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Selenium controls sex-specific immune response and selenoprotein expression during the acute phase response in mice

Mette Stoedter*, Kostja Renko*, Antonia Hög and Lutz Schomburg

Institute for Experimental Endocrinology, Charité - Universitätsmedizin Berlin, Südring 10, D - 13353 Berlin, Germany.

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*equal contribution

Correspondence should be addressed to

Dr. Lutz Schomburg,

Institute for Experimental Endocrinology,

Charité - Universitätsmedizin Berlin, Südring 10,

D - 13353 Berlin, Germany.

phone: +49-30-450 524289, fax: +49-30-450 524922

E-mail: lutz.schomburg@charite.de.

Abbreviations: ERAD, ER-associated protein degradation; GPX, glutathione peroxidase (human); Gpx, glutathione peroxidase (mouse); LPS, lipopolysaccharide (endotoxin); Se, selenium; SEPP, selenoprotein P (human); SepP, selenoprotein P (mouse); SEPS, selenoprotein S (human); SepS, selenoprotein S (mouse); TxnRd, thioredoxin reductase.

Abstract

Selenium modifies inflammatory reactions in rodents and humans. The liver controls metabolism and transport of selenium via hepatically-derived selenoprotein P (SEPP). Intracellular selenoprotein S (SEPS) modifies endoplasmic reticulum function and immune cell activity. Polymorphisms in *SEPS* were associated with cytokine levels and inflammatory diseases in a subset of clinical studies. We hypothesized that sex and selenium represent decisive parameters controlling immune response and regulation of SEPS expression in vivo. Male and female mice on Se-poor diet were supplemented or not with selenite for three days and injected with saline or LPS 24 h before analysis. Selenium supplementation mitigated the LPS-induced rise in circulating cytokines in male mice. Serum SepP and selenium concentrations decreased in response to LPS whereas hepatic SepS was specifically up-regulated despite declining Se concentrations in liver. Hepatic SepS induction was mainly controlled by posttranscriptional mechanisms and attributed to hepatocytes by analysing transgenic mice. Notably, selenium supplementation was essential for an optimal SepS induction. We conclude that selenoprotein biosynthesis becomes redirected in hepatocytes during the acute phase response at the expense of dispensable selenoproteins (e.g. SepP) and in favour of SepS expression thereby causing declining serum selenium and improving liver function. Selenium status and sex control SepS expression and modify cytokine response patterns in serum which might explain contradictory results on associations of *SEPS* genotype and inflammatory diseases in clinical studies.

INTRODUCTION

Selenium (Se) is an essential trace element needed for the biosynthesis of a small number of mammalian selenoproteins [1]. Se intake and personal Se status are implicated in wide-spread human pathologies including cancer, cardiovascular disease or neurodegeneration [2]. Recent research has specifically highlighted the importance of Se status for the activity of the immune system [3-5]. Positive effects of Se supplementation have been observed in a number of clinical trials with patients suffering from sepsis [6], human immunodeficiency virus infection [7] or autoimmune thyroid disease

[8]. The effects of Se are mediated by selenoproteins, a notion which is supported by association studies linking specific selenoprotein genotypes to human diseases [9, 10]. To assess an individual's Se status, glutathione peroxidase activity in blood cells (GPX1) or plasma (GPX3) and total Se or circulating selenoprotein P (SEPP) concentrations are measured, respectively [11]. SEPP is mainly derived from liver and seems to represent the best Se marker for it accounts for the majority of circulating Se in blood and responds over a broad range to Se intake [12]. Serum Se is an established negative acute phase reactant and declining serum Se levels correlate to low circulating SEPP in sepsis [13, 14]. Most importantly, the lower the Se status during critical illness the more likely the patients will not survive [6, 15-17]. Some but not all clinical trials have proven effective to improve outcome of critically ill patients by Se supplementation but the best application regimen, most suitable Se compound and the mechanisms of action are a matter of discussion [18-20].

One possible molecular component linking Se status with the immune system was recently identified when single nucleotide polymorphisms in the human gene encoding selenoprotein S (*SEPS*) were correlated to serum concentrations of proinflammatory cytokines, e.g., IL-6, IL-1 β and TNF- α [21]. *SEPS* encodes an essential factor for retrotranslocation of misfolded proteins from the endoplasmic reticulum (ER) into the cytosol for proteasomal degradation (ERAD, ER-associated protein degradation) [22, 23]. Hereby, *SEPS* is connected to a number of physiological pathways that modulate inflammation, e.g., NF κ B and JNK activation and induction of acute-phase proteins [24]. Insufficient Se supply might cause impaired *SEPS* expression leading to reduced ERAD activity and cell dysfunction upon ER stress. Accordingly, proinflammatory cytokines, glucose deprivation or chemically induced ER stress induce *SEPS* gene expression in vitro [25-27]. A molecular circuit can be envisaged in which inflammatory stimuli induce *SEPS* biosynthesis as a positive acute phase reactant to ensure efficient ERAD activity, stress relaxation and prevention of apoptosis [26]. We wondered about hepatic *SEPS* regulation in vivo during the acute phase response since the hepatic Se status and hepatic enzymes involved in selenoprotein biosynthesis are reduced under these conditions [28]. Moreover, we wanted to test the suitability of a short-term Se supplementation strategy as a preventive measure to positively affect the cytokine response pattern and increase selenoprotein expression in order to prepare an individual for an impending sepsis thread. This scenario can be envisaged for a regular patient in the clinics in face of e.g. a surgical intervention [29]. To this end, we have analysed the endotoxin (LPS)-based murine model of septic shock and studied both male and female mice for their regulation of selenoprotein expression differs between the sexes [30]. Our findings indicate mitigating effects of Se supplementation on the LPS-dependent rise of certain cytokines in blood especially in male mice. This response is paralleled by specific changes in selenoprotein expression in hepatocytes in favour of improved ER function during the acute phase and at the expense of SepP production. We conclude that availability of Se is of prime importance for hepatic SepS expression in vivo. This connection might help to explain some of the discrepant findings on the given or missing associations of *SEPS* genotype with inflammatory diseases in recent clinical studies for these analyses failed to correct for the actual Se status of the probands.

EXPERIMENTAL

Materials

All chemicals were of analytical grade and obtained from Sigma (München, Germany). Oligonucleotides were from Invitrogen, restriction endonucleases were from New England Biolabs (Frankfurt, Germany) and Taq polymerase was from Qiagen (Hilden, Germany).

Animal experimentation

Wild-type C57BL/6J mice were obtained from Charles River (Sulzfeld, Germany) and were bred on regular lab chow (Altromin, Lage, Germany). A time-course analysis of selenoprotein expression after a single injection of LPS was conducted as described before [28]. In addition, transgenic mice on C57BL/6 background carrying a removable gene for the selenocysteine-specific tRNA (floxed *Trsp*) have been generously supplied by D.L. Hatfield et al. [31] and were bred, genotyped and studied as described [32]. In these mice, selenoprotein expression can become specifically impaired in hepatocytes by crossing these mice with a strain expressing Cre recombinase under an albumin-

promoter (*Alb-Cre*) [33]. In a third experiment, groups of male and female mice (n=6 per group) were transferred at the age of 45 days (P45) to a Se-poor diet (C1045, Altromin) containing 0.1 ppm Se on average. Then, they received either regular tap water or 100 μ M Na₂SeO₃ in tap water for three days. 24 h before analysis, all of these mice were injected intraperitoneally once with sterile saline or 100 μ g LPS per animal (*E. coli* 55:B5, Sigma) as shown in Fig. 2. Necropsy, tissue homogenization, RNA-isolation, Northern and Western blot analyses were performed as described [28]. The experimental animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences, and experiments had been approved by the local governmental authorities in Berlin, Germany.

Enzyme assays

Hepatic Gpx and thioredoxin reductase (TxnRd) activities were determined as described [30, 34]. Briefly, livers were homogenized on ice in 250 mM sucrose, 20 mM HEPES, and 1 mM EDTA, pH 7.4 and cleared by centrifugation. Protein concentrations were determined in the supernatants by a modified Bradford assay using IgG as standard (Bio-Rad, Muenchen, Germany). NADPH consumption by GSH reductase was recorded at 340 nm as a measure of oxidized GSH formation in the Gpx reaction with t-butylhydroperoxide as substrate [30]. TxnRd activity was determined by monitoring the NADPH-dependent reduction of 5,5'-dithiobis-(2-nitrobenzoic acid, DTNB) determined as the increase in absorbance at 412 nm as described [34].

Determination of circulating cytokines

Aliquots of serum (10 μ l/sample) were analyzed by a multiplex analyzer system (Luminex 200, Luminex Corp., Austin, TX) in combination with LINCoplex cytokine immunoassays (Linco Research, St. Charles, MO) as described [30]. The tests for murine Il-6, Mcp1 and TNF- α were conducted according to the manufacturer's instructions and calibration curves were generated with seven commercial standard samples for each cytokine covering the concentration range from 0.0 to 10,000 pg/ml. The intra- and inter-assay precision of these cytometric bead assays typically range below 10% [35].

Expression of recombinant SepS

SepS coding sequence was amplified from a murine liver cDNA library (f-primer: AAGCTTAGAGCAGAGAGGAGCAGC, r-primer: CTCGAGCCGCCAGATGATGGGC) with proper restriction sites for in-frame cloning into pcDNA3.1-V5-His-TOPO (Invitrogen). Correct sequence and tag position were verified by DNA sequencing. The resulting construct was transfected or not into human hepatoma Huh7 cells by Ca-phosphate. Cells were harvested 48 h after transfection. The resulting cell lysates containing or not recombinant SepS were used as positive and negative controls in the Western Blot analyses, respectively.

Western Blot Analyses

Serum samples were collected and protein homogenates were prepared from different tissues and separated by 10% (SepP) or 15% (SepS) SDS-PAGE before being transferred to a nitrocellulose membrane (Protran, Schleicher & Schuell, Dassel, Germany) as described [28]. Membranes were stained by Ponceau S (PonS) in order to control complete transfer and equal loading of the lanes. Relative serum albumin concentrations were determined by densitometric quantification of the major band at 67 kD. A polyclonal antiserum (α -SepS) was generated in rabbits by immunization with a synthetic SepS-specific peptide (Ac-GRSYKRNSGRPQEEDGPGC-amid, corresponding to residues 125-142 of murine SepS, *Mus musculus* histocompatibility H47 mRNA, NM_024439.3). Antibodies specific for murine SepP have been characterized before and were used as described [28]. V5-specific antibodies were purchased and used according to the manufacturer's instruction (Invitrogen).

Se determination by total reflection X-ray fluorescence analysis (TXRF)

Aliquots of tissue homogenates (100 μ l) were digested with nitric acid (4 volumes, 65%) and spiked with an internal gallium standard (Sigma). Mouse serum samples (10 μ l) were diluted with distilled water (40 μ l) and spiked as above. Samples were applied to quartz glass carriers and left to dry. TXRF analyses were performed as recommended using a Picofox S2 instrument (Bruker AXS Inc. Madison,

Wisconsin, USA). Accuracy of Se determination was verified with a mouse serum pool and a specified human serum sample (Seronorm, Billigstad, Norway). The inter-assay coefficient of variation was below 10% for samples containing Se concentrations > 100 µg/l.

Statistical analysis

Data are expressed as mean ± SEM. Comparisons of two groups were done with Student's *t*-test. Differences between treatment groups were analyzed by one-way analysis of variance (ANOVA) and Dunnett's or Bonferroni post hoc tests for significance as indicated in the figure legends using Graphpad Prism v4.0 software (Graphpad Software, San Diego, CA, USA). Statistical significance was defined as $p < 0.05$ (*), as $p < 0.01$ (**) or as $p < 0.001$ (***)

RESULTS

Liver-specific induction of SepS during the acute phase response in vivo

SepS has been shown to be dynamically regulated by divergent stressors *in vitro* [25-27]. Therefore, we aimed to test whether SepS expression also changes during the acute phase response *in vivo*. To this end, a SepS-specific antiserum (α -SepS) was raised in rabbits and tested in Western Blot analyses. A single band of approx. 27 kD was detected from cells transfected with a recombinant tagged SepS protein but not in control cells (Fig. 1A). Identity of the band was verified by antibodies directed against the V5 tag of the recombinant SepS protein. *In vivo*, a single injection of LPS induced SepS expression in liver mainly on the protein level (Fig. 1B). Relative transcript concentrations of SepS were determined by densitometric analysis of the Northern blots and remained constant in relation to β -actin signals (β -act.) during the study period of 24 h after LPS injection (Fig. 1B). In comparison to liver, no effects on SepS protein concentrations were observed in brain, spleen or testes of the same mice over the time period analysed (Fig. 1C). In order to identify the cell type responsible for the up-regulation of SepS protein levels in liver, a transgenic mouse line with hepatocyte-specific genetic inactivation of selenoprotein expression was studied [36]. LPS had no effects on SepS protein concentrations in the livers of these mice (Fig. 1D). These results indicate that hepatocytes are responsible for the LPS-dependent induction of SepS in liver during the acute phase response.

Se-dependent sex-specific modulation of the immune response

Se has been shown to modulate the LPS-induced oxidative stress response in murine macrophages *in vitro* [37]. Moreover, mice with altered selenoprotein expression display a different cytokine response than controls upon influenza virus infection [4]. Yet the selenoproteins are sex-specifically expressed [30, 38]. We thus hypothesized that both sex and Se are important parameters for selenoprotein regulation and immune response. Therefore, we conducted a short-term Se supplementation experiment to specifically elucidate the importance of Se availability for the cytokine response and hepatic SepS expression. To this end, male and female mice were raised on a Se-poor diet during the 3 weeks before challenge to develop a marginal Se status. Then, mice were supplemented or not during their last 3 days with sodium selenite (100 µM) via their drinking water and received a single injection of NaCl or LPS (100 µg/animal) 24 h before being sacrificed and analysed (Fig. 2). Circulating levels of Il-6, Mcp1 and TNF- α were analyzed in the individual serum samples. The LPS-injected mice displayed strongly-increased cytokine concentrations compared to the control animals in both the male and female groups (Fig. 3). Se supplementation decreased the fulminant immune response in male mice; here, the LPS-induced TNF- α response was decreased with borderline significance whereas the LPS-dependent inductions of Il-6 and Mcp1 were significantly decreased on average to 50% compared to the Se-poor male individuals. In the female groups, Se had no statistical significant effects on the LPS-induced cytokines under these conditions.

Efficiency of a short term Se supplementation regimen to increase the Se status

In order to test whether this short-term Se supplementation was effective to raise biomarkers of the Se status in the experimental mice, hepatic TxnRd, hepatic Gpx and serum Gpx3 enzyme activities and concentrations of hepatic Sels and serum SepP along with serum and hepatic Se concentrations were analysed. As expected, hepatic Gpx activities, serum and hepatic Se along with circulating SepP

concentrations increased strongly in response to Se supplementation in both female and male mice (Fig. 4A-D). In contrast, hepatic TxnRd remained unaltered. The Se-dependent increase in serum Gpx3 activity was sex-specific under these conditions and statistically significant in males only (Fig. 4E-F). The strong effects on hepatic and circulating Se, hepatic Gpx activity and serum SepP concentrations indicate that application modus, dosage and time were sufficient to allow efficient Se uptake and improved hepatic selenoprotein biosynthesis and secretion under these conditions. Circulating SepP and serum Se turned out to represent robust markers of a short-term Se supplementation effect and faithfully mirrored changes in hepatic Se availability and hepatic Gpx enzyme activities while Gpx3 proved less suitable to monitor supplementation success under these conditions.

Comparison of different Se-dependent biomarkers during the acute phase response

A single sub-lethal LPS-injection represents a reliable model to analyse the acute phase response of Se and selenoproteins in experimental rodents [28, 39]. As observed before, the inflammatory stimulus caused strongly declining serum Se and SepP concentrations but had only marginal effects on serum albumin concentrations (Fig. 4A-B). Hepatic SepP mRNA concentrations remained unaffected under these conditions (data not shown) as studied before in detail [28]. LPS-dependent down regulation of serum Se and SepP concentrations were especially pronounced in the supplemented mice with higher Se status. These results verify that serum Se and SepP represent robust negative acute phase reactants. Hepatic Se concentrations were similarly strongly affected by the LPS stimulus in both sexes (Fig. 4C). In comparison, LPS affected hepatic Gpx activity in Se-supplemented male mice only (Fig. 4D). TxnRd activities in liver were not significantly affected by LPS under these conditions (Fig. 4E). The activity of kidney-derived Gpx3 remained largely unaltered by LPS in the Se-supplemented or Se-poor male or female mice (Fig. 4F). These results are consistent with the different dynamics and half-lives of circulating SepP and Gpx3 [40]. Moreover, this finding is in line with the results from a recent clinical study highlighting circulating SEPP as the presumably best biomarker for Se metabolism in sepsis [14]. In summary, SepP as a mainly hepatic product appears to be directly and strongly affected while Gpx3 as a kidney-derived and SepP-dependent secondary parameter appears to respond only poorly to LPS and the decreasing serum Se and SepP concentrations.

Importance of sex and Se for SelS expression and LPS-dependent maximal induction

In general, ample Se as substrate is needed in the form of selenocysteine-loaded tRNA in order to support biosynthesis of selenoproteins. Accordingly, SepS protein levels increased significantly in Se supplemented mice as compared to Se-poor animals (Fig. 5). In contrast to the declining hepatic Se and circulating SepP concentrations [28], LPS injection increased SepS expression in liver in both male and female animals (Fig. 1B, D and Fig. 5). Notably, a robust induction was observed in the Se-poor animals indicating that certain mechanisms within the hepatocytes can support SepS expression even against overall low Se status in a prioritized manner. In male mice, SepS expression was strongly induced by LPS under Se-poor conditions but not under Se supplemented conditions under which SepS apparently already had reached peak concentrations (Fig. 5). In contrast, maximal SepS expression in females was only observed upon LPS stimulation and Se supplementation, indicating that female mice needed higher Se concentrations or longer supplementation times in order to obtain maximal SepS expression.

DISCUSSION

The acute phase response in sepsis is a life-threatening process aiming to reprogram the organism's metabolism for defence against invading pathogens. Under such conditions, e.g. serum iron becomes strongly reduced as a meaningful reaction to deprive the proliferating microbes from one of their essential trace elements [41]. Likewise, Se in serum is known to decline as a negative acute phase reactant [39, 42]. The physiological purpose for this phenomenon is currently unknown but the absence from the circulation might again deprive the pathogens from an essential component [43]. Alternatively, it might improve the ability of immune cells to fight invaders by e.g. oxidative burst processes whose effects would otherwise be dampened by selenoproteins [44]. These theories still

need experimental proof but are in line with the reduced serum Se and SEPP concentrations under inflammatory conditions in humans [6, 13-16] and declining serum Se and SepP levels in experimental rodents [28, 39]. But what processes control the negative acute phase responses of serum Se and SEPP? Comparing LPS-dependent SepP decline in serum of Se-supplemented and Se-poor mice (Fig.3B), it becomes obvious that a loss of SepP into other tissues or an extensive binding onto the surface of the blood vessels is unlikely. Otherwise a stronger and more uniform decline would have been observed, especially in the male and female animals with low Se status. Since SepP expression depends on the Se status, the declining Se concentrations in liver (Fig. 4C) argue more in favour of a reduced hepatic SepP biosynthesis rate during the acute phase response. This notion is in line with the down-regulation of important factors involved in hepatic SepP biosynthesis under inflammatory conditions [28] and the rapid and specific degradation of another hepatic selenoprotein, i.e., Sep15, during acute ER stress [45]. These findings imply that the reducing Se concentrations in serum rather represent a side-effect secondary to an altered metabolic program in liver. This interpretation is corroborated by the better survival odds of sepsis patients with high Se status and with the positive effects of Se supplementation in critically ill patients [6, 15].

In this light, the concurrently rising SepS concentration in liver as a positive acute phase reactant might offer a causal explanation on the fate of the missing serum Se especially in Se-poor organisms. Of note, the effects of LPS on the biosynthesis of SepP and SelS are elicited largely independent of their transcript concentrations. We have shown before that LPS impairs expression of central components of the selenoprotein biosynthesis machinery and reduces hepatic SepP production without affecting SepP mRNA concentrations [28]. Here, we report a similarly strong effect on SepS expression in liver, albeit in the opposite direction, i.e., increased protein levels again without respective LPS-effects on SepS transcript concentrations (Fig. 1B). Moreover, using the transgenic mice with cell-type specific impaired selenoprotein biosynthesis, both effects proceed simultaneously in the same cells, i.e., in hepatocytes. Obviously, hepatocytes shuttle their available Se into SepS and away from the biosynthesis of other less vital selenoproteins during the acute phase response. Such a posttranslational regulatory circuit nicely complements the diverse molecular pathways known which finally translate ER stress into the inflammatory response, i.e., rearranging the protein biosynthesis pattern via eIF2 α phosphorylation, translational frame-shifting and activation of XBP1 and proteolytic generation of nuclear ATF6-p50 [24]. In contrast to these effects which relay a stress signal from ER into cytoplasm and nucleus, increased biosynthesis of SepS directly improves functioning of the critical cellular compartment, i.e. ER, via its important role during the removal of misfolded proteins during ERAD [25, 27, 46]. It is the nature of SepS as a selenoprotein to depend on a sufficiently high Se status needed for increased biosynthesis. Apparently, the redirection of Se away from other selenoproteins towards SepS expression fulfils this demand both under adverse circumstances as is the case in a poorly supplied individual and during the acute phase response. The concurrent decline of serum Se might represent the price for the prioritized hepatic SepS biosynthesis under inflammatory conditions, i.e., a side-effect.

But an efficient selenoprotein biosynthesis usually profits from increased Se supply. Such support can be provided by a respectively tailored immunonutrition [47]. The positive effects of Se supplementation in the clinics imply that a readily available Se source, e.g. sodium selenite, improves acute phase response and outcome [6]. While the best regimen and selenocompound in terms of application route and dosage is a matter of discussion, a low Se status during septicaemia has uniformly been proven as a negative prognostic marker for survival odds [48, 49]. Therefore, we have attempted a short term preventive Se supplementation effort aiming to improve Se status and selenoprotein biosynthesis fast via oral application in our experiments. The data clearly indicate that this effort was successful and especially the cell-type specific up-regulation of SepS in hepatocytes relied stringently on the Se status. Both male and female mice took advantage of an increased Se supply in order to maximize SepS expression albeit with sexual dimorphic dynamics. In response to supplementation, maximal SepS levels were observed already under basal conditions in male but not in female mice. Likewise, concentrations of circulating cytokines were mitigated by Se especially in male mice.

Even though these mechanistic insights have been deduced from rodent models and not human patients, the interrelation of Se status and SepS expression might help to explain divergent findings in genetic association studies of *SEPS* genotype and inflammatory diseases. In this respect, strong

associations were reported between the well characterized -105 *SEPS* promoter polymorphism [21] and gastric cancer susceptibility [50], coronary heart disease and stroke [51] and preeclampsia [52]. Together with the initial characterization of the interrelation of *SEPS* and inflammatory cytokines [21], a general effect of *SEPS* genotype on inflammatory diseases had been assumed. Therefore, a lack of *SEPS* genotype-disease association as recently reported for type 1 diabetes, rheumatoid arthritis, inflammatory bowel disease or ulcerative colitis [53] was surprising. Here, the generalized conclusion might be misleading since the data have not been corrected for by sex and the actual Se status of the probands. Our results highlight the importance of these parameters for immune response, cytokine patterns and SEPS induction. Congruent data on the interaction of genotype, sex and Se status were recently reported for SEPP isoform expression in human cancer [54]. Since the observed inflammatory effects on SEPP and SELS biosynthesis are mainly exerted on the post-transcriptional level, the Se status and the concentration of selenocysteine-loaded tRNA are decisive parameter for the disease-specific response. According to our results, it seems advisable to determine the Se status along with the *SEPS* genotype in future studies to be better able to compare the genotype-disease association in men and women and gain knowledge on the potential benefit of a personally tailored Se supplementation in a specific pathology. The herein proposed preventive measure to increase the Se status fast via an orally available supplement might prove useful in the clinics to ensuring optimal physiological ER stress response by high SEPS expression prior to a potential challenge. At the same time the unfavourable low circulating Se concentrations can be avoided. Respective clinical studies taking the Se status and sex into account appear necessary and are highly recommended.

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FIGURE LEGENDS**Figure 1 Tissue-specific acute phase regulation of SepS expression in vivo**

(A) The new SepS-antiserum yields strong signals in cells positively transfected with recombinant V5-tagged SepS (lanes 2-5) but not in control cells (lanes 1, 6). Position of immunoreactive SepS co-localized with V5-signals and the theoretical size of the recombinant protein verifying suitability of the antibodies for SepS analysis. (B) Time-course analysis of hepatic SepS protein (top) and mRNA (bottom) concentrations after LPS-injection. Male mice (n = 4 per group) received a single injection of saline (C) or LPS (100 µg/mouse) and were sacrificed after 6 h (all controls) or after the indicated periods of time. Samples were analyzed by two Western and two Northern blots of which a representative result is shown. Liver protein extracts from the individual mice were analyzed by Western blotting (top) and SepS amounts were quantified by densitometry. Ponceau S (PonS) staining served to control for efficient protein transfer onto the membrane and equal loading of the lanes. RNA (bottom) was isolated from the same livers and used for Northern analysis of SepS and β-actin (β-act). *P < 0.05, **P < 0.01 (one-way ANOVA with Dunnett's post hoc test). (C) Tissue-specificity of LPS effects on SepS up-regulation. Protein extracts (n=3) from brain, spleen and testes of the experimental mice in (B) were analyzed by Western blot at different time intervals after injection of LPS. Recombinant SepS served as positive control (+). No significant effects of LPS on SepS protein concentrations were observed in these tissues. (D) Effects of LPS on SepS expression in wild-type (wt) and transgenic (KO) mice. Protein extracts from livers of treated (LPS) and control (C) mice were analyzed by Western blot. Recombinant SepS protein served as positive control (+). Transgenic mice (n=5) with albumin-promoter driven Cre expression causing genetic deletion of the Sec-specific tRNA in hepatocytes express little SepS compared to wt (n=5) animals (wt C vs. KO C). SepS expression is not stimulated by LPS in livers of KO mice (n=3) compared to saline-injected KO controls (n=2) indicating that hepatocytes account for increased SepS expression in liver of wt mice during an acute phase response in vivo. **P < 0.01 (one-way ANOVA with Bonferroni's post hoc test).

Figure 2 Experimentation scheme to study Se-dependent acute phase response

Male and female mice were bred on regular diet and transferred to a Se-poor diet (0.1 ppm Se) at the age of 45 days (P45) on which they remained for the next 3 weeks. Then, groups of animals (n=6 per sex) were supplemented or not with selenite in drinking water (100 µM) for 2 days prior to NaCl (control) or LPS injection (100 µg/animal, i.p.) yielding 4 experimental groups (far right). Acute phase responses of cytokines, Se and selenoproteins were studied 24 h after injection of NaCl or LPS.

Figure 3 Inflammatory cytokines in control and Se supplemented mice after LPS injection

Serum samples from control and experimental female (left row) and male (right row) mice (n=6 per group) were analysed for circulating concentrations of Il-6, Mcp1 and TNF-α. The LPS injection increased serum cytokine concentrations in mice independent of sex. Supplementation of Se mitigated the profound immune response in male but not in female mice, especially pronounced in case of Il-6 and Mcp1. *P < 0.05, **P < 0.01 (Student's *t*-test).

Figure 4 Biomarkers of Se status after Se supplementation and LPS injection

(A) Selenite supplementation increased serum Se concentrations in male and female mice (n=6 per group). LPS injection reduced serum Se strongly in the Se-supplemented mice of both sexes while the effect was not significant in Se-poor animals. (B) Serum levels of SepP increased after Se supplementation as determined by Western blot analysis with antibodies against murine SepP (α-SepP). Ponceau S (PonS) staining (top) verifies constant loading of the serum samples and comparable serum protein amounts in the lanes. Western result of pooled serum samples is shown (top). Quantification of the effects (bottom) was based on additional Western blot analyses with individual samples (n=6 per group). A marginal but significant effect of LPS was observed on albumin concentrations in serum of male mice (*P < 0.05, Student's *t* test). Albumin concentrations in female mice were not affected. LPS caused a significant decline in circulating SepP especially in the Se-supplemented male and female mice. **P < 0.01 (Student's *t*-test). (C) Se concentrations in livers mirror changes in circulating SepP upon selenite and LPS treatment. Se supplementation increased hepatic Se concentrations in all groups of mice. LPS injection caused a strong decline in hepatic Se

concentrations in the Se-supplemented male and female mice but not in Se-poor animals. (D) Hepatic Gpx activity increased by Se supplementation in both female and male mice and declined in response to LPS in male mice only. (E) Hepatic TxnRd activity was similar in male and female mice and remained unaffected by short-term selenite supplementation or LPS treatment in these experiments. (F) Gpx3 activity was determined from the serum samples. Only male mice displayed significantly increased Gpx3 activities in response to the short term oral Se supplementation. LPS had no consistent effect on circulating Gpx3 under these experimental conditions in male or female mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (one-way ANOVA with Bonferroni's post hoc test).

Figure 5 Sex-specific regulation of hepatic SepS in response to selenite and LPS treatment
LPS injection induced maximal SepS concentrations in Se-supplemented mice indicating the importance of the Se status for SepS expression. A Western blot of pooled homogenates from the livers of the experimental mice is depicted (top) in order to provide an overview. SepS protein concentrations were determined from two additional Western analyses from samples of individual mice which are shown (bottom) together with the quantification (graph). Characteristic sex-specific differences were observed with respect to both basal SepS expression levels and responsiveness to Se and LPS. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Student's *t*-test).

Fig. 1A

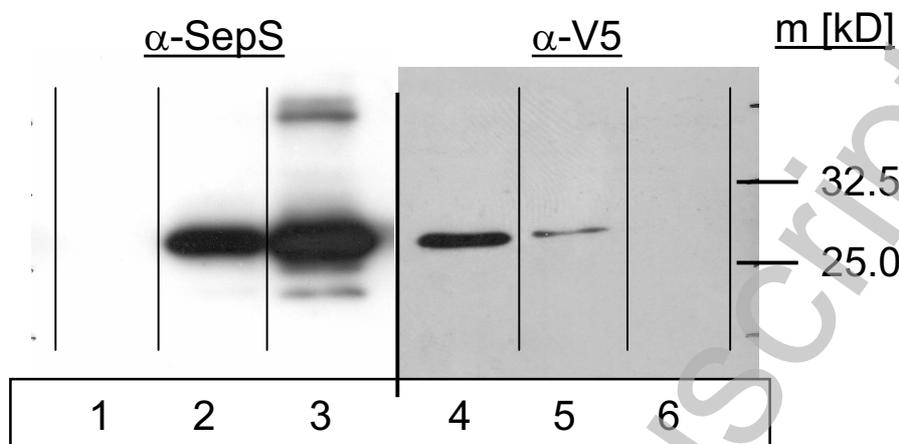
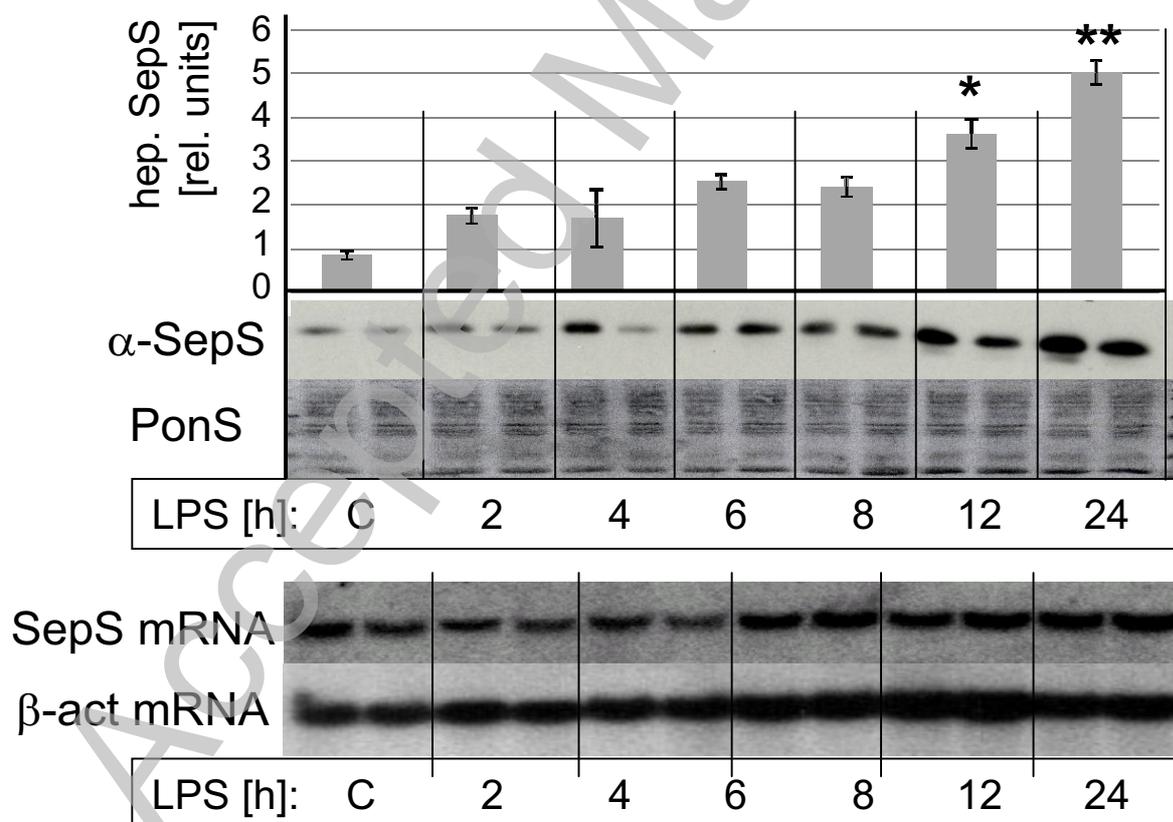


Fig. 1B



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Fig. 1C

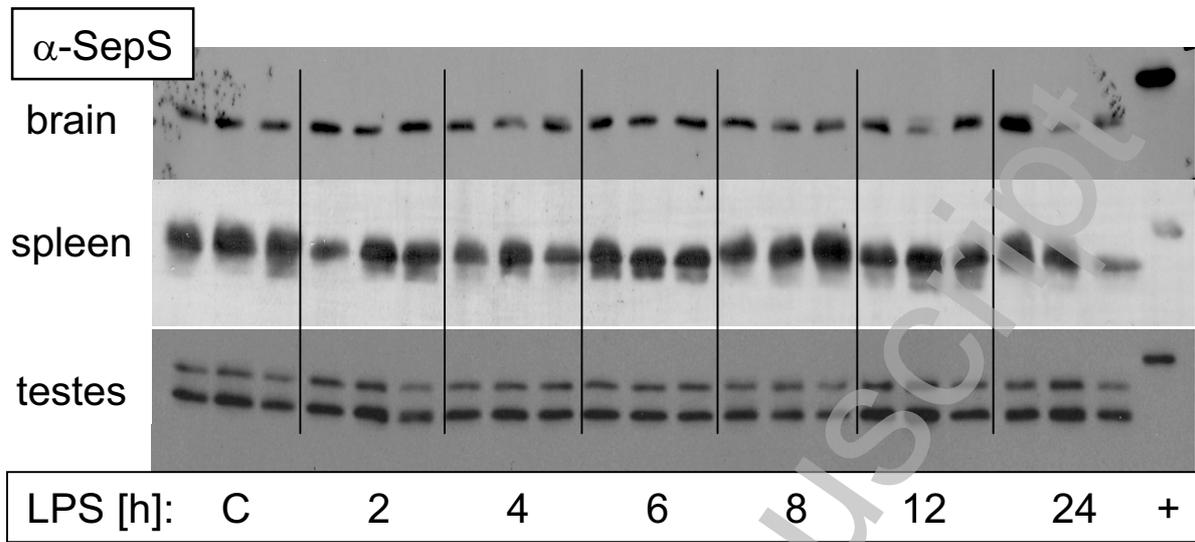


Fig. 1D

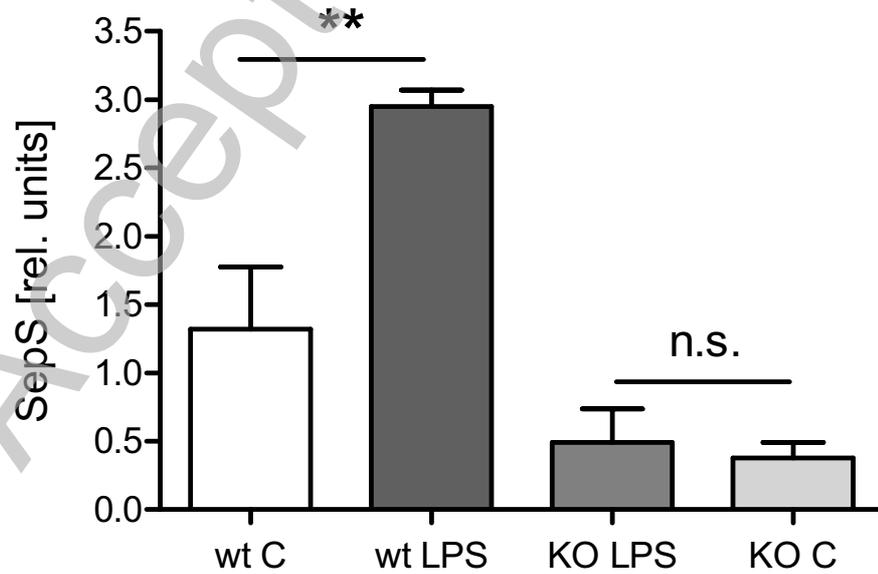
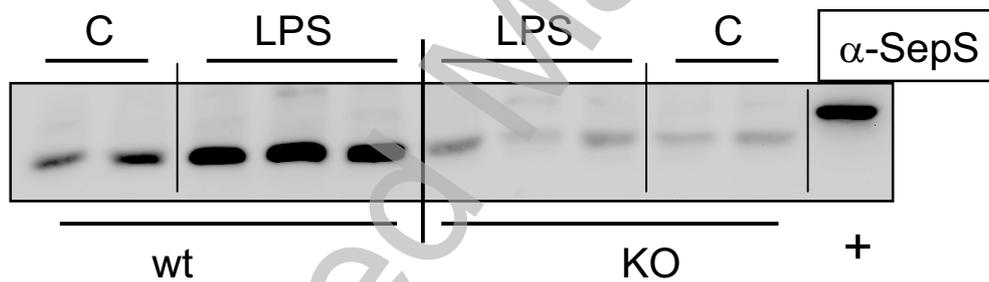
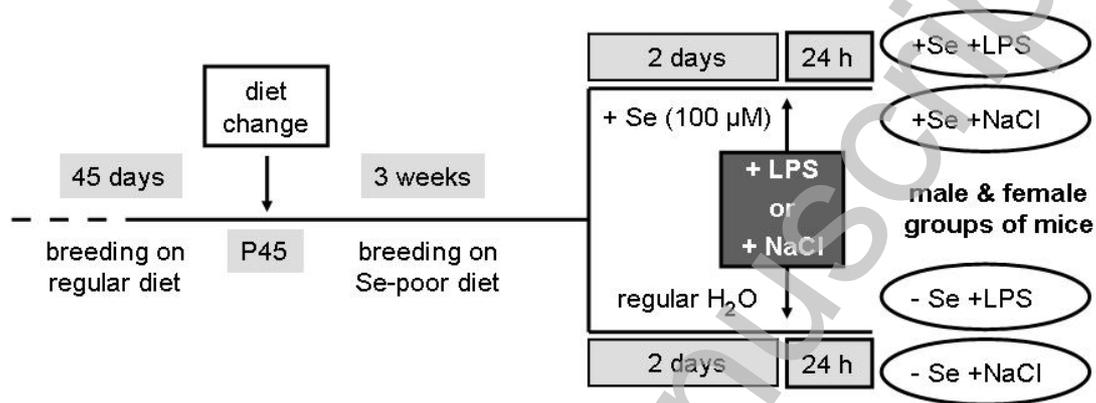


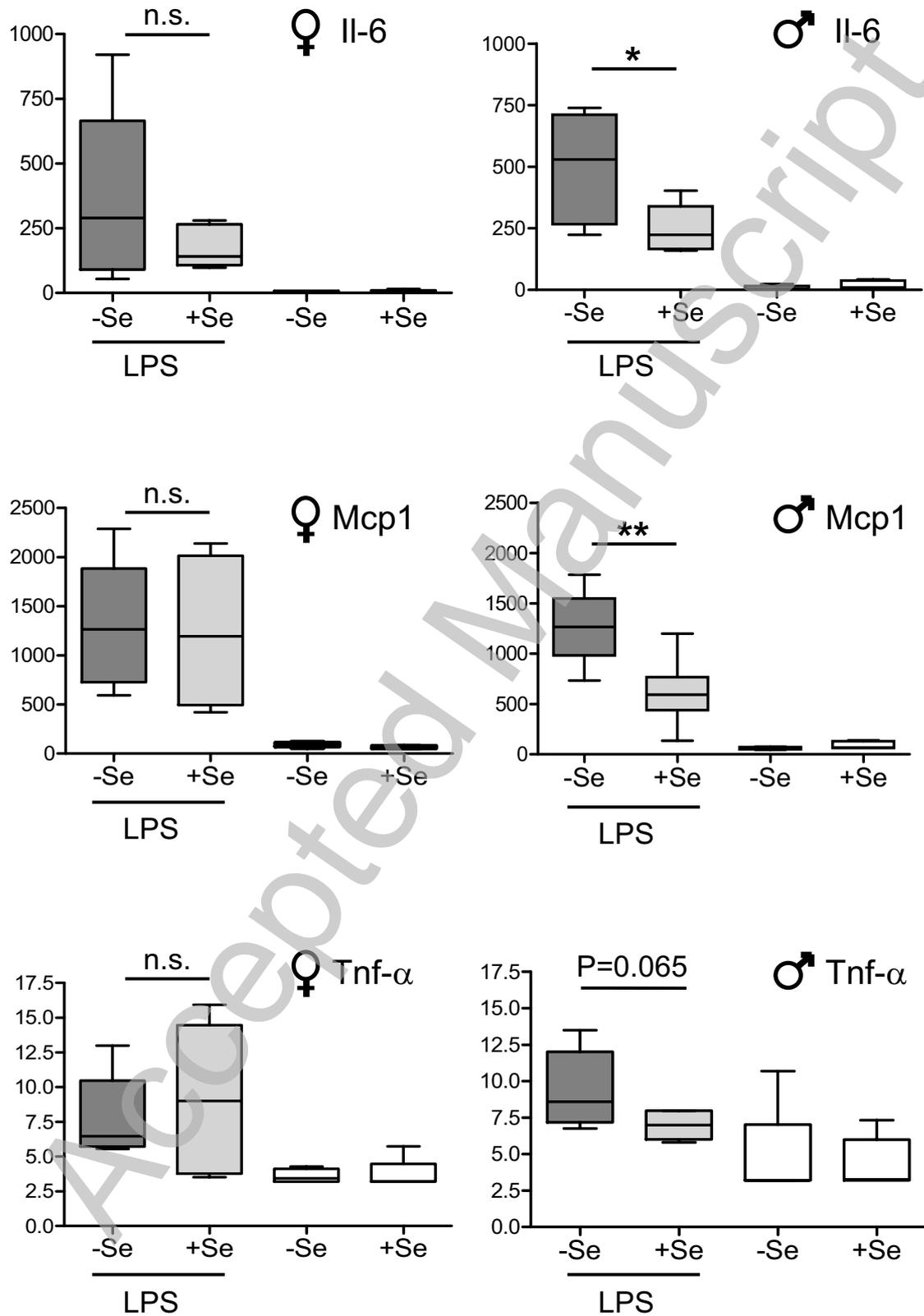
Fig. 2



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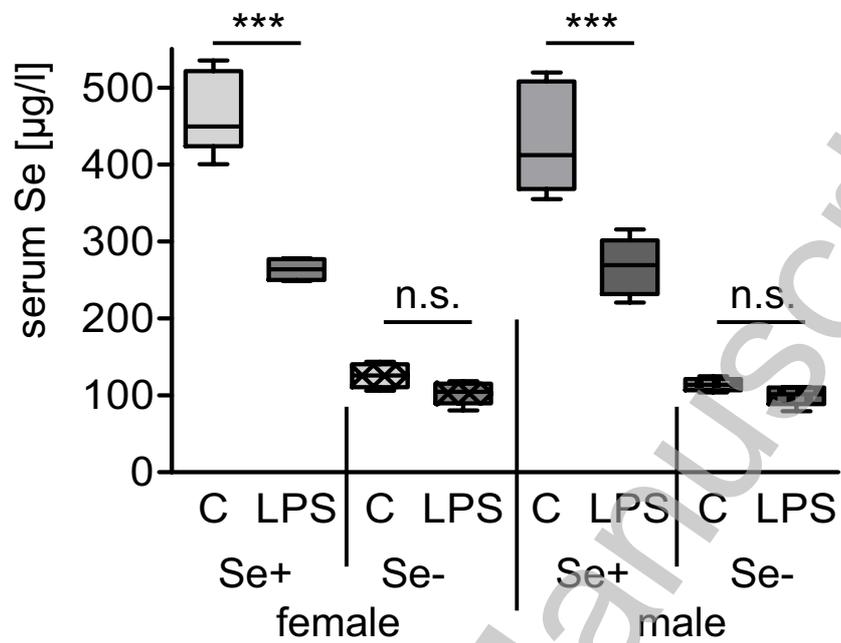
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Fig. 3



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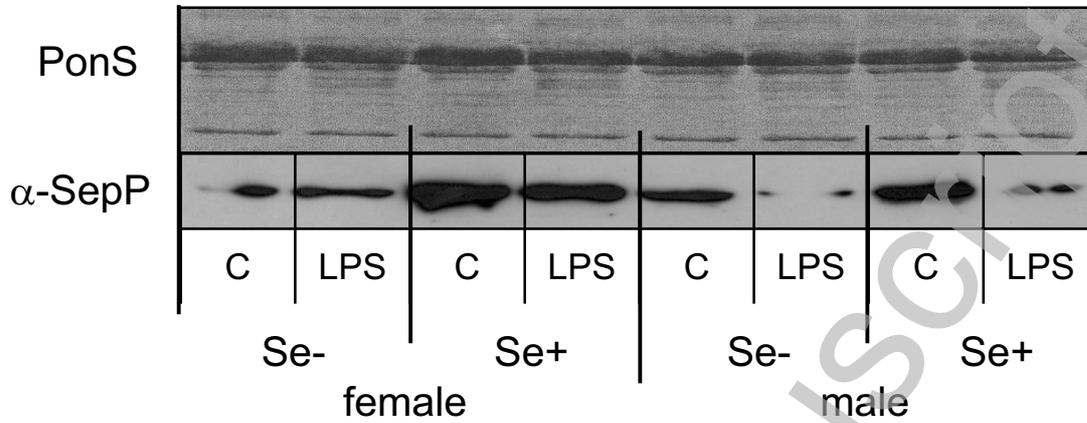
Fig. 4A



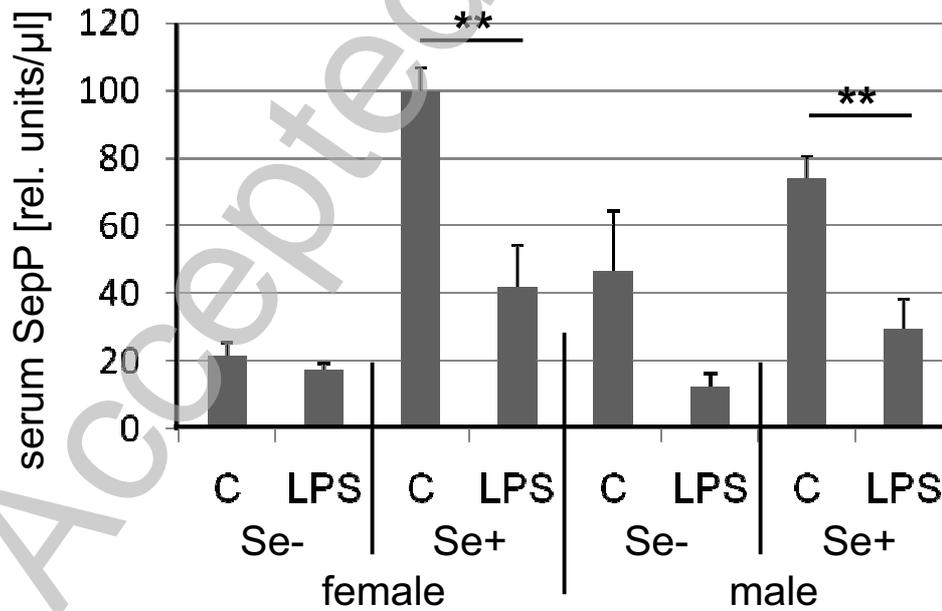
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Fig. 4B



albumin [rel. units/ μ l \pm SEM]	C	+LPS
female Se-	1.00 \pm 0.01	1.02 \pm 0.02
female Se+	1.00 \pm 0.02	1.00 \pm 0.02
male Se-	1.00 \pm 0.01	0.96 \pm 0.03*
male Se+	1.00 \pm 0.02	0.95 \pm 0.02*



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Fig. 4C

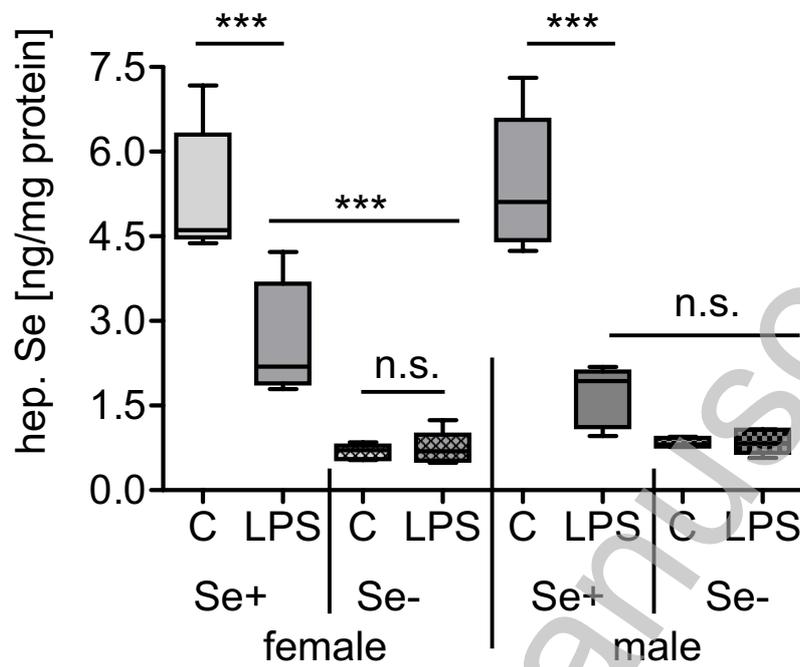


Fig. 4D

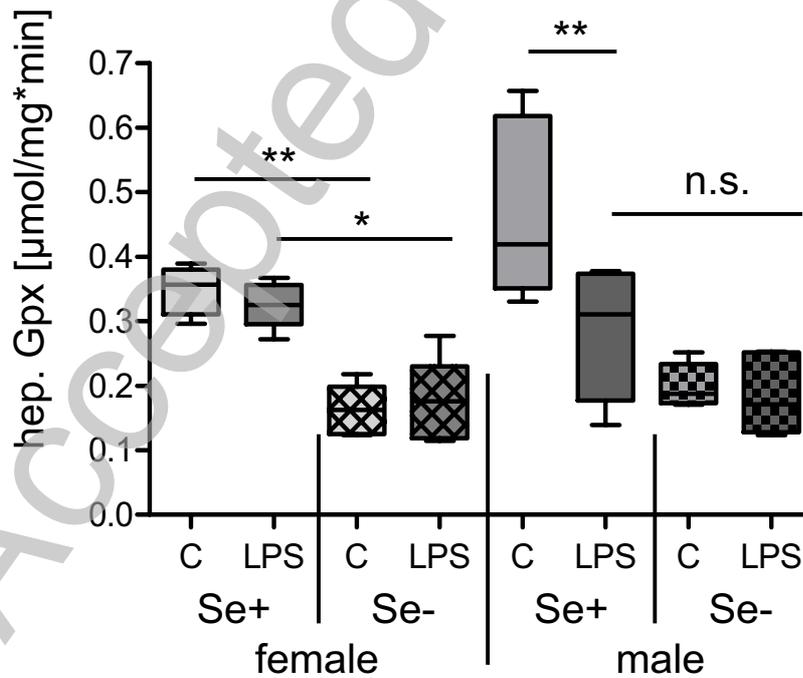


Fig. 4E

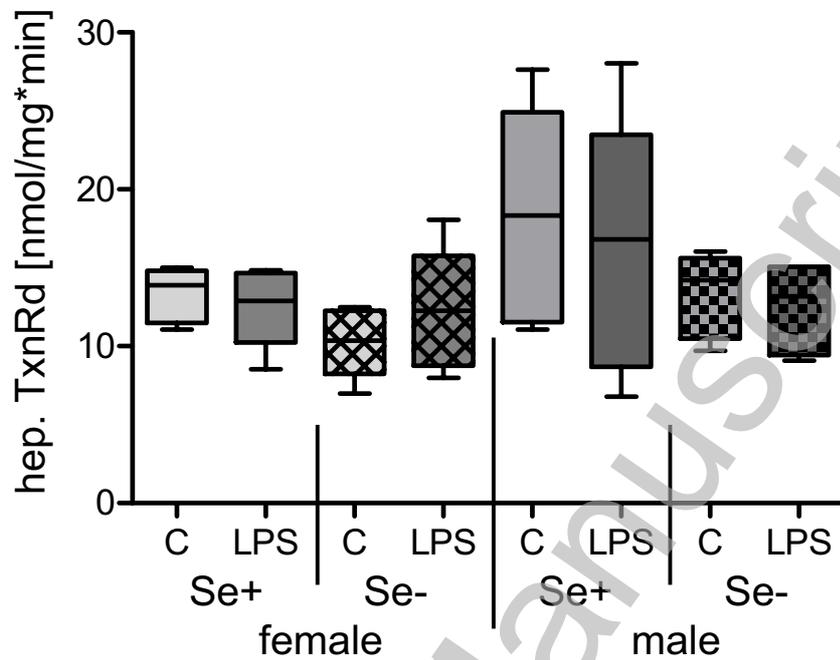
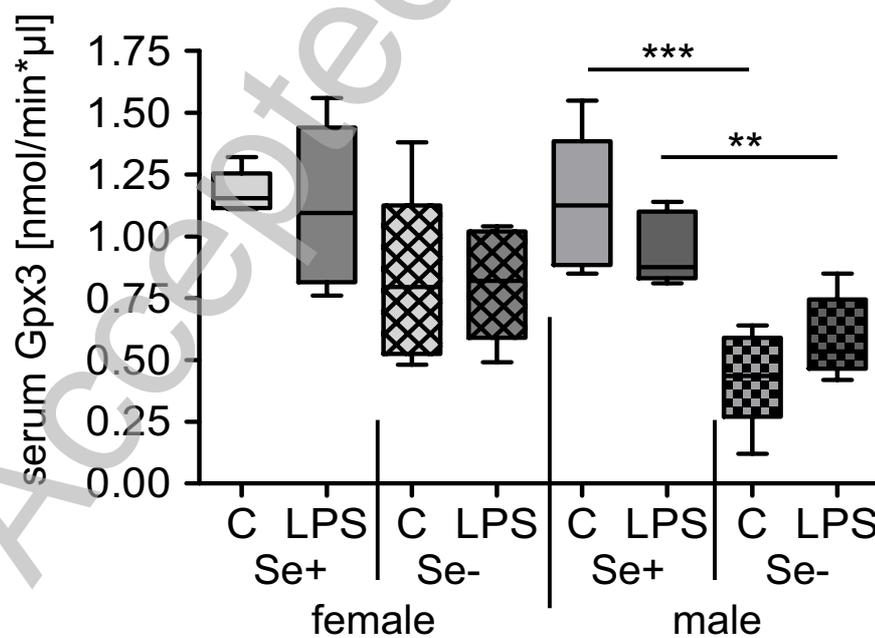
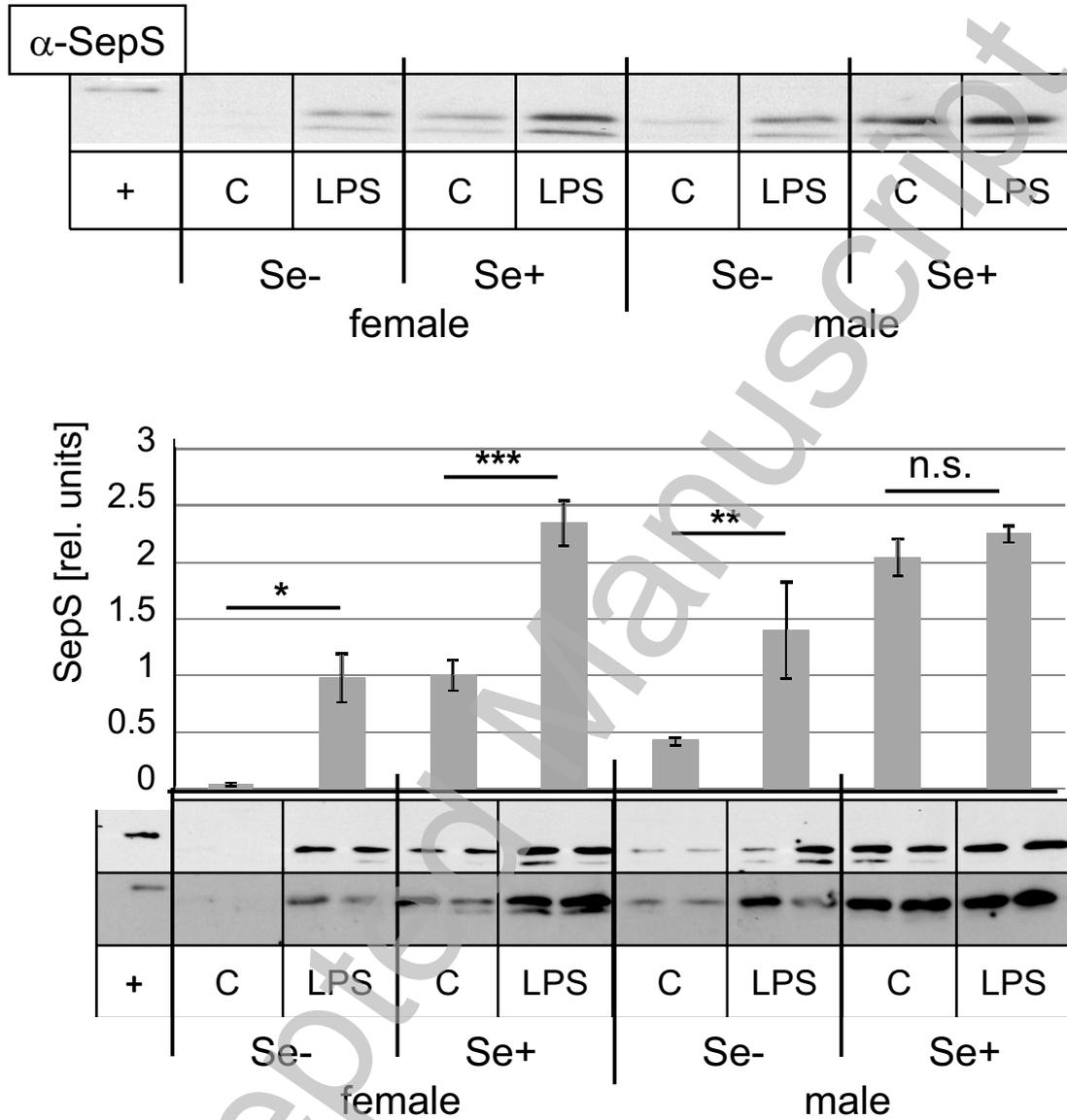


Fig. 4F



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Fig. 5



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