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Human serum proteomics reveal molecular signature after sleep deprivation at night

Protein profile after one night of sleep loss

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INTRODUCTION

Introduction Sleep deprivation is becoming increasingly prevalent. It is caused by several conditions such as night- or alternating shift work and affects cellular processes and molecular mechanisms resulting in altered gene- and protein expression. Circadian rhythm disruption or sleep deprivation are proposed to be involved in the affected cellular mechanisms behind development and progression of various cardiovascular-, immune-, mental- and neurodegenerative disorders as well as cancer[1-6].

We recently revealed human serum proteome changes after a simulated night shift, however, studies associated with partial sleep deprivation using a proteomics and systems biological approach are still sparse (refs. see Bjørkum et al. 2021, BMC, https://doi.org/10.1186/s41606-021-00066-2) [7]. This study aimed to further explore changes in the human blood serum after 6h of sleep deprivation at night using newer proteomic methods identifying another set of serum proteins and exploring their representation and classification in systems biological databases built upon earlier knowledge on the specific proteins.

Material and methods Human blood serum samples from 8 healthy voluntary females aged 22 to 57 years were analyzed using mass spectrometry (Orbitrap Eclipse) and high-pressure liquid chromatography (HPLC Dionex Ultimate 3000). Each subject was their own control, and two samples were taken from each subject, the first sample after 6h of sleep at night from 22:00, and the second sample after 6h of sleep deprivation the following night. Sleep-wake- and other physiological data can be found in Bjørkum et al. 2021[7]. Biological databases and bioinformatic software were used for systems biological- and comparative analysis with other sleep-related datasets.

RESULT

Of the 793 proteins identified by mass spectrometry, 494 were considered for further analysis and 66 were found to be significantly changed after 6h of sleep deprivation at night, 63 upregulated and 3 downregulated. Functional enrichment analysis revealed the associations of these proteins with several biological processes and molecular functions relating to fields such as regulation, binding, or transport. Additionally, our data was found to be linked to different curated gene sets.

Table 1. Significantly changed proteins (66) in human serum after 6h of sleep deprivation

SwissprotID	Gene symbol	Protein name	p-value	Fold-change (log2)	# of peptides
Upregulated proteins (63)					
P06727	**APOA4**	**Apolipoprotein A-IV**	**0.0010**	**1.016**	**45**
P08514	**ITGA2B**	**Integrin alpha-IIb**	**0.0025**	**1.107**	**8**
O43852	**CALU**	**Calumenin**	**0.0028**	**1.054**	**6**
P05556	**ITGB1**	**Integrin beta-1**	**0.0035**	**1.053**	**5**
Q6ZNG0	ZNF620	Zinc finger protein 620	0.0042	1.037	1
Q14515	**SPARCL1**	**SPARC-like protein 1**	**0.0055**	**1.051**	**11**
Q9NPY3	**CD93**	**Complement component C1q receptor**	**0.0073**	**1.099**	**6**
P06733	**ENO1**	**Alpha-enolase**	**0.0075**	**1.070**	**4**
P02549	SPTA1	Spectrin alpha chain, erythrocytic 1	0.0081	1.028	1
A1L4H1	SSC5D	Soluble scavenger receptor cysteine-rich domain-containing protein SSC5D	0.0122	1.044	1
P21926	CD9	CD9 antigen	0.0123	1.095	1
Q13201	**MMRN1**	**Multimerin-1**	**0.0125**	**1.033**	**14**
Q8WUA8	**TSKU**	**Tsukushi**	**0.0125**	**1.063**	**3**
P04180	**LCAT**	**Phosphatidylcholine-sterol acyltransferase**	**0.0133**	**1.030**	**10**
Q14766	**LTBP1**	**Latent-transforming growth factor beta-binding protein 1**	**0.0140**	**1.047**	**18**
P24593	**IGFBP5**	**Insulin-like growth factor-binding protein 5**	**0.0143**	**1.047**	**6**
P09172	**DBH**	**Dopamine beta-hydroxylase**	**0.0146**	**1.034**	**15**
Q15113	**PCOLCE**	**Procollagen C-endopeptidase enhancer 1**	**0.0156**	**1.037**	**8**
P10909	**CLU**	**Clusterin**	**0.0166**	**1.020**	**21**
Q6UXB8	**PI16**	**Peptidase inhibitor 16**	**0.0168**	**1.040**	**8**
P22891	**PROZ**	**Vitamin K-dependent protein Z**	**0.0178**	**1.022**	**10**
Q15582	**TGFBI**	**Transforming growth factor-beta-induced protein ig-h3**	**0.0178**	**1.022**	**16**
Q03591	**CFHR1**	**Complement factor H-related protein 1**	**0.0179**	**1.028**	**12**
O95445	**APOM**	**Apolipoprotein M**	**0.0180**	**1.008**	**11**
P35443	**THBS4**	**Thrombospondin-4**	**0.0213**	**1.070**	**10**
Q12860	**CNTN1**	**Contactin-1**	**0.0214**	**1.056**	**7**
P08253	**MMP2**	**72 kDa type IV collagenase**	**0.0226**	**1.029**	**11**
O00533	**CHL1**	**Neural cell adhesion molecule L1-like protein**	**0.0246**	**1.048**	**22**
P05109	**S100A8**	**Protein S100-A8**	**0.0248**	**1.064**	**2**
P04114	**APOB**	**Apolipoprotein B-100**	**0.0251**	**1.004**	**309**
P10720	**PF4V1**	**Platelet factor 4 variant**	**0.0254**	**1.021**	**5**
Q5SYB0	FRMPD1	FERM and PDZ domain-containing protein 1	0.0262	1.027	1
Q92496	**CFHR4**	**Complement factor H-related protein 4**	**0.0285**	**1.032**	**9**
P35542	**SAA4**	**Serum amyloid A-4 protein**	**0.0290**	**1.017**	**5**
Q06033	**ITIH3**	**Inter-alpha-trypsin inhibitor heavy chain H3**	**0.0292**	**1.019**	**24**
P33151	**CDH5**	**Cadherin-5 OS=Homo sapiens**	**0.0298**	**1.030**	**14**
P07359	**GP1BA**	**Platelet glycoprotein Ib alpha chain**	**0.0298**	**1.038**	**7**
P00746	**CFD**	**Complement factor D**	**0.0309**	**1.028**	**11**
P98160	**HSPG2**	**Basement membrane-specific heparan sulfate proteoglycan core protein**	**0.0311**	**1.059**	**24**
P12111	**COL6A3**	**Collagen alpha-3(VI) chain**	**0.0313**	**1.043**	**27**
Q07954	**LRP1**	**Prolow-density lipoprotein receptor-related protein 1**	**0.0330**	**1.054**	**9**

All significantly changed proteins in human serum after 6h of sleep deprivation with no more than 4 missing values (out of 16), up- (63) and down-regulated (3) with p-values ≤ 0.05. Each protein is listed with their SwissProtID, gene symbol and protein name. The fold-change (log2) shows the degree of change in the protein concentration in the sleep deprivation samples compared to the control samples. The number of peptides indicates how many peptides have been found for the respective protein. Proteins that have been identified by two or more peptides are shown in bold.

Table 2. Top 5 enriched biological processes of the 66 significantly changed proteins

Biological process	Enrichment ratio (ER)	Proteins involved (gene symbol)
Protein activation cascade	36.8	APOH, C1RL, CFD, CFHR1, CFHR4, CLU, GP1BA, F2, F12, FBLN1, KNG1, MASP2, SERPING1
Platelet degranulation	30.8	AHSG, APOH, CD9, CFD, CLU, HRG, ITGA2B, ITIH3, KNG1, LAMP2, MMRN1, PF4, QSOX1, SERPING1, THBS1
Blood coagulation	13.7	APOE, APOH, CD9, F2, F12, FBLN1, GP1BA, HRG, ITGA2B, KNG1, MMRN1, PF4, PF4V1, PROZ, SAA1, SERPING1, THBS1
Coagulation	13.6	APOE, APOH, CD9, F2, F12, FBLN1, GP1BA, HRG, ITGA2B, KNG1, MMRN1, PF4, PF4V1, PROZ, SAA1, SERPING1, THBS1
Hemostasis	13.5	APOE, APOH, CD9, F2, F12, FBLN1, GP1BA, HRG, ITGA2B, KNG1, MMRN1, PF4, PF4V1, PROZ, SAA1, SERPING1, THBS1

The processes are sorted by the highest enrichment ratio (ER). The ER describes the proportion of proteins in our dataset of 66 proteins that belong to a particular biological process divided by the proportion of genes in the background set that belong to the same process. The respective associated significantly changed proteins after sleep deprivation are listed with their gene symbols. The false discovery rate (FDR) describes the proportion of errors among all positive findings (corrected for multiple hypothesis testing) and was ≤ 0.05 for all biological processes.

Table 3. Gene Ontology (GO) biological process, cellular component, molecular function and immune system process groups of the 66 significantly changed proteins

Group	# of terms	% of terms	Most represented term	# of proteins	Associated proteins (gene symbols)
GO Biological Process					
Group11	**63**	**43.15**	**Wound healing**	**20**	**APOE, APOH, CD9, CNTN1, F12, F2, FBLN1, GP1BA, HRG, ITGB1, KNG1, MMRN1, PF4, PF4V1, PROZ, S100A8, SAA1, SERPING1, THBS1, TSKU**
Group10	**38**	**26.03**	**Cholesterol transport**	**9**	**APOA4, APOB, APOE, APOM, CLU, LCAT, LRP1, PCSK9, TSKU**
Group9	10	6.85	Positive regulation of wound healing	7	APOH, CNTN1, F12, F2, HRG, ITGB1, THBS1
Group8	9	6.16	Cholesterol transport	9	APOA4, APOB, APOE, APOM, CLU, LCAT, LRP1, PCSK9, TSKU
Group7	8	5.48	Cholesterol transport	9	APOA4, APOB, APOE, APOM, CLU, LCAT, LRP1, PCSK9, TSKU
Group6	7	4.79	Negative regulation of endothelial cell migration	4	APOE, APOH, HRG, THBS1
Group5	4	2.74	Regulation of transforming growth factor beta1 production	4	LTBP1, LUM, THBS1, TSKU
Group4	2	1.37	Acute inflammatory response	7	AHSG, CNTN1, F12, F2, S100A8, SAA1, SAA4
Group3	2	1.37	Vascular associated smooth muscle cell proliferation	3	DBH, IGFBP5, MMP2
Group2	1	0.68	Complement activation	7	C1RL, CFD, CFHR1, CFHR4, CLU, MASP2, SERPING1
Group1	1	0.68	Cell adhesion mediated by integrin	4	HRG, ITGA2B, ITGB1, MMRN1
Group0	1	0.68	Cysteine-type endopeptidase inhibitor activity	3	AHSG, HRG, KNG1
GO Cellular Compartment					
Group6	**8**	**40.00**	**High-density lipoprotein particle**	**10**	**APOA4, APOB, APOE, APOH, APOM, CLU, LCAT, PLA2G7, SAA1, SAA4**
Group5	**5**	**25.00**	**Vesicle lumen**	**16**	**AHSG, APOB, APOH, CFD, CLU, DBH, HRG, ITIH3, KNG1, MMRN1, PF4, QSOX1, S100A8, SERPING1, THBS1, TTR**
Group4	3	15.00	Extracellular matrix	28	ADAMTSL4, AHSG, APOA4, APOE, APOH, CLU, CNTN1, COL6A3, F12, F2, FBLN1, GP1BA, HRG, HSPG2, KNG1, LTBP1, LUM, MMP2, MMRN1, PCOLCE, PF4, S100A8, SERPING1, SPARCL1, SSC5D, TGFBI, THBS1, THBS4
Group3	1	5.00	Ficolin-1-rich granule membrane	3	CD93, CLU, LAMP2
Group2	1	5.00	Blood microparticle	11	AHSG, APOA4, APOE, C1RL, CFHR1, CLU, F2, HRG, ITGA2B, KNG1, SERPING1
Group1	1	5.00	Platelet dense granule	3	APOH, ITIH3, LAMP2
Group0	1	5.00	Endoplasmic reticulum lumen	18	ADAMTSL4, AHSG, APOA4, APOB, APOE, CALU, CLU, COL6A3, F2, IGFBP5, KNG1, LTBP1, PCSK9, PROZ, QSOX1, SERPING1, SPARCL1, THBS1
GO Molecular Function					
Group11	**11**	**33.33**	**Lipoprotein particle receptor binding**	**6**	**APOB, APOE, CLU, HSPG2, LRP1, PCSK9**
Group10	**7**	**21.21**	**Lipoprotein particle receptor binding**	**6**	**APOB, APOE, CLU, HSPG2, LRP1, PCSK9**
Group9	2	6.06	Opsonin binding	4	CD93, CFHR1, CFHR4, MASP2
Group8	2	6.06	Fibronectin binding	6	FBLN1, IGFBP5, ITGB1, MMP2, SSC5D, THBS1
Group7	2	6.06	Apolipoprotein binding	3	LCAT, LRP1, PCSK9
Group6	2	6.06	Serine-type endopeptidase activity	13	C1RL, CFD, CNTN1, COL6A3, F12, F2, HRG, ITIH3, MASP2, MMP2, PCSK9, PROZ, SERPING1

Functional analysis performed with the Cytoscape plug-in ClueGO. The number of terms describes how many processes, components or functions are represented in each respective group of the GO category and the percentage of terms describes the relative representation of each group. Groups that have ≥ 20% of the terms assigned to them are marked in bold. The most represented term in every group of each GO category is the term with the greatest number of significantly changed proteins involved of all the terms in the group. The respective gene symbols of the proteins involved are listed in the associated proteins column. If there were multiple terms with the same number of proteins involved in a group, the term with the higher percentage of associated genes found in ClueGO was listed. All p-values were adjusted for multiple hypothesis testing (Bonferroni correction) and ≤ 0.05.

CONCLUSION

Our study was able to reveal another set of human serum proteins altered by sleep deprivation and to connect similar biological processes to sleep deprivation that have been identified before with slightly different methods in Bjørkum et al. 2021.

Based on the differentially expressed proteins in this study, our results not only support these previous findings, but also those from other sleep-related studies, that sleep deprivation affects several biological functions such as regulation, binding or transport, and thereby also associates to protein changes in clinically relevant pathological conditions like altered platelet function and coagulation, oxidative stress, impaired immune function, cardiovascular and neurodegenerative diseases, and cancer.

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