CLINICAL STUDY

Skeletal muscle 11 β hydroxysteroid dehydrogenase type 1 activity is upregulated following elective abdominal surgery

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Abstract

Objective: Cortisol has been traditionally implicated in the causation of peri-operative skeletal muscle (SkM) insulin resistance, but cortisol levels return to normal within 72 h of surgery. Tissue cortisol bioactivity may be prolonged by local upregulation of the enzyme 11 β HSD1. We aimed to investigate the changes of SkM 11 β HSD1 enzyme activity and mRNA expression, relative to plasma cortisol, insulin and glucose levels following elective abdominal surgery.

Patients and design: Eight non-diabetic subjects (two male, six female) underwent serial plasma hormone sampling and muscle biopsy of vastus lateralis at baseline and on day 5 following elective laparoscopic cholecystectomy.

Methods: SkM 11 β HSD1 and H6PDH mRNA levels were measured by quantitative RT-PCR and enzyme activity by % conversion of ³H cortisone to cortisol. Plasma glucose, insulin, free fatty acids (FFA), tumour necrosis factor- α and cortisol by standardised assays.

Results: Compared with baseline, SkM 11 β HSD1 activity was significantly increased on day 5 after surgery (14.7 ± 2.1 vs $20.4 \pm 3.2\%$, $P=0.005$). Neither 11 β HSD1 nor H6PDH mRNA levels were altered after surgery. Plasma cortisol ($P=0.027$), FFA ($P=0.01$) and glucose ($P=0.004$) rose rapidly following surgery and had returned to baseline values by 24 h post-surgery. There was no significant change in plasma insulin.

Conclusions: This is the first study to demonstrate an upregulation of SkM 11 β HSD1 activity in response to a physiological stressor. Sustained activation of this enzyme may increase tissue cortisol bioactivity.

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Introduction

The hypothalamic–pituitary–adrenal (HPA) axis constitutes a major part of the neuroendocrine system that controls the response to surgical stress (1). Both the HPA axis and sympathetic nervous system are activated by afferent nerve input from the area of surgical trauma (2). During elective abdominal surgery, circulating cortisol increases rapidly shortly after surgical incision, peaks at 6 h and remains elevated for up to 72 h post-operatively (3). No significant difference in the responses of cortisol or adrenaline has been observed between laparoscopic versus open cholecystectomy (4). Induction of anaesthesia does not appear to impact upon the plasma levels of ACTH, cortisol or the catecholamines (4).

Surgical trauma induces insulin resistance in normal and glucose intolerant individuals, which lasts post-operatively for at least one week (5). For example, following elective open cholecystectomy, insulin sensitivity is reduced maximally (~50%) on day 1 and by

~30% still by day 5 (5). Laparoscopic cholecystectomy is also associated with an increase in insulin resistance (6), albeit to a lesser extent than open surgery (7). Skeletal muscle is thought to be the main site of insulin resistance, and abnormalities of the insulin signalling cascade that have been demonstrated in the acute post-operative period (8). However, the causes or triggers for the development of this intracellular insulin resistance are unknown. Traditionally, the counter-regulatory hormones, particularly cortisol, but also glucagon, catecholamines and GH have been implicated in the pathogenesis of this insulin resistance.

Cytokines are also thought to be important mediators of insulin resistance (9). Both tumour necrosis factor- α (TNF α) and interleukin-6 (IL-6) activate the HPA axis to stimulate ACTH and cortisol production (1). During abdominal surgery under general anaesthesia, cytokine levels increase gradually after skin incision and during surgery, peak on the first post-operative morning and return to baseline by the 7th post-operative day. Following open cholecystectomy, both TNF α (10) and

IL-6 levels increase, but laparoscopic surgery does not induce a cytokine response that is detectable in the systemic circulation (11).

11 β hydroxysteroid dehydrogenase 1 (11 β HSD1) regulates local tissue exposure to active glucocorticoid (12). Oxoreductase activity of 11 β HSD1 is conferred by the endoplasmic reticulum enzyme hexose-6-phosphate dehydrogenase (H6PDH). We have recently demonstrated an increased activity of skeletal muscle (SkM) 11 β HSD1 in diabetic subjects following exogenous administration of the synthetic glucocorticoid dexamethasone, which induced a state of insulin resistant metabolic stress (13). There are no previous data describing whether SkM 11 β HSD1 expression or activity is altered at the time of a physiological stress. We postulated that an increase in SkM 11 β HSD1-mediated generation of intracellular cortisol may occur in response to the physiological stress of surgery, which could prolong local cortisol bioactivity and an insulin resistant state at tissue level in the absence of a sustained plasma cortisol increase. In this study, we sought to examine in normal subjects the time course of plasma substrates (glucose and free fatty acids (FFA)), hormones (cortisol and insulin), cytokine (TNF α) and SkM 11 β HSD1 enzyme activity and mRNA expression following elective cholecystectomy.

Subjects and methods

Study subjects

The study was approved by the St Vincent's Hospital Human Research Ethics Committee. Six women and two men undergoing elective laparoscopic cholecystectomy were recruited at pre-admission clinic. Seven of the patients had known cholelithiasis, while one woman had a thickened gall-bladder wall requiring removal. She was subsequently found to have an indolent lymphoma confined to the gall bladder and has not had any further specific treatment. Five of the subjects were on no medication, two were taking proton pump inhibitors, while one patient (a 33-year-old female) was on a maintenance dose of thyroxine for primary hypothyroidism and metformin 750 mg twice daily for treatment of polycystic ovary syndrome. We had previously demonstrated that the use of metformin in subjects with type 2 diabetes did not alter SkM 11 β HSD1 activity compared with subjects on diet alone (13). During surgery, one subject required conversion to an open cholecystectomy because the gall bladder was adherent to omentum and colon. Surgery was uncomplicated for the remaining subjects. The mean age of the subjects was 51.5 ± 5.1 years; the mean body mass index (BMI) 35.5 ± 2.7 kg/m² (range 25.0–47.6), and mean waist:hip ratio was 0.91 ± 0.04 (range 0.74–1.08). No subject was taking any medications known to affect the HPA axis. None of the

subjects had significantly abnormal (twice upper limit of normal) liver function tests. Two subjects fulfilled criteria for the metabolic syndrome (14).

Experimental design

Biopsy of vastus lateralis muscle was performed using a 5 mm Bergstrom needle as previously described (15). Subjects underwent an initial muscle biopsy in the operation theatre after induction of anaesthesia and prior to the cholecystectomy. Surgery commenced in all subjects before 1000 h. Subjects did not receive i.v. dextrose prior to surgery. The second biopsy was performed 4–6 days (median day 5) after the day of surgery on the contralateral vastus lateralis under sedation with i.v. midazolam and local anaesthetic after overnight fasting (15). No subject received any glucocorticoid or etomidate as part of their anaesthetic protocol. Fresh muscle (mean weight 192 mg, range 120–353 mg) was dissected free of visible adipose and connective tissue and placed immediately in serum-free DMEM (Invitrogen) for 11 β HSD1 activity studies (13). Separate samples were immediately snap frozen in liquid nitrogen (within 7 s) for mRNA analysis and stored at -70°C .

Blood sampling was performed on the following days in relation to the day of surgery (day 0 of protocol): days -2 , 0, 1, 5 and 28. Morning blood sampling was done after overnight fasting from 2200 h. On the day of surgery, sampling was performed both prior to and 5 h after the commencement of surgery.

Laboratory assays

Plasma glucose was analysed by a glucose oxidase method employing YSI 1500 Sidekick analyser (Yellow Springs Instrument Company, Yellow Springs, OH, USA), coefficient of variation (CV) 2.4%. Insulin was measured with an established RIA, with $<1\%$ cross-reactivity to proinsulin and sensitivity of 0.5 mU/l (16). Serum cortisol was measured with a competitive immunoassay using chemiluminescent technology (sensitivity 6 nmol/l, intra-assay CV 6.3, 7.2 and 6.3% at concentrations 153, 492 and 782 nmol/l respectively, Bayer ADVIA Centaur). FFA were determined by an enzymatic colorimetric method using a kit (Wako Pure Chemical Industries, Osaka, Japan). TNF α was measured using a high sensitivity sandwich ELISA (sensitivity 0.12 pg/ml, Quantikine, R&D, Minneapolis, MN, USA). Homeostatic model assessment (HOMA-R) was calculated using the computer program of Levy *et al.* (17). The β -cell function index was calculated in the fasting basal steady state as follows: $20 \times (\text{fasting insulin } (\mu\text{U/ml}) / \text{fasting glucose } (\text{mmol/l})) - 3.5$ (18).

Quantitative real time RT-PCR

11 β HSD1 and H6PDH mRNA expression in skeletal muscle were determined using quantitative real-time RT-PCR (Taqman Chemistry) as previously described (19). 11 β HSD1 specific primers and a probe were designed using Primer express 1.5 software (ABI, Foster City, CA, USA). Forward primer-5'-GCAAAGGGATCG-GAAGAGAGA-3', reverse primer-5'-GCT GAGGCTG-CTCCAAGCT-3', MGB FAM probe-5'-CCA CAT GGG CTC CCA-3'. For human H6PDH, a specific assay-on-demand gene expression assay was obtained from Applied Biosystems (Foster City, CA, USA). Singleplex reactions were performed in triplicate and normalised to the endogenous control human 18s rRNA (Applied Biosystems). Data were obtained as C_t values, the cycle number at which logarithmic PCR plots cross a calculated threshold line, and expressed as ΔC_t , the difference between C_t target and C_t endogenous control (mean ΔC_t values were obtained from two runs). Relative 11 β HSD1 mRNA levels and H6PDH levels were calculated using the $\Delta\Delta C_t$ methodology (Formula = $2^{-\Delta\Delta C_t}$). Total RNA from commercially obtained human skeletal muscle (BD Biosciences, Palo Alto, CA, USA) was reverse transcribed to cDNA and was used as the calibrator to obtain $\Delta\Delta C_t$ values in all experiments.

11 β HSD1 activity

Skeletal muscle 11 β HSD1 oxoreductase activity was quantified by the conversion of ^3H cortisone to cortisol as previously described (19). Within 4 h of the biopsy, fresh muscle was incubated in serum-free DMEM containing ^3H cortisone (1 nM, specific activity 2.6 TBq/mmol), 100 nM cold cortisone and 5 $\mu\text{g}/\text{ml}$ insulin at 37 °C with 5% CO_2 for 24 h. Small clumps of muscle tissue were incubated to minimise disruption of cells. Steroids were then extracted with ethyl acetate, dried, resuspended in ethanol, and separated by thin layer chromatography (TLC) on plastic-backed silica gel 60 plates (Merck) using chloroform/ethanol (92:8) as solvent. The labelled cortisone and its converted product were visualised by phospho-imaging (Fujix BAS 1000; Fuji Film Company, Tokyo, Japan) and calculated as a percentage conversion (19).

Statistical analysis

SPSS v13.0 statistical software was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Data are expressed as mean $\pm$ S.E.M. Paired Students t -test was used to analyse 11 β HSD1 mRNA expression and enzyme activity data before and after surgery. One-way repeated measures ANOVA was used to assess differences in hormonal and biochemical parameters over the peri-operative course. Pearson correlation analysis was employed to test the relationships between variables.

For one subject, a fasting blood sample was not obtained on the first post-operative day. Results for glucose, insulin and FFA were therefore imputed from the average of the other 7 patients' results at this time point.

Results

Demographic data of the subjects are shown in Table 1. At baseline, the surgical subjects had a high BMI, ranging from 25.0–47.6 kg/m^2 (Table 1). None of the surgical subjects were diabetic, but as expected, they had raised fasting insulin levels and were insulin resistant as measured by HOMA-R and QUICKI.

Skeletal muscle 11 β HSD1 mRNA expression and enzyme activity at baseline

11 β HSD1 mRNA baseline levels and oxoreductase enzyme activity are shown in Table 1. As expected (19), the relative abundance of SkM H6PDH was $\sim$ 40-fold higher compared with 11 β HSD1 mRNA in skeletal muscle and this relationship did not change with surgery (data not shown).

Skeletal muscle 11 β HSD1 mRNA expression and enzyme activity after surgery

11 β HSD1 mRNA levels did not significantly change after surgery; mean ΔC_t 19.60 ± 0.52 (before surgery) compared with 18.98 ± 0.67 (after surgery), $P = 0.26$. The relative 11 β HSD1 expression (compared with the commercial calibrator) before surgery was 3.27 ± 0.99 compared with 6.32 ± 2.23 , $P = 0.133$ (Fig. 1a). H6PDH was also unchanged before and after surgery; mean ΔC_t 14.50 ± 0.52 (before surgery) compared with 14.24 ± 0.50 (after surgery), $P = 0.66$.

SkM 11 β HSD1 enzyme activity significantly increased after surgery: % conversion ^3H cortisone to cortisol per 200 mg muscle/24 h $14.7 \pm 2.1\%$ before surgery compared with $20.4 \pm 3.2\%$ 5 days after surgery, $P = 0.005$ (Fig. 1b). This increase of 11 β HSD1 activity occurred in all subjects, whereas an increase in 11 β HSD1 mRNA levels occurred in 5 out of

Table 1 Baseline data for surgical subjects.

n (M/F)	2/6
Age (years)	51.5 ± 5.1
BMI (kg/m^2)	35.5 ± 2.7
WHR	0.91 ± 0.04
Glucose (mmol/l)	4.9 ± 0.27
Insulin ($\mu\text{U}/\text{ml}$)	12.0 ± 1.2
Cortisol (nmol/l)	381 ± 45
FFA (mmol/l)	0.39 ± 0.07
11 β HSD1 activity (% conversion/200 mg/24 h)	14.7 ± 2.10
11 β HSD1 mRNA (ΔC_t)	19.60 ± 0.52
H6PDH mRNA (ΔC_t)	14.50 ± 0.52

Figure 1 11βHSD1 relative mRNA levels and enzyme activity at baseline and 4–6 days after surgery. * $P < 0.05$ compared with before surgery.

8 subjects. There was no difference observed in 11βHSD1 mRNA or activity with respect to the timing of the post-operative biopsy (day 4, 5 or 6).

Biochemical data

Cortisol changed significantly across the measured time points ($P = 0.027$), with a mean peak at 5 h post-operatively (Fig. 2a). However, this clearly defined peak was seen only in 50% of the subjects. The subjects who had no cortisol peak did not differ in age or BMI, but did have a higher HOMA-R ($P = 0.045$). There was no correlation between the change in SkM 11βHSD1 activity and the change in cortisol from pre-operatively to their peak level ($r = -0.08$, $P = 0.85$).

Glucose and FFA also rose significantly (glucose $P = 0.004$, FFA $P = 0.01$), both peaking at 5 h post-operatively (Fig. 2b and c). The β-cell function index fell significantly, with the nadir on the first post-operative day ($P = 0.014$, Fig. 2d). By contrast, there was no significant change in TNFα ($P = 0.09$), insulin ($P = 0.14$), HOMA-R ($P = 0.10$) or QUICKI ($P = 0.21$) (Table 2).

There was no correlation between the change in 11βHSD1 activity and the change in cortisol ($r = 0.05$,

Figure 2 Mean levels of (a) cortisol, (b) FFA, (c) glucose and (d) β-cell function index during the perioperative time course. * $P < 0.05$ and ** $P < 0.01$ for a change across surgery by one-way repeated measures ANOVA for all parameters.

$P = 0.90$), glucose ($r = 0.37$, $P = 0.37$) or change in FFA ($r = -0.46$, $P = 0.25$) from pre-operative to 5 h post-operatively.

Discussion

This study presents the novel finding that a physiological stressor, laparoscopic abdominal surgery, significantly stimulates 11βHSD1 oxoreductase activity in skeletal muscle, despite no significant change in

Table 2 Mean biochemical data at each time point.

	Pre-op	Day 0	Day 0 + 5 h	Day 1	Day 5	Day 28
Insulin (μ U/ml)	16.1 $\pm$ 2.6	14.9 $\pm$ 2.4	12.0 $\pm$ 2.01	9.5 $\pm$ 1.25	13.1 $\pm$ 2.40	13.1 $\pm$ 2.11
HOMA-R	2.1 $\pm$ 0.32	2.0 $\pm$ 0.30	1.6 $\pm$ 0.27	1.3 $\pm$ 0.17	1.7 $\pm$ 0.31	1.7 $\pm$ 0.29
QUICKI	0.32 $\pm$ 0.009	0.32 $\pm$ 0.007	0.33 $\pm$ 0.008	0.34 $\pm$ 0.009	0.33 $\pm$ 0.007	0.33 $\pm$ 0.009
TNF α (pg/ml)	2.45 $\pm$ 1.5	2.52 $\pm$ 1.79	1.81 $\pm$ 1.21	1.80 $\pm$ 0.96	1.80 $\pm$ 0.80	2.63 $\pm$ 1.7

11 β HSD1 mRNA content. The disparity between the significant increase in 11 β HSD1 oxoreductase activity without a significant increase in mRNA expression is consistent with our previously published work (13). The change in enzyme activity was observed in all subjects, while five out of eight demonstrated a rise in mRNA expression. This highlights a lack of correlation between mRNA expression and enzyme activity which has been reported in other biological enzyme systems (20, 21). While given the small number of subjects studied, and the possibility that a type 2 error exists, important factors other than gene expression affect functional enzyme activity, including protein translation, post-translational modification, protein–protein interactions and substrate availability. The study was statistically powered to show a difference in enzyme activity as the primary end point.

Post surgery, at a time when plasma cortisol had returned to basal levels, upregulation of SkM 11 β HSD1 activity may potentially increase local tissue levels of active cortisol in skeletal muscle. Using isotopically labelled cortisol, it is possible to examine 11 β HSD1 and 2 activity *in vivo* (22). This technique has been utilised to measure 11 β HSD1 and 2 activity in splanchnic and lower limb tissues (23). Such a measurement in our study would have been a valuable addition to the data, but is invasive, requiring femoral artery and vein cannulation in an angiography suite. In addition, we lacked the facilities to measure deuterium-labelled cortisol and cortisone. While further measures of SkM 11 β HSD1 oxoreductase activity across the post-operative period would have been desirable to map out a time course, it is difficult to justify ethically given the need for multiple invasive muscle biopsies. Day 5 was chosen because it was a time point where plasma cortisol would have been normal for several days. In an attempt to determine the mechanism of the observed SkM 11 β HSD1 activity upregulation, we have measured across the time of surgery, factors known to stimulate 11 β HSD1 in other tissues.

The maximal mean cortisol response in this study was seen 5 h after the commencement of surgery. This is consistent with published literature that reports maximal cortisol levels occurred within 6 h of abdominal surgery (1, 24). However, in only half our subjects was there a clearly defined cortisol peak at this time point. Importantly, in the patients whose cortisol did not peak at 5 h, an increase in SkM 11 β HSD1 oxoreductase activity was still consistently demonstrated. We found

these subjects had a significantly higher HOMA-R as a marker of insulin resistance, but the relevance of this is unclear. One possible explanation for the lack of a cortisol peak at 5 h may be that the maximum peri-operative cortisol for these subjects occurred prior to this time point. Donald *et al.* who compared the response of the entire HPA axis in subjects undergoing laparoscopic versus open cholecystectomy (4), reported that the maximal response in the laparoscopic subgroup was seen at 1 h post-operatively, which equates to $\sim$ 2 h after commencement of surgery (25). Therefore, there may still have been a significant increase in plasma cortisol above baseline in all subjects, which may have been identified by a more intensified blood sampling protocol.

Abdominal surgery has been associated with the development of insulin resistance (5–7). Although stress hormones have been implicated in the pathogenesis of this catabolic state, they return to normal within the first 72 h of surgery while the insulin-resistance may persist for at least 5 days. Certainly, prolonged intracellular cortisol action may cause insulin resistance by inhibiting skeletal muscle GLUT4 translocation to the cell surface and reduce insulin-stimulated glycogen synthesis (26). We hypothesised that there would be an increase in plasma insulin and HOMA-R across the time of surgery, but this proved not to be the case. One possible explanation for this is that the HOMA-R and QUICKI methods lacked the sensitivity to measure a change in insulin resistance in our small number of patients. Also, our surgical patients were already insulin resistant prior to surgery, which may have limited the extent of any further change in insulin resistance following surgery. Few data exist on the expected changes in fasting insulin, glucose and HOMA-R in response to a surgical stress. In a study comparing the metabolic response to open versus laparoscopic cholecystectomy, Ortega *et al.* demonstrated a reduced insulin and glucose response 4 and 24 h post-operatively following the laparoscopic procedure (24). In Thorell's study which mapped out a peri-operative course of insulin resistance, the euglycaemic clamp was performed at several time points, including less than 5 days prior to surgery and post-operatively on days 1, 5, 9 and 20 after surgery (5). Insulin resistance was maximal on the first post-operative day but still present on the 5th post-operative day being $\sim$ 30% lower compared with the pre-operative values. In the decade since these studies were published, it is possible that

refinements in laparoscopic surgical technique and anaesthesia may have further diminished the stress response, leading to less insulin resistance in the post-operative state.

Our data are more consistent with a reduction in β -cell function, as demonstrated by the significant fall in the β -cell index, rather than an increase in insulin resistance. A significant increase in plasma glucose was observed post-operatively while there was a non-significant trend for mean insulin levels to fall after surgery, with the lowest levels on the first post-operative day. A simultaneous reduction in insulin and rise in glucose, as seen in this study, have been previously demonstrated in response to direct sympathetic nerve stimulation (27). Adrenaline has been shown to directly inhibit the acute insulin response, and to stimulate hepatic glucose output (28). Therefore, the increased sympathetic drive seen during cholecystectomy (4, 24) may account for the alterations of glucose and insulin in this study. These observations suggest that while insulin resistance is one possible mechanism underlying the metabolic perturbations associated with abdominal surgery, relative insulin deficiency may also be an important factor. This may be particularly relevant in the presence of co-existing insulin resistance in this patient group. Although, insulin is a stimulus to adipose tissue 11 β HSD1 (29), the observed time course of plasma insulin levels do not account for the increased 11 β HSD1 activity in skeletal muscle.

A rise in the level of circulating FFA occurred around the time of surgery, with peak levels at 5 h after the commencement of surgery. There are limited data examining the changes in FFA specifically during laparoscopic cholecystectomy. One study comparing the lipid responses of patients undergoing laparoscopic compared with open cholecystectomy found a significant rise in FFA in both groups post-operatively (30), while another found that FFA fell significantly with surgery (31). However, glucocorticoids and increased sympathetic tone are known to induce lipolysis (32) which could account for the increase in FFA. While a direct effect of elevated FFA on skeletal muscle 11 β HSD1 has not to our knowledge been examined, the possibility arises that the FFA elevation noted in the current study could in part cause the upregulation of 11 β HSD1 activity, given that it has been noted that intralipid infusion upregulated 11 β HSD1 mRNA in adipose tissue (29). While we acknowledge that the peak in FFA occurred in the early post-operative period, it cannot be excluded that a short lived increase in a stimulatory factor might have a longer term effect on SkM 11 β HSD1 activity.

The role of inflammatory cytokines in the stress response to the surgical procedure was examined because TNF α has been shown to increase 11 β HSD1 activity in primary cultures of adipose stromal cells *in vitro* (33). Cytokine production in adipocytes may stimulate 11 β HSD1-mediated cortisol generation

within adipose tissue that then promotes adipocyte differentiation (34). TNF α also upregulates 11 β HSD1 in glomerular mesangial cells (35). Our study found no significant increase in plasma TNF α across the period of surgery, but this does not exclude a local tissue effect on SkM 11 β HSD1 oxoreductase activity. This lack of a significant change in plasma TNF α is consistent with previous reports comparing laparoscopic and open cholecystectomy (11, 36).

In conclusion, this study has demonstrated a significant upregulation of skeletal muscle 11 β HSD1 oxoreductase activity in subjects undergoing elective laparoscopic cholecystectomy when assessed at day 5 post-operatively, when plasma cortisol had returned to baseline. This increase was not associated with consistent changes in any of the factors known to upregulate 11 β HSD1 in other tissues. This is the first study to report an acute physiological stressor increasing activity of 11 β HSD1 in human tissue.

Declaration of interest

C Jang has received lecture fees from Novo Nordisk. W J Inder has received lecture fees from Novartis, Novo Nordisk and Eli Lilly. V R Obeyesekere and F P Alford have nothing to disclose.

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