (left) and gastrocnemius (right) muscle mass of WT and Apc mice at 13 (WT n = 11; Apc n = 10) and 23 (WT n = 12; Apc n = 8) weeks. (F) Representative laminin immunostaining (left) of gastrocnemius muscle transverse sections in WT and Apc mice at 13 and 23 weeks. Scale bar, 100 µm. Frequency distribution of gastrocnemius muscle fibre cross-sectional area (centre) in WT and Apc mice at 13 (WT n = 7; Apc n = 6) and 23 (WT n = 5; Apc n = 4) weeks. A mean of 540 ± 247 fibres per muscle were counted. (G) Absolute maximal isometric tetanic force (P0, left) of tibialis anterior muscle recorded in 23-week-old WT (n = 14) and Apc (n = 8) mice. Specific maximal isometric tetanic force (ς0, right) of tibialis anterior muscle recorded at 100 Hz in 23-week-old WT (n = 14) and Apc (n = 8) mice. (H) Tibialis anterior muscle force during a 120 s fatigue protocol at 50 Hz in 23-week-old WT (n = 12) and Apc (n = 8) mice. (I) RT-qPCR analysis of Murf1 and Mafbx mRNA levels in the quadriceps muscle of WT and Apc mice at 13 (WT n = 11; Apc n = 9) and 23 (WT n = 12; Apc n = 7) weeks. (J) Representative immunoblot (left) of phosphorylated ribosomal protein S6 (rpS6Ser235/236), DDIT4 protein, and 4EBP1 protein content in the quadriceps muscle of WT and Apc mice at 13 and 23 weeks. The hypophosphorylated active form of 4EBP1 migrates faster than the hyperphosphorylated inactive form. Quantitative analysis (right) of rpS6Ser235/236 and 4EBP1 protein content in the quadriceps muscle of WT and Apc mice at 13 (WT n = 8; Apc n = 8) and 23 (WT n = 8; Apc n = 9) weeks. 4EBP1 protein level was not quantified as expression level in WT mice was barely detectable. Data are means ± SEM. (A, B, C, E, F right, H, I) Data were analysed by a two-way ANOVA with Sidak’s multiple comparison test. (D) Data were analysed by a log-rank (Mantel–Cox) test. (G, J) Data were analysed by a two-tailed unpaired t-test with Welch’s correction when necessary. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

Transcriptional rewiring of hepatic metabolism in 23-week-old Apc mice

Hepatic gluconeogenesis is increased in cancer patients,15–17 suggesting that molecular adaptations should occur to sustain hepatic glucose production. In the present study, transcript levels of key gluconeogenic enzymes, Pcx and Pck1, were increased in 23-week-old cachetic mice (Figure 2A and 2C). The increased expression of Pck1 was also confirmed at the protein level (Figure 2D). Conversely, the mRNA levels of Mixkpi (ChREBP), a transcription factor that drives the expression of glycolytic genes such as Gk and Pklr, as well as that of the glucose transporter Sclc2a2,34 were decreased in 23-week-old Apc mice (Figure 2B and 2C). Transcript level of Gpd2, a component of the glycerol phosphate shuttle that boots glucose oxidation,35 was also down-regulated (Figure 2B and 2C). Expression of gluconeogenic genes can be activated in response to glucagon by stimulation of CREB transcription factor.14 Here, the active phosphorylated form of CREB was decreased in the liver of Apc mice and phosphorylation of the CREB-regulated transcription coactivator 2 (CRT2C) remained unchanged (Figure 2D), suggesting that this mechanism was not activated. The expression of gluconeogenic genes can be also activated by the coordinate interplay of the transcriptional co-activator PPARGC1A (PGC1A) and FOXO1 transcription factor.36 The mRNA levels of Pgc1a and Foxo1, but also that of Foxo3, were increased in the liver of 23-week-old Apc mice (Figure 2A). The increased expression of Pgc1a was also confirmed at the protein level (Figure 2D), suggesting an activation of a PGC1A/FOXO1 axis in cachectic mice. These adaptations in 23-week-old Apc mice were accompanied by a decrease in blood glucose concentration and an increase in blood lactate concentration (Figure 2E).

Transcriptional rewiring of hepatic metabolism was not limited to glucose metabolism. Ketone bodies, which are produced by the liver from acetyl-CoA during excessive fatty acid oxidation, become important substrates for energy production by the brain, kidneys, and skeletal muscle when others substrates are missing or less used. Serum concentration of β-hydroxybutyrate, the most prevalent circulating ketone body, was decreased in 23-week-old Apc mice (Figure 3A). Accordingly, the transcript level of Ppara, a master transcriptional regulator of fatty acid oxidation and ketogenic gene expression,37,38 was down-regulated in 23-week-old Apc mice, as well as the transcript level of ketogenesis rate-limiting enzymes (Hmgcs2 and Bdh1) and fatty acid oxidation genes (Figure 3B). Therefore, 23-week-old Apc mice display a hypo-ketonemic phenotype associated with a reduction in the expression of hepatic fatty acid oxidation genes, consistent with the reduction in PPARα gene expression. Expression of genes encoding lipolytic (Lipe, Mglil) and lipogenesis (Acyl, Acacb, Fasn, Scd1) proteins were also down-regulated (Figure 3C–3D). Finally, disturbed expression of transcripts encoding urea cycle enzymes (Cps1, Otc, Ass1, Asl) was also observed (Figure 3E). Collectively, these data indicate that the liver of Apc mice experience a major transcriptional rewiring that affects glucose, ketone body, lipid, and nitrogen metabolism.

The HPA axis is activated in 23-week-old Apc mice

The secretion of GC is regulated by the HPA axis. Hypothalamic mRNA level of corticotropin-releasing hormone (CRH) that activates the pituitary secretion of adrenocorticotropic hormone (ACTH) was increased in 23-week-old Apc mice (Figure 4A). Accordingly, hypothalamic expression of Fkbb5,
(A) mRNA level fold change vs WT 13 wk

(B) mRNA level fold change vs WT 13 wk

(C) Glucose → G6pc → G3-P → DHAP → GAP → F-6-P → F-1,6-BP → PFK1 → FBP1 → G-6-P → Glucose

(D) Protein level (fold change vs corresponding WT)

(E) Glycemia and Lactatemia
Figure 2 Transcriptional rewiring of gluconeogenic and glycolytic gene expression in Apc mice. (A) Transcript level of gluconeogenic genes in liver of WT and Apc mice at 13 (WT n = 11; Apc n = 10) and 23 (WT n = 12; Apc n = 7) weeks. (B) Transcript level of glycolytic genes in liver of WT and Apc mice at 13 (WT n = 11; Apc n = 10) and 23 (WT n = 12; Apc n = 7) weeks. (C) Schematic view of glycolysis and gluconeogenesis. Decreased, unchanged, or increased transcript levels are indicated in blue, grey, and red, respectively. (D) Representative immunoblot (left) and quantitative analysis (right) of PGClαA, phosphonylated CRTC2 (CRTC2Ser121), PCK1, phosphonylated CREB (CREBSer133), and total CREB protein content in the quadriceps muscle of WT and Apc mice at 13 (WT n = 8; Apc n = 8) and 23 (WT n = 8; Apc n = 8) weeks. (E) Blood glucose concentration (left) in WT and Apc mice at 13 (WT n = 12; Apc n = 12) and 23 (WT n = 12; Apc n = 12) weeks. Blood lactate concentration (right) in WT and Apc mice at 13 (WT n = 11; Apc n = 11) and 23 (WT n = 11; Apc n = 11) weeks. Data are means ± SEM. (A, B, E) Data were analysed by two-way ANOVA with Sidák’s multiple comparison test. § indicates a main genotype effect. (D) Data were analysed by a two-tailed unpaired t-test with Welch’s correction when necessary. * or $P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. DHAP, dihydroxyacetone phosphate; F-1,6-BP, fructose-1,6-bisphosphate; F-6-P, fructose-6-phosphate; G-3-P, glycerol-3-phosphate; G-6-P, glucose-6-phosphate; GAP, glycolaldehyde-3-phosphate; OAA, oxaloacetic acid; PEP, phosphoenolpyruvate.

indictative of the hyperactive function of the hypothalamus in response to GC injection, was also increased (Figure 4A). ACTH stimulates the release of GC by adrenal glands. mRNA levels of ACTH receptor (Mc2y), as well as those of Cyp21a1 and Cyp11b1, which encode enzymes involved in adrenal corticosterone biosynthesis, were increased in 23-week-old Apc mice (Figure 4B). Adrenomegaly was also evident (Figure 4C), further supporting a hyperactivation of adrenal glands. Accordingly, the circulating level of corticosterone, the predominant GC in mouse, was higher in Apc mice than in WT littermates (Figure 4D). Hepatic transcript levels encoding steroid detoxifying enzymes, such as Papss2, Srd5a1, Ugt2b35, and Ugt2b36, were also significantly decreased in 23-week-old Apc mice compared with WT mice (Figure 4E–4F). Finally, in agreement with the hypogonadotropic hypogonadism found in male Cushing’s syndrome, testis mass was also significantly decreased in Apc mice (Figure 4G). Together, these data indicate that an activation of the HPA axis and a lower capacity of hepatic GC detoxification increase circulating corticosterone level in 23-week-old Apc mice.

The expression of GC-responsive genes is regulated in skeletal muscle and liver of 23-week-old Apc mice

Prompted by the finding of HPA axis activation, we pursued our investigations to determine whether expression of GC-responsive genes is regulated in skeletal muscle and liver of Apc mice. Although the transcript level encoding GC receptor (Nsr3C2) remained unchanged, mRNA level encoding HSD11B1, a dehydrogenase that converts the inactive 11-dehydrocorticosterone to the active corticosterone was increased in the quadriceps muscle of 23-week-old Apc mice (Figure 5A), suggesting an increased conversion of inactive 11-dehydrocorticosterone into active corticosterone. Accordingly, corticosterone content was increased in the quadriceps muscle of 23-week-old Apc mice (Figure 5B). Muscle corticosterone level also negatively correlated with body mass (Figure 5C). Previous studies indicated that administration of dexamethasone, a synthetic GC, triggers a typical transcriptional response in skeletal muscle characterized by an increase in the transcript level of Bnip3, Ddit4, Fkbp5, Foxo1, Foxo3, Klf15, Lc3b, Mucf2, Mafbx, Mafb1, Mc2r, Mt1, Mt2, Mucf1, and Slc39a14 (Figure 5D and Table S2). Most importantly, the quadriceps muscle of 23-week-old Apc mice also displayed an increase in the expression of these GC-responsive genes, as evidenced by the marked and significant increases in the transcript level of Bnip3, Cebpd, Ddit4, Fkbp5, Foxo1, Foxo3, Klf15, MAFbx, Mt1, Mt2, Mucf1, and Slc39a14 (Figure 5D and Table S2). Out of 15 genes that were either up-regulated or down-regulated in dexamethasone-treated mice, 13 behaved similarly in 23-week-old Apc mice. Overall, the overlap of the transcriptional signature in skeletal muscle of dexamethasone-treated and 23-week-old Apc mice was significant (Figure 5E). Importantly, the GC-dependent transcriptional response was partially shared by skeletal muscle of 13-week-old Apc mice.

We next hypothesized that if GC are systemic regulators of cancer cachexia, hepatic metabolic rewiring observed in 23-week-old Apc mice should sign a GC action and should be therefore reproduced in the liver of dexamethasone-treated mice. First, liver corticosterone content was increased in 23-week-old Apc mice compared with age-matched WT mice (Figure 5F). Expression levels of genes involved in glucose, nitrogen, ketone body, and lipid metabolisms described in Apc mice were then compared with those of dexamethasone-treated mice. We also included genes that were identified as GC-responsive (Cebpd, Ddit4, Fkbp5, Klf15, Mt1, Mt2, and Slc39a14) and non-responsive (Foxo4) in skeletal muscle. Nineteen out of 28 genes that were differentially expressed in the liver of 23-week-old Apc mice were similarly regulated in dexamethasone-treated mice.

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Figure 3  F Transcriptional rewiring of ketogenic, lipid, and nitrogen metabolisms in \( Apc \) mice. (A) Serum \( \beta \)-hydroxybutyrate concentration in WT and \( Apc \) mice at 13 (WT \( n = 10; Apc \ n = 13 \)) and 23 (WT \( n = 9; Apc \ n = 11 \)) weeks. (B) Transcript level of ketogenic genes and fatty acid oxidation genes (left) in the liver of WT and \( Apc \) mice at 13 (WT \( n = 11; Apc \ n = 10 \)) and 23 (WT \( n = 12; Apc \ n = 7 \)) weeks. Schematic view of ketogenesis in the liver (right). Decreased and unchanged transcript levels are indicated in blue and grey, respectively. (C) Transcript level of lipolytic genes (left) in the liver of WT and \( Apc \) mice at 13 (WT \( n = 11; Apc \ n = 10 \)) and 23 (WT \( n = 12; Apc \ n = 7 \)) weeks. Schematic representation of triglyceride hydrolysis (right). Decreased or unchanged transcript levels are indicated in blue and grey, respectively. (D) Transcript level of lipogenic genes (left) in the liver of WT and \( Apc \) mice at 13 (WT \( n = 11; Apc \ n = 10 \)) and 23 (WT \( n = 12; Apc \ n = 7 \)) weeks. Schematic view of lipogenesis (right). Decreased and increased transcript levels are indicated in blue and red, respectively. (E) Transcript level of genes encoding urea cycle enzymes (left) in liver and WT and \( Apc \) mice at 13 (WT \( n = 11; Apc \ n = 10 \)) and 23 (WT \( n = 12; Apc \ n = 7 \)) weeks. Schematic view of the urea cycle (right). Decreased, unchanged, and increased transcript levels are indicated in blue, grey, and red, respectively. Data are means ± SEM. (A, B, C, D, E) Data were analysed by two-way ANOVA with Sidak’s multiple comparison test. § indicates a main genotype effect. (B) Data were analysed by a two-tailed unpaired t-test with Welch’s correction when necessary. * or \( \#P < 0.05, ** or \#\#P < 0.01, \#\#\#P < 0.001, \) and \#\#\#\#P < 0.0001. AA, acetoacetate; AA-CoA, acetoacetyl-CoA; Arg, arginine; asp, aspartate; BHB, beta-hydroxybutyrate; CP, carbamoyl phosphate; DG, diglyceride; FA, fatty acid; HMG-CoA, beta-hydroxy beta-methylglutaryl-CoA; MG, monoglyceride; MUFA, monounsaturated fatty acid; OAA, oxaloacetic acid; TG, triglyceride.

(\textit{Figure 5G} and Table S3). The overlap between transcriptional profiles was significant both for up-regulated and down-regulated transcripts (\textit{Figure 5H}). Of note, only seven genes were similarly expressed in 13-week-old \( Apc \) mice and dexamethasone-treated mice (\textit{Figure 5G}), suggesting that the transcriptional regulation of GC-responsive genes in the liver progressively takes place during the progression through the disease.

The expression of GC-responsive genes is regulated in other animal models of cancer

We next wanted to further establish and strengthen the biological relevance of our observations in other models of cancer cachexia. Lewis lung carcinoma (LLC) tumour-bearing mice, a fast tumour burden model of cancer,\(^{46}\) were first used (Data S1). Thirty-five days after LLC cell inoculation, tumour-bearing mice displayed pronounced weight loss (\textit{Figure S2A}; Data S1) and marked decrease in skeletal muscle mass (\textit{Figure S2B}; Data S1). The expression pattern of GC-responsive genes in the \textit{gastrocnemius} muscle of LLC-tumour bearing mice was similar to the one reported in 23-week-old \( Apc \) mice, as shown by the increased expression of \( 4ebp1, Cebp \), \( Ddit4, Fkbp5, Fxo1, Mafbx, MuRF1, Mt1, Mt2, \) and \( Slc39a14 \) (\textit{Figure S2C}).

The expression of GC-responsive genes was also investigated in the established \( KPC \) mouse model of pancreatic ductal adenocarcinoma (PDAC).\(^{29}\) As \( KPC \) mice showed heterogeneity in disease progression and mortality, mice were stratified as moderate (MOD) and severe (SEV) \( KPC \) animals. SEV \( KPC \) mice were euthanized at age ranging from 98 to 198 days (\textit{Figure 6A}) upon reaching at least one of the following endpoints, body weight loss (based on the kinetic analysis of body weight), adipose tissue loss, abdominal distension, and/or lethargy. MOD \( KPC \) mice were euthanized before the onset of one of these cachetic features at age ranging from 96 to 146 days (\textit{Figure 6A}). Despite heterogeneity in body weight at analysis partly due to the presence of large pancreatic tumours in some SEV \( KPC \) mice, body weight was significantly decreased in SEV \( KPC \) mice compared with control mice (\textit{Figure 6B}). MOD and SEV \( KPC \) mice develop pancreatic lesions ranging from pancreatic intraepithelial neoplasia (PanIN) to PDAC (\textit{Figure 6C}). No difference in the relative occurrence of PanIN and PDAC was observed between MOD and SEV \( KPC \) mice, indicating that the severity of the pancreatic lesions was not necessarily associated with the severity of the disease. SEV \( KPC \) mice exhibited lower masses of the \textit{tibialis anterior} and \textit{gastrocnemius} skeletal muscles compared with control mice (\textit{Figure 6D}). Consistent with the data obtained in 23-week-old \( Apc \) mice, a significant increase in the transcript level of \( Cebp \), \( Ddit4, Fkbp5, Fxo1, Fxo3, Klf15, Mafbx, Mstn, Mt1, Mt2, MuRF1, \) and \( Slc39a14 \) (\textit{Figure 6E}) was also observed in the \textit{tibialis anterior} muscle of SEV \( KPC \) mice. Importantly, this was not observed in MOD \( KPC \) mice, confirming that the signature was associated with the severity of the disease. In the liver, and with the notable exception of the transcripts of genes encoding gluconeogenic proteins whose expression remained unchanged (\textit{Pcx, Pck1, Fbp2}) or even decreased (\textit{G6pc}), the expression profiles of GC-responsive genes (\textit{Ceabp, Ddit4, Fkbp5, Mt1, Mt2, Slc39a14}) (\textit{Figure 6F}), as well as that of glycolytic (\textit{Mxpl, G6pd2, Pklr}), ketogenic (\textit{Hmgcs2, Bh4l}), and fatty acid synthesis (\textit{Acly, Fasn}) genes (\textit{Figure 6G}) were similar in SEV \( KPC \) mice and 23-week-old \( Apc \) mice. This response was not observed in MOD \( KPC \) mice.

Discussion

In the present study, we demonstrate a unique phenotype in 23-week-old \( Apc \) mice characterized by an activation of the HPA axis, a lower potential for the clearance of GC by the liver, and elevated GC levels in the serum, skeletal muscle, and liver. Using molecular, biological, and biochemical assays, we show that HPA axis activation was associated with a transcriptional response of GC-responsive genes involved in skeletal muscle catabolism and hepatic metabolism.

\cite{10.1002/jcsm.12939}
**Diagram A**

- mRNA level (fold change vs WT 23 wk)
  - WT
  - Apc
  - Cth
  - Fkbp5

**Diagram B**

- mRNA level (fold change vs WT 23 wk)
  - WT
  - Apc
  - Mc2r
  - Cyp21a1
  - Cyp11b1

**Diagram C**

- Adrenal gland mass (mg)
  - WT
  - Apc

**Diagram D**

- Serum corticosterone concentration (nmol.L⁻¹)
  - WT
  - Apc
  - 13 wk
  - 23 wk

**Diagram E**

- mRNA level (fold change vs WT 13 wk)
  - Akr1D1
  - Papss2
  - Srd5a1
  - Ugt2b35
  - Ugt2b36

**Diagram F**

- SRD5A1, SRD5A2 (5α-reductase)
- Corticosterone
- 5α-dihydrocorticosterone
- Akr1D1 (5β-reductase)
- 5β-dihydrocorticosterone
- 3α,5α-tetrahydrocorticosterone
- 3α,5β-tetrahydrocorticosterone
- Ugt2b35, Ugt2b36 (UDP-glucuronyl transferase)
- Glucuronidated steroids
- Sulfate ester of steroids
- Bile, urine

**Diagram G**

- Testis mass (mg)
  - WT
  - Apc
The hepatic expression of glucose metabolism-related genes in 23-week-old Apc mice was rearranged in the direction of increased gluconeogenesis and reduced glycolysis, what with previous studies showing marked depletion in liver glycogen store in cancer cachectic mice.\textsuperscript{27,47,48} indicate a greater reliance on hepatic glucose production for gluconeogenesis. Collectively, with the changes in glycaemia and lactatemia, these data are consistent with an increased systemic demand for glucose. Amino acids coming from skeletal muscle catabolism and the lactate produced by tumour metabolism can be used as substrates for hepatic gluconeogenesis to produce glucose for tumour growth.\textsuperscript{4} Supporting this hypothesis, increased expression of glucose transporter 1\textsuperscript{49} and increased lactate content\textsuperscript{50} has been reported in the tumour of cancer mice, suggesting active glucose uptake and increased lactate production by the tumour. As the cost of partial glucose oxidation into lactate by glycolysis (2 ATP) is less than the cost of glucose synthesis by gluconeogenesis (6 ATP), this metabolic cycle could contribute to energy loss in cachectic mice. One may also consider metabolic cycles between skeletal muscle and tumour, or between skeletal muscle and liver, whereby lactate produced by skeletal muscle from glucose could be used by the tumour (lactate oxidation for tumour growth) or by the liver for gluconeogenesis. However, data indicate a reduction in the whole energy metabolism of cachectic skeletal muscle,\textsuperscript{47,50–52} suggesting that these metabolic cycles would be less relevant in cancer mouse.

As previously described,\textsuperscript{27} cachetic cancer mice displayed a hypo-ketogenic phenotype characterized by a decrease in β-hydroxybutyrate circulating level. Production of ketone bodies increases when acetyl-CoA production by fatty acid oxidation exceeds the capacity of citrate to be incorporated into the tricarboxylic acid cycle. Here, the complete exhaustion of adipose tissue store and the decreased expression of fatty oxidation genes (Cpt1, Hadh1), Ppara, and PPARA ketogenic target genes (Hmgcs2, Bdh1) in the liver of 23-week-old Apc mice agree with the hypo-ketonomic phenotype. Conversely, one may expect that ketone body production has transiently increased during the time course of adipose tissue store depletion earlier during the development of cachexia. Accordingly, increased ketogenesis has been reported during the time course of the disease in C26 tumour-bearing mice,\textsuperscript{48} a fast tumour growth model. One may also question the fate of ketogenic amino acids produced by skeletal muscle catabolism. However, as most of the ketogenic amino acids (phenylalanine, isoleucine, threonine, tryptophan, and tyrosine) are also gluconeogenic, our data suggest that amino acids coming from skeletal muscle would be mainly used to sustain liver gluconeogenesis. In support of this assumption, an increase in the expression of many genes involved in the conversion of amino acids into pyruvate has been reported in the liver of cachectic cancer mice.\textsuperscript{27} Therefore, hypo-ketonaemia would be a hallmark of an advanced cachectic state characterized by depleted adipose tissue store. Our findings complement previous works,\textsuperscript{27} showing that fenofibrate, a PPARA agonist, restores β-hydroxybutyrate circulating level and prevents cancer cachexia in tumour-bearing mice, and that ketone bodies attenuate skeletal muscle atrophy in cachectic mice.\textsuperscript{53} Maintaining ketone production could be thus favourable to limit the progression of cancer cachexia.

Our findings demonstrate that HPA axis activation in 23-week-old Apc mice was associated with a decrease in the transcript level of liver GC detoxifying enzymes, an increase in corticosterone content (serum, skeletal muscle, and liver), and the induction of a typical transcriptional response in skeletal muscle and liver that was recapitulated by dexamethasone injection in WT mice. In skeletal muscle, this signature was more specifically associated with the regulation of GC metabolism (Cebpδ, Fkbp5, Klf15), the activation of metal cation transport and binding (Slc39a14, Mt1, Mt2), and the negative regulation of proteostasis (Bnip3, Ddit4, Foxo1, Foxo3, Lc3b, MuRF1, 4ebp1). We also showed that this transcriptional signature was shared by other models of cancer cachexia (LLC tumour-bearing mice and KPC mice). Finally, the analysis of published transcriptomic lists of differentially expressed genes indicate that the transcriptional signature of GC-responsive genes in skeletal muscle was also largely shared by multiple animal models of cancer cachexia (Figure S3; Data S1), further establishing the relevance of our observations. Although not investigated in the present study, GC may also exert indirect effects on skeletal muscle proteostasis by modulating testosterone biosynthesis. Indeed, GC are known to inhibit testosterone biosynthesis by Leydig cells,\textsuperscript{54} and circulating testosterone level is decreased in Apc mice.\textsuperscript{55}
Overlapping p-value = 0.037
Odds ratio = 15.2
Jaccard Index = 0.8

1312

(A)

(B)

(C)

(D)

(E)

(F)

(G)

Up-regulated genes

Down-regulated genes

Overlapping p-value = 7.1x10^{-5}
Odds ratio = 34.1
Jaccard Index = 0.6

Overlapping p-value = 5.5x10^{-5}
Odds ratio = 9.8
Jaccard Index = 0.4

(Apc 13wk) WT-DEX

(Apc 23wk) WT-DEX

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together with a decrease in gonad size\textsuperscript{55} (also observed in the present study). GC may thus indirectly contribute to muscle loss by decreasing testosterone production/secretion and its subsequent anabolic action. In the liver, GC-responsive genes were involved in glycolysis and gluconeogenesis (\textit{Foxo1}, \textit{Gpd2}, \textit{Mxl1}, \textit{Pck1}, \textit{Pcx}, \textit{Pgc2a1}, \textit{Slc2a2}, \textit{Zk}), ketogenesis (\textit{Bdh1}), lipid metabolism (\textit{Acly}, \textit{Fasn}, \textit{Hadh}, \textit{Lpe}, \textit{Pnpla2}), GC metabolism (\textit{Cebp}, \textit{Fkbp5}), and metal cation transport and binding (\textit{Slc39a1}, \textit{Mti1}, \textit{Mti2}). Some transcripts, such as \textit{Arg1}, \textit{Asl}, \textit{Ass1}, and \textit{Cps1} encoding urea cycle enzymes, were not coordinately regulated between 23-week-old \textit{Apc} mice and dexamethasone-treated mice, indicating that GC-independent mechanisms also transcriptionally regulated liver metabolism.

This transcriptional signature was also shared by SEV \textit{KPC} mice, with the exception of genes encoding gluconeogenic proteins. The non-responsiveness of gluconeogenic genes could be explained by the selective erosion of \textit{\beta}-cells (the endocrine cells that produce insulin) that occur in \textit{KPC} mice, whereas the pool of alpha-cells (the endocrine cells that produce glucagon) appear to be relatively preserved.\textsuperscript{56} This is expected to create an imbalance between the production of insulin and glucagon, which should conceivably alter the regulation of hepatic glucose metabolism in this mouse model. Overall, our data strongly support a role for GC in targeting transcriptional responses in skeletal muscle and liver of cachectic \textit{Apc} mice. Importantly, animal models of cancer cachexia have been shown to only partially recapitulate the phenotype of human cancers.\textsuperscript{57} Therefore, a challenging aspect will be then to translate these advances to the clinic and determine whether this response occurs in human cancer patients, and if so, for what type of cancers and at what stage of the disease.

HPA axis activation and increased GC level may have potential impact on the development of insulin resistance, a condition that is frequently observed in human cancer patients and in animal models of cancer cachexia.\textsuperscript{58} Physiological doses of GC have thus been shown to inhibit the insulin signal through genomic and non-genomic effects,\textsuperscript{59,60} suggesting that HPA axis activation in \textit{Apc} mice may also contribute to insulin resistance. Corollary, GC-induced impaired insulin signalling may also contribute to increase protein degradation and stimulate muscle wasting.\textsuperscript{61} Characterizing the mechanisms that contribute to the regulation of insulin pathway in relation to the HPA axis will help to better understand systemic metabolic issues of cancer cachexia, but also to delineate the genomic and non-genomic effects of GC during cancer cachexia.

One may also question the existence of a gender effect in the regulation of HPA axis in \textit{Apc} mice. Female mice have been described to produce a more robust neuroendocrine response to acute stress, evidenced by higher ACTH and corticosterone levels compared with males.\textsuperscript{62} Whether or not this can contribute to a gender difference in the sensitivity to cancer cachexia is currently unknown, but clearly needs to be investigated.

Our data raise an important question: How is the HPA axis regulated to control the transcription of GC-responsive genes during cancer cachexia? Previous studies indicated that intracerebroventricular injection of IL-1\textbeta activated the HPA axis, increased the circulating corticosterone concentration, triggered the expression of GC-responsive genes in skeletal muscle, and skeletal muscle atrophy.\textsuperscript{63} Accordingly, a hypothalamic inflammation has also been consistently reported in animal models of cancer cachexia,\textsuperscript{64–67} together with the invasion of circulating immune cells\textsuperscript{68,69} and astrogliosis,\textsuperscript{69} which also testify of hypothalamic inflammation. Although the existence of central inflammation in cancer cachectic patients is currently unknown, a chronic systemic inflammation state has also been associated with skeletal muscle mass loss in human cancer patients,\textsuperscript{70} and the intravenous injection of cytokines in cancer patients increases circulating cortisol level by activating the HPA axis.\textsuperscript{71,72} Tumour-induced production of cytokines, together with the host immune response to tumour growth, can be thus centrally sensed and relayed within the hypothalamus to drive the activation of the HPA axis and increase the circulating level of GC during disease.

\textbf{Figure 5} The expression of GC-responsive genes is regulated in the \textit{quadriceps} muscle and liver of \textit{Apc} mice during cancer cachexia. (A) RT-qPCR analysis of \textit{Hsd11b1} and \textit{Nr3c2} mRNA levels in the \textit{quadriceps} muscle of WT and \textit{Apc} mice at 13 (\textit{WT n = 11; Apc n = 10}) and 23 (\textit{WT n = 12; Apc n = 7}) weeks. (B) Corticosterone level in the \textit{quadriceps} muscle of WT and \textit{Apc} mice at 13 (\textit{WT n = 11; Apc n = 10}) and 23 (\textit{WT n = 11; Apc n = 7}) weeks (left). (C) Pearson correlation between \textit{quadriceps} muscle corticosterone level and body mass in WT and \textit{Apc} mice at 13 (\textit{WT n = 11; Apc n = 10}) and 23 (\textit{WT n = 11; Apc n = 7}) weeks. (D) Heatmap showing a GC-responsive transcriptional signature in the \textit{quadriceps} muscle of 13-week-old (\textit{n = 10}) and 23-week-old (\textit{n = 7}) \textit{Apc} mice, and dexamethasone-treated (\textit{WT-DEX n = 10}) mice. Data were obtained by RT-qPCR analysis and normalized to corresponding WT mice (13-week-old WT mice \textit{n = 11}; 23-week-old WT mice \textit{n = 12}, and vehicle-treated WT mice \textit{n = 10}). For Log\textsubscript{2}(fold change) > 5, cells are set by default in the most intense red. (E) Venn diagram of differentially expressed genes in the \textit{quadriceps} muscle of 23-week-old \textit{Apc} and WT-DEX mice. (F) Corticosterone level in the liver of WT and \textit{Apc} mice at 13 (\textit{WT n = 11; Apc n = 10}) and 23 (\textit{WT n = 12; Apc n = 7}) weeks. (G) Heatmap displaying a GC-responsive transcriptional signature in the liver of 13-week-old (\textit{n = 10}) and 23-week-old (\textit{n = 7}) \textit{Apc} mice, and dexamethasone-treated WT mice (\textit{WT-DEX n = 10}). Data were obtained by RT-qPCR analysis and normalized to corresponding WT mice (13-week-old WT mice \textit{n = 11}; 23-week-old WT mice \textit{n = 12}, and vehicle-treated mice \textit{n = 10}). For Log\textsubscript{2}(fold change) > 6, cells are set by default in the most intense red. (H) Venn diagram of up-regulated (left) and down-regulated (right) genes in the liver of 23-week-old \textit{Apc} and WT-DEX mice. Data are means ± SEM. (A, B, F) Data were analysed by two-way ANOVA with Sidak’s multiple comparison test. (C) Data were analysed with a Pearson correlation test. (D, G) Differentially expressed genes were identified after Mann–Whitney test or two-tailed unpaired t-test with Welch’s correction if necessary. (E, H) Overlapping significance, odds ratio, and Jaccard index were calculated with Fisher’s exact test. *\textit{p} < 0.05, **\textit{p} < 0.01, and ***\textit{p} < 0.001.
progression. In light of our findings, this mechanism may also function under other pathological situations associated with inflammation, such as sepsis. Thus, the physiological and molecular framework that we propose in the context of cancer cachexia could also be investigated in other cachexia-associated diseases.

As the syndrome is firmly established in 23-week-old animals, data obtained at this time point only testify to the activation of the HPA axis. Similar ascertainments can also be drawn from the data obtained with SEV KPC mice. But one may question whether HPA axis activation occurs early during the pathogenic process to trigger cachexia, or later to main-

As we highlight the implication of HPA axis activation and GC in the course of cancer cachexia, the current work does not investigate the upstream mechanisms by which the HPA axis is activated and does not determine whether GC are drivers and/or amplifiers of cancer cachexia. Anyhow, it is likely that additional local or systemic signals also contribu-

Finally, our data also question the relevance of GC-inhibiting strategies to combat cancer cachexia. Pioneering studies indicate that adrenalectomy of tumour-bearing animals did not attenuate cancer cachexia. However, this surgical approach to the treatment of cancer cachexia induces weight loss by itself. The steroid hormone inhibitor RU486 has provided contrasting results, with some positive effects on the attenuation of body weight loss, or not. However, in addition to having anti-GC effects, RU486 also exerts antiapoptotic and antiangiogenic effects, which may mitigate the potential antitumour effects of GC inhibition. By contrast, the potential relevance of GC-based therapy has been convincingly reported in LLC-tumour bearing mice with targeted deletion of GC receptor in skeletal muscle. This single targeted tissue approach showed that skeletal muscle mass loss was abrogated in tumour-bearing mice. One may thus speculate that targeting GC at the systemic level by specific molecular tools may be promising in preclinical models of cancer cachexia.

While we highlight the implication of HPA axis activation and GC in the course of cancer cachexia, the current work does not investigate the upstream mechanisms by which the HPA axis is activated and does not determine whether GC are drivers and/or amplifiers of cancer cachexia. Anyhow, it is likely that additional local or systemic signals also contribu-

In summary, our results demonstrate that HPA axis activation and increased corticosterone levels in Apc mice are associated with the transcription of GC-responsive genes in skeletal muscle and liver of cachectic Apc mice. These results highlight a neuroendocrine mechanism that involves the HPA-GC pathway in contributing to the transcriptional regulation of skeletal muscle catabolism and hepatic
metabolism during cancer cachexia in Apc mice. Understanding the mechanisms regulating the HPA axis and GC secretion during cancer cachexia, together with the knowledge of the molecular basis of the GC-induced effects in skeletal muscle and liver will offer the potential to develop strategies that may contribute to minimize the debilitating effects of cancer cachexia.

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Conflict of interest

The authors declare no competing interest.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.
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