Manuscript category: Original article

Short running header: Humoral surgical stress response

Perioperative humoral stress response to laparoscopic vs open aortobifemoral bypass surgery

Anne H. Krog^{a,b}, Per M. Thorsby^{a,c}, Mehdi Sahba^d, Erik M. Pettersen^e, Irene Sandven^f,
Jørgen J. Jørgensen^{a,b}, Jon O. Sundhagen^b & Syed S.H. Kazmi^b

^aInstitute of Clinical Medicine, University of Oslo, Oslo, Norway; ^bDepartment of Vascular Surgery, Division of Cardiovascular and Pulmonary Diseases, Oslo University Hospital, Oslo, Norway; ^cHormone Laboratory, Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway; ^dDepartment of Vascular Surgery, Østfold Central Hospital, Kalnes, Norway; ^eDepartment of Vascular Surgery, Sørlandet Hospital HF, Kristiansand, Norway; ^fOslo Center for Biostatistics and Epidemiology (OCBE), Oslo University Hospital, Oslo, Norway.

Corresponding author:

Anne Helene Krog MD

Institute of clinical medicine

University of Oslo

P.O box 1171, Blindern

0318 OSLO

Norway

E-mail address: a.h.krog@medisin.uio.no

Abstract

Background

Minimally invasive surgery seems to reduce hormonal stress response to surgery, but has not previously been examined in major abdominal vascular surgery. Aortic crossclamping time and operation time is known to be longer in the totally laparoscopic aortobifemoral bypass (LABF) as compared to open aortobifemoral bypass (OABF). The main objective of our study was to measure the hormonal stress response during surgery and aortic crossclamping in patients undergoing a totally laparoscopic versus an open aortobifemoral bypass.

Methods

This was a substudy of a larger randomized controlled multicentre trial. Thirty consecutive patients with severe aortoiliac occlusive disease were randomized to either a laparoscopic (LABF) or an open (OABF) procedure. The surgical stress response was measured by perioperative monitoring of adrenocorticotrophic hormone (ACTH), aldosterone, metanephrine and cortisol at eight different timepoints.

Results

During surgery there was an increase in all humoral stress markers in both groups. The analysis of covariance showed increased levels of cortisol and ACTH in open group at 24 hours timepoint as compared to the baseline and this difference was statistically significant between the two groups, which indicate an earlier return to baseline levels in the laparoscopic group. Results from the General Estimated Equations (GEE) model indicate that LABF generates lower level of metanephrine and higher level of aldosterone as compared to OABF.

Conclusion

Although they have higher levels of ACTH, aldosterone and cortisol during surgery, the patients operated with a laparoscopic aortobifemoral bypass achieve earlier hormonal homeostasis after surgery compared to open aortobifemoral bypass.

Key words: *adrenocorticotrophic hormone, aldosterone, hydrocortisone, laparoscopy, metanephrine,*

Introduction

There are a number of responses to surgical trauma and injury: physiological, psychological and biochemical [1-3]. The purpose of these stress responses is to bring the patient back to a homeostatic plateau [1]. A part of this reaction is the humoral response [4]. Corticotropin releasing hormone (CRH) is released from the hypothalamus which stimulates the pituitary synthesis of ACTH. ACTH regulates the release of glucocorticoids. Glucocorticoids in turn initiates catecholamine synthesis, gluconeogenesis, glycogenesis, etc. [1].

The hormonal stress response has been used as measure of surgical trauma [5, 6]. Some studies have shown that laparoscopic surgery may lead to a decreased hormonal stress response in patients, which may result in decreased complication rates, morbidity and mortality [7,8]. The evidence has, however, not been conclusive and many studies in other fields of surgery show no statistically significant difference [5,9,10]. It has been suggested that the effect might be different in major surgery and this has been pointed out as an area for further research[5].

Aortoiliac occlusive disease (AIOD) is amongst several expressions of atherosclerosis. One treatment for AIOD is the aortobifemoral bypass, which can be performed laparoscopically as well as with the traditional open technique [11-13]. The procedure, either laparoscopic or open, is a major abdominal surgical intervention and involves crossclamping. In that sense it potentially derives a large hormonal stress response [14]. Aortic crossclamping has previously been shown to be responsible for hemodynamic changes at the time of crossclamping and declamping [14]. A longer aortic cross-clamping time during laparoscopic aortobifemoral bypass has been a point of concern with this type of surgery [15]. Besides the hemodynamic changes, crossclamping initiates the release of catecholamines. Other metabolites are released

from the ischemic tissue, which again can affect the systemic circulation and have implications for the perioperative morbidity and mortality [14]. However, in patients with severe aortoiliac occlusive disease the changes in the hemodynamics have been shown to be less prominent probably due to a gradual development of collateral vessels secondary to AIOD [14]. Laparoscopic aortic surgery was introduced at Oslo University Hospital, Aker, in 2005 and after successfully conducting a prospective comparative cohort study during the earlier experience, a randomized multicentre trial, Norwegian Laparoscopic Aortic Surgery Trial (NLAST) is being performed [16].

As far as the authors' know there are no previous studies measuring the hormonal stress response in laparoscopic versus open aortobifemoral bypass surgery. The aim of this substudy was to investigate whether laparoscopic aortobifemoral bypass surgery induces a reduced hormonal stress response during surgery and crossclamping of the aorta compared to open aortobifemoral bypass.

Materials and methods

Design

The substudy described in this article is a part of the NLAST, a randomized controlled trial where patients with severe symptomatic aortoiliac occlusive disease (AIOD) classified according to the TASC-II (Trans-Atlantic Inter-Society Consensus) [17] as type D lesions were randomized to either totally laparoscopic aortobifemoral bypass (LABF) or open aortobifemoral bypass (OABF) surgery.

Participants

Three vascular surgery departments in the South-East region of Norway participated in the study. Patients were included based on the following inclusion and exclusion criteria.

Inclusion criteria

- Patient with AIOD, TASC type D lesion [17], and symptoms in form of:
 - Intermittent claudication, with pain-free walking distance < 200 meters and/or,
 - Chronic critical lower limb ischemia with rest pain or ischemic ulcers (duration of symptoms > 2 weeks)

Exclusion criteria

- Eligible for endovascular procedure
- COPD (Chronic Obstructive Pulmonary disease) $\geq$ stage IV, GOLD classification [18]
- Symptomatic coronary heart disease
- Chronic heart failure, EF (ejection fraction) < 40%
- Active cancer disease
- Hostile abdomen, previous major abdominal surgery
- Abdominal aortic aneurysm (AAA) $\geq$ 3.0 cm (defined by TASC-II [17])
- Acute critical limb ischemia (duration of symptoms $\leq$ 2 weeks)

Intervention

The patients underwent aortobifemoral bypass surgery with either a totally laparoscopic or an open approach under general anaesthesia. We used aortobifemoral silver coated Dacron grafts. Both techniques begin with the dissection of the femoral artery.

Open technique: The surgery was performed through a vertical midline abdominal incision.

The aorta was dissected free, before crossclamping above the site of anastomosis. The

proximal anastomosis was performed in an end-to-side fashion. Afterwards the clamp was moved to the proximal end of the graft. The distal end-to-side anastomosis was constructed, clamp removed and blood flow re-established to the lower extremities.

Laparoscopic technique: The procedure was performed totally laparoscopically and according to the technique described by Coggia et al [19], using six small incisions for the laparoscopic equipment. The pneumoperitoneum was obtained by CO₂ insufflation. Standard pressure was maintained at 12 mmHg. The aorta was dissected free using a transperitoneal retrocolic prerenal approach and then following the same steps as the open technique.

Outcomes

Primary outcome was hormonal stress response due to surgical trauma measured by perioperative monitoring of adrenocorticotrophic hormone (ACTH), aldosterone, metanephrines and cortisol. We chose these markers as they reflect the body's endocrine response to surgery and/or trauma [2,20]. Blood samples were taken before, during and after surgery at the following eight timepoints;

1. After anaesthesia and administration of central venous access (baseline)
2. Right after first groin incision
3. Right after first incision for laparotomy/laparoscopy
4. Immediately before crossclamping of the aorta
5. Immediately after the placement of the aortic crossclamp
6. After reestablishment of flow through first graft limb (clamp removed)
7. Wound closure
8. 24 hours after the beginning of surgery

Blood samples were primarily taken from arterial catheter, and if not possible from central venous catheter. ACTH and metanephrines were analysed from ethylenediaminetetraacetic acid (EDTA) plasma. EDTA tubes were placed on ice before sampling, and centrifuged at 4°C, 3000 turns/min, within 30 min. Cortisol and aldosterone were analysed from serum, which had been centrifuged at 4°C, 3000 turns/min and frozen within two hours from sampling. Both plasma and serum samples were frozen in polypropylene nunc vials and stored at - 80°C until analysis. The samples were analysed by the Hormone Laboratory at Oslo University Hospital.

Changes in outcome

In the protocol we planned to measure epinephrine and norepinephrine. But due to paracetamol interference with analysis of epinephrine [21], and the occasional use of artificial norepinephrine during surgery, these could not be measured directly. Metanephrine which is a physiological breakdown product of and correlates with epinephrine was chosen as a substitute.

Procedure for analysis

Aldosterone was analysed using the LIAISON Aldosteron assay, a chemiluminescent immunoassay (CLIA), on the LIAISON Analyzer (Diasorin Inc, USA). Cortisol was analysed using a competitive luminoimmunoassay (LIA), and ACTH a non-competitive immunoluminometric assay (ILMA). Both analysed on kits from Siemens on the Immulite 2000 from Siemens Healthcare (Siemens Healthcare, Germany). Metanephrines was analysed using a competitive radioimmunoassay (RIA), on a kit from LDN (Labor Diagnostika Nord GMBH, Germany).

Sample size

This was a substudy in a larger randomized trial, and an individual power analysis was not conducted on these outcomes.

Randomization and blinding

The patients were enrolled and included by clinicians and researchers before they were randomized to either open or laparoscopic surgery. We used block randomization, with six patients in each block and different sequences for the three participating hospitals. The sequence was random and kept contained in opaque sequentially numbered envelopes. It was not known by the researchers. Laboratory technicians were blinded, but blinding of surgeon and/or participants after randomization was not possible.

Statistics

The four markers of operative stress; cortisol, aldosterone, ACTH and metanephrine were considered as continuous outcomes and measured at eight timepoints for all subjects in the two groups. Categorical variables were summarized as frequencies and continuous variables by the median and interquartile range. Comparisons between the two treatment groups were performed using the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. The serial measurements of the hormonal markers were presented graphically, and analysed using the General Estimated Equations (GEE) model [22]. All markers were analysed in their logged form due to skewed distribution of data. Since the number of clusters were scars (30 patients), we considered measures at only three timepoints (i.e. after intubation and establishment of central venous access, immediately before aortic cross clamping, and 24 hours after start of surgery) to meet the conditions for the adequacy of

the GEE model. In addition we evaluated change in serum levels from baseline (after intubation and establishment of central venous access) to follow-up (24 hours after start of surgery). Comparison between the two treatment groups was performed using analysis of covariance (ANCOVA) [23]. Statistical significance was set at a 5 % level ($p < 0.05$). The software used for statistical analyses were Epi Info (Epi Info TM software, Center for Disease Control and Prevention, Atlanta, USA), IBM SPSS statistics version 22.0 (IBM corporation, New York, USA) and STATA 13.0 (StataCorp LP, College Station, TX, USA).

Ethics

The patients were not exposed to any additional unnecessary harm or strain as a result of their participation. The participants gave an informed, written consent. The trial was approved by the Regional Committee for Medical and Health Research Ethics (registration number 2012/1367), and establishment of a biological bank was approved by the Norwegian Data Inspectorate. Ethical standards were in accordance with the Helsinki Declaration of 1975. The trial was registered at www.clinicaltrials.gov, with the registration number NCT01793662.

Results

Participant flow and recruitment

Thirty consecutive patients from the participating hospitals were included from February 2013- January 2015 and randomized to either LABF (n=14) or OABF (n=16) (figure I). The two groups had comparable patient baseline characteristics (table I). One patient was converted from laparoscopic to open surgery, due to bleeding. The patient was analysed in the laparoscopic group, as intention to treat. No patients were excluded after randomization or lost to follow up at the time of analysis. There were a total of 5 missing blood samples out of 240. This substudy trial was completed after the inclusion of 30 patients.

Outcomes and estimation

We provide a graphical presentation of the serum/plasma levels of cortisol, aldosterone, ACTH and metanephrine during and after surgery in figure II-V, and a numerical presentation in table II. When purely describing the absolute values, all the hormone levels increase following the beginning of surgery and decline again postoperatively. Cortisol, ACTH and Aldosterone were higher in the laparoscopy group during surgery, but returned earlier to baseline as compared to the open surgery group. However, metanephrine was lower in the laparoscopic group. All stress markers declined during the aortic crossclamping in both LABF and OABF group (table II).

Further analysis with the GEE model (table III) also indicates that laparoscopic aortobifemoral bypass (LABF) generates lower level of metanephrine and higher level of aldosterone as compared to open aortobifemoral bypass (OABF) during follow-up from after intubation and establishment of central venous access until 24 hours after operation. For the biomarkers cortisol and ACTH we cannot be conclusive because the effect was not significant, probably due to power deficiency.

The estimated mean difference between the two treatment groups from baseline (after central venous line, before surgery) until follow-up at 24 hours indicates that LABF have an earlier return to baseline in the serum level of cortisol and ACTH as compared to OABF. The analysis of covariance finds that the difference between the groups regarding changes in serum level of cortisol and ACTH during follow up is statistically significant. For aldosterone the reduction was borderline significant, and for metanephrine the reduction was non-significant. The results from the ANCOVA analysis are presented in table IV.

Surgery related parameters are given in table V. Patients treated with a laparoscopic procedure had a longer anastomosis time as compared with open surgery, but significantly less bleeding. One patient in each group had suprarenal aortic crossclamping.

Clinical outcomes are described in table VI. The patients had significantly shorter hospital stay in the laparoscopic group. There was no difference in postoperative morbidity or mortality.

Discussion

Both laparoscopic and open aortobifemoral bypass surgery causes an increased hormonal stress response as compared to baseline measured by the serum levels of ACTH, cortisol, aldosterone and metanephrine. We found higher level of serum aldosterone in the laparoscopic aortobifemoral bypass (LABF) group, and lower plasma levels of metanephrine compared to open aortobifemoral bypass (OABF) (table III). The serum level of cortisol and ACTH in the LABF group returned earlier to baseline compared to OABF group.

Although other studies have shown lower or non-significant changes in the stress hormones among the patients operated with laparoscopic surgery as compared to open surgery [9,10,24], earlier correction of hormonal homeostasis has been observed in some studies for the laparoscopy patients [20,25,26]. Laparoscopic surgery may have a positive effect on hormonal stress response, but it has also been argued that this effect is lost in major aortic surgery [27]. The aortic crossclamping differentiates this type of surgery from other areas of laparoscopic surgery, and results from other fields of surgery may not be applicable. An animal study found no difference in the humoral response to open and laparoscopic aortic surgery, but there are no known research on human subjects [27].

In our study there were higher serum levels of cortisol, ACTH and aldosterone in the laparoscopic group during surgery, however, in the GEE model only the difference in aldosterone was statistically significant. A possible cause of higher levels of cortisol, ACTH and aldosterone may be the pneumoperitoneum, which is previously known to initiate an inflammatory reaction [28,29] and hormonal stress response [30-32]. The increase in these hormones is measurable after only a few minutes after the introduction of pneumoperitoneum, and stays high during the pneumoperitoneum time. An animal study found a similar cortisol reaction to both laparoscopic surgery and pneumoperitoneum alone, which indicates that the

surgical stress after laparoscopic surgery may be mainly attributable to the pneumoperitoneum [33]. The operating time in LABF is longer than in a lot of other laparoscopic minor surgery e.g. laparoscopic appendectomy or cholecystectomy, hence there is a prolonged pneumoperitoneum. The pneumoperitoneum causes hypercapnia, reduced venous blood return and reduced cardiac output [34-36]. It also reduce renal blood flow, which results in a decreased renal function, increased antidiuretic hormone, aldosterone and sometimes oliguria [37,38], and could explain the increase in aldosterone during LABF. Gasless pneumoperitoneum induces less cortisol release than CO₂ [32], and low pressure sufflation is recommended [36].

Aldosterone release is also inhibited by the administration of saline [39]. The OABF group in our study had a significant higher blood loss (see table V) and it is possible that the OABF group as a result have been given more fluid supplements during surgery than the laparoscopic group, consequently resulting in much lower aldosterone in the open group.

The aortic anastomosis time is longer in the laparoscopic group (se table V) which is associated with an increased stress response [14]. There is also a synergic effect combined with the pneumoperitoneum [40,41]. However, in our study the hormone levels decrease in both groups during aortic crossclamping, and the effect of prolonged aortic anastomosis time in the laparoscopic group does not seem to increase the hormonal stress markers.

Limitations

Timing of blood samples is essential [24]. Studying previous research we found a wide array of blood sample protocols, and none in our field of surgery. One could argue that the blood samples could have been taken at fixed timepoints equal in the two groups. This would have

made it possible to measure area under the curve (AUC) which is superior to the comparison of single points in repeated correlated measurements [42]. However, the stress hormones have a rapid release and short half life time [43]. We identified eight different timepoints deemed to potentially be moments of large alterations in physiological stress. To capture the difference at these timepoints we decided to take the samples at specific events during surgery rather than at fixed points in time.

Blood samples were primarily taken from an arterial catheter, and if not possible from central venous catheter. Catecholamines, especially norepinephrine should not be measured in plasma taken from a peripheral vein due to peripheral production [44]. However metanephrine has a longer half life time and is more evenly distributed in the vascular system.

In an endocrine perspective the most stressful point is the anaesthesia reversal and extubation [45]; this was not measured in our study since our main focus was the differences between the surgical procedures. Using spinal or epidural anaesthesia reduce hormonal stress [46,47].

Fourteen patients in the open surgery group had an epidural catheter placed before surgery, compared to only three in the laparoscopic group ($p = 0.0003$). However, the administration of drugs through the epidural catheter was not initiated until the end of surgery, and should not affect samples taken during surgery. Any effect at 24 hours would only be in favour of open surgery and would probably not alter the results.

The increase in aldosterone during LABF, does not seem to have a negative effect on the clinical outcome (table IV), but this sub-study was neither aimed nor designed or powered to investigate any correlation.

Power analysis was not performed on these substudy endpoints as our study was part of a larger randomized trial, NLAST. There is also an issue of multiple testing due to the large number repeated measurements and limited number of participants. For further research in this field a power analysis should be conducted. Due to our small sample, we considered only three timepoints (i.e. after intubation and establishment of central venous access, immediately before aortic cross clamping, and 24 hours after start of surgery) to meet the conditions for the adequacy of the GEE model.

In most studies published on this topic there is described less inflammation after laparoscopic surgery. But when it comes to hormonal changes the advantage of laparoscopy might be less in major vascular surgery. However, the lower values of ACTH, cortisol and aldosterone 24 hours after laparoscopic surgery, may indicate that open surgery generates a prolonged endocrine stress reaction, compared to the acute endocrine stress seen during laparoscopy. This may influence on restitution time. A larger trial based on a power analysis is necessary to draw any certain conclusions and examine the effect on clinical outcome.

The strengths of this study are the randomized nature, the number of measurements and the novelty of the study population.

Conclusion

Patients operated with laparoscopic aortobifemoral bypass have higher levels of ACTH, aldosterone and cortisol during surgery, lower perioperative levels of metanephrine, but achieve earlier hormonal homeostasis after surgery as compared to open aortobifemoral bypass.

Acknowledgements

We thank all participating hospitals, and especially the local hospital biochemical laboratories and the Hormone Laboratory at Oslo University Hospital for all help.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. There was no external funding.

Author contributions

Conception and design: SSK, AHK, PMT, JJJ, JOS

Data collection: AHK, MS, EMP, SSK

Analysis and interpretation: AHK, PMT, IS

Statistical analysis: IS, AHK

Writing the article: AHK, IS, SSK

Critical revision of the article: AHK, SSK, IS, PMT, JJJ, MS, EMP, JOS

Final approval of the article: AHK, SSK, IS, PMT, JJJ, MS, EMP, JOS

Overall responsibility: AHK, SSK

Tables

Table I Baseline characteristics of patients treated with either totally laparoscopic or open aortobifemoral bypass for severe aortoiliac occlusive disease

	Laparoscopy (N=14)	Open surgery (N=16)
Age in years, median (IQR)	60.0 (54.0-64.0)	66.0 (58.5-69.5)

Female gender N (%)	7 (50)	9 (56)
Body mass index (BMI), median (IQR)	26.1 (22.5-29.7)	23.1 (19.2-27.2)
Current smoker N (%)	7 (50)	10 (63)
Hypertension (HT) N (%)	10 (71)	11 (69)
Renal failure N (%)	0 (0)	1 (6)
COPD N (%)	4 (29)	3 (19)
Hyperlipidaemia N (%)	5 (36)	2 (13)
Diabetes mellitus (DM) N (%)	1 (7.1)	0 (0)
Coronary heart disease (CHD) N (%)	2 (14)	5 (31)
ASA classification ^a		
ASA class 2. N (%)	0 (0)	1 (6)
ASA class 3. N (%)	14 (100)	15 (94)
Fontaine classification ^b		
Fontaine class 2. N (%)	11 (79)	12 (75)
Fontaine class 3. N (%)	3 (21)	4 (25)

Definitions:

^a American Society of Anesthesiologists (ASA) classification

^b Fontaine classification (classification of symptoms in peripheral atherosclerotic disease)

Table II Median serum/plasma levels of the hormonal markers at the different timepoints after laparoscopic aortobifemoral bypass surgery (LABF) compared with open aortobifemoral bypass surgery (OABF).

Hormone	After central venous line	At incision in groin	Straight after laparotomy/laparoscopy incision	Before aortic cross clamping	Immediately after aortic cross clamping	Open flow through first graft limb	Closing of the incision	24 hours after beginning of surgery
---------	---------------------------	----------------------	--	------------------------------	---	------------------------------------	-------------------------	-------------------------------------

	Median (IQR)		Median (IQR)		Median (IQR)		Median (IQR)		Median (IQR)		Median (IQR)		Median (IQR)		Median (IQR)	
	LABF	OABF	LABF	OABF	LABF	OABF	LABF	OABF	LABF	OABF	LABF	OABF	LABF	OABF	LABF	OABF
Cortisol (nmol/L)	245 (204-374)	268 (210-388)	210 (141-259)	230 (176-293)	282 (192-447)	244 (145-340)	939 (754-1061)	607 (432-784)	868 (775-1041)	631 (447-807)	876 (808-934)	541 (388-670)	818 (689-921)	591 (345-860)	68 (40-198)	591 (104-677)
Aldosterone (pmol/L)	243 (157-1850)	165 (118-188)	240 (158-523)	160 (109-200)	267 (196-633)	149 (121-232)	838 (603-1245)	445 (310-564)	1070 (638-1410)	457 (293-609)	790 (449-1060)	353(214-540)	731 (376-1100)	397 (267-639)	176 (132-287)	232 (124-486)
ACTH (pmol/L)	2.0 (1.0-2.0)	2.0 (1.5-3.5)	1.0 (1.0-2.0)	2.0 (1.0-2.0)	3.5 (2.0-7.0)	2.0 (1.0-6.0)	71.5 (27.5-109.0)	25.5 (7.5-82.5)	75.5 (30.0-104.0)	22.0 (5.0-61.5)	22.0 (7.0-77.0)	6.5 (3.0-22.0)	12.0 (6.0-60.0)	18.5 (3.5-65.5)	1.0 (1.0-1.0)	3.0 (1.0-8.0)
Meta-nephrine (nmol/L)	0.26 (0.18-0.30)	0.32 (0.26-0.40)	0.28 (0.19-0.33)	0.34 (0.27-0.36)	0.23 (0.19-0.35)	0.32 (0.28-0.39)	0.36 (0.22-0.48)	0.45 (0.36-0.50)	0.37 (0.21-0.45)	0.39 (0.33-0.54)	0.36 (0.23-0.38)	0.39 (0.31-0.46)	0.31 (0.21-0.33)	0.42 (0.37-0.50)	0.33 (0.21-0.48)	0.46 (0.35-0.58)

Statistics: IQR=interquartile range

Table III The effect of laparoscopic aortobifemoral bypass (LABF) versus open aortobifemoral bypass (OABF) on serum levels of cortisol, aldosterone, ACTH and metanephrine, using the GEE model

Biomarker	Coefficient	95% Confidence Interval	Standard error	Z	p-value
Cortisol	-0.1008	-0.4023 to 0.2007	0.1538	-0.66	0.512
Aldosterone	0.3985	0.0045 to 0.7924	0.2010	1.98	0.047
ACTH	0.0146	-0.3671 to 0.3962	0.1947	0.07	0.940
Metanephrine	-0.2498	-0.5037 to 0.0041	0.1296	-1.93	0.054

Statistics: General Estimated Equations (GEE)

Table IV Comparing serum/plasma level of cortisol, aldosterone, ACTH and metanephrine in patients undergoing laparoscopic aortobifemoral bypass (LABF) versus open aortobifemoral bypass (OABF) at baseline (after central venous line, before surgery) and at follow-up (24 hours after beginning of surgery)

		Serum/plasma level			
		Mean (standard deviation)			
Biomarker		Laparoscopic group (n=14)	Open surgery group (n=16)	Difference between means (95% CI)	p-value*
Cortisol (nmol/L)	Baseline	295.14 (167.09)	285.88 (101.07)	9.26 (-92.51;111.05)	
	Follow-up	161.86 (169.97)	473.47 (337.02)	-311.61 (-517.35; -105.87)	
	Change	-133.29 (245.08)	195.07 (351.25)	-328.35 (-560.75; -95.96)	
	ANCOVA			-310.95 (-591.37; -100.53)	0.016
Aldosterone (pmol/L)	Baseline	376.07 (442.26)	171.94 (83.28)	240.13 (-53.62; 461.88)	
	Follow-up	284.50 (236.84)	431.73 (560.22)	-147.23 (479.37; 184.90)	
	Change	-91.57 (396.08)	254 (533.45)	-345.84 (-705.98; 14.30)	
	ANCOVA			-212.66 (-559.48; 134.16)	0.089
ACTH (pmol/L)	Baseline	1.71 (0.73)	2.50 (1.41)	-0.79 (-1.62; 0.05)	
	Follow-up	1.14 (0.63)	16.50 (43.91)	-15.36 (-40.7; 9.99)	
	Change	-0.57 (0.93)	13.93 (44.03)	-14.50 (-39.93; 10.93)	
	ANCOVA			-16.79 (-43.17; 9.59)	0.010
Metanephrine (nmol/L)	Baseline	0.27 (0.09)	0.36 (0.15)	-0.09 (-0.18; 0.01)	
	Follow-up	0.36 (0.17)	0.47 (0.18)	-0.11 (-0.25; 0.02)	
	Change	0.09 (0.14)	0.12 (0.24)	-0.03 (-0.18; 0.12)	
	ANCOVA			-0.09 (-0.23; 0.05)	0.149

Notes: * p-value on logged data

Statistics: Comparison between the two treatment groups was performed using analysis of covariance (ANCOVA)

Table V Surgery related parameters for patients treated with either totally laparoscopic or open aortobifemoral bypass for severe aortoiliac occlusive disease.

	Laparoscopy (N=14)	Open surgery (N=16)	P-value
Operation time(minutes), median(IQR)	216 (195-235)	202 (169-230)	0.2613 ^a
Blood loss during surgery(ml) median(IQR)	275 (150-600)	1000 (575-1150)	0.0285 ^a
Supra renal clamping (patients), N	1	1	0.9234 ^b
Anastomosis time^a (minutes), median(IQR)	43 (41-52)	30 (20-38)	0.0002 ^a
Aortic crossclamping time^b (minutes), median(IQR)	90 (77-109)	76 (61-97)	0.0881 ^a
Epidural anaesthesia, N (%)	3 (21)	14 (88)	0.0003 ^b

Statistics: ^aMann Whitney U test, ^bFishers exact test, IQR=interquartile range

Definitions: ^aAnastomosis time is defined as time from aortic crossclamping until the clamp is moved to the proximal end of the graft. ^bAortic crossclamping time is defined as time from aortic crossclamping until the clamp is removed the graft and flow to one of the legs is re-established.

Table VI Clinical outcome after laparoscopic aortobifemoral bypass (LABF) vs open aortobifemoral bypass (OABF)

	Laparoscopy N= 14	Open surgery N=16	P-value
Length of stay in hospital(days), Median (IQR)	5.0 (4.0-6.0)	9.0 (6.5-11.0)	0.0010 ^a
30-day mortality, N	0	0	
30-day systemic morbidity^c, N (%)	1 (7)	4 (25)	0.2094 ^b

Statistics: ^aMann Whitney U test, ^bFishers exact test, IQR=interquartile range

Definitions: ^cSystemic morbidity was defined as: Systemic complication/morbidity including systemic infection, ileus, sepsis, acute respiratory distress syndrome, multiple organ failure, renal failure, myocardial infarction and/or cerebral infarction/haemorrhage. Not including local morbidity defined as local wound complications or complications related to the vascular graft.

Figure legends

Figure I Flow chart of patient population with aortoiliac occlusive disease (AIOD) treated with totally laparoscopic aortobifemoral bypass (LABF) or open aortobifemoral bypass (OABF)

Figure II Comparing median serum cortisol (nmol/L) during and after totally laparoscopic aortobifemoral bypass (LABF) vs open aortobifemoral bypass (OABF). Boxes representing the interquartile range (IQR). o representing outliers. * representing extreme values.

Timepoints:

1. After anaesthesia and administration of central venous access
2. Right after first groin incision
3. Right after laparotomy/laparoscopy
4. Immediately before crossclamping of the aorta
5. Immediately after the placement of the aortic crossclamp
6. After reestablishment of flow through first graft limb.
7. Wound closure
8. 24 hours after the beginning of surgery

Figure III Comparing median serum aldosterone (pmol/L) during and after totally laparoscopic aortobifemoral bypass (LABF) vs open aortobifemoral bypass (OABF). Boxes representing the interquartile range (IQR). o representing outliers. * representing extreme values.

Timepoints:

1. After anaesthesia and administration of central venous access
2. Right after first groin incision
3. Right after laparotomy/laparoscopy
4. Immediately before crossclamping of the aorta
5. Immediately after the placement of the aortic crossclamp
6. After reestablishment of flow through first graft limb.
7. Wound closure

8. 24 hours after the beginning of surgery

Figure IV Comparing median plasma ACTH (adrenocorticotrophic hormone)(pmol/L) during and after totally laparoscopic aortobifemoral bypass (LABF) vs open aortobifemoral bypass (OABF). Boxes representing the interquartile range (IQR). o representing outliers. * representing extreme values.

Timepoints:

1. After anaesthesia and administration of central venous access
2. Right after first groin incision
3. Right after laparotomy/laparoscopy
4. Immediately before crossclamping of the aorta

5. *Immediately after the placement of the aortic crossclamp*
6. *After reestablishment of flow through first graft limb.*
7. *Wound closure*
8. *24 hours after the beginning of surgery*

Figure V Comparing median plasma metanephrine(nmol/L) during and after totally laparoscopic aortobifemoral bypass (LABF) vs open aortobifemoral bypass (OABF). Boxes representing the interquartile range (IQR). o representing outliers. * representing extreme values.

Timepoints:

1. *After anaesthesia and administration of central venous access*
2. *Right after first groin incision*

3. *Right after laparotomy/laparoscopy*
4. *Immediately before crossclamping of the aorta*
5. *Immediately after the placement of the aortic crossclamp*
6. *After reestablishment of flow through first graft limb.*
7. *Wound closure*
8. *24 hours after the beginning of surgery*

- [1] Udelsman R, Holbrook NJ. Endocrine and molecular responses to surgical stress. *Curr Probl Surg* 1994;31:662-720.
- [2] Desborough JP. The stress response to trauma and surgery. *Br J Anaesth* 2000;85:109-17.
- [3] Halter JB, Pflug AE, Porte D, Jr. Mechanism of plasma catecholamine increases during surgical stress in man. *J Clin Endocrinol Metab* 1977;45:936-44.
- [4] Barton RN. The neuroendocrinology of physical injury. *Baillieres Clin Endocrinol Metab* 1987;1:355-74.
- [5] Kehlet H. Surgical Stress Response: Does Endoscopic Surgery Confer an Advantage? *World J Surg* 1999;23:801-7.
- [6] Chernow B, Alexander HR, Smallridge RC, Thompson WR, Cook D, Beardsley D, Fink MP, Lake CR, Fletcher JR. Hormonal responses to graded surgical stress. *Arch Intern Med* 1987;147:1273-8.
- [7] Radunović M, Lazović R, Panić N, Bulajić M. Biohumoral and endocrine parameters in assessment of surgical trauma in open and laparoscopic cholecystectomy. *Vojnosanit Pregl* 2013;70:555-60.
- [8] Nguyen NT, Goldman CD, Ho HS, Gosselin RC, Singh A, Wolfe BM. Systemic stress response after laparoscopic and open gastric bypass. *J Am Coll Surg* 2002;194:557-66.
- [9] Kim TK, Yoon JR. Comparison of the neuroendocrine and inflammatory responses after laparoscopic and abdominal hysterectomy. *Korean J Anesthesiol* 2010;59:265-9.
- [10] Rahr H, Bendix J, Ahlburg P, Gjedsted J, Funch-Jensen P, Tønnesen E. Coagulation, inflammatory, and stress responses in a randomized comparison of open and laparoscopic repair of recurrent inguinal hernia. *Surg Endosc* 2006;20:468-72.
- [11] Bruls S, Quaniers J, Tromme P, Lavigne JP, Van Damme H, Defraigne JO. Comparison of laparoscopic and open aortobifemoral bypass in the treatment of aortoiliac disease. Results of a contemporary series (2003-2009). *Acta Chir Belg* 2012;112:51-8.

- [12] Tieck J, Remy P, Sabbe T, D'Hont C, Houthoofd S, Daenens K, Fourneau I. Laparoscopic versus open approach for aortobifemoral bypass for severe aorto-iliac occlusive disease--a multicentre randomised controlled trial. *Eur J Vasc Endovasc Surg* 2012;43:711-5.
- [13] Lecot F, Sabbe T, Houthoofd S, Daenens K, Fourneau I. Long-term Results of Totally Laparoscopic Aortobifemoral Bypass. *Eur J Vasc Endovasc Surg*.
- [14] Gelman S. The pathophysiology of aortic cross-clamping and unclamping. *Anesthesiology* 1995;82:1026-60.
- [15] Fourneau I, Lerut P, Sabbe T, Houthoofd S, Daenens K, Nevelsteen A. The Learning Curve of Totally Laparoscopic Aortobifemoral Bypass for Occlusive Disease. How Many Cases and How Safe? *Eur J Vasc Endovasc Surg* 2008;35:723-9.
- [16] Kazmi SSH, Jørgensen JJ, Sundhagen JO, Krog AH, Flørenes TL, Kollerøs D, Abdelnoor M. A comparative cohort study of totally laparoscopic and open aortobifemoral bypass for the treatment of advanced atherosclerosis. *Vasc Health Risk Manag* 2015;11:541-7.
- [17] Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, Bell K, Caporusso J, Durand-Zaleski I, Komori K, Lammer J, Liapis C, Novo S, Razavi M, Robbs J, Schaper N, Shigematsu H, Sapoval M, White C, White J, Clement D, Creager M, Jaff M, Mohler E, 3rd, Rutherford RB, Sheehan P, Sillesen H, Rosenfield K. Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). *Eur J Vasc Endovasc Surg* 2007;33 Suppl 1:S1-75.
- [18] Gomez. Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines for chronic obstructive pulmonary disease. *Curr Opin Pulm Med* 2002;8:81-6.
- [19] Coggia M, Bourriez A, Javerliat I, Goeau-Brissonniere O. Totally laparoscopic aortobifemoral bypass: a new and simplified approach. *Eur J Vasc Endovasc Surg* 2002;24:274-5.

- [20] Le Blanc-Louvry I, Coquerel A, Koning E, Maillot C, Ducrotté P. Operative Stress Response Is Reduced After Laparoscopic Compared to Open Cholecystectomy. *Dig Dis Sci* 2000;45:1703-13.
- [21] Davidson FD. Paracetamol-associated interference in an HPLC-ECD assay for urinary free metadrenalines and catecholamines. *Ann Clin Biochem* 2004;41:316-20.
- [22] Zeger SL, Liang K-Y, Albert PS. Models for Longitudinal Data: A Generalized Estimating Equation Approach. *Biometrics* 1988;44:1049-60.
- [23] Vickers AJ, Altman DG. Analysing controlled trials with baseline and follow up measurements. *Brit Med J* 2001;323:1123-4.
- [24] Watt DG, Horgan PG, McMillan DC. Routine clinical markers of the magnitude of the systemic inflammatory response after elective operation: A systematic review. *Surgery* 2015;157:362-80.
- [25] Crema E, Ribeiro EN, Hial AM, Alves Junior JT, Pastore R, Silva AA. Evaluation of the response of cortisol, corticotropin and blood platelets kinetics after laparoscopic and open cholecystectomy. *Acta Cir Bras* 2005;20:364-7.
- [26] Karayiannakis AJ, Makri GG, Mantzioka A, Karousos D, Karatzas G. Systemic stress response after laparoscopic or open cholecystectomy: a randomized trial. *Br J Surg* 1997;84:467-71.
- [27] Byrne J, Hallett JW, Jr., Ilstrup DM. Physiologic responses to laparoscopic aortofemoral bypass grafting in an animal model. *Ann Surg* 2000;231:512-8.
- [28] Schietroma M, Carlei F, Cecilia EM, Piccione F, Sista F, De Vita F, Amicucci G. A prospective randomized study of systemic inflammation and immune response after laparoscopic nissen fundoplication performed with standard and low-pressure pneumoperitoneum. *Surg Laparosc Endosc Percutan Tech* 2013;23:189-96.

- [29] Ozmen MM, Zulfikaroglu B, Col C, Cinel I, Isman FK, Cinel L, Besler TH. Effect of increased abdominal pressure on cytokines (IL1 beta, IL6, TNFalpha), C-reactive protein (CRP), free radicals (NO, MDA), and histology. *Surg Laparosc Endosc Percutan Tech* 2009;19:142-7.
- [30] Mikami O, Fujise K, Matsumoto S, Shingu K, Ashida M, Matsuda T. High intra-abdominal pressure increases plasma catecholamine concentrations during pneumoperitoneum for laparoscopic procedures. *Arch Surg* 1998;133:39-43.
- [31] Mikami O, Kawakita S, Fujise K, Shingu K, Takahashi H, Matsuda T. Catecholamine release caused by carbon dioxide insufflation during laparoscopic surgery. *J Urol* 1996;155:1368-71.
- [32] Larsen J, Ejstrup P, Svendsen F, Pedersen V, Redke F. Systemic response in patients undergoing laparoscopic cholecystectomy using gasless or carbon dioxide pneumoperitoneum: a randomized study. *J Gastrointest Surg* 2002;6:582-6.
- [33] Marcovich R, Williams AL, Seifman BD, Wolf JS. A canine model to assess the biochemical stress response to laparoscopic and open surgery. *J Endourol* 2001;15:1005-8.
- [34] O'Leary E, Hubbard K, Tormey W, Cunningham AJ. Laparoscopic cholecystectomy: haemodynamic and neuroendocrine responses after pneumoperitoneum and changes in position. *Br J Anaesth* 1996;76:640-4.
- [35] Safran DB, Orlando R, 3rd. Physiologic effects of pneumoperitoneum. *Am J Surg* 1994;167:281-6.
- [36] Neudecker J, Sauerland S, Neugebauer E, Bergamaschi R, Bonjer HJ, Cuschieri A, Fuchs K-H, Jacobi C, Jansen FW, Koivusalo A-M, Lacy A, McMahon MJ, Millat B, Schwenk W. The European Association for Endoscopic Surgery clinical practice guideline on the pneumoperitoneum for laparoscopic surgery. *Surg Endosc* 2002;16:1121-43.

- [37] Demyttenaere S, Feldman L, Fried G. Effect of pneumoperitoneum on renal perfusion and function: a systematic review. *Surg Endosc* 2007;21:152-60.
- [38] Nguyen NT, Perez RV, Fleming N, Rivers R, Wolfe BM. Effect of prolonged pneumoperitoneum on intraoperative urine output during laparoscopic gastric bypass. *J Am Coll Surg* 2002;195:476-83.
- [39] Engquist A, Blichert-Toft M, Olgaard K, Brandt MR, Kehlet H. Inhibition of aldosterone response to surgery by saline administration. *Br J Surg* 1978;65:224-7.
- [40] Martin-Cancho MF, Crisostomo V, Soria F, Calles C, Sanchez-Margallo FM, Diaz-Guemes I, Uson-Gargallo J. Physiologic Responses to Infrarenal Aortic Cross-Clamping during Laparoscopic or Conventional Vascular Surgery in Experimental Animal Model: Comparative Study. *Anesthesiol Res Pract* 2008;2008:581948.
- [41] Martin-Cancho MF, Sanchez-Margallo FM, Soria F, Diaz-Guemes I, Crisostomo V, Calles C, Lima JR, Uson-Gargallo J. Physiological responses to different ischemic periods during laparoscopic infrarenal aortic cross-clamping: evaluation in an experimental animal model. *Ann Vasc Surg* 2009;23:506-18.
- [42] Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. *Brit Med J* 1990;300:230-5.
- [43] Labhart A. *Clinical Endocrinology: Theory and Practice*: Springer Science & Business Media 1974.
- [44] Hjemdahl P. Plasma catecholamines—analytical challenges and physiological limitations. *Baillieres Clin Endocrinol Metab* 1993;7:307-53.
- [45] Udelsman R, Norton JA, Jelenich SE, Goldstein DS, Linehan WM, Loriaux DL, Chrousos GP. Responses of the hypothalamic-pituitary-adrenal and renin-angiotensin axes and the sympathetic system during controlled surgical and anesthetic stress. *J Clin Endocrinol Metab* 1987;64:986-94.

- [46] Milosavljevic SB, Pavlovic AP, Trpkovic SV, Ilic AN, Sekulic AD. Influence of spinal and general anesthesia on the metabolic, hormonal, and hemodynamic response in elective surgical patients. *Med Sci Monit* 2014;20:1833-40.
- [47] Barr J, Boulind C, Foster JD, Ewings P, Reid J, Jenkins JT, Williams-Yesson B, Francis NK. Impact of analgesic modality on stress response following laparoscopic colorectal surgery: a post-hoc analysis of a randomised controlled trial. *Tech Coloproctol* 2015;19:231-9.
- [48] Krog AH, Sahba M, Pettersen EM, Sandven I, Thorsby PM, Jorgensen JJ, Sundhagen JO, Kazmi SS. Comparison of the acute-phase response after laparoscopic versus open aortobifemoral bypass surgery: a substudy of a randomized controlled trial. *Vasc Health Risk Manag* 2016;12:371-8.