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The cortisol stress response induced by surgery: a systematic review and meta-analysis

Running title: The cortisol response to surgery.

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SUMMARY

Objective: Surgery is a stressor that can be categorized by duration and severity, and induces a systemic stress response that includes increased adrenal cortisol production. However, the precise impact of surgical stress on the cortisol response remains to be defined.

Design: We performed a systematic review and meta-analysis to assess the cortisol stress response induced by surgery and to stratify this response according to different parameters.

Methods: We conducted a comprehensive search in several databases from 1990 to 2016. Pairs of reviewers independently selected studies, extracted data and evaluated the risk of bias. Cortisol concentrations were standardized, pooled in meta-analysis and plotted over time.

Results: We included 71 studies reporting peri-operative serum cortisol measurements in 2,953 patients. The cortisol response differed substantially between moderately/highly invasive and minimally invasive surgical procedures. Minimally invasive procedures did not show a peri-operative cortisol peak, whereas more invasive surgeries caused a cortisol surge that was more pronounced in older subjects, women and patients undergoing open surgery and general anaesthesia. The duration of the procedure and the use of etomidate for induction of anaesthesia did not affect the cortisol response.

Conclusions: The peri-operative cortisol stress response is dynamic and influenced by patient-specific, surgical and anaesthetic features. However, the available evidence is derived from highly heterogeneous studies, with only two of 71 studies measuring cortisol by mass spectrometry, which currently prevents a precise and reproducible definition of this response.

Key words: Cortisol; Surgery; Stress; Pituitary-Adrenal System; Adrenal Insufficiency; Hydrocortisone; Adrenal cortex.

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INTRODUCTION

The controlled trauma of a surgical insult activates adaptive changes in the neuro-hormonal system and the inflammation response.[1] The primary mechanism responsible for cortisol hypersecretion in response to stress is executed by the afferent nerve signals derived from the surgical site, which in turn stimulate the hypothalamus to release corticotropin-releasing hormone and arginine vasopressin.[2] These two peptides then stimulate secretion of adrenocorticotropic hormone from the anterior pituitary, which stimulates cortisol secretion by the adrenal cortex. Surgery can be considered a standardized model for assessing the cortisol response to stress and relevant modifying factors.

Understanding the expected magnitude of cortisol production during surgical stress is very relevant, especially for patients with adrenal insufficiency who require a dose increase in exogenous cortisol replacement during stress. Primary and secondary adrenal insufficiency affects up to 424/1,000,000 people and comes with the need for lifelong glucocorticoid replacement.[3] Furthermore, according to a recent meta-analysis,[4] 2.4-21.5% of patients receiving chronic treatment with supraphysiological exogenous steroids, e.g. for chronic obstructive pulmonary disease, develop tertiary adrenal insufficiency after glucocorticoid discontinuation. In all these patients, surgical stress required an increase in glucocorticoid replacement dose to avoid a life-threatening adrenal crisis.

Recommendations regarding the dose, administration route, timing, tapering and duration of glucocorticoid therapy vary substantially.[5] Moreover, the recommended doses of perioperative steroid dosing is based on the long-held notion that the activation of the hypothalamic-pituitary-adrenal (HPA) axis, including the adrenal cortisol output in response to surgery, is directly proportionate to the severity of the surgical stress.[6-8] However, most of the data underpinning this assumption derive from works published before 1990 and are based on limited case series. Surgical and anaesthetic techniques, as well as the rigour of

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cortisol assays have changed significantly since then, in particular following the now more widespread introduction of the current diagnostic reference standard mass spectrometry.

Therefore, our objective was to perform a systematic review and meta-analysis of published studies investigating the cortisol response to surgery in order to estimate the expected range of perioperative cortisol concentrations and to identify factors that potentially affect this response.

METHODS AND EVIDENCE ACQUISITION

This systematic review and meta-analysis was performed based on an *a priori* protocol. The methods and results of the review are reported according to the PRISMA statement (preferred reporting items for systematic reviews and meta-analyses).[9]

Inclusion and exclusion criteria

We included original studies published after 1990 that enrolled adult humans undergoing any type of surgical procedure (under local or general anaesthesia), with a sample size of at least 5, and which reported at least one measurement of serum cortisol by any method during surgery and within 10 days from surgery. Studies published before 1990 were not considered representative of the recent advances in surgical and anaesthetic techniques, as well as of current methods for cortisol estimation used in clinical practice. Studies reporting cortisol measurements only after the administration of exogenous glucocorticoids or corticotropic agents (i.e. tetracosactide) were excluded. Surgical procedures involving the brain were also excluded to remove the potential confounding factor of surgically-induced central adrenal insufficiency.[10]

Data sources and search strategy

A comprehensive search of several databases was conducted for records published between 1990 and May 26th, 2016. The databases included Medline In-Process & Other

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Non-Indexed Citations, MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, and Cochrane Database of Systematic Reviews. The search strategy was designed and conducted by an experienced librarian (LJP) with input from the corresponding author (IB). Controlled vocabulary supplemented with keywords was used to search for articles of interest. The full search strategy is available in **Supplementary Table 1**.

Study selection

Initial screening of the identified studies was performed by four independent reviewers (AP, KA, HKA and IB). Titles and abstracts of the identified studies were screened in duplicate, taking into consideration the predefined inclusion criteria. Many of the identified studies retrieved by our search were not relevant or not original and were excluded at this phase. The full-text screening was then performed in duplicate to assess eligibility for final inclusion and discrepancies were resolved through discussion and consensus. The reference lists of the original studies and narrative reviews were searched for any additional relevant studies that were not identified by our literature search. The list and characteristics of the included studies is reported in **Table 1**.

Data extraction and variable and outcome definitions

Data extraction of the eligible studies was carried out independently and in duplicate (AP, KA, HKA and IB) using the DistillerSR online platform, Evidence Partners.[11] Any disagreements or discrepancies in extracted data were resolved by consensus.

The data initially extracted included the last name of the first author and year of publication, the country where the study was conducted, demographics of participants, patient inclusion and exclusion criteria, and study design. The reviewers then extracted the cortisol measurements from all the eligible studies, dividing them by the time since the baseline assessment (prior to surgery). When exact figures were not reported in the text, cortisol

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measurements and descriptive statistics were extracted from graphs. All cortisol measurements were converted to SI units (nmol/L).

Finally, we considered various parameters potentially affecting cortisol concentrations during and after surgery (age, gender, grade and duration of surgical procedure, type of anaesthesia use and type of serum total cortisol assay) and extracted these data from the studies for which this information was available with the plan to perform subgroup analyses.

The surgical procedures were classified according to the modified Johns Hopkins surgical criteria, which define three grades of invasiveness (**Supplementary Table 2**): minimally invasive (grade I), moderately invasive (grade II), and highly invasive (grade III).[12] **Supplementary Table 3** reports the parameters that have been extracted and used to generate specific subsets of patients, as well as the number of cohorts and sample sizes used for meta-analysis of the cortisol measurements. The latter are reported according to the time of measurement, ranging from baseline to the 7th post-operative day (POD-7).

Methodological quality and risk of bias assessment

Critical appraisal of the included studies was assessed independently and in duplicate (AP, KA, HKA and IB). Therefore, we derived the following parameter from an existing guide on appraising uncontrolled studies [13]: (i) inclusion criteria; (ii) exclusion criteria; (iii) cortisol assay; (iv) timing of cortisol measurements; (v) reporting of cortisol measurements; (vi) time points of cortisol measurements. Any disagreements or differences in the quality assessments were resolved by consensus.

Statistical analysis

We conducted a meta-analysis using the random-effects model to pool estimates from the included studies. A random-effects model was used, rather than a fixed-effects model, in order to account for heterogeneity between-study and within-study variability. We used the I^2 statistic to estimate the percentage of total between-study variation due to heterogeneity

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rather than chance (ranging from 0 to 100%). I^2 values of 25, 50 and 75% are considered to represent low, moderate and high heterogeneity, respectively. Statistical analyses were conducted using OpenMeta[Analyst].[14-16] The outcome of interest was total serum cortisol concentration, a continuous outcome that was extracted from the studies as means and standard deviations when possible. If the standard deviations were not reported, we converted the standard errors or interquartile ranges to standard deviations. Medians were used in place of means when the means were not reported and the sample size was 25 or more. The area under the curve (AUC) was calculated using the trapezoidal rule. AUCs have been then divided by 24-h intervals and compared either to the pre-operative cortisol measurements or to a standard AUC 0-24-h for cortisol derived from the literature (data from cortisol profiling of 33 healthy adults sampled over 24 hours every 20 minutes).[17,18] Most studies in this systematic review reported multiple cortisol measurements during the day of surgery (i.e. the first 24 hours after the baseline assessment). From the 1st post-operative day (POD-1) onwards only single measurements every 24 hours were reported. To compare post-surgical cortisol secretion to the day of surgery cortisol secretion, we calculated the cortisol AUCs of each 24-h-interval.

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RESULTS

Characteristics of included studies

The search yielded 7,634 references for the abstract screening of which 71 studies [19-89] were included in the analysis (**Table 1**). The PRISMA flow diagram (**Figure 1**) details the number of records identified, included and excluded, and the reasons for exclusions. Eligible studies included are predominantly prospective (n=34 interventional and n=36 observational), while one study is of retrospective-prospective nature. Most of the studies are from European (n=39) and Asian-Pacific centres (n=22) while ten studies originated from American centres.

A total of 2,953 patients (mean age 54 years, 50.2% women) undergoing surgery (n=223 grade I minimally invasive surgery, n=1,568 grade II moderately invasive surgery, and n=1,162 grade III highly invasive surgery, **Table 1**) were included. The mean duration of surgery was 154 min (range 24-495) min as reported by 42/71 studies.

Peri-operative cortisol measurements were extracted from the studies and included baseline assessment (prior to surgery) up to 7th post-operative day (POD-7). As described in **Supplementary Table 3**, the number of observations up to POD-2 was high (>9,000 single cortisol measurements). However, the number of patient cohorts with available cortisol measurement between POD-3 and POD-7 available for analysis dropped considerably (<600 single cortisol measurements).

Most of the studies were judged to be of good quality (**Supplementary Table 4**); low quality scores were mainly due to absent or inadequate description of inclusion and exclusion criteria (especially the latter). Overall, the included studies clearly reported the methodology and the timing of cortisol measurements. For those studies reporting more than one patient cohort, the reviewers were almost always able to discriminate the cortisol measurements among the different cohorts and perform sub-group analyses (**Supplementary Tables 3 and 5**).

Grade of surgery and peri- and post-operative cortisol concentrations

Four studies (n=194 patients) reported data for grade I (minimally invasive) procedures, while the cortisol response during grade II and grade III surgeries has been investigated in 41 (n=1,494 patients) and 22 studies (n=1,095 patients), respectively. Four studies (n=170 patients) reported cortisol measurements from mixed cohorts, ranging across the three grades of surgery (**Table 1 and Supplementary Table 3**). Whilst grade II surgical procedures were heterogeneous in terms of the type of surgery and surgical techniques, most

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of the studies on grade III procedures (16 out of 24) focused on cardiac surgery, mainly coronary artery bypass grafting (CABG), **Table 1**.

Cortisol measurements from patients undergoing grade I surgery have been performed only over a period of up to 24 hours after surgery, while patients undergoing grade II and III surgeries have been evaluated for cortisol up to POD-7 (**Table 2 and Supplementary Table 3**). In order to increase the number of observations, we have also merged data from grade II and III procedures and these results are reported in **Table 2** and **Figure 2B+F**.

In our systematic review, we found that grade of surgery significantly affected cortisol secretion. Patients undergoing grade I surgery did not show an intraoperative cortisol peak, and even demonstrated lower intraoperative cortisol measurements when compared to baseline; post-surgical cortisol concentrations were again similar to baseline when measured within 6 hours after the procedure (**Figure 2C**). Nevertheless, when compared to published data on healthy, unstressed adults,[17,18] the mean cortisol output over the first 24 hours after grade I surgical procedure is approximately doubled. On the other hand, patients undergoing grade II and III surgery had 1.9-fold and 1.7-fold, respectively, higher cortisol output within the first 24-h period than patients with grade I surgery, and 4-fold and 3.5-fold higher cortisol output than healthy, unstressed individuals. Total serum cortisol peaked around the time of extubation (grade II procedures) and between 6 and 18h after the end of surgery (grade III procedures) (**Figure 2A**). Moreover, in both grade II and III surgeries, mean cortisol values remained elevated in comparison to the baseline measurements up to POD-7 (**Figure 2D-F**).

Demographics and peri- and post-operative cortisol concentrations

After stratifying the combined cohorts of grade II and III procedures according to age (cut-off 60 years), we observed that older patients demonstrated higher cortisol concentrations (**Figure 3A**). In comparison to cohorts with female predominance (79% on average), in studies with >50% male patients (78% on average) the cortisol output was lower

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the first 24-h from surgery but higher on POD1 (**Figure 3B**). The AUCs for total cortisol measurements, stratified by age and gender, are reported in **Supplementary Table 5**.

Duration of surgery and peri- and post-operative cortisol concentrations

When dividing the cohorts according not only to the grade but also the mean duration of surgery (≤180 or >180 min, 884 patients vs. 875 patients, respectively), we found that duration of surgery did not substantially affect the cortisol response within 48 hours of surgery (**Figure 3C+D**). The AUCs for total cortisol measurements are reported in **Supplementary Table 5**.

Surgical and anaesthetic techniques and peri- and post-operative cortisol concentrations

A laparoscopic surgical approach (n=446 patients) was associated with a slightly lower cortisol response during and after surgery as compared to open surgery (n=654 patients) (**Figure 4A**). Conversely, studies with >50% of patients treated preoperatively with the anaesthetic agent etomidate, a known inhibitor of CYP11B1 and hence cortisol synthesis,[90,91] have not demonstrated significant differences in cortisol response (n=502 patients vs. n=1,444 patients who did not receive etomidate) (**Figure 4B**). Three studies (n=134 patients) compared general anaesthesia to regional (spinal or epidural) anaesthesia; the latter group had a 17% lower AUC for total cortisol over the first 24 hours after the initiation of surgery (**Figure 4C**). The AUCs for total cortisol measurements, stratified by surgical and anaesthetic techniques, are reported in **Supplementary Table 5**.

Effect of cortisol assay on peri- and post-operative cortisol concentrations

As expected, the types of cortisol assays used in the studies varied, with 63 studies using immunoassays, two liquid chromatography-tandem mass spectrometry (LC-MS/MS), two spectrophotometry and one double isotope dilution (**Table 1**). Three studies did not report the assay used. Perioperative total cortisol concentrations measured by immunoassay were higher when compared to cortisol measurements by LC-MS/MS – **Figure 4D**. While we

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found no significant differences based on the type of immunoassay used, total cortisol measurements with ELISA demonstrated the highest measurements up to POD-1. The AUCs for total cortisol measurements stratified by the assay are reported in **Supplementary Table 5**. None of the included studies utilised LC-MS/MS to measure total cortisol between baseline (prior to the initiation of surgery) and POD-1; thus, the AUCs for the first 24-h could not be compared between immunoassays and LC-MS/MS.

DISCUSSION

Surgery causes a controlled systemic stress response that encompasses a wide range of endocrine, immune and cardiovascular effects.[2] In this systematic review and meta-analysis, we focused on the surgery-induced HPA axis activation with an objective to estimate cortisol output and their dependence on several defined parameters.

We found that the cortisol response to surgical stress varies significantly in patients undergoing minimally invasive surgeries (grade I) in comparison to patients undergoing moderate and highly invasive surgeries (grade II and III). On the day of surgery, the cortisol output increases by 2-fold (grade I), 4-fold (grade II) and 3.5-fold (grade III) in comparison to a reference population of healthy individuals.

Interestingly, we found that grade I procedures do not cause a peri-operative cortisol peak. Moreover, cortisol actually decreases in comparison to pre-surgical measurements, possibly explained by the fact that the (minor) stress stimulus is counteracted by other factors, such as anaesthesia and sedation. It is important to keep in mind that only 6 studies on 223 patients reported on cortisol measurements after minor surgeries, and strong conclusions are not possible.

We found that the cortisol response does not differ substantially between moderately (grade II) and highly invasive (grade III) surgeries with serum cortisol peaking between the

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time of extubation and 18h after surgery, and increased cortisol concentrations noted up to 1 week after surgery. Studies included in the current systematic review were highly heterogeneous in terms of patient selection, surgical and anaesthetic techniques, peri-operative care and methodological aspects – all these factors can affect the cortisol stress response to surgery (**Supplementary Table 6**).[92-98] Recently, Khoo B., et al. [99] reported on the peri-operative cortisol response after stratifying surgical procedures according to the POSSUM scoring system [100] and observed a positive correlation of cortisol peak to the invasiveness of the surgical procedure.[99] We believe that several elements can account for the differences compared to the results of our systematic review, including (i) a different classification system, (ii) difference in demographics, and (iii) the difference in procedures.

Studies published before 1990 suggested that serum cortisol during major surgery rises rapidly but usually returns to baseline values within 24-48-h.[7] Our data, on the contrary, show that mean cortisol can remain higher than baseline measurements for a longer duration, up to POD-7. For example, the study by Naito Y., et al. (included in this systematic-review and meta-analysis) measured cortisol at 1-h intervals in patients undergoing elective upper abdominal surgery up to POD-7 and showed that cortisol levels remained elevated for more than 3 days after surgery, with a loss of the circadian rhythm.[23] However, it is important to highlight that our results regarding the cortisol response after POD-3 derive only from a limited number of studies with small sample sizes.

Our data show that older subjects tend to have a higher peri-operative cortisol response. This can partly be explained by the fact that these patients (i) can have multiple comorbidities and a poorer pre-operative performance status, (ii) have a higher risk of post-operative complications and (iii) often require non-elective surgery. In addition, some age-related physiological changes of the HPA axis should be taken into account. In fact, older, non-stressed patients show a flattened circadian cortisol rhythm, and are likely to have higher

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cortisol values in comparison to younger controls.[101] A further confounding factor is that cortisol measurements might underestimate the actual cortisol production in older patients. In fact, some authors observed a reduction of cortisol-binding globulin (CBG) with age, especially in males,[102] although this has not been confirmed by others.[103] Further studies are needed to investigate which mechanisms actually affect the age-related cortisol response induced by surgery. In fact, the number of older patients undergoing surgical procedures is increasing over recent decades, and higher post-operative cortisol values in the older patient population with an increased risk of complications (e.g. cognitive dysfunction).[65,83] This may also have a bearing when considering glucocorticoid stress supplementation in older hypoadrenal patients undergoing surgery.

Laparoscopic surgery triggers a less robust acute phase and immune response in comparison to open procedures, and is also associated with a reduction of post-operative pain and hospital stay.[104-107] Our data on the cortisol response from abdominal and pelvic laparoscopic procedures are in line with these observations, although large-scale, prospective studies are lacking. Similarly, our results regarding the effects of etomidate on the post-operative cortisol response are based on a quite disproportionate comparison of only 8 studies in comparison to 38 studies without etomidate. Moreover, the regimens and doses of etomidate used throughout the studies varied considerably, and inhibition of the major cortisol biosynthesis enzyme CYP11B1 by cortisol will invariably result in accumulation of 11-deoxycortisol, which can cross-react with cortisol immunoassays, leading to 'falsely' elevated cortisol values.[108] These factors may explain why we did not observe the well-known inhibitory effect of etomidate on cortisol production.[109] Our data obtained from 3 prospective randomized studies, show that regional anaesthetic techniques are associated with a 17% lower cortisol response than that of general anaesthesia over the first 24-h. Epidural anaesthesia techniques (especially when performed below the umbilicus) can lead to blunted

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cortisol responses after surgery, in comparison to general anaesthesia. This effect seems more prominent when epidural anaesthetics are used instead of epidural opioids, probably because the first affect both sensory and sympathetic afferent stimuli, whereas opioids act on nociceptive pathways only.[110]

A limitation of this systematic review and meta-analysis is that serum cortisol has been measured with different assays. Immunoassays are known to have different degrees of cross-reactivity to endogenous and exogenous steroids other than cortisol,[108,111,112] and this cross-reactivity can be exacerbated during stress, as the HPA axis activation leads to increased output of cortisol and its precursors.[113] Moreover, inter-assay and inter-laboratory variability of serum cortisol measurements in patients with septic shock can be substantial for both immunoassays and LC-MS/MS-based techniques.[114] If we refer to cortisol measurements through LC-MS/MS as the 'gold standard', our results confirm that immunoassays lead to an over-estimation of cortisol after surgery. However, data from LC-MS/MS are very limited (only 2 studies from the same study group) and no cortisol measurements are available during or immediately after surgery, when the cortisol response is at its highest and the disagreement between LC-MS/MS and immunoassays is expected to be more apparent.[115]

Another limitation is that we could only compile data on total cortisol. In healthy subjects, 95% of serum cortisol is protein-bound, primarily to CBG and secondarily to albumin.[116] Five percent of serum cortisol is free (not protein-bound) and this is its bioactive form.[117] Several authors have observed a drop of CBG after surgery (up to 23-44% compared to baseline).[57,71,99] This drop correlates with the severity of surgery,[99] and can be interpreted as an adaptive mechanism to increase the delivery of bioactive cortisol to target tissues.[118] The direct measurement of free cortisol is cumbersome and not routinely available in clinical settings, moreover, formulas to indirectly calculate unbound cortisol from total cortisol and

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CBG values have been proven to be inaccurate after surgery, because of the effects of post-operative hypoalbuminaemia and haemodilution.[8,46,119] Notably, the drop of albumin can be substantial after CABG.[71] The unavailability of free cortisol measurements in the included studies could have affected some of our observations. First, we did not observe a stratification of the total cortisol stress response between grade II and III surgical procedures – the high prevalence of CABG among grade III surgery (and thus the confounding effect of haemodilution, hypoalbuminaemia and CBG drop) may partially explain this. Moreover, studies including predominantly male patients mainly report total cortisol measurements from grade III procedures (CABG in most of the cases), and this may account for the differences that we observed among men and women.

There is general agreement that the daily dose of glucocorticoids needs to be increased in patients with adrenal insufficiency undergoing surgery to prevent adrenal crisis, and that hydrocortisone is the treatment of choice.[5,120] Nonetheless, no randomized controlled studies have assessed the precise glucocorticoid dose requirements in these patients and no consensus exists regarding the optimal management of perioperative glucocorticoid therapy. The perioperative glucocorticoid requirements detailed in the recent Endocrine Society guideline on diagnosis and treatment of primary adrenal insufficiency rely only on the severity of the surgical stress.[120] The guideline recommends hydrocortisone 25-75mg/24h in adults for 1 to 2 days after minor to moderate surgery and intravenous bolus of 100mg hydrocortisone followed by continuous infusion of 200mg/24h on the day of major surgery, to be tapered rapidly switching to oral regimen depending on clinical state.[120] The results of this systematic review and meta-analysis suggest that other factors should be taken into account in addition to the magnitude of the surgical stress when deciding for the perioperative steroid coverage. According to the serum cortisol response, older subjects,

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women and patients undergoing open surgery and general anaesthesia may require higher doses of hydrocortisone. Moreover, extra steroid coverage might be required at least up to one week following moderately to highly invasive procedures.

CONCLUSIONS

This systematic review and meta-analysis of the modern literature of cortisol response during surgery demonstrated significant differences of cortisol response in relation to the grade of surgery, with significantly higher cortisol response to grade II and III than to grade I surgery. We found that increased cortisol secretion continues up to the post-operative day 7 after surgery and is affected by age, sex and invasiveness of the surgical or anaesthetic technique. Current evidence stems from highly heterogeneous studies of mostly moderate quality. Of note, only two of 71 included studies have used LC-MS/MS, which, however, is now considered state-of-the-art for the measurement of cortisol.[121] Large prospective studies, entailing rigorous patient selection, stratification, and standardized outcome measurement by LC-MS/MS are needed to clarify how the adrenal gland responds to surgery and which factors shape this response. Such data would help to inform and tailor the current guidelines on glucocorticoid stress dose cover in hypoadrenal patients undergoing surgery.

DECLARATION OF CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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FIGURE LEGENDS

Figure 1 – PRISMA flow diagram.

Figure 2 – Total serum cortisol concentrations according to the grade of surgery. All values are expressed in nmol/L and are reported as means ± 95% CI, obtained after meta-analysis of all included studies. The first value in each panel corresponds to baseline cortisol measurements before initiation of surgery. The cortisol measurement at time 0 corresponds to the initiation of surgery (Knife-To-Skin, KTS). **Panel A)** Comparison between the three grades of surgery over the first 24-h – the numbers 1 to 7 indicate the specific time points at which cortisol has been measured (1: baseline; 2: KTS; 3: during surgery; 4: end of surgery; 5: first 6h after surgery; 6: between 6 and 18h after surgery; 7: 24-h after surgery). **Panel B)** Cortisol response over the first 24-h, after combining the data of surgery grade II and III (n=67 studies). **Panel C)** Surgery grade I (n=4 studies). **Panel D)** Surgery grade II (n=41 studies). **Panel E)** Surgery grade III (n=22 studies). **Panel F)** Combined data of surgery grade II and III (n=67 studies).

Figure 3 – Impact of age, sex and mean duration of surgery on total serum cortisol up to 48h after surgery. All values are expressed in nmol/L and are reported as means ± 95% CI, obtained after meta-analysis of all included studies. The first value in each panel corresponds to baseline cortisol measurements before initiation of surgery. The cortisol measurement at time 0 corresponds to the initiation of surgery. **Panel A)** Impact of patient age after combining data of surgery II and III – comparison between cohorts with a mean age of ≤60 years (n=32 studies) or >60 years (n=21 studies). **Panel B)** Impact of gender after combining data of surgery II and III – comparison between cohorts including either >50% females (n=26

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studies) or >50% males (n=25 studies). **Panel C)** Impact of the mean duration of surgery grade II – either ≤180 min (n=21 studies) or >180 min (n=4 studies). **Panel D)** Impact of the mean duration of surgery grade III – either ≤180 min (n=2 studies) or >180 min (n=10 studies).

Figure 4 – Total serum cortisol measurements according to differences in the surgical approach, anaesthetic techniques and four cortisol assays. All values are expressed in nmol/L and are reported as means ± 95% CI, obtained after meta-analysis of all included studies. The first value in each panel corresponds to baseline cortisol measurements before initiation of surgery. The cortisol measurement at time 0 corresponds to the initiation of surgery. **Panel A)** Studies comparing laparotomy (n=24 studies) and laparoscopic techniques (n=18 studies). **Panel B)** Studies either not using etomidate as an anaesthetic agent (n=38) or administering it to >50% of the patients (n=8). **Panel C)** Studies comparing general and regional anaesthesia (n=3). **Panel D)** Comparison of four cortisol assays (RIA, radioimmunoassay – n=33 studies; ECLIA, electrochemiluminescence immunoassay – n=17 studies; ELISA, enzyme-linked immunosorbent assays – n=6 studies; LC-MS/MS, liquid chromatography–tandem mass spectrometry – n=2 studies).

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Table 1 – Included studies.

Author (year)	Country	Study type	Surgical procedure	Grade of surgery [a]	Population Sample size	Total cortisol assay
Høgevold (1990) [19]	Norway	Prospective, observational	Total hip replacement for osteoarthritis	II	7	RIA
Bartalena (1990) [20]	Italy	Prospective, observational	Surgery for breast cancer, non-functioning adrenal cancer, adenocarcinoma of the colon, lung cancer, urethral obstruction	II	10	RIA
Hosokawa (1990) [21]	Japan	Prospective, observational	Minor surgeries (e.g. tympanoplasty) and major surgeries (cholecystectomy)	I, II	22	Double isotope dilution
Bickel (1991) [22]	Germany	Prospective, randomized	Elective cholecystectomy	II	26	RIA
Naito (1991) [23]	Japan	Prospective, observational	Elective upper abdominal surgery	II	15	RIA
Juma (1991) [24]	Saudi Arabia	Prospective, observational	Elective major orthopaedic surgery	II	16	RIA
Harris (1991) [25]	USA	Prospective, observational	Surgery for traumatic thoracic and/or abdominal injuries	III	10	RIA
Donald (1993) [26]	New Zealand	Prospective, interventional	Elective cholecystectomy	II	12	ELISA
McMahon (1993) [27]	UK	Prospective, randomized	Elective cholecystectomy	II	20	Fluorescent immunoassay
Deuss (1994) [28]	Germany	Prospective, observational	Elective cholecystectomy	II	65	ELISA
Crozier (1994) [29]	Germany	Prospective, randomized	Elective hysterectomy for non-malignant diseases	II	20	RIA
Jakeways (1994) [30]	UK	Prospective, non-randomized	Elective cholecystectomy	II	24	RIA
Dionigi (1994) [31]	Italy	Prospective, randomized	Elective cholecystectomy	II	57	RIA
Frank (1995) [32]	USA	Prospective, randomized	Vascular, abdominal and thoracic surgery	II, III	74	RIA
Ellstrom (1996) [33]	Sweden	Prospective, randomized	Hysterectomy for benign disorders	II	24	Not reported
Jameson (1997) [34]	UK	Prospective, randomized	Hysterectomy	II	16	RIA
Karayiannakis (1997) [35]	Greece	Prospective, randomized	Elective cholecystectomy	II	83	RIA
Roth-Isigkeit (1997) [36]	Germany	Prospective, observational	Coronary artery bypass grafting surgery	III	50	RIA
Stratton (1997) [37]	UK	Prospective, observational	Total hip replacement for osteoarthritis	II	6	RIA
Plunkett (1997) [38]	USA	Prospective, randomized	Coronary artery bypass grafting surgery	III	121	Fluorescent immunoassay

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Eriksson-Mjöberg (1997) [39]	Sweden	Prospective, randomized	Surgery for rectocele and cystocele	II	30	RIA
Bellón (1998) [40]	Spain	Prospective, observational	Elective cholecystectomy	II	28	RIA
Engin (1998) [41]	Turkey	Prospective, randomized	Elective cholecystectomy	II	32	RIA
Yamauchi (1998) [42]	Japan	Prospective, observational	Laparoscopic cholecystectomy, modified radical-mastectomy, pulmonary lobectomy, thoracic oesophagectomy	II, III	24	IRMA
Delogu (1999) [43]	Italy	Prospective, observational	Elective cholecystectomy	II	46	RIA
Friedrich (1998) [44]	Germany	Prospective, observational	Surgery for either uterine leiomyomas, chronic pelvic discomfort or desire for definitive contraception	II	20	RIA
Vogeser (1999) [45]	Germany	Prospective, observational	Cardiac surgery	III	14	ECLIA
Roth-Isigkeit (2000) [46]	Germany	Prospective, observational	Coronary artery bypass grafting surgery	III	28	RIA
Schricker (2000) [47]	Germany	Prospective, randomized	Hysterectomy	II	20	RIA
Cho (2000) [48]	South Korea	Prospective, observational	Elective radical gastrectomy for gastric adenocarcinoma	II	31	RIA
Ogata (2000) [49]	Japan	Prospective, observational	Elective partial gastrectomy	II	16	RIA
Mahla (2000) [50]	Austria	Prospective, observational	Non-cardiac elective surgeries	II	67	ELISA
Nguyen (2002) [51]	USA	Prospective, randomized	Gastric bypass	II	48	RIA
Ozarda Ilçöl (2002) [52]	Turkey	Prospective, observational	Major abdominal or gynaecological surgery	II	16	ECLIA
Holub (2002) [53]	Czech Republic	Prospective, observational	Hysterectomy for benign or premalignant pelvic disorders	II	77	Fluorescent immunoassay
Marana (2003) [54]	Italy	Prospective, randomized	Ovarian cyst removal	II	20	RIA
Vogeser (2003) [55]	Germany	Prospective, observational	Coronary artery bypass grafting surgery	III	12	LC-MS/MS
Vogeser (2003) [56]	Germany	Prospective, observational	Coronary artery bypass grafting surgery	III	16	LC-MS/MS
le Roux (2003) [57]	UK	Prospective, observational	Major elective surgery	III	31	ELISA
Lattermann (2003) [58]	Canada	Prospective, randomized	Elective colorectal surgery	II	16	RIA
Shahbazi (2004) [59]	Iran	Prospective, interventional	Coronary artery bypass grafting surgery	III	30	RIA
Maciejczyk-Pencula (2004) [60]	Poland	Prospective, randomized	Hysterectomy for leiomyomas	II	49	ELISA
Haque (2004) [61]	Bangladesh	Prospective, interventional	Elective cholecystectomy	II	30	Not reported
Ledowski (2005) [62]	Germany	Prospective, randomized	Minor elective ear-nose-throat surgery	I	43	ECLIA
Crema (2005) [63]	Brazil	Prospective, interventional	Elective cholecystectomy	II	31	ECLIA

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Yeager (2005) [64]	USA	Prospective, randomized	Coronary artery bypass grafting surgery and/or cardiac valve replacement	III	20	RIA
Kudoh (2005) [65]	Japan	Prospective, observational	Abdominal surgery	II	115	Spectrophotometry
Nicholson (2005) [66]	UK	Prospective, observational	Pelvic and/or acetabular reconstruction surgery for extensive fractures	III	20	ELISA
Rahr (2006) [67]	Denmark	Prospective, randomized	Inguinal hernia repair	I	61	ECLIA
Buyukkocak (2006) [68]	Turkey	Prospective, randomized	Anorectal surgery for benign conditions	II	58	ECLIA
Kashiwabara (2007) [69]	Japan	Prospective, observational	Surgery of the digestive tract	I, II, III	50	IRMA
Baričević (2007) [70]	Serbia	Prospective, observational	Surgery for malignant gastric or pancreatic tumours	II	21	RIA
Christ-Crain (2007) [71]	UK	Prospective, observational	Coronary artery bypass grafting surgery	III	24	ECLIA
Rapp-Kesek (2007) [72]	Sweden	Prospective, randomized	Coronary artery bypass grafting surgery	III	18	Fluorescent immunoassay
Spratt (2008) [73]	USA	Prospective, observational	Abdominal aortic aneurysm resection and coronary artery bypass grafting surgery	III	29	RIA
Mujagić (2008) [74]	Bosnia and Herzegovina, Croatia	Prospective, randomized	Low abdominal surgery (colon cancer, hysterectomy)	II	50	Fluorescent immunoassay
Bender (2009) [75]	Turkey	Prospective, randomized	Inguinal hernia repair	I	40	ECLIA
Kim (2010) [76]	South Korea	Prospective, randomized	Hysterectomy for non-malignant disease	II	24	RIA
Mu (2010) [77]	China	Prospective, observational	Coronary artery bypass grafting surgery	III	243	ECLIA
Engin (2010) [78]	Turkey	Prospective, observational	Elective abdominal surgery (for cholelithiasis, for gastroesophageal reflux or for gastric carcinoma)	II	106	ECLIA
Celic-Spuzic (2011) [79]	Bosnia and Herzegovina	Retrospective-prospective	Prostatectomy for benign conditions	III	100	RIA
Mello (2011) [80]	Brasil	Prospective, observational	Surgery requiring intensive care unit stay	III	20	ECLIA
Debono (2011) [81]	UK	Prospective, observational	Coronary artery bypass grafting surgery	III	30	ECLIA
Adas (2013) [82]	Turkey	Prospective, observational	Endoscopic retrograde cholangiopancreatography for bile duct stones	I	50	Spectrophotometry
Mu (2013) [83]	China	Prospective, observational	Coronary artery bypass grafting surgery	III	166	ECLIA
Ferreira (2014) [84]	Brasil	Prospective, interventional	Cardiac surgery with cardiopulmonary bypass	III	27	Not reported

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Kahveci (2014) [85]	Turkey	Prospective, randomized	Lower extremity surgery	II	60	ECLIA
Maas (2014) [86]	The Netherlands	Prospective, randomized	Oesophagectomy for oesophageal carcinoma	III	27	ECLIA
Gu (2015) [87]	China	Prospective, randomized	Oesophagectomy for oesophageal carcinoma	III	57	RIA
Lebherz (2016) [88]	Germany	Prospective, observational	Cardiac surgery with cardiopulmonary bypass	III	22	ECLIA
Możański (2016) [89]	Poland	Prospective, randomized	Elective abdominal laparoscopic surgery	II	62	ECLIA

[a] Refer to Supplementary Table 2 for the classification of the surgical procedures. When a subgroup analysis could not be performed within the study cohort(s), the grade of surgery was assigned according to the most frequent surgical procedure(s).

Abbreviations: ECLIA: electrochemiluminescence; ELISA: enzyme-linked immunosorbent assay; IRMA: immunoradiometric assay; LC-MS/MS: liquid chromatography-tandem mass spectrometry; RIA: radioimmunoassay.

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Table 2 – Areas under the curve (AUCs) for total cortisol values, divided by 24-h periods and by the grade of surgery.

		AUC 0-24h	AUC Day 1 post-op.	AUC Day 2 post-op.	AUC Day 3 post-op.	AUC Day 4 post-op.	AUC Day 5 post-op.	AUC Day 6 post-op.	AUC Day 7 post-op.
Surgery grade I	**Absolute value (nmol*h/L)**	9,541	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	%difference to surgical baseline [a]	+11%	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	%difference to a standard 24-h AUC [b]	+116%	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Surgery grade II	**Absolute value (nmol*h/L)**	18,066	13,152	13,032	13,272	13,320	12,972	10,980	10,392
	%difference to surgical baseline [a]	+102%	+47%	+46%	+48%	+49%	+45%	+23%	+16%
	%difference to a standard 24-h AUC [b]	+309%	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Surgery grade III	**Absolute value (nmol*h/L)**	16,010	14,904	12,636	11,820	n/a	n/a	13,056	n/a
	%difference to surgical baseline [a]	+77%	+65%	+40%	+31%	n/a	n/a	+44%	n/a
	%difference to a standard 24-h AUC [b]	+262%	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Surgery grade II+III (combined)	**Absolute value (nmol*h/L)**	17,567	13,812	12,864	12,528	12,972	12,360	12,348	12,372
	%difference to surgical baseline [a]	+95%	+53%	+43%	+39%	+44%	+37%	+37%	+37%
	%difference to a standard 24-h AUC [b]	+297%	n/a	n/a	n/a	n/a	n/a	n/a	n/a

During the first 24-h after surgery, multiple total cortisol measurements are available; therefore, the AUCs have been compared to a standard 24-h AUC for cortisol derived from the literature.[17,18] From the first post-operative day onwards only measurements every 24-h are available, therefore the AUCs have been compared to AUCs calculated according to the baseline cortisol values.

[a] Percentage difference between the AUC and the pre-surgical AUC. The pre-operative AUC has been calculated by considering a 24-h period between two identical total cortisol values, derived from the baseline cortisol measurements (prior to the initiation of surgery). The calculated pre-operative AUCs for surgery grade I, II, III and II+III are 8,616 nmol*h/L, 8,952 nmol*h/L, 9,048 nmol*h/L, 9,000 nmol*h/L, respectively.

[b] Percentage difference between the AUC and a standard 24-h AUC for cortisol obtained by 24-h cortisol profiling of 33 healthy adults (4,420 nmol*h/L).[17,18]

Abbreviations: AUC: area under the curve for serum cortisol values; n/a: not available or not applicable.

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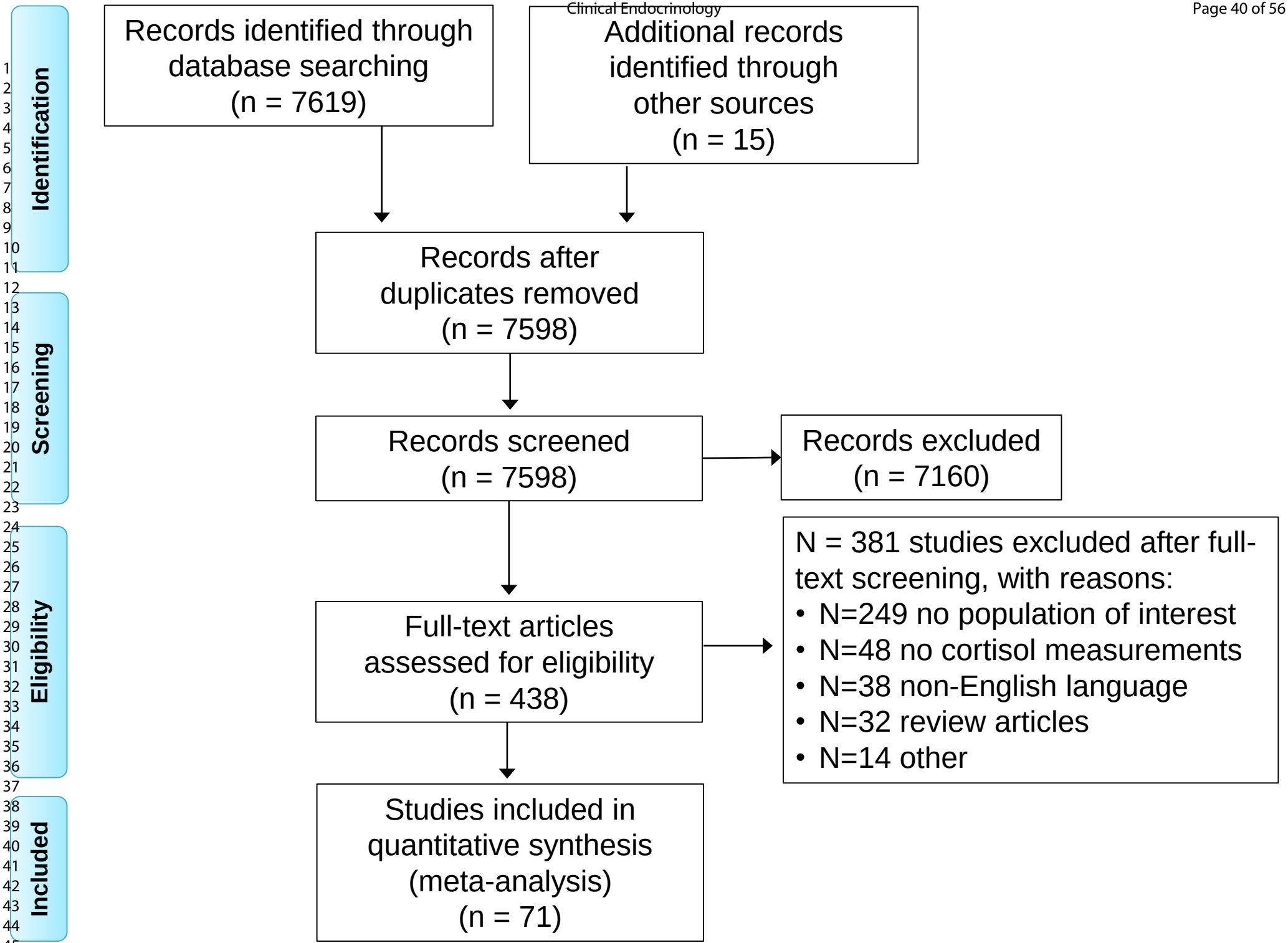
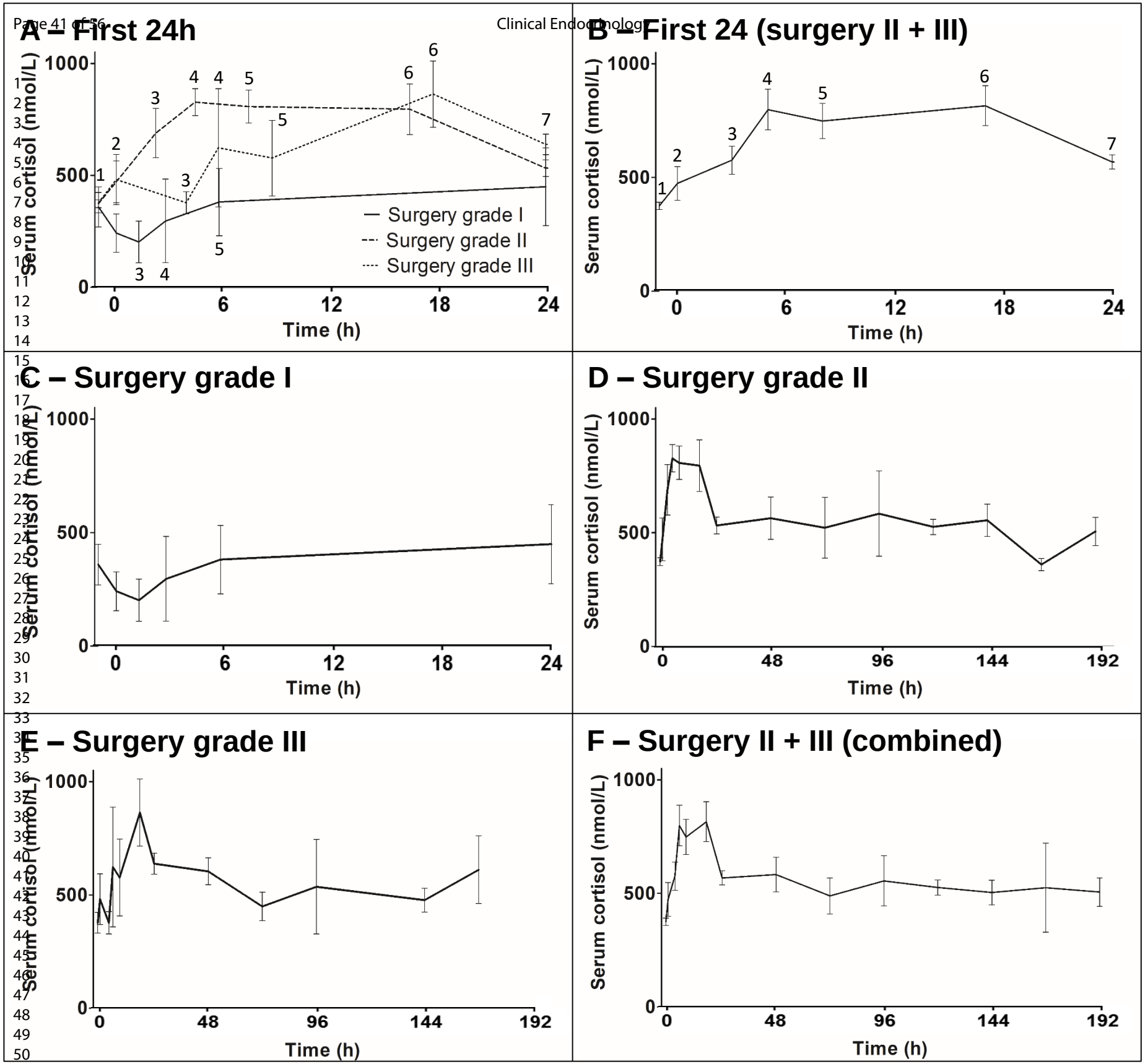
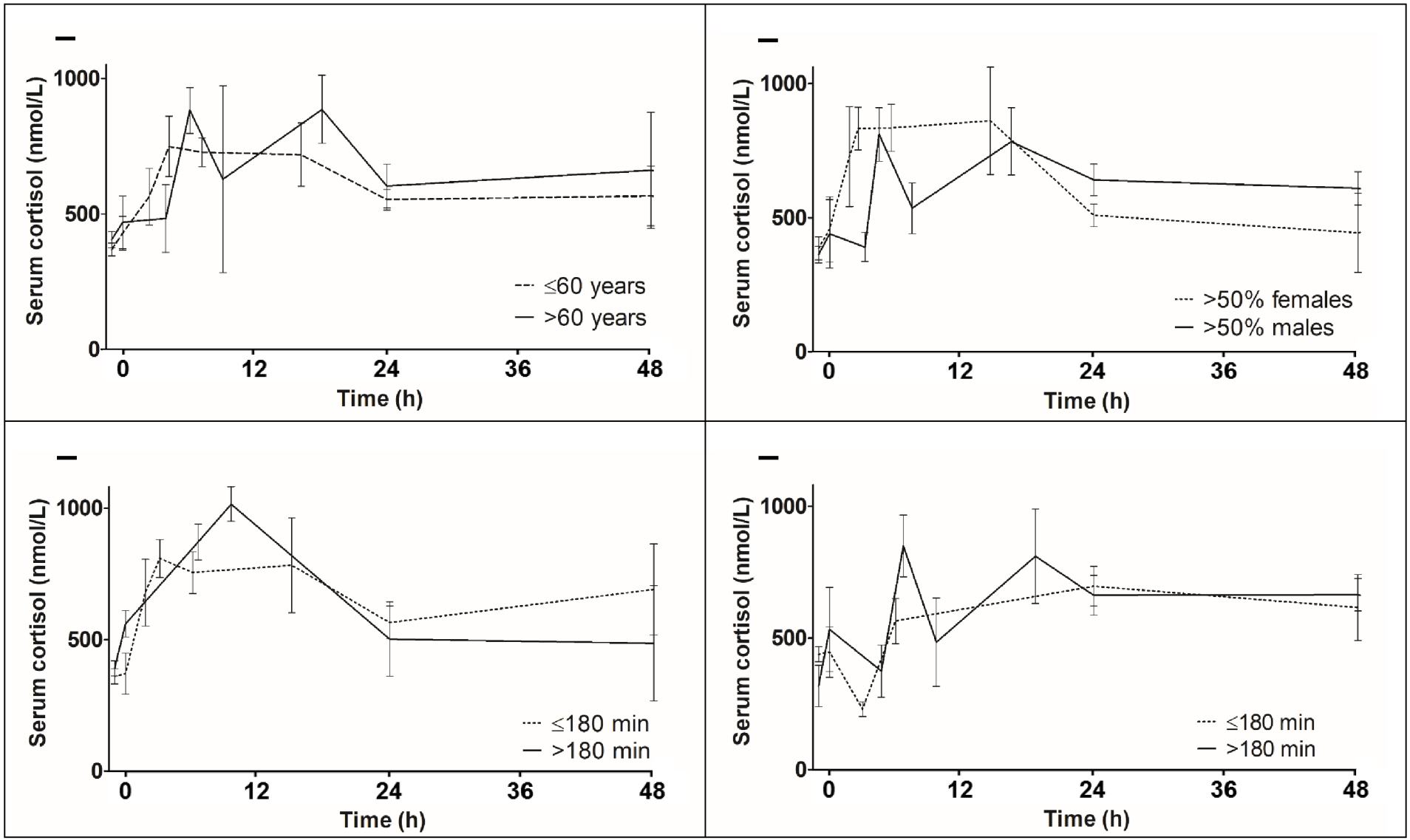
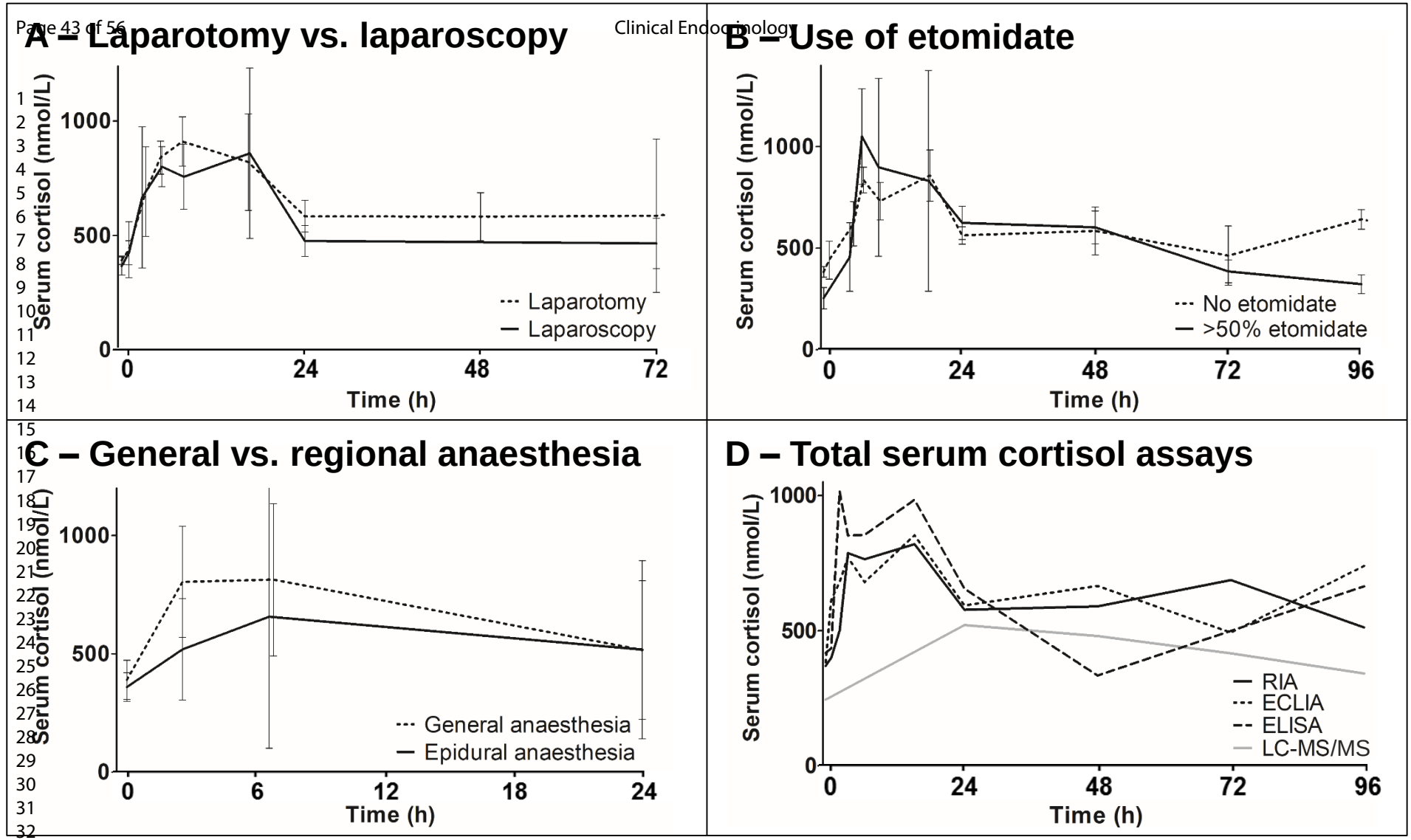

Identification
Screening
Eligibility
Included
Records identified through database searching (n = 7619)
Additional records identified through other sources (n = 15)
Records after duplicates removed (n = 7598)
Records screened (n = 7598)
Records excluded (n = 7160)
Full-text articles assessed for eligibility (n = 438)
N = 381 studies excluded after full-text screening, with reasons:
• N=249 no population of interest
• N=48 no cortisol measurements
• N=38 non-English language
• N=32 review articles
• N=14 other
Studies included in quantitative synthesis (meta-analysis) (n = 71)


A – First 24h
Serum cortisol (nmol/L)
Time (h)
Surgery grade I
Surgery grade II
Surgery grade III
B – First 24 (surgery II + III)
Serum cortisol (nmol/L)
Time (h)
C – Surgery grade I
Serum cortisol (nmol/L)
Time (h)
D – Surgery grade II
Serum cortisol (nmol/L)
Time (h)
E – Surgery grade III
Serum cortisol (nmol/L)
Time (h)
F – Surgery II + III (combined)
Serum cortisol (nmol/L)
Time (h)


Serum cortisol (nmol/L)
1000
500
0
0
12
24
36
48
Time (h)
≤60 years
>60 years
>50% females
>50% males
≤180 min
>180 min


A – Laparotomy vs. laparoscopy
Serum cortisol (nmol/L)
1000
500
0
0
24
48
72
Time (h)
Laparotomy
Laparoscopy
B – Use of etomidate
Serum cortisol (nmol/L)
1000
500
0
0
24
48
72
96
Time (h)
No etomidate
>50% etomidate
C – General vs. regional anaesthesia
Serum cortisol (nmol/L)
1000
500
0
0
6
12
18
24
Time (h)
General anaesthesia
Epidural anaesthesia
D – Total serum cortisol assays
Serum cortisol (nmol/L)
1000
500
0
0
24
48
72
96
Time (h)
RIA
ECLIA
ELISA
LC-MS/MS

Supplementary Table 1 – Full search strategy.

Ovid

Database(s): Embase 1988 to 2016 Week 21, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to Present, EBM Reviews - Cochrane Central Register of Controlled Trials April 2016, EBM Reviews - Cochrane Database of Systematic Reviews 2005 to May 25, 2016

Search Strategy:

#	Searches	Results	Annotations
1	exp Hydrocortisone/	149584	
2	exp Cortisone/	29070	
3	cortisone acetate/	1061	
4	Glucocorticoids/	106016	
5	Dehydroepiandrosterone Sulfate/	11203	
6	(adreson or corticosteroid* or cortifair or cortisol or cortisone or cortril or dehydrocortisone or "Dehydroepiandrosterone Sulfate" or DHEAS or epicortisol or Glucocorticoid* or hydrocortisone).mp.	658684	
7	1 or 2 or 3 or 4 or 5 or 6	661993	
8	blood.fs.	1554351	
9	metabolism.fs.	4636381	
10	("adrenal function*" or "adrenal reserve" or "adrenal response" or analys* or analyz* or assay* or assess* or blood or concentration* or measur* or pharmacokinetic* or saliva or serum or urine).mp.	23423278	
11	8 or 9 or 10	25720523	
12	exp *Hydrocortisone/ or exp *Cortisone/ or *cortisone acetate/ or *Glucocorticoids/ or *Dehydroepiandrosterone Sulfate/ or (adreson or corticosteroid* or cortifair or cortisol or cortisone or cortril or dehydrocortisone or "Dehydroepiandrosterone Sulfate" or DHEAS or epicortisol or Glucocorticoid* or hydrocortisone).ti.	184113	
13	12 and ("adrenal function*" or "adrenal reserve" or "adrenal response" or analys* or analyz* or assay* or assess* or blood or concentration* or measur* or pharmacokinetic* or saliva or serum or urine).ti.	18392	
14	((critical* adj3 ill*) or burn or burns or "multi-organ dysfunction*" or "multi-organ failure" or "multiple organ dysfunction*" or "multiple organ failure" or polytrauma* or trauma*).mp.	919041	
15	7 and 11 and 14	12792	
16	13 or 15	30747	
17	16 not "traumatic brain injur*".ti.	30284	
18	17 not "conference abstract".pt.	28307	
19	limit 18 to yr="1980 -Current"	25076	

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20	(exp animals/ or exp nonhuman/) not exp humans/	8423008
21	((alpaca or alpacas or amphibian or amphibians or animal or animals or antelope or armadillo or armadillos or avian or baboon or baboons or beagle or beagles or bee or bees or bird or birds or bison or bovine or buffalo or buffaloes or buffalos or "c elegans" or "Caenorhabditis elegans" or camel or camels or canine or canines or carp or cats or cattle or chick or chicken or chickens or chicks or chimp or chimpanze or chimpanzees or chimps or cow or cows or "D melanogaster" or "dairy calf" or "dairy calves" or deer or dog or dogs or donkey or donkeys or drosophila or "Drosophila melanogaster" or duck or duckling or ducklings or ducks or equid or equids or equine or equines or feline or felines or ferret or ferrets or finch or finches or fish or flatworm or flatworms or fox or foxes or frog or frogs or "fruit flies" or "fruit fly" or "G mellonella" or "Galleria mellonella" or geese or gerbil or gerbils or goat or goats or goose or gorilla or gorillas or hamster or hamsters or hare or hares or heifer or heifers or horse or horses or insect or insects or jellyfish or kangaroo or kangaroos or kitten or kittens or lagomorph or lagomorphs or lamb or lambs or llama or llamas or macaque or macaques or macaw or macaws or marmoset or marmosets or mice or minipig or minipigs or mink or minks or monkey or monkeys or mouse or mule or mules or nematode or nematodes or octopus or octopuses or orangutan or "orang-utan" or orangutans or "orang-utans" or oxen or parrot or parrots or pig or pigeon or pigeons or piglet or piglets or pigs or porcine or primate or primates or quail or rabbit or rabbits or rat or rats or reptile or reptiles or rodent or rodents or ruminant or ruminants or salmon or sheep or shrimp or slug or slugs or swine or tamarin or tamarins or toad or toads or trout or urchin or urchins or vole or voles or waxworm or waxworms or worm or worms or xenopus or "zebra fish" or zebrafish) not (human or humans)).mp.	7384547
22	19 not (20 or 21)	20693
23	limit 22 to ("all adult (19 plus years)" or "young adult (19 to 24 years)" or "adult (19 to 44 years)" or "young adult and adult (19-24 and 19-44)" or "middle age (45 to 64 years)" or "middle aged (45 plus years)" or "all aged (65 and over)" or "aged (80 and over)") [Limit not valid in Embase,CCTR,CDSR; records were retained]	16937
24	limit 23 to (adult <18 to 64 years> or aged <65+ years>) [Limit not valid in Ovid MEDLINE(R),Ovid MEDLINE(R) In-Process,CCTR,CDSR; records were retained]	11411
25	limit 22 to ("all infant (birth to 23 months)" or "all child (0 to 18 years)" or "newborn infant (birth to 1 month)" or "infant (1 to 23 months)" or "preschool child (2 to 5 years)" or "child (6 to 12 years)" or "adolescent (13 to 18 years)") [Limit not valid in Embase,CCTR,CDSR; records were retained]	14125
26	limit 25 to (embryo or infant or child or preschool child <1 to 6 years> or school child <7 to 12 years> or adolescent <13 to 17 years>) [Limit not valid in Ovid MEDLINE(R),Ovid MEDLINE(R) In-Process,CCTR,CDSR; records were retained]	3963
27	26 not 24	1469
28	22 not 27	19224
29	limit 28 to (editorial or erratum or letter or note or addresses or autobiography or bibliography or biography or blogs or comment or dictionary or directory or interactive tutorial or interview or lectures or legal cases or legislation or news or newspaper article or overall or patient education handout or periodical index or portraits or published erratum or video-audio media or webcasts) [Limit not valid in Embase,Ovid MEDLINE(R),Ovid MEDLINE(R) In-Process,CCTR,CDSR; records were retained]	1178
30	from 29 keep 1-1066	1066
31	28 not 30	18158
32	limit 31 to yr="2012 -Current"	4498

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33 remove duplicates from 32	3067
34 limit 31 to yr="2007 -2011"	4237
35 remove duplicates from 34	2957
36 limit 31 to yr="2001 -2006"	3749
37 remove duplicates from 36	2499
38 limit 31 to yr="1993 -2000"	3075
39 remove duplicates from 38	1945
40 31 not (32 or 34 or 36 or 38)	2599
41 remove duplicates from 40	1945
42 33 or 35 or 37 or 39 or 41	12413
43 (case adj3 report*).mp,pt.	3575111
44 (systematic adj3 review).mp,pt.	235991
45 review.mp,pt.	5358492
46 45 not 44	5122501
47 42 not 46	9903
48 47 not 43	9131
49 limit 48 to yr="1990 -Current"	7919

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Supplementary Table 2 – Classification of surgical procedures (adapted from the modified Johns Hopkins surgical criteria).[12]

Grade	General description	Includes	Excludes
I	Minimal to mild risk independent to anaesthesia. Minimal to moderately invasive procedure. Potential blood loss less than 500 ml.	Breast biopsy; removal of minor skin or subcutaneous lesions; myringotomy tubes; hysteroscopy; cystoscopy; vasectomy; circumcision; fibre-optic bronchoscopy; diagnostic laparoscopy; dilatation and curettage; fallopian tube ligation; arthroscopy; inguinal hernia repair; laparoscopic lysis of adhesion; tonsillectomy; rhinoplasty.	Open exposure of internal body organs; repair of vascular or neurological structures; placement of prosthetic devices; post-operative monitored care setting; open exposure of abdomen, thorax, neck, cranium; resection of major body organs.
II	Moderately to significantly invasive procedures. Potential blood loss 500–1500 ml. Moderate risk to patient independent of anaesthesia.	Thyroidectomy; hysterectomy; myomectomy; cystectomy; cholecystectomy; laminectomy; hip/knee replacement; nephrectomy; major laparoscopic procedures; resection/reconstructive surgery of the digestive tract.	Open thoracic or intracranial procedure; major vascular repair (e.g. aorto-femoral bypass); planned post-operative monitored care setting (intensive care unit, post anaesthesia care unit).
III	Highly invasive procedures. Potential blood loss greater than 1500 ml. Major to critical risk to patient independent of anaesthesia. Usual post-operative intensive care unit stay with invasive monitoring.	Major orthopaedic-spinal reconstruction; major reconstruction of the gastrointestinal tract; major genitourinary surgery (e.g. radical retro-pubic prostatectomy); cardiothoracic procedures; intracranial procedures; major procedures on the oropharynx; major vascular, skeletal, neurological repair.	

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Supplementary Table 3 – Subgroup analyses performed for meta-analysis.

Parameters		Day of surgery [a]	Day 1 post-op.	Day 2 post-op.	Day 3 post-op.	Day 4 post-op.	Day 5 post-op.	Day 6 post-op.	Day 7 post-op.	References
Grade of surgery [b]	Grade I	SS: 659	SS: 90	n/a	n/a	n/a	n/a	n/a	n/a	21, 62, 67, 69, 75, 82
	Grade II	SS: 3,829	SS: 981	SS: 366	SS: 121	SS: 75	SS: 16	SS: 10	SS: 71	19-24, 26-35, 37, 39-44, 47-54, 58, 60, 61, 63, 65, 66, 68-70, 74, 76, 78, 85, 89
	Grade III	SS: 1,749	SS: 871	SS: 209	SS: 67	SS: 55	n/a	SS: 18	SS: 27	25, 32, 36, 38, 42, 45, 46, 55-57, 64, 69, 71-73, 77, 79-81, 83, 84, 86-88
Age [c]	Grade II, ≤60 years	SS: 2,987	SS: 601	SS: 150	SS: 69	n/a	n/a	n/a	n/a	22, 28, 30, 31, 34, 35, 40, 42-44, 47, 48, 51, 53, 54, 58, 61, 63, 65, 66, 68, 76, 78, 85, 89
	Grade II, >60 years	SS: 533	SS: 158	SS: 98	SS: 6	SS: 6	n/a	n/a	n/a	28, 37, 39, 50, 58, 65, 69, 78, 85
	Grade III, ≤60 years	SS: 597	SS: 320	SS: 135	n/a	n/a	n/a	n/a	n/a	36, 46, 57, 73, 83, 84, 87
	Grade III, >60 years	SS: 809	SS: 472	SS: 22	SS: 12	n/a	n/a	SS: 18	n/a	38, 42, 59, 64, 69, 71-73, 77, 80, 81, 88
Gender [d]	Grade II+III, >50% females	SS: 2,205	SS: 588	SS: 119	SS: 77	SS: 49	n/a	n/a	SS: 49	19, 23, 25, 27-32, 36, 37, 40, 41, 44, 48, 50, 51, 54, 57, 58, 60, 67, 71, 73, 77
	Grade II+III, >50% males	SS: 2,143	SS: 1,088	SS: 345	SS: 39	SS: 55	n/a	n/a	SS: 43	42, 45, 47, 52, 53, 56, 62, 65, 66, 68, 74, 76, 80, 82, 83, 85
Duration of surgery [e]	Grade II, ≤180 min	SS: 2,278	SS: 558	SS: 115	SS: 89	n/a	n/a	n/a	n/a	19, 23, 24, 27, 28, 31, 32, 38-41, 44, 50, 51, 55, 62, 65, 73, 82, 86
	Grade II, >180 min	SS: 221	SS: 31	SS: 31	n/a	n/a	n/a	n/a	n/a	45, 48, 66
	Grade III, ≤180 min	SS: 279	SS: 57	SS: 57	n/a	n/a	n/a	n/a	n/a	84, 87
	Grade III, >180 min	SS: 865	SS: 668	SS: 100	SS: 12	n/a	n/a	n/a	n/a	36, 38, 42, 46, 69, 71, 77, 81, 83, 88
Surgical technique [f]	Laparoscopy	SS: 1,086	SS: 270	n/a	SS: 64	n/a	n/a	n/a	n/a	26-28, 30, 31, 33, 35, 40-44, 51, 53, 61, 63, 76, 89
	Laparotomy	SS: 1,676	SS: 411	SS: 221	SS: 34	SS: 49	n/a	n/a	n/a	23-25, 27, 28, 30, 32, 37, 38, 40, 41, 45, 46, 48-50, 57, 58, 60, 62, 66, 73, 75

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Anaesthesia [g]	Grade II+III, etomidate used in >50% of patients	SS: 320	SS: 523	SS: 104	SS: 12	SS: 12	n/a	n/a	n/a	34, 36, 45, 46, 55, 77, 83, 89
	Grade II+III, no etomidate used	SS: 3,761	SS: 884	SS: 268	SS: 40	SS: 10	SS: 16	SS: 10	SS: 6	16, 17, 19, 20, 23, 25, 27-32, 34-40, 44, 51, 54-56, 61-63, 65, 66, 68, 71, 76-78, 82, 84, 86
	General anaesthesia	SS: 142	SS: 59	n/a	n/a	n/a	n/a	n/a	n/a	58, 68, 85
	Regional anaesthesia	SS: 142	SS: 59	n/a	n/a	n/a	n/a	n/a	n/a	
Assay [h]	RIA	SS: 2,715	SS: 548	SS: 223	SS: 16	SS: 26	SS: 16	SS: 10	SS: 22	19, 20, 22-25, 29-32, 34-37, 39-41, 43, 44, 46-49, 51, 54, 58, 59, 64, 70, 73, 76, 79, 87
	ECLIA	SS: 1,117	SS: 683	SS: 67	SS: 43	SS: 27	n/a	n/a	SS: 27	45, 52, 63, 68, 71, 77, 78, 80, 81, 83, 85, 86, 88, 89
	ELISA	SS: 547	SS: 229	SS: 114	n/a	SS: 49	n/a	n/a	SS: 49	26, 28, 50, 57, 60, 66
	LC-MS/MS	SS: 28	SS: 28	SS: 28	SS: 28	SS: 28	n/a	n/a	n/a	55, 56
ALL STUDIES n=2,953 patients		SS: 6,460	SS: 2,149	SS: 590	SS: 257	SS: 130	SS: 16	SS: 28	SS: 141	Listed in Table 1

The studies included in each subgroup analysis are reported in the last column. For each parameter assessed the cumulative sample size (SS – number of single cortisol measurements) is reported. These are divided by time points, which cover from the baseline assessment (prior to surgery) up to the 7th post-operative day. The cortisol responses for each subgroup analysis are reported in Figures 2 to 4.

[a] Serum cortisol measurements during the day of surgery have been captured according to six time points: time 1, baseline (before surgery); time 2, initiation of surgery; time 3, during surgery; time 4, end of surgery; time 5, first 6h after surgery; time 6, between 6 and 18h after surgery. The SSs reported in this column correspond to the sum of all the six time points.
[b] The grade of surgery has been defined according the classification reported in Supplementary Table 2.
[c] The cohorts have been divided according to the grade of surgery and to the mean age of patients being ≤ or >60 years.
[d] The cohorts have been divided according to the percentage of males and females. Only cohorts with either >50% females or >50% males have been included.
[e] The cohorts have been divided according to the grade and the mean duration of surgery (either ≤180 or >180 minutes).
[f] Only the cohorts of patients who underwent either laparotomies or abdominal/pelvic laparoscopies have been included for analysis.
[g] We included only cohorts for which the anaesthetic technique was described in detail. As for the use of etomidate, we compared cohorts in which etomidate was not used as an anaesthetic agent to cohorts where this was used in >50% of patients. As far as the anaesthetic technique is concerned, we used data derived from prospective interventional studied comparing general and regional anaesthesia (either spinal or epidural thoracolumbar anaesthesia).
[h] We have divided studies according to the cortisol assays used.

Abbreviations: ECLIA: electrochemiluminescence; ELISA: enzyme-linked immunosorbent assay; IRMA: immunoradiometric assay; LC-MS/MS: liquid chromatography-tandem mass spectrometry; n=number of studies; SS: sample size; RIA: radioimmunoassay; n/a: not available.

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Supplementary Table 4 – Methodological quality assessment of the included studies.

First author (year of publication)	Patients (inclusion criteria) [a]	Patients (exclusion criteria) [b]	Cortisol measurement (methodology) [c]	Cortisol measurement (timing) [d]	Cortisol measurement (reporting) [e]	Cortisol measurement (time points) [f]
Høgevold (1990) [19]	Yes	Unclear	Yes	Yes	Yes	Yes
Bartalena (1990) [20]	Yes	Unclear	Yes	Yes	Yes	Yes
Hosokawa (1990) [21]	Yes	Unclear	Yes	Yes	Yes	Yes
Bickel (1991) [22]	Somewhat	Yes	Yes	Yes	Yes	Yes
Naito (1991) [23]	Somewhat	Somewhat	Yes	Somewhat	Somewhat	Yes
Juma (1991) [24]	Yes	Unclear	Yes	Yes	Yes	Yes
Harris (1991) [25]	Somewhat	Unclear	Yes	Yes	Yes	Yes
Donald (1993) [26]	No	Unclear	Yes	Yes	Yes	Yes
McMahon (1993) [27]	Somewhat	Unclear	Yes	Yes	Yes	Somewhat
Deuss (1994) [28]	Somewhat	Unclear	Yes	Yes	Yes	Yes
Crozier (1994) [29]	Yes	Unclear	Yes	Yes	Yes	Yes
Jakeways (1994) [30]	Yes	Unclear	Somewhat	Yes	Yes	Yes
Dionigi (1994) [31]	Yes	Unclear	Somewhat	Yes	Yes	Somewhat
Frank (1995) [32]	Unclear	Unclear	Yes	Somewhat	Yes	Yes
Ellstrom (1996) [33]	Yes	Unclear	No	Yes	Yes	Yes
Jameson (1997) [34]	Somewhat	Unclear	Yes	Yes	Yes	Yes
Karayiannakis (1997) [35]	Yes	Yes	Yes	Yes	Yes	Yes
Roth-Isigkeit (1997) [36]	Yes	Yes	Yes	Yes	Yes	Yes
Stratton (1997) [37]	Yes	Unclear	Yes	Yes	Yes	Yes
Plunkett (1997) [38]	Yes	Unclear	Yes	Yes	Yes	Yes
Eriksson-Mjöberg (1997) [39]	Yes	Yes	Yes	Yes	Yes	Somewhat
Bellón (1998) [40]	Somewhat	Unclear	Yes	Yes	Yes	Yes
Engin (1998) [41]	Yes	Yes	Yes	Somewhat	Yes	Yes
Yamauchi (1998) [42]	Yes	Yes	Yes	Yes	Yes	Yes
Delogu (1999) [43]	Yes	Yes	Yes	Somewhat	Yes	Yes
Friedrich (1998) [44]	Yes	Yes	Somewhat	Yes	Yes	Yes
Vogeser (1999) [45]	Yes	Yes	Yes	Yes	Yes	Yes
Roth-Isigkeit (2000) [46]	Yes	Somewhat	Yes	Yes	Yes	Yes
Schricker (2000) [47]	Yes	Yes	Yes	Yes	Yes	Yes

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Cho (2000) [48]	Yes	Yes	Yes	Somewhat	Yes	Yes
Ogata (2000) [49]	Yes	Unclear	Yes	Yes	Yes	Yes
Mahla (2000) [50]	Yes	Unclear	Yes	Yes	Yes	Yes
Nguyen (2002) [51]	Yes	Unclear	Yes	Yes	Yes	Yes
Ozarda Ilçöl (2002) [52]	Yes	Unclear	Yes	Yes	Yes	Yes
Holub (2002) [53]	Yes	Unclear	Yes	Yes	Yes	Yes
Marana (2003) [54]	Yes	Yes	Yes	Yes	Yes	Yes
Vogeser (2003) [55]	Yes	Unclear	Yes	Yes	Yes	Yes
Vogeser (2003) [56]	Yes	Unclear	Yes	Somewhat	Yes	Yes
le Roux (2003) [57]	Yes	Yes	Yes	Yes	Yes	Yes
Lattermann (2003) [58]	Yes	Yes	Yes	Somewhat	Yes	Yes
Shahbazi (2004) [59]	No	Unclear	Yes	Yes	Yes	Yes
Maciejczyk-Pencula (2004) [60]	Yes	Unclear	Yes	Yes	Yes	Yes
Haque (2004) [61]	Yes	Unclear	No	Yes	Yes	Yes
Ledowski (2005) [62]	Yes	Yes	Yes	Yes	Yes	Yes
Crema (2005) [63]	Yes	Unclear	Yes	Yes	Yes	Yes
Yeager (2005) [64]	Yes	Yes	Yes	Yes	Yes	Yes
Kudoh (2005) [65]	Yes	Unclear	Somewhat	Yes	Yes	Yes
Nicholson (2005) [66]	Yes	Yes	Yes	Yes	Yes	Yes
Rahr (2006) [67]	Somewhat	Unclear	Yes	Yes	Yes	Yes
Buyukkocak (2006) [68]	Yes	Yes	Yes	Yes	Yes	Yes
Kashiwabara (2007) [69]	Yes	Yes	Yes	Yes	Yes	Yes
Baričević (2007) [70]	Yes	Unclear	Yes	Yes	Yes	Yes
Christ-Crain (2007) [71]	Yes	Yes	Yes	Yes	Yes	Yes
Rapp-Kesek (2007) [72]	Yes	Unclear	Yes	Yes	Yes	Yes
Spratt (2008) [73]	Yes	Yes	Yes	Yes	Yes	Yes
Mujagić (2008) [74]	Yes	Yes	Yes	Yes	Yes	Yes
Bender (2009) [75]	Yes	Unclear	Yes	Yes	Yes	Yes
Kim (2010) [76]	Yes	Yes	Yes	Yes	Yes	Yes
Mu (2010) [77]	Yes	Yes	Yes	Yes	Yes	No
Engin (2010) [78]	Yes	Yes	Yes	Somewhat	Yes	Somewhat
Celic-Spuzic (2011) [79]	Yes	Unclear	Yes	Yes	Yes	Somewhat
Mello (2011) [80]	Yes	Yes	Yes	Yes	Yes	Yes
Debono (2011) [81]	Yes	Yes	Yes	Yes	Yes	Yes

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Adas (2013) [82]	Yes	Unclear	Somewhat	Yes	Yes	Yes
Mu (2013) [83]	Yes	Yes	Yes	Yes	Yes	Yes
Ferreira (2014) [84]	Yes	Unclear	No	Yes	Yes	Yes
Kahveci (2014) [85]	Yes	Unclear	Yes	Yes	Yes	Yes
Maas (2014) [86]	Somewhat	Unclear	Yes	Yes	Yes	Yes
Gu (2015) [87]	Yes	Unclear	Yes	Yes	Yes	Yes
Lebherz (2016) [88]	Yes	Yes	Yes	Yes	Yes	Yes
Możański (2016) [89]	Yes	Yes	Yes	Yes	Yes	Yes

[a] Question answered to assess the quality of the study – Are inclusion criteria for cohort selection described in sufficient detail?
[b] Question answered to assess the quality of the study – Are patients on steroids or any other disease influencing endogenous steroid production excluded?
[c] Question answered to assess the quality of the study – Is the cortisol assay used clearly reported?
[d] Question answered to assess the quality of the study – Is the timing of cortisol measurement clearly reported?
[e] Question answered to assess the quality of the study – Is the cortisol measurement reported for the whole cohort?
[f] Question answered to assess the quality of the study – Is it possible to correlate the cortisol measurement to a specific time point for the cohort reported?

eer Review

Supplementary Table 5 – Areas under the curve (AUCs) for total cortisol values, divided by 24-h periods and by different parameters (age, gender, duration of surgery, surgical technique, anaesthesia, serum cortisol assays).

Parameters		Number of studies	AUC 0-24-h (nmol*h/L)	AUC Day 1 post-op. (nmol*h/L)	AUC Day 2 post-op. (nmol*h/L)	AUC Day 3 post-op. (nmol*h/L)	AUC Day 4 post-op. (nmol*h/L)	AUC Day 5 post-op. (nmol*h/L)	AUC Day 6 post-op. (nmol*h/L)	AUC Day 7 post-op. (nmol*h/L)
Age	Grade II, ≤60 years	25	17,130	12,276	11,724	n/a	n/a	n/a	n/a	n/a
	Grade II, >60 years	9	17,448	14,268	14,796	n/a	n/a	n/a	n/a	n/a
	Grade III, ≤60 years	7	13,658	15,456	n/a	n/a	n/a	n/a	n/a	n/a
	Grade III, >60 years	12	16,574	18,372	15,816	n/a	n/a	n/a	n/a	n/a
	Grade II+III (combined), ≤60 years	32	16,443	13,428	12,480	n/a	n/a	n/a	n/a	n/a
	Grade II+III (combined), >60 years	21	17,258	15,168	13,620	n/a	n/a	n/a	n/a	n/a
Gender	Grade II+III (combined), >50% females	26	18,696	11,436	12,972	15,600	n/a	n/a	n/a	n/a
	Grade II+III (combined), >50% males	25	15,817	15,012	13,092	12,168	n/a	n/a	n/a	n/a
Duration of surgery	Grade II, ≤180 min	21	17,542	15,072	13,800	n/a	n/a	n/a	n/a	n/a
	Grade II, >180 min	4	18,954	11,856	n/a	n/a	n/a	n/a	n/a	n/a
	Grade III, ≤180 min	2	14,005	15,752	n/a	n/a	n/a	n/a	n/a	n/a
	Grade III, >180 min	10	15,516	15,917	12,791	n/a	n/a	n/a	n/a	n/a
	Grade II+III (combined), ≤180 min	23	17,203	14,793	13,458	n/a	n/a	n/a	n/a	n/a
	Grade II+III (combined), >180 min	14	16,451	14,995	12,172	n/a	n/a	n/a	n/a	n/a

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Surgical technique	Laparoscopy	18	17,968	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Laparotomy	24	19,063	13,987	14,016	14,994	n/a	n/a	n/a	n/a
Anaesthesia	Grade II+III (combined), etomidate used in >50% of patients	8	18,480	14,729	11,862	8,508	n/a	n/a	n/a	n/a
	Grade II+III (combined), no etomidate used	38	17,677	13,772	12,576	13,260	14,008	12,963	n/a	n/a
	General anaesthesia	3	16,270	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Regional anaesthesia	3	13,517	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Assays	RIA	33	17,765	14,007	15,326	14,389	12,448	12,963	10,976	9,936
	ECLIA	17	18,127	15,098	13,895	14,782	n/a	n/a	n/a	n/a
	ELISA	6	21,114	11,865	n/a	n/a	n/a	n/a	n/a	n/a
	LC-MS/MS	2	9,569	12,005	10,750	9,091	n/a	n/a	n/a	n/a

Abbreviations: AUC: area under the curve for serum cortisol values; n/a: not available or not applicable.

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Supplementary Table 6 – Factors potentially affecting the perioperative cortisol response and the measurement of total serum cortisol.

Factors	Assessed in this article	Other factors

Patient characteristics	• Patient age;
	• Patient gender.
		• Comorbidities: these are not always clearly reported in the inclusion/exclusion criteria of the articles included. Some disorders can alter the cortisol metabolism (e.g. liver disease, nephrotic syndrome, thyrotoxicosis, hypothyroidism and diabetes mellitus). The exclusion of patients with adrenal or pituitary disorders is not always specified in the articles included in this systematic review;
		• ASA physical status: not all the studies included in this article specify the ASA physical status. When it is specified, it can vary considerably among the included cohorts. The ASA physical status correlates with the duration of hospital stay and post-operative complications, that can affect the cortisol response;
		• Nutrition status: malnutrition can enhance the cortisol output;
		• Studies including patients undergoing surgery for traumatic lesions do not always specify if some of the patients experienced head trauma. Head trauma can lead to temporary or long-term central adrenal insufficiency;
		• Several medications can potentially affect the cortisol metabolism and measurement, and their use is not always specified in the included articles (medications specifically used for the treatment of endogenous hypercortisolism and adrenocortical cancer are not reported):
		  o Increase of CBG – e.g. Oestrogens; Tamoxifen.
		  o Medications enhancing the CYP3A4 activity – e.g. Barbiturates; Carbamazepine; Oxcarbazepine; Phenytoin; Reverse transcriptase inhibitors; Rifampicin;
		  o Medications inhibiting the CYP3A4 activity – e.g. Amiodarone; Azole antifungals; Chloramphenicol; Clarithromycin; Nefazodone; Nicardipine; Protease inhibitors;
		  o Medications binding to the glucocorticoid receptor: Medroxyprogesterone acetate; Megestrol acetate;
		  o Medications having an action on pituitary ACTH production: Glitazones;
		  o Cross-reactivity with cortisol assays: Biotin; Quinine; Quinidine; Spironolactone.

Features of surgery	• Grade of surgery (as per the modified Johns Hopkins surgical criteria); • Laparotomy vs. laparoscopy;	• Surgery for malignant conditions: radical resection for cancer is often associated with greater surgical trauma and longer operative time; • Surgery for post-traumatic lesions: surgery after trauma can be associated with longer operative time, greater blood loss and higher risk of complications; • Non-elective surgery: it is associated with higher rates of complications; • Perioperative haemodilution and/or hypoproteinaemia (due to blood loss or intravenous fluid administration): it can causes to falsely lower cortisol values; • Post-operative drop of CBG.
Anaesthetic techniques	• General vs. regional anaesthesia; • Use of etomidate.	• Epidural anaesthesia techniques (especially when performed below the umbilicus) can lead to blunted cortisol responses after surgery, in comparison to general anaesthesia. When epidural anaesthesia is used for thoracic and upper abdominal surgeries, the modulatory effect on cortisol secretion is less evident.[114] However, a recent study suggests that the combination of general anaesthesia and thoracic epidural blockade is more efficient in determining a reduction of the cortisol response in comparison to general anaesthesia alone;[87]

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		• Opiates are known to suppress cortisol secretion. General anaesthesia with opiates (especially when high doses are used) can blunt the cortisol response,[47,115-117] but not all authors confirmed these observations.[43,92,118] In cardiac surgery, the cortisol response induced by the cardiopulmonary bypass appears to overcome the inhibitory effects of high-dose opiates;[114] • Sevoflurane anesthesia in patients undergoing laparoscopic pelvic surgery was associated with significantly lower concentrations of ACTH and cortisol (during and immediately after surgery), when compared to Isoflurane;[54] • Patients receiving a balanced anaesthesia (Sevoflurane/Remifentanil) showed higher cortisol levels after extubation, when compared to a total intravenous anaesthesia (Propofol/Remifentanil).[62] • Midazolam: it is a benzodiazepine containing an imidazole ring (like Etomidate). It can attenuate the cortisol response after surgery;[2,119] • Perioperative use of steroids: not always specified as an exclusion criterion in the articles included in this systematic review and meta-analysis.
Post-operative complications		Post-operative complications (e.g. fever, haemorrhage, infections and sepsis, acute confusion) are a cause of stress and can affect the cortisol output. Not all studies included in this article specify post-operative complications. One study reported data from patients with post-operative sepsis.[70]
Post-operative care		Several factors can potentially affect the stress cortisol response after surgery, for example: • Admission to the intensive care unit; • Intraoperative normothermia: the fall of core body temperature can stimulate the stress response to surgery (cortisol and catecholamines), while post-operative forced air warming can blunt the cortisol surge;[32] • Post-operative sedation: it helps reducing the surgical stress index post-operatively.[120] The use of sedatives and hypnotics can interfere with the sleeping patterns and the circadian rhythm; • Post-operative management of pain, nausea and vomiting; • Management of drains, tubes and catheters; • Timing of post-operative nutrition and mobilization.
Methodological factors	Total cortisol assays	Several factors may have an impact on the total cortisol values that have been extracted for this systematic review and meta-analysis: • The timing of cortisol measurements is not always clearly reported; • Most of pre- and post-operative cortisol measurements have been performed during the morning. However, this is not always specified; • Some of the included articles did not report the exact cortisol values, which have been therefore extracted from graphs.
Abbreviations: ACTH: adrenocorticotropic hormone; ASA: American Society of Anesthesiologists; CBG: cortisol-binding globulin; CYP3A4: Cytochrome P450 3A4.		

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