

Therapeutic glucocorticoids mechanisms of actions in rheumatic diseases

Raza, Karim; Hardy, Rowan; Cooper, Mark S.

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Therapeutic glucocorticoids: mechanisms of actions in rheumatic diseases

[Au: Shortened title OK? (as the character limit for titles is 90 characters, including spaces). Also changed to "rheumatic" to make the rheumatology aspect more clear]

Comment [MC1]: This is ok.

Rowan S. Hardy¹, Karim Raza¹ and Mark S. Cooper² [Au: Author names OK - with the order and abbreviations as you would like to them appear on the article?]

Comment [MC2]: Yes all ok and accurate

¹University of Birmingham, Birmingham, UK

²ANZAC Research Institute, University of Sydney, Sydney, Australia

[Au: Affiliations correct?]

Comment [MC3]: Correct

Abstract

Therapeutic glucocorticoids have been widely used in rheumatic diseases since they became available over 60 years ago. Despite the advent of more specific biologic therapies, a notable proportion of individuals with chronic rheumatic diseases continue to be treated with these drugs. Glucocorticoids are powerful, broad spectrum anti-inflammatory agents but their use is complicated by an equally broad range of adverse effects. The specific cellular mechanisms by which glucocorticoids have their therapeutic action have been difficult to identify, and attempts to develop more selective drugs on the basis of the action of [Au: OK? is this what you meant? Or the structure?] glucocorticoids have proven difficult. The actions of glucocorticoid seem to be highly cell type and context dependent. Despite emerging data on the effect of tissue-specific manipulation of glucocorticoid receptors in rodent [Au: rodent meaning mouse?] models of inflammation, the cell types and intracellular targets of glucocorticoids in rheumatic diseases have not been fully identified. Although showing some signs of decline, the use of systemic glucocorticoids in rheumatology is likely to continue to be widespread and careful consideration is required by rheumatologists to balance the beneficial effects and deleterious effects of these agents [Au: OK?].

Comment [MC4]: This is ok. The structure is not important, it is the action that is relevant

Comment [MC5]: Yes. All the models have used mice rather than rats. I would be happy with the term mouse instead of rodent.

Comment [MC6]: Yes, ok with me.

29 **[Au: For your information, H1 and H2 refer to the level of heading and will be removed**
 30 **before proofs are made. H1 subheadings can have max 38 characters including spaces. H2**
 31 **subheadings can have a max 80 characters including spaces. Subheads have been edited**
 32 **to fit these limits, where indicated]**

33 **[H1] Introduction**

34 The introduction of glucocorticoids and their notable effects in the treatment of patients
 35 with rheumatoid arthritis (RA) led to the award of the Nobel Prize for Physiology or
 36 Medicine in 1950¹. Subsequently, systemic glucocorticoid therapy has been employed in a
 37 range of rheumatic diseases. For many of these conditions, the evidence for glucocorticoid
 38 therapy remains based on clinical experience rather than rigorous clinical trials. Despite the
 39 introduction of biologic drugs, which have a much greater specificity for components of the
 40 immune system than glucocorticoids, systemic glucocorticoid therapy continues to be
 41 widely used².

42 In the general population, ~1% of individuals are treated with oral glucocorticoids on a long-
 43 term basis, and this figure rises to around 3% in elderly individuals^{3,4}. In individuals with RA,
 44 oral glucocorticoid usage continues to be widespread, although is potentially declining **[Au:**
 45 **OK?]**². For some conditions (such as systemic vasculitis, systemic lupus erythematosus and
 46 polymyalgia rheumatica), the use of glucocorticoids for long periods of time remains an
 47 **important** part of current treatment approaches.

48 The adverse effects of prolonged glucocorticoid therapy are well established and extremely
 49 common⁵. Glucocorticoids have effects on **[Au: OK? Is this what you meant?]** almost all
 50 tissues and bodily systems (the selected effects of glucocorticoids are represented in
 51 supplementary figure 1). Adverse effects of particular medical importance include
 52 osteoporosis and fracture, glucose intolerance and diabetes, central obesity, **[Au: What do**
 53 **you mean by central obesity?]** muscle wasting, increased risk of infection, depression and
 54 cataracts. **[Au: Please reference this statement.]** The effect of glucocorticoids on bone is
 55 related to the dose and duration of therapy but treatment durations beyond 3 months are
 56 associated with a 30% increase in overall fracture risk and at least a doubled risk of vertebral
 57 fracture⁶. The risk of developing diabetes is doubled in patients with RA taking prednisolone
 58 doses of 7.5mg or above⁵. Although adverse effects on bone can be mitigated
 59 pharmacologically⁷ **[Au: With what therapies?]**, the effects on other tissues such as muscle
 60 wasting, skin thinning, obesity and increased risk of diabetes have no specific treatment.
 61 Patient perceptions of adverse effects often differ from those of their treating clinician^{8,9}
 62 with features such as weight gain and insomnia rated very important by patients whereas
 63 clinicians focussed on features such as diabetes and infection risk (considered less important
 64 by patients). **[Au: Could you elaborate a little more (to clarify the relevance to**
 65 **glucocorticoid therapy specifically?).]**

Comment [MC7]: Yes, this is what the data shows.

Comment [MC8]: Typo. Probably from the original version

Comment [MC9]: Yes this is ok.

Comment [MC10]: This is a common term in endocrinology but maybe not rheumatology. It refers to the accumulation of fat in the abdomen (and neck) rather than in the limbs. 'Visceral' obesity would be a synonym. Alternatively 'accumulation of fat in the abdomen' would be accurate.

Comment [MC11]: Much of this literature is classical but I would suggest: Strehl C, Bijlsma JW, de Wit M, Boers M, Caeyers N, Cutolo M, Dasgupta B, Dixon WG, Geenen R, Huizinga TW, Kent A, de Thurah AL, Listing J, Mariette X, Ray DW, Scherer HU, Seror R, Spies CM, Tarp S, Wiek D, Winthrop KL, Buttgereit F. Defining conditions where long-term glucocorticoid treatment has an acceptably low level of harm to facilitate implementation of existing recommendations: viewpoints from an EULAR task force. *Ann Rheum Dis.* 2016 Jun;75(6):952-7.

Comment [MC12]: This is discussed later but if needed the phrase 'with bisphosphonates or denosumab therapy' could be added.

Comment [MC13]: Attempted to do so.

66 Despite their widespread use over many decades the mechanisms by which glucocorticoids
 67 have their desired anti-inflammatory effects remain unclear. Many cell types and cellular
 68 pathways have been proposed as the key targets for these effects. Experimental evidence
 69 suggests that there is likely to be a diversity of cell types and pathways involved and these
 70 targets are likely to differ between different diseases treated. An improved understanding
 71 of these targets in specific disease states opens up the potential to design novel
 72 therapeutics that retain anti-inflammatory effects with less risk of adverse consequences.

73 **[Au: So far you have introduced the use of glucocorticoids and their adverse effects, but**
 74 **haven't mentioned anything about mechanisms of action (until the paragraph below,**
 75 **describing what this Review will cover). Perhaps you could have a short paragraph here to**
 76 **outline the outstanding questions surrounding mechanisms of action of glucocorticoids**
 77 **and why understanding these mechanisms is useful (to help introduce the precise of this**
 78 **Review)]**

Comment [MC14]: Completely agree. Paragraph added.

79 In this Review, we summarise the current understanding of the basis by which
 80 glucocorticoids have therapeutic effects in inflammatory, and in particular rheumatic,
 81 diseases. We consider the pharmacological properties that enable glucocorticoids to have
 82 useful effects in a wide range of conditions and the probable cellular targets of these
 83 actions. We also consider the molecular mechanisms underlying the adverse effects of
 84 glucocorticoids and assess the prospects for developing novel therapeutics that retain
 85 beneficial properties with reduced risks of adverse effects.

87 **[H1] Glucocorticoids and their receptors [Au: OK?]**

Comment [MC15]: Yes ok

88 Cortisol (referred to as hydrocortisone when used as a therapeutic) is the main endogenous
 89 glucocorticoid in humans. Cortisol secretion is essential to life **[Au: Is it possible to**
 90 **reference this statement?]**. This steroid hormone is released in a pronounced circadian
 91 rhythm (high in the morning before waking and very low around midnight) and its synthesis
 92 is considerably upregulated during states of stress. Cortisol is synthesised in the adrenal
 93 cortex from cholesterol and retains the cyclopentanoperhydrophenanthrene 'steroid'
 94 backbone structure. The synthetic glucocorticoids most commonly used to treat systemic
 95 inflammation in rheumatology (prednisolone, methylprednisolone and dexamethasone) are
 96 very similar in structure to cortisol, with only relatively modest modifications (figure 1) **[Au:**
 97 **OK?]**. These changes variously reduce enzymatic breakdown of the molecule to increase the
 98 ability of the steroid to bind to the glucocorticoid receptor **[Au: our journal style is to avoid**
 99 **2-letter abbreviations (with some rare exceptions), and so I have removed the**
 100 **abbreviation GR throughout (unless referring to particular isoforms) OK?]**, and reduce or
 101 eliminate the intrinsic mineralocorticoid (salt retaining) activity. **[Au: Is there a reference**
 102 **you can provide for this background information on glucocorticoids?]**

Comment [MC16]: This is tricky. Addison described this condition in 1855 and it was well known from then on that if the adrenal glands failed death was inevitable. I could propose one of my previous references but this is quite general (and I'm aware that I've quoted my work in other areas already). The reference would be: Cooper MS1, Stewart PM. Corticosteroid insufficiency in acutely ill patients. N Engl J Med. 2003 Feb 20;348(8):727-34.

Comment [MC17]: Yes ok

Comment [MC18]: Yes ok

Comment [MC19]: I would suggest ref: Fuller PJ, Lim-Tio SS, Brennan FE. Specificity in mineralocorticoid versus glucocorticoid action. Kidney Int. 2000 Apr;57(4):1256-64.

103 **[Au: Paragraph break OK here?]** Endogenous glucocorticoids can bind to the glucocorticoid
 104 receptor (encoded by *NR3C1*) and the mineralocorticoid receptor **[Au: encoded by? (to**
 105 **match the previous statement?)].** The glucocorticoid receptor is expressed in most cells
 106 within the body and is thought to mediate most of the anti-inflammatory and negative
 107 consequences of therapeutic glucocorticoids. The glucocorticoid receptor contains various
 108 structural domains important for ligand binding, nuclear localisation, DNA binding and
 109 activation functions¹⁰. The un-bound glucocorticoid receptor is found within the cytoplasm
 110 but is transported to the nucleus after binding of the receptor by glucocorticoid. The
 111 molecular actions arising from the glucocorticoid-bound glucocorticoid receptor are
 112 discussed in a later section **[Au: OK?]**.

Comment [MC20]: Yes ok

Comment [MC21]: Encoded by NR3C2. Agree should be added

113 **[Au: Paragraph break OK here?]** The mineralocorticoid receptor is expressed primarily in
 114 cells that regulate salt and water balance such as the distal tubule of the kidney, the salivary
 115 and sweat glands and the colonic epithelium. Even though glucocorticoids such as cortisol
 116 and prednisolone have an affinity for the mineralocorticoid receptor, the interaction
 117 between these glucocorticoids and the mineralocorticoid receptor is prevented by the
 118 presence of an enzyme (corticosteroid 11 β -dehydrogenase isozyme 2 **[Au: OK?]**
 119 (11 β HSD2)); this enzyme inactivates these glucocorticoids **[Au: these glucocorticoids**
 120 **specifically, or glucocorticoids in general?]** in mineralocorticoid-sensitive cells¹¹.
 121 Glucocorticoids such as dexamethasone and triamcinolone do not bind the
 122 mineralocorticoid receptor and thus have no mineralocorticoid activity.

Comment [MC22]: Yes ok

Comment [MC23]: Yes ok

Comment [MC24]: I don't think this is ok. I have never seen this terminology in the medical literature or used it myself. This might be the term used by pure chemists (and Wikipedia!) but would prefer to stick with 11b-hydroxysteroid dehydrogenase type 2.

Comment [MC25]: These glucocorticoids is correct. The enzyme is selective for certain glucocorticoids so 'glucocorticoids' would be incorrect.

Comment [MC26]: Yes ok

124 **[H1] Pharmacokinetics [Au: Shortened title (to fit character limits) OK?]**

125 A fundamental structural property of therapeutic glucocorticoids is that they can pass
 126 through biological membranes to access intracellular receptors (figure 2). Glucocorticoids
 127 such as prednisone and prednisolone are efficiently absorbed through the gastrointestinal
 128 tract. Although poorly soluble in water owing to their lipophilic nature, glucocorticoids can
 129 be carried effectively in the circulation through their association with plasma proteins
 130 (primarily globulin and albumin **[Au: OK?]**). Orally or intravenously **[Au: OK?]** administered
 131 glucocorticoids can thus penetrate most tissues. Coupled with the almost universal
 132 distribution of glucocorticoid receptors within tissues, this high degree of penetration
 133 means that glucocorticoid therapy can target cells that mediate inflammation at a systemic
 134 level. This high bioavailability, however, comes at the price of considerable 'off target'
 135 exposure of tissues unrelated to the condition being treated. Some therapeutic
 136 glucocorticoids, such as cortisone and prednisone, lack intrinsic glucocorticoid receptor
 137 binding activity but when given orally **[Au: why orally and not intravenously?]** are
 138 converted to their active counterparts, cortisol and prednisolone, by 11 β -hydroxysteroid
 139 dehydrogenase type 1 (11 β -HSD1), an enzyme that is highly expressed in the liver (figure 2).
 140 Expression of 11 β -HSD1 in various stromal and immune cells can also amplify the actions of

Comment [MC27]: No. There is a specific type of 'globulin' than has high affinity for cortisol. It is termed 'corticosteroid binding globulin' so the phrase such be 'primarily corticosteroid binding globulin and albumin'

Comment [MC28]: Yes ok

Comment [MC29]: Cortisone and prednisone are only available in oral formulations. The key point here is that these can be given orally because they are converted to active forms on first pass through the liver (most oral drugs have to go through the liver before getting into the circulation). The sentence could be changed to include the phrase 'by first pass metabolism in the liver' since 'first pass metabolism is the technical term for this phenomena.

141 these glucocorticoids locally through conversion of these glucocorticoids from their inactive
142 to their active forms^{12,13}.

143 The pharmacokinetic properties of glucocorticoids have been successfully modified to
144 improve their therapeutic properties. The development of depot injections for joint and
145 muscle injection has proven to be highly successful¹⁴. These depot injections are formulated
146 such that the glucocorticoid is released at a much slower rate than typical glucocorticoid
147 preparations. Another approach to improving glucocorticoid effectiveness through changes
148 in their pharmacokinetics is the development of timed release preparations of
149 glucocorticoids designed to mimic the circadian rhythm of cortisol release¹⁵. A formulation
150 of prednisone has been developed that involves drug encapsulation. The drug is taken at
151 night and the tablet releases prednisone ~4 hours after ingestion (that is, at approximately
152 2am if given at 10pm, thus mimicking the pattern of release of endogenous glucocorticoids).
153 The timed approach seems to particularly benefit the early morning stiffness that occurs in
154 RA¹⁶. Furthermore, this treatment approach has the potential to minimise the adverse
155 metabolic effects of glucocorticoids that seem to be greater when administered at times
156 when the normal circadian levels of cortisol should be low^{17,18}. In addition to the overall
157 circadian rhythm, cortisol synthesis also follows an ultradian (minute to minute) rhythm. In
158 human studies, the pattern of cortisol exposure seems important in determining the
159 cognitive and emotional response to glucocorticoids¹⁹. Any such patterning will be absent
160 during treatment with therapeutic glucocorticoids.

161 Considerable scope remains to further develop glucocorticoid-based therapies on the basis
162 of manipulation of their pharmacokinetic properties such that these agents more selectively
163 target specific tissues of interest. Examples of new strategies include the development and
164 clinical evaluation of novel liposomal-based or nanoparticle-based treatments^{20,21}. In these
165 preparations, glucocorticoids are attached to or incorporated within molecules that can be
166 selectively taken up by specific cell types such as macrophages or that have better
167 penetration [Au: Can I just check you don't mean better targeting to sites of
168 inflammation? Or retention in site of inflammation? Or do you actually mean that have
169 better penetrate into these sites?] at sites of inflammation. One study in a mouse model of
170 arthritis showed the feasibility of loading glucocorticoids into a hydrogel that was sensitive
171 to breakdown by enzymes released into the joint during inflammation²². After injection into
172 the joint, this hydrogel–glucocorticoid complex reduced arthritis whereas the equivalent
173 dose of free glucocorticoid did not, presumably because free glucocorticoid was rapidly lost
174 from the joint. Although these strategies offer new opportunities for local and systemic
175 treatments, they are likely to only fulfil their potential when targeted to the cells that
176 directly mediate the beneficial effects of glucocorticoids [Au: OK?].

177

178 [H1] Mechanisms of action

Comment [MC30]: Better penetration is what we were getting at. Liposomes are large structures that cannot escape the circulation except where there is a breakdown of the vascular endothelium. Such a breakdown occurs at sites of inflammation. I agree that the sentence is a bit ambiguous as various approaches are used to try to get selectively greater action of glucocorticoids at specific tissues. None of them however involve retention.

Comment [MC31]: Yes ok

179 [H2] Glucocorticoid– glucocorticoid receptor interactions [Au: OK?]

Comment [MC32]: Yes ok

180 The majority of the therapeutic actions of glucocorticoids are thought to occur through
 181 interaction of glucocorticoids with the glucocorticoid receptor. ~~It is now clear recent years~~
 182 [Au: We prefer to avoid the word “recent” as it can be construed by different readers
 183 differently. What time frame are you referring to here? The last few years? The last
 184 decade?] ~~it has become clear~~ that the glucocorticoid receptor can have a variety of forms
 185 [Au: OK?], which can influence glucocorticoid signalling. The complexity of the mechanism
 186 of action of the glucocorticoid receptor [Au: Is this what you meant?] is still being clarified
 187 and a diverse array of glucocorticoid receptor isoforms (such as splice variants and isoforms
 188 with different translational start sites) can be present and differ between different tissues
 189 and between cells within the same tissue [Au: OK?] ²³. From the single *NR3C1* gene locus,
 190 two main transcriptional variants of the glucocorticoid receptor have been identified
 191 (termed GR α and GR β [Au: OK?]) ^{24, 25} (figure 3a). GR α is the primary transcript in most cells
 192 and contains all the domains required for glucocorticoid receptor signalling. The GR β
 193 transcript lacks the ability to bind endogenous glucocorticoids and is produced at a lower
 194 rate in most cells [Au: lower than what? Meaning lower than the GR α isoform?] but in
 195 certain contexts production can be upregulated. In vitro studies suggest that GR β can have a
 196 dominant negative action on GR α through the formation of GR α –GR β heterodimers or
 197 GR β –GR β homodimers rather than GR α –GR α homodimers. A function for GR β in the
 198 development of resistance to glucocorticoid therapy in the clinical setting has been
 199 proposed ^{25, 26}. Although these studies are intriguing, a concrete link between GR β and
 200 rheumatic disease pathophysiology or response to therapeutic glucocorticoids has yet to be
 201 established.

Comment [MC33]: In light of this point I have changed the text. There are two different aspects to the isoform issue with different time frames so it would be confusing to specify a time frame.

Comment [MC34]: Yes ok.

Comment [MC35]: Yes ok

Comment [MC36]: Yes ok

Comment [MC37]: Yes ok

Comment [MC38]: Yes the GR α isoform

202 Additional glucocorticoid receptor diversity arises from variation in glucocorticoid receptor
 203 isoform protein translation. At least 8 translational variants can be produced from the GR α
 204 transcript (termed GRA, GRB, GRC1, GRC2, GRC3, GRD1, GRD2 and GRD3). Importantly,
 205 these glucocorticoid receptor variants seem to differ in their ability to regulate gene
 206 expression ^{27,28}. Although GRA is the classical glucocorticoid receptor isoform that has been
 207 extensively studied, and is the isoform discussed almost exclusively below, the other protein
 208 isoforms can also be expressed at notable levels and this expression considerably varies
 209 between tissues [Au: OK?]. The glucocorticoid receptor isoforms can undergo a range of
 210 post-translational modifications including phosphorylation, acetylation, sumoylation and
 211 ubiquitination, which also influence the function of the glucocorticoid receptor ²⁹⁻³¹. The
 212 implications of translational isoforms and post-translational modifications of the
 213 glucocorticoid receptor in rheumatic diseases has not been examined, although a role for
 214 translational isoforms has been identified in the immune response to lipopolysaccharide in
 215 mice ²⁷.

Comment [MC39]: Yes ok

216

217 [H2] *Glucocorticoid receptor signalling*

218 The mechanisms by which glucocorticoid receptor complexes function are complex and still
 219 poorly understood. Although many variations of glucocorticoid receptor signalling
 220 mechanisms exist, these mechanisms can be broadly divided into transactivation and
 221 transrepression (Figure 3b). In transactivation, direct binding of the glucocorticoid receptor
 222 to specific DNA sequences, referred to as glucocorticoid response elements (GREs), causes
 223 an increase in gene transcription; this process generally occurs with glucocorticoid receptor
 224 dimers [Au: OK?]. In transrepression, monomeric glucocorticoid receptor 'tether' to specific
 225 factors in such a way that they cannot bind to DNA, interfering with downstream
 226 proinflammatory signalling pathways [Au: OK?]. As such, gene transcription is reduced
 227 without the glucocorticoid receptor directly interacting with the DNA. The term
 228 transrepression is also used for an additional mechanism in which glucocorticoid receptor
 229 homodimers bind to DNA GREs (so called negative GREs) such that gene transcription is
 230 inhibited [Au: Is this non-classical transrepression, as later you refer to "classical negative
 231 GREs" and "classical GREs". Or later, when you refer to "classical negative GREs", are you
 232 simply referring to all negative GREs?]. Other, less well characterised mechanisms by which
 233 glucocorticoids can signal include the release of chaperone proteins during binding of the
 234 glucocorticoid to the glucocorticoid receptor and through binding of glucocorticoids to cell
 235 membrane-associated glucocorticoid receptors^{32, 33}. A schematic overview of some of the
 236 ways in which the glucocorticoid receptor can function at a cellular level is outlined in figure
 237 4. [Au: I moved this sentence down from the start of the paragraph so that figure 3 could
 238 be discussed fully before figure 4 is mentioned]

Comment [MC40]: Yes ok

Comment [MC41]: Yes ok

Comment [MC42]: noted

239 The classical view of glucocorticoid receptor signalling was that the metabolic actions of
 240 glucocorticoids (such as those leading to induction of hepatic gluconeogenetic enzymes)
 241 were primarily mediated via transactivation through binding of glucocorticoid receptor
 242 homodimers to the promoter regions of target genes. By contrast, the anti-inflammatory
 243 actions of glucocorticoids were thought to be caused by transrepressive interactions of
 244 monomeric glucocorticoid receptors with components of proinflammatory signalling
 245 pathways (most importantly NF- κ B and AP-1) in ways not generally involving DNA binding,
 246 such that these pathways were suppressed. This model of glucocorticoid receptor signalling
 247 led to the concept that glucocorticoid-like molecules could be developed with reduced
 248 transactivation potential (and thus reduced adverse effects) but with retained or increased
 249 transrepression activity. This class of drugs has been labelled selective glucocorticoid
 250 receptor agonists (SEGRAs) or 'dissociated' glucocorticoid agonists. [Au: Please provide
 251 references for your description of this concept and class of drugs] Although providing a
 252 framework for commercial drug development, a range of experimental findings have shown
 253 that this concept has limitations. For example it is now established that some important
 254 anti-inflammatory genes such as *DUSP1*, *SPHK1* and *ANXA1* [Au: OK? Are you referring to
 255 genes in mice or humans here?] rely on classical transactivation [Au: What do you mean by
 256 "classical transactivation" (i.e. what would be non-classical transactivation)? I'm not sure

Comment [MC43]: A recent review is: Cooper MS, Zhou H, Seibel MJ. Selective glucocorticoid receptor agonists: glucocorticoid therapy with no regrets? J Bone Miner Res. 2012 Nov;27(11):2238-41. doi: 10.1002/jbmr.1753.

Comment [MC44]: These would be mouse studies

Comment [MC45]: I suggest removing the term classical as it is too confusing

257 **this has been introduced)** for glucocorticoid-mediated transcription **[Au: OK?]**³⁴⁻³⁶. Mice
 258 have been generated that have a targeted mutation of the glucocorticoid receptor such that
 259 the glucocorticoid receptor retains ligand-binding and DNA-binding capacity but has a
 260 reduced ability to form homodimers and thus transactivate gene promoters **[Au: This**
 261 **sentence was a little long so I've split into two, OK?]**. These mice, known as GR^{dim/dim} mice
 262 **[Au: OK?]**, have been used to examine the probable contribution that transactivation and
 263 transrepression make to the actions of glucocorticoids³⁷. GR^{dim/dim} mice were less
 264 responsive **[Au: meaning less responsive than wild-type mice?]** to the protective effects of
 265 glucocorticoid therapy during experimental sepsis indicating a protective role for
 266 glucocorticoid receptor transactivation in systemic inflammatory illness³⁸. Additionally,
 267 glucocorticoids still have detrimental effects on the bones of dimerization-deficient mice
 268 **[Au: Still referring to 'dim-dim' mice?]**, suggesting that the adverse effects on bone are
 269 mediated through transrepression³⁹.

Comment [MC46]: Yes ok

Comment [MC47]: Yes ok

Comment [MC48]: Yes ok

Comment [MC49]: Yes, than wild type mice

Comment [MC50]: Yes these are the 'dim/dim' mice

270 Our understanding of the complexity of glucocorticoid receptor signalling has increased
 271 substantially with the advent of techniques that enable examination of in vivo glucocorticoid
 272 receptor binding across the whole genome. These techniques surprisingly show that the
 273 majority of glucocorticoid receptor DNA binding sites are not within ~~classical-predicted~~ gene
 274 promoters and that monomeric glucocorticoid receptors commonly bind to DNA in
 275 association with other transcription factors; furthermore, many of these responses are
 276 through binding of ~~non-classical~~ negative GREs ~~with structures unlike previously described~~
 277 ~~negative GREs~~ **[Au: As mentioned above, could you clarify what you mean by "non-**
 278 **classical" here (i.e. what would be a classical negative GRE"?)**⁴⁰. During treatment with
 279 pharmacological levels of glucocorticoids **[Au: meaning that the affects you describe in the**
 280 **previous sentence are of non-pharmacological (/lower) levels of glucocorticoids?]**, there
 281 seems to be a shift ~~from binding to these unexpected GREs~~ **[Au: from what?]** to increased
 282 ~~binding use of expected classical~~ GREs by glucocorticoid receptor homodimers⁴¹. The ability
 283 of glucocorticoid receptors to bind to specific areas of DNA varies considerably between cell
 284 types and even within the cell type, depending on the developmental stage and chromatin
 285 organisation of the cell⁴². For example, in cells of the monocyte lineage, glucocorticoid
 286 treatment affects the expression of more mRNAs in monocytes than in differentiated
 287 macrophages⁴³. Of the mRNAs affected in monocytes, the majority are related to cell
 288 differentiation. This finding is perhaps not a surprise given that the glucocorticoid receptor
 289 has to coordinate between determining cell lineage (glucocorticoids being important for the
 290 differentiation of many cell types) and regulating acute metabolic and stress responses **[Au:**
 291 **OK? Is this what you meant?]**.

Comment [MC51]: Yes. It might be made clear clearer by saying 'with higher levels of glucocorticoids in the pharmacological range'

Comment [MC52]: Yes it is

292 Research in this area has been complicated by a reliance on experiments that involve
 293 cultured cells of mouse origin or transformed human cell lines. However, current studies are
 294 attempting to use primary cells isolated from humans, which should provide data that is
 295 generalizable and applicability to the clinical situation **[Au: OK?]**⁴⁴.

Comment [MC53]: Yes ok

296

297 [H2] Cellular targets of glucocorticoid signalling

298 This diversity of genomic targeting between cells is also paralleled by a diversity of
 299 transcriptional responses in various cell types. Glucocorticoids can affect the expression
 300 levels of up to 20% of all genes in immune cells⁴⁵. However, the number of genes affected
 301 differs considerably between cell types. In 2019, one study examined the glucocorticoid-
 302 induced changes in the transcriptome of various human primary cells (that is, monocytes,
 303 CD4 T cells, B cells, neutrophils, fibroblasts, myoblasts, preadipocytes, osteoblasts and
 304 endothelial cells). The cells were treated with therapeutic levels of glucocorticoids for
 305 between 2 and 6 hours⁴⁴. Notably, only a small number of genes were influenced by
 306 glucocorticoids in the same way across the different cell types. By contrast, each lineage had
 307 a distinct expression profile that only partially overlapped with the other cell types
 308 examined. This finding suggests that identifying some of the critical anti-inflammatory
 309 actions of glucocorticoids on specific cell types might lead to the development of more
 310 selectively targeted medications [Au: OK?]. However, these results also imply that
 311 therapeutic glucocorticoids in clinical practice target a range of cell types and cellular targets
 312 such that determining the exact mechanism by which glucocorticoids have beneficial
 313 effects, and testing these effects [Au: OK? Is this what you meant? Or else, this what (i.e.
 314 "this" requires a noun)] in adequately powered clinical trials, might prove extremely
 315 difficult.

Comment [MC54]: Yes ok

Comment [MC55]: Yes exactly what is meant

316 Attempts to identify the exact target cells (for example, T cells, macrophages, dendritic cells
 317 and stromal cells [Au: Are these examples of target cells, or examples of potential target
 318 cells?]) of glucocorticoids in various rheumatic diseases has proven difficult. Even for cells
 319 that have been implicated in mediating glucocorticoid effects, identifying the specific
 320 cellular targets [Au: meaning molecular targets?] and/or consequences in these cells have
 321 proven difficult to dissect. In various contexts glucocorticoids have possible anti-
 322 inflammatory effects that can be mediated through changes in cellular proliferation, survival
 323 or differentiation, reduced expression of inflammatory mediators or increased expression of
 324 anti-inflammatory factors. Specific cellular factors, including nuclear factor-κB (NF-κB), AP-1,
 325 annexins, dual-specific phosphatases, glucocorticoid-induced leucine zipper and microRNAs,
 326 are considered important glucocorticoid targets in a variety of cell types⁴⁶. None of these
 327 factors have proven to be a dominant mechanism by which glucocorticoids exert their anti-
 328 inflammatory action. Selected examples of molecular pathways and processes thought to be
 329 important targets of glucocorticoid actions in specific tissues are highlighted in Table 1.

Comment [MC56]: These are examples of potential targets. The actual targets are not known

Comment [MC57]: Yes molecular is better

330

331 [H2] Insights from mouse models

332 Additional insights into the specific targets of glucocorticoids in inflammatory arthritis have
333 come from genetically modified mice that have targeted alterations of glucocorticoid
334 receptor expression or signalling capacity⁴⁷. These studies (summarised in Table 2) have
335 examined the critical target cells for therapeutic glucocorticoids in mouse [Au: OK?] models
336 of acute and chronic polyarthritis. These inflammatory models include adjuvant-induced
337 arthritis (AIA) and serum transfer induced arthritis (STIA). One study examined the effect of
338 glucocorticoid receptor deletion in various cell types on the ability of glucocorticoids to
339 suppress inflammation and joint swelling in the AIA model⁴⁸. Deletion of the glucocorticoid
340 receptor in T cells prevented the therapeutic effects of glucocorticoids but deletion of the
341 glucocorticoid receptor in stromal cells (which includes synovial fibroblasts, chondrocytes
342 and osteoblasts) did not. Notably, glucocorticoids suppressed T helper 17 (T_H17) type
343 responses [Au: OK? Referring to in the mice with T-cell specific GR deletion].

Comment [MC58]: Yes ok

344 In 2018, the same group of researchers examined the mediators of glucocorticoid therapy in
345 the STIA model.⁴⁹ Using a combination of approaches (including inducible gene deletion
346 using Cre-*lox* technology and bone marrow chimeras) they demonstrated that the anti-
347 inflammatory action of glucocorticoids in this model was not mediated via T cells or other
348 cells of haematopoietic origin. Only when the glucocorticoid receptor was present in the
349 stromal cell compartment was dexamethasone treatment able to reduce inflammation. As
350 such, glucocorticoids seem to mediate different anti-inflammatory effects via two entirely
351 separate cell types in two mouse models commonly used to model inflammatory arthritides
352 such as RA. Further adding to the diversity of cellular targets of glucocorticoids, in a model
353 of allergic dermatitis, the presence of glucocorticoid receptor in cells of the monocyte-
354 macrophage or neutrophil lineages seemed essential for maintaining the beneficial effects
355 of [Au: OK? Is this what you meant?] glucocorticoids⁵⁰. These studies clearly show that the
356 effects of glucocorticoids are probably mediated by different cell types in different diseases
357 and that the exact targets cannot be reliably predicted without experimental testing using
358 approaches such as those described above.

Comment [MC59]: No. Glucocorticoids suppressed responses in the wild type mice but not in the T cell specific GR deletion indicating that the GR in T cells regulated Th17 responses.

359 Although the anti-inflammatory actions of glucocorticoids are generally assumed to be
360 mediated by effects on immune cells, other experiments also suggest a role for the stromal
361 compartment in modulating inflammation. Glucocorticoid signalling in specific cell types can
362 be blocked by ectopic expression of the 11 β HSD2 enzyme. Artificial expression [Au:
363 expression or overexpression?] of 11 β HSD2 in chondrocytes resulted in increased levels of
364 joint inflammation in the AIA and STIA mouse models⁵¹. This finding suggests that
365 glucocorticoids can mediate anti-inflammatory effects via targeting chondrocytes. By
366 contrast, 11 β HSD2 expression in osteoblasts resulted in reduced joint inflammation in mice
367 with STIA⁵². These results indicate that, paradoxically, glucocorticoid signalling in some
368 stromal cells can have pro-inflammatory effects in arthritis. Glucocorticoids are also
369 recognised to have pro-inflammatory effects in other cell types such as microglia⁵³.

Comment [MC60]: Yes ok

Comment [MC61]: Expression. The enzyme is not normally present in chondrocytes so this is referring to the artificial expression of an enzyme that is not normally there

370

371 **[H1] Mechanisms underlying adverse effects [Au: Shortened title OK?]**

Comment [MC62]: Yes ok

372 Long term use of therapeutic glucocorticoids is associated with a range of adverse effects.
373 The effects that have been studied most extensively are the actions of glucocorticoids on
374 bone, muscle and glucose metabolism (figure 5).

375 *[H2] Effects of glucocorticoids on bone*

376 An excess of glucocorticoids **[Au: OK? What would be considered “excess”? Or is this not**
377 **quantifiable?]** is associated with reduced bone mineral density, impaired bone quality and
378 an increased risk of fracture⁷. The relationship between glucocorticoids and bone is
379 particularly complicated in rheumatic diseases as inflammation itself has a detrimental
380 effect on bone⁵⁴. It is likely that glucocorticoid suppression of inflammation has a net
381 positive effect on bone metabolism but that the continued use of glucocorticoids when
382 inflammation is low is detrimental. Glucocorticoids have effects on all the major cells
383 involved in bone metabolism (the specific targets are highlighted in figure 5 and have been
384 reviewed elsewhere^{7, 54}). Glucocorticoids impair osteoblast proliferation and reduce their
385 ability to produce bone matrix proteins³⁹. High doses of glucocorticoids can cause apoptosis
386 of osteocytes, the most abundant cell type within the skeleton and a critical mediator of the
387 balance between bone resorption and formation⁵⁵. Glucocorticoids also stimulate the
388 activity of osteoclasts but during long term treatment the production of osteoclasts **[Au: by**
389 **“production of osteoclasts” are you referring to osteoclast differentiation?]** is suppressed
390 by glucocorticoids⁵⁵. At a molecular level, the changes that occur in bone in response to
391 glucocorticoids can be largely explained by the effects of glucocorticoids on inhibiting
392 anabolic bone signalling in osteocytes and osteoblasts; in these cells **[Au: OK? Do**
393 **glucocorticoids stimulate the next effects in both cells types? or just one of these cell**
394 **types?]**, glucocorticoids stimulate the expression of inhibitors of anabolic bone signalling
395 and thus reduce bone formation and increase the expression of the pro-osteoclastogenic
396 factor receptor activator of NF-κB ligand (RANKL)⁵⁶. In GR^{dim/dim} mice, the adverse effects of
397 glucocorticoids on the skeleton still occur, despite the reduced capacity of the
398 glucocorticoid receptor to dimerise³⁹. This finding suggests that these adverse effects on
399 bone are mediated through glucocorticoid receptor transrepression **[Au:OK?]**.

Comment [MC63]: Good point. Glucocorticoids are normally present but if levels are too high from tumours or drugs then these problems develop. It might be phrased ‘Exposure to supraphysiological levels of glucocorticoids (either endogenous or pharmacological is associated....’

Comment [MC64]: Glucocorticoids stimulate differentiation of mature osteoclasts but inhibit differentiation of very early precursors. It might be changed to ‘long term treatment the differentiation of early precursors to mature osteoclasts’

Comment [MC65]:

Comment [MC66]: This applies to both cell types (the cell types are hard to tell apart in reality)

Comment [MC67]: Yes ok

401 *[H2] Effects of glucocorticoids on muscle*

402 Long term glucocorticoid use is associated with reduced muscle mass and strength **[Au:**
403 **Could you please reference this statement?]**. **In patients with RA receiving therapeutic**
404 **glucocorticoids, muscle wasting is rapid, long lasting and is a considerable morbidity factor**
405 **that increases the risk of subsequent falls and fractures^{59, 62, 65-67} [Au: I moved this**
406 **highlighted sentence to here to improve the flow, OK?]**. The specific pathways involved in
407 glucocorticoid-induced muscle wasting **[Au: OK?]** are highlighted in figure 5. In patients

Comment [MC68]: I would suggest original references 59 and 60 (Lofberg et al, wang R et al)

Comment [MC69]: Yes ok

Comment [MC70]: Yes ok

408 receiving therapeutic glucocorticoids, muscle wasting is mediated by both a robust
 409 reduction in anabolic protein synthesis and an increase in protein degradation [Au:OK? (I
 410 split up this sentence as it was fairly long)]. The reduction in anabolic protein synthesis is
 411 secondary to suppression of the PI3K–AKT–mTOR pathway and downstream targets
 412 ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein 1
 413 (4EBP1, also known as eIF4EBP1) [Au: OK? Do these targets mediate the anti-anabolic
 414 effects mentioned next? (or else, how to the two sentences connect?)]. The anti-anabolic
 415 effects are mediated by a plethora of changes, including the suppression of anabolic
 416 signalling via insulin-like growth factor I (IGF1) and insulin receptor substrate 1 (IRS1), and
 417 the induction of the anti-anabolic factors myostatin and DNA damage-inducible transcript 4
 418 protein (DDIT4, also known as REDD1) [Au: Edits OK? I moved this sentence here to
 419 improve the flow, OK?]^{57, 60-62}. By contrast, increased protein degradation is mediated by
 420 E3-protein ligases, E3 ubiquitin-protein ligase TRIM63 and F-box only protein 32 [Au:OK?],
 421 increased activity of the ubiquitin proteasomal degradation pathway, and increased
 422 autophagy⁵⁷⁻⁵⁹. The induction of catabolic proteosomal degradation and autophagy seem to
 423 be driven through an direct induction of forkhead box protein O1 (FOXO1) by
 424 glucocorticoids and an indirect induction [Au: An indirect induction of what? FOXO1?]
 425 secondary to suppression of the PI3K–AKT–mTOR pathway^{63, 64}.

Comment [MC71]: Yes ok

Comment [MC72]: The other way around. The most downstream effects are on pi3k etc and these effects are secondary to changes in the pathways described next.

Comment [MC73]: ok

Comment [MC74]: ok

Comment [MC75]: should be 'a' rather than an

Comment [MC76]: yes FOXO1

426

427 [H2] Effects of glucocorticoids on glucose and lipid metabolism

428 Glucocorticoids have complex effects on the distribution of fat and the regulation of energy
 429 substrates at a systemic level^{68, 69}. Glucocorticoids have effects on all the tissues involved in
 430 glucose and lipid metabolism (including the liver, muscle, adipose tissue and endocrine
 431 pancreas). A coordinated change in systemic energy metabolism is a feature of the stress
 432 response; hence, glucocorticoid-induced changes are probably simply a magnification of
 433 these changes. Given the diversity of targets of glucocorticoids, no single mechanism or
 434 cellular target within these tissues has been identified. Interestingly, some evidence from
 435 rodent [Au: meaning mouse?] studies suggest that glucocorticoid-induced changes in bone
 436 [Au:OK?] might mediate, at least in part, some of the effects of glucocorticoids on systemic
 437 energy metabolism⁷⁰. Mice with ectopic expression of 11 β HSD2 in osteoblasts and
 438 osteocytes, and thus an abrogation of glucocorticoid signalling selectively in these tissues,
 439 [Au: By these tissues do you mean bone? Or do you mean these cells?] do not develop
 440 insulin resistance and glucose intolerance in response to glucocorticoid therapy, unlike their
 441 wild-type counterparts. The osteoblast-specific protein osteocalcin [Au: Meaning
 442 osteocalcin produced by osteoblasts?] is a potential mediator between bone and energy
 443 metabolism [Au: Ok? On the basis of what study(s)? Could you reference the study here?].
 444 In other contexts, osteocalcin can improve insulin sensitivity through a variety of actions on
 445 the liver and pancreas⁷¹, but osteocalcin levels [Au: meaning expression or activity?] are
 446 notably inhibited by glucocorticoids. In support of this concept, mice with heterotopic

Comment [MC77]: yes mouse

Comment [MC78]: yes ok

Comment [MC79]: cells is more accurate please change

Comment [MC80]: meaning that osteocalcin is only made by osteoblasts. It is not made anywhere else under normal circumstances

Comment [MC81]: please use reference 71 (lee et al.)

Comment [MC82]: actually this means the levels in the blood. Maybe 'the concentration of osteocalcin in the blood is notably' would be better

447 expression of osteocalcin in the liver are protected against the effects of glucocorticoids on
448 metabolism⁷⁰.

449

450 **[H1] Therapeutic implications [Au: H1 subheading OK?]**

451 Although the mechanisms underlying the adverse effects of glucocorticoids are well defined
452 (figure 5), this information has only resulted in approaches for reducing the effects of
453 glucocorticoids on bone [Au: OK? is this what you meant? Or did you mean in the context
454 of bone diseases (in which case, which diseases are you referring to)?]^{7, 12, 70}. Notably, the
455 cessation of therapeutic glucocorticoids results in a gradual recovery of bone mass and a
456 return of normal anabolic bone formation over time⁷². By contrast, although further
457 confirmatory studies are required, muscle loss associated with intra-muscular glucocorticoid
458 injections seems to occur rapidly and has a limited capacity to return to pre-treatment levels
459 ⁶⁵. Currently, anabolic and anti-catabolic treatments to manage muscle loss in this context
460 are limited to exercise interventions, for which limited evidence of their efficacy in rheumatic
461 diseases is available [Au: OK?]. A hope for many years has been that SEGRAs might retain
462 the anti-inflammatory activity of glucocorticoids but have reduced effects on metabolism.
463 The development of systemically-administered SEGRAs has proven difficult and no SEGRA
464 has yet made its way into clinical use in rheumatic diseases⁷³⁻⁷⁵. However, one potential
465 SEGRA, fosdagrocorat, is currently being evaluated in the context of treatment of RA⁷⁵. In
466 the phase 2a study, fosdagrocorat [Au: OK?] treatment resulted in a reduction in disease
467 activity [Au: reduction compared with baseline, or reduction compared with a control
468 group?] with no notable adverse effects after 2 weeks. A follow up 12 weeks study in 323
469 patients with moderate to severe RA has compared various doses of fosdagrocorat and
470 prednisone against a placebo, and has had encouraging results [Au: Please reference this
471 study here]. At doses that gave equivalent ACR response rates [Au: Which ACR response
472 rate? ACR20?] to prednisone [Au: Predidone at what dose - the standard recommended
473 dose?], fosdagrocorat was associated with a reduction in levels of glycosylated haemoglobin
474 subunit β [Au: OK?] whereas prednisone treatment was not [Au: What is the relevance of
475 reduced levels of glycosylated haemoglobin? Please clarify for the non-specialist reader
476 (i.e. how this is linked to glycaemia)]. This finding suggests that this drug has the potential
477 to suppress inflammation (through repression) but has less effect on glycaemic control than
478 currently-used glucocorticoids [Au: OK? Or prednisone specifically?] (owing to reduced
479 transactivation). Further trials of this medication in RA and other conditions are awaited.

480 Another notable adverse effect of glucocorticoid therapy is suppression of adrenal function.
481 This effect is caused by feedback of glucocorticoids on the hypothalamus and pituitary
482 glands, resulting in inhibition of corticotropin-releasing hormone and adrenocorticotrophic
483 hormone protein synthesis [Au: Clarify what these enzymes are involved in (such as
484 cortisol production) (for non-specialist readers)?]. Up to one third of patients with RA
485 treated with low-dose glucocorticoids have clinically notable suppression of adrenal

Comment [MC83]: yes ok

Comment [MC84]: this is the correct meaning

Comment [MC85]: yes ok

Comment [MC86]: yes ok

Comment [MC87]: compared to baseline

Comment [MC88]: Buttgerit F, Strand V, Lee EB, Simon-Campos A, McCabe D, Genet A, Tammara B, Rojo R, Hey-Hadavi J. Fosdagrocorat (PF-04171327) versus prednisone or placebo in rheumatoid arthritis: a randomised, double-blind, multicentre, phase IIb study. RMD Open. 2019 Apr 16;5(1):e000889. doi: 10.1136/rmdopen-2018-000889. eCollection 2019.

Comment [MC89]: Yes ACR20

Comment [MC90]: The study was complex using various doses but for these analyses the prednisone dose was 10mg per day

Comment [MC91]: No. This is actually HbA1c so it would be the A chain. However, the standard term used is glycosylated haemoglobin or HbA1c.

Comment [MC92]: I think any clinician will be familiar with HbA1c etc. However, after haemoglobin you could add (an indicator of average blood glucose levels over time)

Comment [MC93]: I would stick with glucocorticoids since highly related forms such as prednisolone are used in some parts of the world.

Comment [MC94]: This would be too complex. There are about 8 enzymes involved in the adrenal alone. The sites of negative feedback for CRH are complex involving lots of brain areas. Most clinicians know that glucocorticoids switch off the adrenals.

486 function⁷⁶. Glucocorticoid feedback at the hypothalamus and the pituitary seem to depend
 487 on the transrepression function of glucocorticoids^{77,78} and thus SEGRAs would be expected
 488 to also suppress endogenous cortisol production. In this situation, patients treated with
 489 SEGRAs would have no glucocorticoid available for transactivation functions. This effect
 490 could potentially lead to glucocorticoid deficiency in tissues that depend on transactivation
 491 for their normal metabolic function, the consequences of which are unclear [Au: OK?].

Comment [MC95]: Yes ok

492 A clinical problem that remains unaddressed is that of 'glucocorticoid resistance'. This term
 493 is generally used to describe situations in which particular diseases or inflammatory
 494 pathways that are usually responsive to glucocorticoids fail to respond to glucocorticoid
 495 treatment or lose sensitivity to glucocorticoids over time. The mechanistic basis for
 496 glucocorticoid resistance is unclear but possible mechanisms involve reduced glucocorticoid
 497 receptor expression in target tissues, upregulated expression of specific glucocorticoid
 498 receptor isoforms that are less effective in suppressing inflammation in the target tissues
 499 [Au:OK?], disease-induced changes to chromatin structures that result in reduced access of
 500 glucocorticoid receptors to GREs or the switching on of inflammatory pathways that are
 501 intrinsically resistant to glucocorticoid suppression⁷⁹. Unfortunately, attempts to reverse or
 502 overcome glucocorticoid resistance have been unsuccessful to date. One approach that has
 503 been attempted has been to pharmacologically open chromatin to enable access of the
 504 glucocorticoid receptor to otherwise hidden GREs in patients with chronic obstructive
 505 pulmonary disease [Au: Could you please reference this statement?]. This approach was
 506 tested on the basis of in vitro data indicating that low-dose theophylline (a methylxanthine
 507 drug currently used in the treatment of respiratory diseases [Au: Addition OK?]) could
 508 stimulate the activity of histone deacetylases important for promoting glucocorticoid
 509 sensitivity [Au: OK?]⁸⁰. Unfortunately, in a large randomised controlled trial, theophylline
 510 did not improve the effectiveness of inhaled glucocorticoids in patients with chronic
 511 obstructive pulmonary disease⁸¹.

Comment [MC96]: Yes ok

Comment [MC97]: This would be the same as below ref 80 (Ito et al)

Comment [MC98]: Yes ok

Comment [MC99]: Yes ok

512

513 [H1] Conclusion

514 In the first two decades after the introduction of cortisol and cortisone, structural
 515 modification of glucocorticoids led to the successful introduction of oral and intravenous
 516 therapeutics that we still use today. These glucocorticoids have a high tissue penetration, a
 517 prolonged half-life and high affinity for the glucocorticoid receptor. Subsequent advances in
 518 modulating glucocorticoid properties in other medical disciplines have generally been on
 519 the basis of manipulating the pharmacokinetic properties of glucocorticoids to better target
 520 drugs to specific tissues (and thus limit systemic adverse effects) or to limit their activity to
 521 specific times of the day, rather than manipulating the molecular mechanisms of action of
 522 these drugs. This trend looks set to continue given the difficulty in unravelling the complex
 523 effects of glucocorticoids at a cellular level and the advances in development of novel drug
 524 delivery systems.

525 The actual cells and cellular targets most important to the action of glucocorticoids remain
 526 obscure for most rheumatic diseases. This lack of knowledge prevents the development of
 527 more specific therapeutic agents on the basis of how glucocorticoids work. Without these
 528 more specific agents, glucocorticoid use in these conditions is likely to continue. The
 529 research approaches most likely to lead to specific targets include genetically modified mice
 530 with tissue specific alteration of glucocorticoid sensitivity and transcriptome studies using
 531 primary human cells. [Au: Could you perhaps comment on what the future direction is for
 532 this aspect of glucocorticoid therapy - i.e. are efforts still ongoing to understand these
 533 underlying mechanisms that might still be informative in future?]

Comment [MC100]:

Comment [MC101]: Hopefully addressed this weakness

534 In the meantime, glucocorticoids will probably remain important drugs, particularly during
 535 initial disease management, for rapid control of disease flare and, for some people, for long
 536 term maintenance therapy at a low dose. In these situations, structured approaches to
 537 'glucocorticoid stewardship' will be needed to ensure patients are treated with the
 538 minimum dose of glucocorticoids required to achieve the beneficial effects.

539

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877

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 880 Inflammatory Arthritis Centre at the University of Birmingham. ~~xxxx~~ [Au: This is an OPTIONAL
 881 section. Space is available to note any acknowledgements you would like to be included with the
 882 article, such as grant support or editorial assistance]

883

884 **Author contributions**

885 The authors contributed equally to all aspects of the article.

886

887

888 **Competing interests**

889 The authors declare no competing interests. [Au:OK?]

890

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895 **Supplementary information**

896 Supplementary information is available for this paper at <https://doi.org/10.1038/s415XX-XXX-XXXX-X>

897

898 **Key points**

- 899 • Therapeutic glucocorticoids are powerful, broad spectrum anti-inflammatory agents
 900 that are limited by a wide range of adverse effects.
- 901 • The specific mechanisms of action by which glucocorticoids mediate anti-
 902 inflammatory effects in rheumatic diseases are still unclear, hindering the
 903 development of novel therapeutic agents [Au: OK (i.e. I merged the last bullet point
 904 you had to this one, as they were very similar)]

Comment [MC102]: Yes ok

Comment [MC103]: Yes ok

- Approaches to the study of glucocorticoid actions have been complicated by the widespread use of animal tissues and transformed cell lines rather than human primary cells.
- The development of novel glucocorticoids that ‘dissociate’ molecular transrepression from transactivation have proven difficult, however, one such dissociated glucocorticoid is undergoing clinical trials in patients with inflammatory arthritis.
- The use of genetically modified mice with altered glucocorticoid sensitivity in specific tissues and transcriptomic studies using primary human cells are the most promising approaches to define the most important cellular and molecular targets of glucocorticoids.

[Au: Ideally, you should have 4-6 key points that mention the main aspects covered in this Review. Currently, these points only mentioned the unknown mechanisms and limitations of mouse models. Could you include some additional key points - perhaps one on glucocorticoid receptor signalling, one on cellular targets and one on therapeutic implications - to help give a nice coverage of the different aspects covered in this Review?]

Comment [MC104]: Hopefully addressed

Figure 1: Natural and synthetic glucocorticoids [Au: OK?]

Comment [MC105]: Yes ok

The molecular structures of the endogenous glucocorticoid cortisol and common synthetic glucocorticoid derivatives prednisolone, methylprednisolone, triamcinolone, dexamethasone and betamethasone. A hydroxyl group at position 11 of the steroid ring (highlighted in red) is critical to the activity of these glucocorticoids.

[Au: For part A, why is one of the "HO"s in red? Is this a key position shared by all the derivatives?]

Comment [MC106]: Hopefully addressed

Figure 2: Systemic and local metabolism and inactivation of circulating glucocorticoids.

Circulating glucocorticoids shuttle between their inactive form, mediated by dehydrogenase inactivation by corticosteroid 11 β -dehydrogenase isozyme 2 (11 β HSD2) in the kidneys, and their active form, mediated by oxoreductase activation by 11 β HSD1 in the liver. Intracellular pre-receptor metabolism determines local activation and inactivation of glucocorticoids. Cells expressing 11 β HSD1 increase local glucocorticoid activation and glucocorticoid receptor ligand binding. By contrast, cells expressing 11 β HSD2 rapidly inactivate glucocorticoids, protecting the mineralocorticoid receptor from inappropriate glucocorticoid ligand binding and activation. 11keto and 11 β -hydroxyl steroids are irreversibly 5 α or 5 β reduced to their inactive metabolites tetrahydrocortisone, 5 α -tetrahydrocortisol and tetrahydrocortisol by the actions 5 α and 5 β reductase. Further metabolism by 20 α and 20 β reductase yields inactive α and β cortolones and cortols. THE, tetrahydrocortisone;

[Au: What does "ALDO" refer to in this figure?]

Comment [MC107]: ALDO is aldosterone

941

942 **Figure 3: Glucocorticoid receptors**

943 A) The structural domains of the glucocorticoid receptor isoforms glucocorticoid receptor α
944 (GR α) and GR β . B) During glucocorticoid receptor binding, homodimers of GR α bind to the
945 glucocorticoid response element (GRE) to regulate gene expression whereas GR α -GR β
946 heterodimers function as dominant negative inhibitors, antagonising the activity of GR α .
947 DBD (DNA binding domain); LBD (ligand binding domain)

948

949 **Figure 4: Molecular mechanisms of glucocorticoid receptor signalling.**

950 Glucocorticoid receptor signalling can involve transactivation (a), transrepression (b) or other
951 mechanisms (c). These mechanisms can involve either dimeric or monomeric receptors; can
952 involve direct binding of these receptor complexes to DNA or indirect effects on other DNA
953 binding factors; or can sometimes involve interactions in the cytoplasm or cell membrane.
954 For direct dimer transactivation and transrepression, ligand-bound GR α homodimers bind
955 to glucocorticoid response elements (GREs) to elicit either direct induction or suppression of
956 downstream gene expression. For monomer signalling, ligand-bound monomeric GR α bind
957 to GREs and recruit co-activators or co-repressors to influence secondary transcription
958 factor regulation of gene expression (mediating transactivation or transrepression,
959 respectively). For monomeric tethering, ligand-bound monomeric GR α bind directly to a
960 secondary transcription factor to either positively or negatively regulate downstream gene
961 expression (transactivation or transrepression, respectively). For cell membrane receptor
962 signalling, glucocorticoids bind to cell membrane-bound receptors and mediate
963 transmembrane activity resulting in non-genomic signalling. For chaperone protein
964 signalling, the disassociation of chaperone proteins from the GR α on the binding of
965 ligand liberates the chaperone proteins such they can influence changes in intracellular
966 signalling pathways mediated by unbound chaperone proteins secondary to their
967 disassociation from ligand bound GR α . For PI3K competition, ligand bound GR α can
968 sequester PI3K modifying preventing its ability to activate AKT and regulate downstream
969 AKT signalling the ability of PI3K to influence AKT signalling. [Au: Could you please clarify
970 what you mean by this last sentence?].

971 [Au: Does "RE" (following XY and Nk κ B) refer to "response elements"?)

972 [Au: You've also mentioned "competition for PI3K" in the figure - could you briefly
973 mention this aspect in the figure legend?]

974

975 **Figure 5: The deleterious actions of glucocorticoids in muscle and bone.**

Comment [RH(oMaSR108): Mark. The figure for this implies that PI3K bound to GR can still signal. I don't think this is the case. Can you make a note for artistic editor that GR binding blocks PI3K signalling (Figure 4)

Comment [MC109]: Hopefully done

Comment [MC110]: This is not correct. It should be NF-kappaB with kappa as a symbol. It stands for nuclear factor kappa B.

Comment [MC111]: Yes RE is response element.

Comment [MC112]: Hopefully done

976 In muscle, glucocorticoids induce catabolic (E3 ligase and FOXO1) and anti-anabolic
 977 (Myostatin and DDIT4) signalling pathways and suppress anabolic signalling pathways (IGF-
 978 1, S6K1, 4E-BP, PI3K, AKT and mTOR), resulting in muscle wasting. In osteocytes,
 979 glucocorticoids directly induce the release of the anti-anabolic Wnt inhibitor sclerostin and
 980 induce osteocyte autophagy and apoptosis through increased BIM, ER stress and ATG7
 981 signalling. Glucocorticoids suppress bone formation by inhibiting factors that regulate
 982 osteoblast differentiation and proliferation (Wnt, BMP, TGFβ [Au: OK?], DKK1, sex steroids,
 983 AP-1) and inducing factors that induce osteoblast apoptosis and autophagy (PyK2, JNK, Bim,
 984 E4BP4, ER stress). Osteoclastic Bone resorption is directly upregulated by glucocorticoids via
 985 osteoclasts [Au: by glucocorticoids?] through the direct suppression of OPG in combination
 986 with- induction of proteolytic enzymes in osteoclasts such as collagenase 3. Glucocorticoids
 987 can also have secondary effects on bone metabolism (green boxes). Briefly, glucocorticoids
 988 directly suppress lower OPG secondary to the reduction in mature osteoblasts and
 989 osteocyte numbers, whilst the reduction in muscle mass results in reduced loading and
 990 mechanosensing by osteoclasts, which further suppresses OPG production by these cells. .
 991 suppression of osteoblast differentiation and maturation [Au: If they directly suppress
 992 OPG, isn't this a primary (rather than secondary) mechanism that should instead be
 993 mentioned above?] in osteocytes and osteoblasts, as well as indirectly suppress OPG in
 994 response to decreased mechanosensing in patients with muscle wasting [Au: Could you
 995 explain this mechanism a little more (I'm not sure I understand the link between muscle
 996 mass, mechanosensing and OPG production) - perhaps you could expand on this slightly
 997 for clarity?]. This occurs in conjunction with the direct induction of RANKL in osteoblasts.
 998 Together these shift the OPG to RANKL ratio in favour of increased osteoclast maturation
 999 and activation and increased bone resorption. Therapeutic interventions are able to
 1000 prevent bone loss through their targeting of either anabolic or catabolic bone metabolism
 1001 in osteoblasts and osteoclasts. The parathyroid hormone analogue tTeriparatide promotes
 1002 anabolic bone formation in osteoclasts, promoting their differentiation and survival. Anti-
 1003 catabolic agents such as bBisphosphonates directly promote cell death and apoptosis in
 1004 osteoclasts, whilst dDenosumab blocks RANKL signalling in osteoclasts, preventing their
 1005 maturation and activation.

1006 [Au: You also include therapeutic interventions in this figure (bisphosphates, denosumab
 1007 and teriparatide). Could you mention these aspects in the figure legend?]

1009 **Table 1:** Effects of therapeutic glucocorticoids on different cell types [Au: Shortened title
 1010 OK?]

Cell type	Effect of therapeutic glucocorticoids on cellular responses	References [Au: I've created row for each effect as we no longer have bullet points in our tables (and so this seemed to be the best way to display this information). However, I wasn't sure which reference referred to which effect - could you

Comment [MC113]: yes

Comment [RH(oMaSR114): addressed

Comment [RH(oMaSR115): this has been expanded

Comment [RH(oMaSR116): These have been included

Comment [MC117]: yes ok

		please move each to the correct row (making sure there is at least one reference for each?)
Adaptive immune cells		
T helper cells	Decreased Th1 and Th17 differentiation and cytokine production Decrease cytokine production	Lieberman AC et al. Cytokine Growth Factor Rev. (2007)⁸² Franchimont D et al. J Immunol. (2000)⁸⁴
	Increase apoptosis	Reichardt HM et al. Cell. (1998)³⁷
	Decrease T cell signalling	Franchimont D et al. J Immunol. (2000)⁸⁴
	Increased T _H 2 differentiation and cytokine production	Franchimont D et al. J Immunol. (2000)⁸⁴ Ramírez F et al. J Immunol. (1996)⁸³
	Increased T _H 2 effects	
Cytotoxic T cells	Decreased cytokine production	Schleimer RP et al. J Immunol. (1984)⁸⁶
	Increased apoptosis	Migliorati G et al. Pharmacol. Res. (1992)⁸⁵
	Decreased T cell signalling	Schleimer RP et al. J Immunol. (1984)⁸⁶
	Decreased cytotoxic capacity	Schleimer RP et al. J Immunol. (1984)⁸⁶
B cells	Decreased B cell receptor signalling	Cupps TR et al. J Clin Invest. (1985)⁸⁸ Franco LM et al. J Exp Med. (2019)⁴⁴
	Increased apoptosis	Lill-Elghanian D et al. Exp Biol Med. (2002)⁸⁷
	Decreased TLR7 and BCR signalling	Franco LM et al. J Exp Med. (2019)⁴⁴
	Upregulation of BLIMP1 and IL-10	Franco LM et al. J Exp Med. (2019)⁴⁴
Innate immune cells		
Mast cells	Decreased Toll like receptor signalling	Zhou J et al. Allergy. (2008)⁸⁹
	Increased histamine release	Zhou J et al. Allergy. (2008)⁸⁹
Macrophages	Decreased pro-inflammatory cytokines	Franchimont D. Ann N Y Acad Sci. (2004)⁹² Zhou JY et al. Br J Surg. (2010)⁹³
	Increased pro-resolution cytokines	Barczyk K et al. Blood. (2010)⁹⁰ Franchimont D. Ann N Y Acad Sci. (2004)⁹²
	Increased efferocytosis phagocytosis	McColl A et al. J Immunol. (2009)⁹¹ Zhou JY et al. Br J Surg. (2010)⁹³
	Increased M2 polarisation	Barczyk K et al. Blood. (2010)⁹⁰
	Decreased Toll like receptor signalling	Franchimont D. Ann N Y Acad Sci. (2004)⁹²
Neutrophils	Increased production	Cavalcanti DM et al. Pharmacol. (2007)⁹⁵
	Decreased extravasation	Filep, JG et al. Circulation (1997)⁹⁴ Cavalcanti DM et al. Pharmacol. (2007)⁹⁵
Basophils or Eosinophils	Decreased Toll like receptor signalling	Mogensen TH et al. Infect Immun. (2008)⁹⁸
	Increased apoptosis	Sivertson KL et al. Cell Immunol. (2007)⁹⁷
	Increased expression of CXCR4 and migration to the spleen, bone marrow and lymph	Khoury P et al. Allergy. (2018)⁹⁶
Resident mesenchymal cells		
Osteoblasts or osteocytes	Decreased differentiation	Rauch A et al. Cell (2010)³⁹
	Increased apoptosis	Chen F et al. Calcif Tissue Int. (2014)⁹⁹
	Increased RANKL	Swanson C et al. Endocrinology (2006)¹⁰⁰

Comment [RH(oMaSR118): I have amended the headings and deleted one column to address the point on T cell function

		Humphrey EL et al. Bone. (2006)¹⁰¹
	Decreased OPG	Swanson C et al. Endocrinology (2006)¹⁰⁰ Humphrey EL et al. Bone. (2006)¹⁰¹
Chondrocytes	Increased MMP activity	Huang Y et al. J Steroid Biochem Mol Biol. (2018)¹⁰²
	Decreased GAC production	Huang Y et al. J Steroid Biochem Mol Biol. (2018)¹⁰²
Myoblasts and fused Myotubes Myotubes [Au: What cells are you referring to?]	Increased proteolysis	Braun TP et al. Front Physiol. (2015)¹⁰³
	Increased autophagy	Troncoso R et al. Cell Cycle. (2014)¹⁰⁴
Stromal fibroblasts	Decreased cytokine and chemokine production	Hardy RS et al, Arth Res Ther (2006) ¹⁰⁵
	Decreased invasiveness	Durmus M et al. Anesth Analg. (2003)¹⁰⁶
	Decreased lymphocyte adhesion	Durmus M et al. Anesth Analg. (2003)¹⁰⁶
	Decreased wound healing	Durmus M et al. Anesth Analg. (2003)¹⁰⁶
Cells linking innate & adaptive immunity Innate/adaptive link immune cells [Au: What do you mean by "adaptive link immune cells"?)		
Natural killer cells	Increased activation	Pitzalis C et al. J Immunol. (1997)¹⁰⁷ Pitzalis C et al. Ann N Y Acad Sci. (2002)¹⁰⁸
Dendritic cells	Decreased cytokine production	Cao Y et al. Blood. (2013)²⁸
	Decreased maturation	Elftman MD et al. Immunology. (2007)¹⁰⁹ Cao Y et al. Blood. (2013)²⁸
	Increased apoptosis	Cao Y et al. Blood. (2013)²⁸
	Decreased antigen presentation	Elftman MD et al. Immunology. (2007)¹⁰⁹

Comment [RH(oMaSR119): Hopefully this clarifies. Blasts fuse to form multinucleate myotubes (muscle fibres)

Comment [RH(oMaSR120): Clarified

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Table 2: Effects of therapeutic glucocorticoids on mouse models of inflammatory arthritis
[Au: Shortened title OK?]

Comment [MC121]: ok

Study	Inflammatory mouse model [Au: OK?]	Transgenic mouse	Treatment with dexamethasone investigated?	Inflammatory outcome
Koenen et al. Ann Rheum Dis, 2018 ⁴⁹	STIA	Haematopoietic glucocorticoid receptor deletion	Yes	Normal response to glucocorticoid [Au: OK?]
		Stromal glucocorticoid receptor deletion	Yes	Resistant to glucocorticoid [Au: OK?]
Baschant et al. PNAS, 2011 ⁴⁸	AIA	Macrophage glucocorticoid receptor deletion	Yes	Normal response to glucocorticoid
		Dendritic cell	Yes	Normal

Comment [MC122]: yes ok

Comment [MC123]: yes

Comment [MC124]: yes

		GR deletion		response to glucocorticoid
		B cell glucocorticoid receptor deletion	Yes	Normal response to glucocorticoid
		T cell glucocorticoid receptor deletion	Yes	Resistant to glucocorticoids
Tu et al. FASEB, 2018 ⁵¹	STIA	Chondrocyte blockade of glucocorticoid	No	Exaggerated inflammation and joint destruction [Au: OK?]
	AIA	Chondrocyte blockade of glucocorticoid	No	Exaggerated inflammation and joint destruction [Au: OK?]
Buttgereit et al. Arthritis Rheum, 2009 ⁵²	STIA	Osteoblast blockade of glucocorticoid signalling	No	Attenuated inflammation and joint destruction [Au: OK?]
Hardy et al. J Autoimm, 2018 ¹²	TNF-transgenic model of arthritis [Au: OK?]	Global blockade of 11 β -HSD1 glucocorticoid activation	No	Exaggerated inflammation and joint destruction [Au: OK?]
		Stromal blockade of 11 β -HSD1 glucocorticoid activation	No	Attenuated inflammation and joint destruction [Au: OK?]
Coutinho et al. Endocrinology, 2012 ¹³	STIA	Global blockade of 11 β -HSD1 glucocorticoid activation	No	Exaggerated inflammation and joint destruction

Comment [MC125]: yes

Comment [MC126]: yes

Comment [MC127]: yes ok

Comment [MC128]: yes ok

Comment [MC129]: yes ok

Comment [MC130]: yes ok

1014 AIA, antigen-induced arthritis; STIA, Serum transfer induced arthritis

1015 **Table of contents blurb**1016 Glucocorticoids are anti-inflammatory therapies commonly used in rheumatology but have
1017 wide-ranging adverse effects. Understanding the pharmacokinetic properties and

Hardy et al.,

Therapeutic Glucocorticoids

1018 mechanisms of action of glucocorticoids could inform in the development of novel therapies
1019 with fewer adverse effects. [Au: OK?]

Comment [MC131]: yes