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1           **Carbonyl reductase 1 catalyzes 20 $\beta$ -reduction of glucocorticoids,**  
2           **modulating receptor activation and metabolic complications of obesity**

3  
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39 **Supplementary Materials:**

40 **SM1 Quantification of urinary glucocorticoid metabolites**

41 Glucocorticoids were extracted from equine urine (20mL) by solid phase extraction on Bond  
42 Elut Nexus mixed mode Large Reservoir Capacity, 60 mg columns (Agilent Technologies,  
43 Santa Clara, CA, USA). Glucocorticoids were extracted from human urine (10mL) by solid  
44 phase extraction on Sep-Pak columns (Waters, Milford, MA, USA).

45 Steroid conjugates were hydrolysed using  $\beta$ -glucuronidase followed by re-extraction. The  
46 steroids obtained were derivatized to form methoxime-trimethylsilyl (MO-TMS) derivatives.  
47 Steroidal derivatives were separated by gas chromatography using the TRACE GC Ultra Gas  
48 Chromatograph (Thermo Fisher Scientific). Analysis was performed on a TSQ Quantum Triple  
49 Quadrupole GC-tandem mass spectrometer (Thermo Fisher Scientific) using a 35HT  
50 Phenomenex column (30m, 0.25mm, 0.25 $\mu$ m, Agilent Technologies) as previously described  
51 (45, 46). Epi-cortisol and epi-tetrahydrocortisol were used as internal standards (Steraloids,  
52 Newport, RI, USA). The steroids analyzed were cortisol (F), cortisone (E), 5 $\beta$ -  
53 tetrahydrocortisol (5 $\beta$ -THF), 5 $\beta$ -tetrahydrocortisone (5 $\beta$ -THE), 5 $\alpha$ -tetrahydrocortisol (5 $\alpha$ -  
54 THF) (45, 46) with the inclusion of the following transitions (collision energy)  $\alpha$ -cortol  
55 (535 $\rightarrow$ 355, 20V) and  $\beta$ -cortol (535 $\rightarrow$ 455, 10V),  $\alpha$ - and  $\beta$ -cortolone (449 $\rightarrow$ 269, 10V), 6 $\beta$ -  
56 hydroxycortisol (693 $\rightarrow$ 513, 10V), 20 $\alpha$ - dihydrocortisol (578 $\rightarrow$ 488, 10V) and 20 $\beta$ -  
57 dihydrocortisol (681 $\rightarrow$ 578, 10V).

58

59 Steroid quantities in equine urine were expressed as a ratio to creatinine, which was measured  
60 using a colorimetric method based on the modified Jaffe's reaction (IL650 analyser,  
61 Instrumentation Laboratories, Barcelona, Spain).

62 **SM2 Quantification of glucocorticoids in plasma**

63 Plasma samples (1 mL Equine, 200  $\mu$ L Human) enriched with internal standard (D4-F, D4-E  
64 and D8-B; 250 ng of each) were extracted by liquid-liquid extraction. Chloroform (10 volumes)  
65 was added to each sample, mixed and the organic layer was dried under nitrogen (60  $^{\circ}$ C). The  
66 extracts were re-suspended in mobile phase (60  $\mu$ L, water: methanol 70:30 v/v) for  
67 quantification of steroids by LC-MS/MS. The injection volume was 30  $\mu$ L.

### 68 **SM3 Quantification of glucocorticoids in adipose tissue**

69 Adipose samples (100mg) were homogenized in ethyl acetate (1 mL) and enriched with internal  
70 standard (D4-F, D8-E, D8-B; 250ng of each). The homogenate was slowly dripped onto chilled  
71 ethanol: glacial acetic acid: water (95:3:2 v/v, 10 mL) and frozen at -80  $^{\circ}$ C overnight. Samples  
72 were thawed (4  $^{\circ}$ C) prior to sonication (8 x 15 second bursts) and centrifugation (3000 x g, 30  
73 mins, 4  $^{\circ}$ C). The supernatant was dried under nitrogen (60  $^{\circ}$ C), re-suspended in methanol (10  
74 mL) and frozen at -80  $^{\circ}$ C overnight. Samples were thawed (RT) and hexane (10 mL) added  
75 and mixed. The hexane layer was removed, the remaining methanol dried down under nitrogen  
76 (60  $^{\circ}$ C) and re-suspended in water (400  $\mu$ L) and ethyl acetate (4 mL). The organic layer was  
77 removed, dried under nitrogen (60  $^{\circ}$ C) and re-suspended in 30 % methanol (5 mL). C18 Bond  
78 Elut columns (Agilent Technologies, Santa Clara, CA, USA) were conditioned (methanol 5  
79 mL) and equilibrated (water 5 mL), samples were loaded and steroids eluted with methanol (2  
80 mL). Eluates were dried down under nitrogen (60  $^{\circ}$ C) and re-suspended in 60  $\mu$ L mobile phase  
81 (water: methanol, 70:30 v/v) for quantification of steroids by LC-MS/MS. Injection volume  
82 was 30  $\mu$ L.

### 83 **SM4 Quantification of mRNA by RT-qPCR**

84 Total RNA was extracted from adipose and liver using the RNAeasy Mini Kit (Qiagen Inc,  
85 Valencia, CA, USA). The tissue was mechanically disrupted in either QIAzol (Qiagen) for

86 adipose tissue or RLT buffer (Qiagen) for liver tissue. Total RNA was extracted from cells in  
87 QIAzol lysis reagent using an RNeasy Mini Kit according to the manufacturer's instructions.

88 Quantitative real-time polymerase chain reaction was performed using a Light-cycler 480  
89 (Roche Applied Science, Indianapolis, IN, USA). Primers were designed using sequences from  
90 the National Centre of Biotechnological Information and the Roche Universal Probe Library  
91 (see Table S7-9) for details of primers for genes of interest and housekeeping genes). Samples  
92 were analysed in triplicate and amplification curves plotted (y axis fluorescence, x axis cycle  
93 number). Triplicates were deemed acceptable if the standard deviation of the crossing point  
94 was  $< 0.5$  cycles. A standard curve (y axis crossing point, x axis log concentration) for each  
95 gene was generated by serial dilution of cDNA pooled from different samples and fitted with  
96 a straight line and deemed acceptable if reaction efficiency was between 1.7 and 2.1.

## Supplementary Tables and Figures

*Table S 1 Characteristics of equine study subjects*

	<b>Lean (n=14)</b>	<b>Obese (n=14)</b>
<b>Age (years)</b>	15.6 ± 5.6	13.8 ± 7.8
<b>Sex</b>	4 Females 10 Castrated males	8 Female 5 Castrated males
<b>Breeds</b>	11 Thoroughbred 2 Native pony 1 Percheron	2 Thoroughbred 3 Cob 7 Native ponies 1 Arab pony 1 Clydesdale
<b>Body condition score (5)</b>	2.3 ± 0.3	3.8 ± 0.7*

Data are expressed as mean ± SEM. Student's t-test or Mann-Whitney U test: \*p<0.05

*Table S 2 Characteristics of obese human participants with type 2 diabetes providing 24 hour urine samples*

<b>n</b>	19
<b>Age (years)</b>	58.9 ± 1.5
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	32.60 ± 1.2
<b>Concurrent medications</b>	4 no medication 15 metformin

All participants were male and diagnosed with diabetes. Participants provided 24 hour urine samples. The additional lean and obese participants providing urine were recruited as part of a different study (Upreti et al 2014). Data are expressed as mean ± SEM.



*Table S 3 Characteristics of lean and obese study participants providing plasma samples*

	<b>Lean (n=10)</b>	<b>Obese (n=10)</b>
<b>Age (years)</b>	50.5 ± 10.4	50.0 ± 11.8
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	23.8 ± 1.2	32.9 ± 2.7
<b>Concurrent medications</b>	<ul style="list-style-type: none"> <li>• 6 No medication</li> <li>• 1 perindopril, nifedipine,</li> <li>• 1 citalopram, simvastatin, clopidogrel, ranitidine</li> <li>• 1 clomipramine</li> <li>• 1 ranitidine</li> </ul>	<ul style="list-style-type: none"> <li>• 8 No medication</li> <li>• 1 tamsulosin, lansoprazole</li> <li>• 1 pantoprazole</li> </ul>

Plasma was collected (between 8 and 9am) from lean and obese but otherwise healthy men. Participants with diabetes or prior corticosteroid treatment were excluded prior to recruitment. Data are expressed as mean ± SEM.

*Table S 4 Characteristics of lean and obese men providing adipose biopsy samples during surgery.*

	<b>Lean (n=8)</b>	<b>Obese (n=8)</b>
<b>Age (years)</b>	55.7 ± 12.7	52.8 ± 13.7
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	23.0 ± 1.7	38.8 ± 6.7
<b>Surgery</b>	<ul style="list-style-type: none"> <li>• Cholecystectomy</li> <li>• Laparoscopic cholecystectomy</li> <li>• Removal of gastric band</li> <li>• Hernia repair</li> <li>• Open cholecystectomy</li> <li>• 3 Abdominal hernia repairs</li> </ul>	<ul style="list-style-type: none"> <li>• Gastric bypass</li> <li>• Laparoscopic sleeve gastrectomy</li> <li>• Laparoscopic cholecystectomy</li> <li>• Cholecystectomy</li> <li>• 2 Laparoscopic funduplications</li> <li>• Laparoscopic cholecystectomy</li> <li>• Laparoscopic removal of gastric band and gastric bypass</li> </ul>

All the participants were male. Samples were obtained at the time of surgery. Data are expressed as mean ± SEM.

*Table S 5 Estimates of the phenotypic associations with CBR1 expression in the liver based on Mendelian Randomisation*

<b>Outcome</b>	<b>Beta</b>	<b>Se</b>	<b>P value</b>	<b>N</b>	<b>Sample</b>
BMI	-0.01	0.01	0.25	339224	(55)
Body Fat	-0.01	0.01	0.06	100716	(56)
2hr glucose adjusted for BMI	<0.01	0.03	0.89	15234	(57)
Fasting glucose	0.01	<0.01	0.02	58074	(58)
Fasting insulin	<0.01	<0.01	0.45	51750	(58)
HOMA-B	<0.01	<0.01	0.59	46186	(59)
HOMA-IR	0.01	0.01	0.27	46186	(59)
HbA1c	0.01	<0.01	0.01	46368	(60)

These analyses used rs1005696 as the instrument for CBR1 expression in liver and associations were analysed using MR-base. See supplementary references for further details of studies used.

Table S 6 Human primer sequences for PCR

<b>Gene Symbol, full name</b>	<b>Forward Primer (3'→ 5')</b>	<b>Reverse Primer (5'→ 3')</b>
<b><i>RNA18s</i></b> (ribosomal RNA 18s)	CTTCCACAGGAGGCCTACA C	CGCAAAATATGCTGGAAC TT
<b><i>DUSP1</i></b> (dual specificity phosphatase 1)	TTCAAGAGGCCATTGACTT	CCTGGCAGTGGACAAACA C
<b><i>GILZ</i></b> (glucocorticoid- induced leucine zipper)	CCGTTAAGCTGGACAACAG TG	ATGGCCTGTTTCGATCTTGT T
<b><i>FKBP51</i></b> (FK506-binding protein 51)	GGATATACGCCAACATGTT CAA	CCATTGCTTTATTGGCCTCT
<b><i>IGFBP1</i></b> (insulin-like growth factor binding protein 1)	GCCTTGGCTAAACTCTCTA CGA	CCATGTCACCAACATCAAA AA
<b><i>IL-1β</i></b> (Interleukin 1β)	TGTAATGAAAGACGGCACA CC	TCTTCTTTGGGTATTGCTTG G
<b><i>CBR1</i></b> (Carbonyl Reductase 1)	TCCCTCTAATAAAACCCCA AGG	GGTCTCACTGCGGAACTTC T

Table S 7 Equine primer sequences for PCR

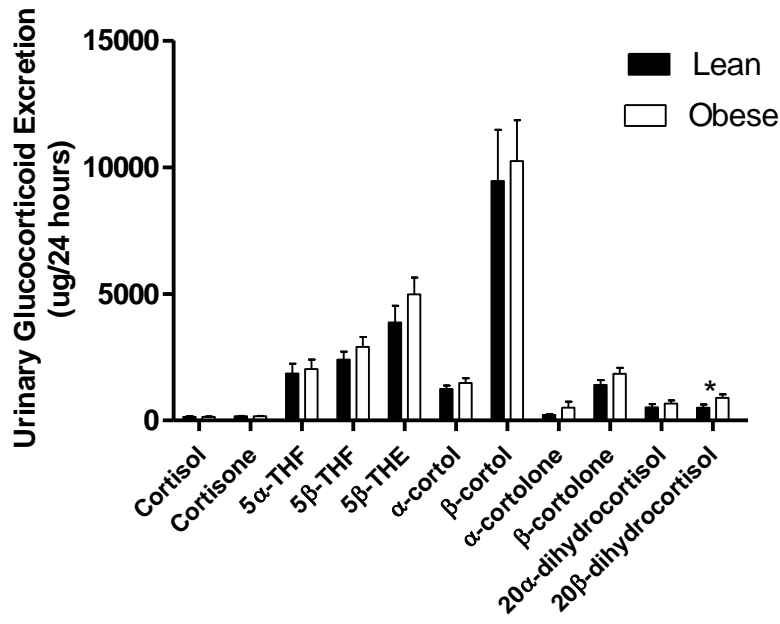
<b>Gene Symbol, full name</b>	<b>Forward Primer (3'→ 5')</b>	<b>Reverse Primer (5'→ 3')</b>
<b><i>RNA18s</i></b> (ribosomal RNA 18s)	TGACCCAAGGCTAGTAGCT GA	TTCAACACATCACCCACCA T
<b><i>SDHA</i></b> (Succinate)	CTACGGAGACCTTAAGCAT CTGA	GGGTCTCCACCAGGTCAGT A

dehydrogenase complex)		
<b><i>Equine CBRI</i></b> (Carbonyl Reductase 1)	ACCCAGCCATGTCTTACAC C	CAGGATAGTGAAGCCGAT GC

Table S 8 Murine primer sequences for PCR

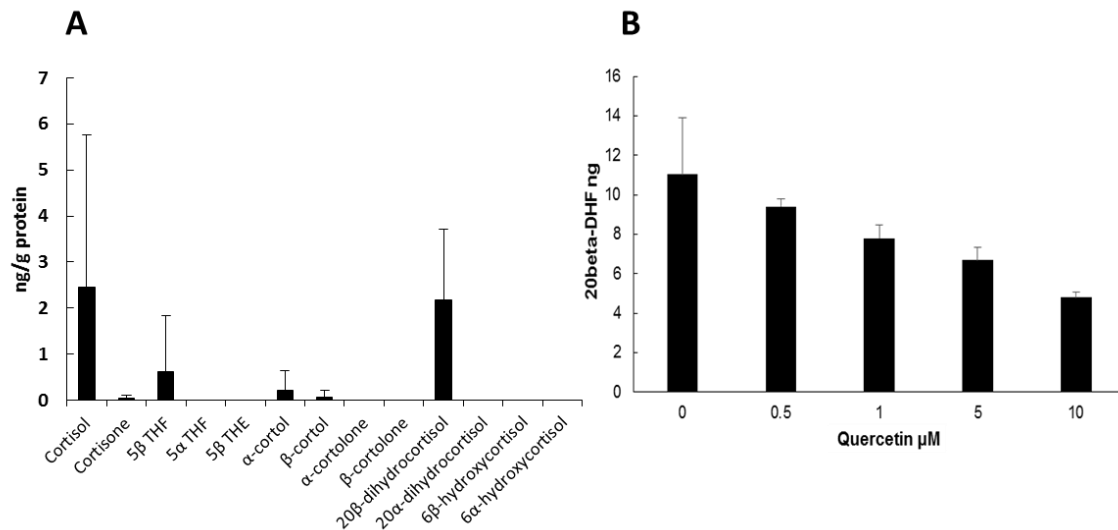
<b>Gene Symbol, full name</b>	<b>Forward Primer (3'→ 5')</b>	<b>Reverse Primer (5'→ 3')</b>
<b><i>RNA18s</i></b> ( <i>ribosomal RNA 18s</i> )	CTCAACACGGGAAACCT CAC	CGCTCCACCAACTAAGA ACG
<b><i>Tbp</i></b> ( <i>TATA-binding protein</i> )	GGGAGAATCATGGACCA GAA	GATGGGAATTCCAGGAG TCA
<b><i>Per1</i></b> ( <i>Period 1</i> )	GCTTCGTGGACTTGACAC CT	TGCTTTAGATCGGCAGT GGT
<b><i>Pepck</i></b> (phosphoenolpyruvate carboxykinase)	GAGGCACAGGTCCTTTTC AG	GTTCTGGGCCTTTGTG AC
<b><i>Adipq</i></b> ( <i>Adiponectin</i> )	GGTGAGAAGGGTGAGAA AGGA	TTTCACCGATGTCTCCCT TAG
<b><i>Lpl</i></b> ( <i>Lipoprotein lipase</i> )	CTCGCTCTCAGATGCCCT AC	GGTTGTGTTGCTTGCCAT T
<b><i>Sgk1</i></b> ( <i>serum glucocorticoid kinase 1</i> )	TTTCCAAAGGGGGATGC T	TGTTGGCATGATTACAT TGTTCT
<b><i>ENaC1</i></b> ( <i>Epithelial sodium channel 1</i> )	AGCACAGAGAACACCCC TGT	TGGCTCTTCCTACCCTCT CTC
<b><i>Cbr1</i></b> (Carbonyl Reductase 1)	AGGTGACAATGAAAACG AACTTT	GGACACATTCACCACTC TGC

**Figure S 1** The human urinary glucocorticoid metabolite profile in lean and obese individuals



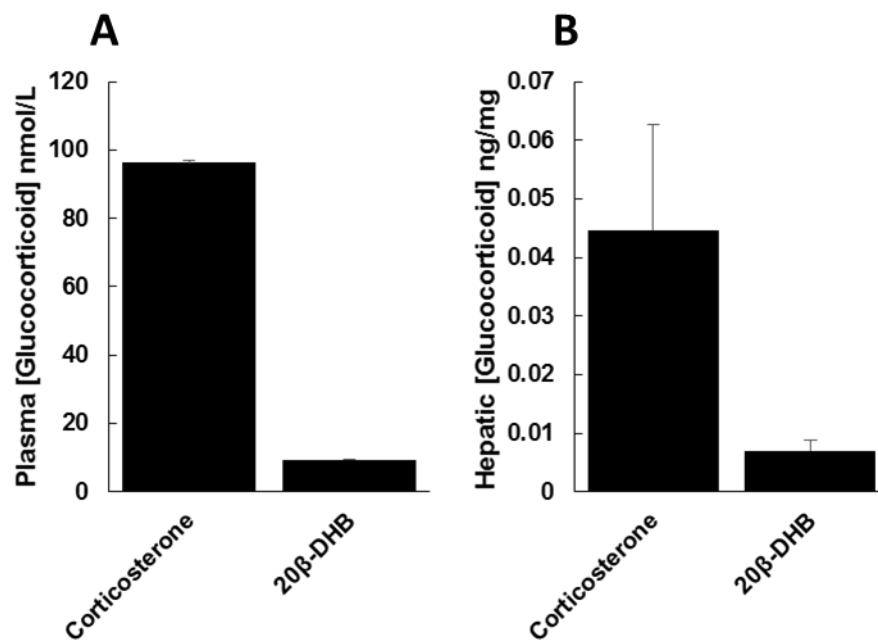
Urinary glucocorticoids of lean and obese participants were extracted, derivitized and quantified by GC-MS/MS. Urinary excretion of 20β-dihydrocortisol was higher in the obese group.

**Figure S 2** Equine liver cytosol metabolises cortisol to 20β-dihydrocortisol and this production is inhibited by quercetin



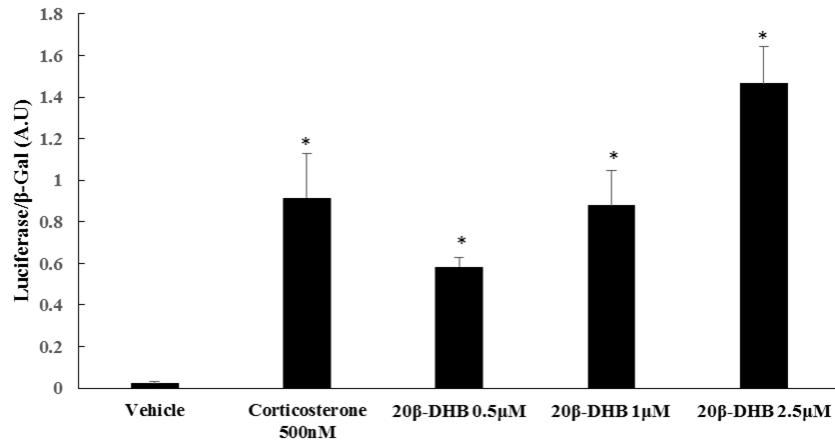
[A] In the presence of NADPH equine liver cytosol produced 20 $\beta$ -DHF as the predominant metabolite. (THF = tetrahydrocortisol, THE = tetrahydrocortisone). [B] CBR1 inhibitor quercetin prevented production of 20 $\beta$ -DHF by equine liver cytosol. Data are mean  $\pm$  SEM.

Figure S 3 20 $\beta$ -Dihydrocorticosterone is present in murine tissues



20 $\beta$ -Dihydrocortisone (the murine equivalent of 20 $\beta$ -dihydrocortisol) was measured by LC-MS/MS in murine [A] plasma and [B] liver. Data are mean  $\pm$  SEM.

Figure S 4 20 $\beta$ -Dihydrocorticosterone induces murine glucocorticoid receptor activation



HEK293 cells transfected with murine GR-MMTV-Luc were incubated with increasing concentrations of 20 $\beta$ -dihydrocorticosterone (20 $\beta$ -DHB) the murine equivalent of 20 $\beta$ -dihydrocortisol. Data are mean  $\pm$  SEM, \*P<0.05 compared to vehicle.

## Supplementary References

- Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518(7538):197-206.
- Lu Y, Day FR, Gustafsson S, Buchkovich ML, Na J, Bataille V, Cousminer DL, Dastani Z, Drong AW, Esko T, et al. New loci for body fat percentage reveal link between adiposity and cardiometabolic disease risk. *Nat Commun*. 2016;7(
- Saxena R, Hivert MF, Langenberg C, Tanaka T, Pankow JS, Vollenweider P, Lyssenko V, Bouatia-Naji N, Dupuis J, Jackson AU, et al. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. *Nat Genet*. 2010;42(2):142-8.
- Manning AK, Hivert MF, Scott RA, Grimsby JL, Bouatia-Naji N, Chen H, Rybin D, Liu CT, Bielak LF, Prokopenko I, et al. A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycaemic traits and insulin resistance. *Nat Genet*. 2012;44(6):659-69.
- Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet*. 2010;42(2):105-16.
- Soranzo N, Sanna S, Wheeler E, Gieger C, Radke D, Dupuis J, Bouatia-Naji N, Langenberg C, Prokopenko I, Stolerman E, et al. Common variants at 10 genomic loci influence hemoglobin A1C levels via glycaemic and nonglycaemic pathways. *Diabetes*. 2010;59(12):3229-39.