Gender- and menstrual phase dependent regulation of inflammatory gene expression in response to aerobic exercise

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ABSTRACT

The immunological reaction to exercise has been investigated with increasing intensity in the last 10-20 years, with most human studies performed in male subjects. Recently, gender-specific aspects have received growing attention, but studies carefully monitoring the influence of gender, including the menstrual cycle, are rare. Here, we report gene expression patterns in response to a run at 93% of the individual anaerobic threshold of 9 women with regular menstrual cycles and no use of oral contraceptives who ran both at day 10 (follicular phase, F) and at day 25 (luteal phase, L) of their cycle. 12 male subjects (M) served as controls. The mRNA was pooled group wise and processed on a gene expression microarray encompassing 789 genes, including major genes of the inflammatory and anti-inflammatory reaction. The differences of gene expression between time points t_0 (before run) and t_1 (after run) were analyzed. Females in L showed a higher extent of regulation than females in F or men. Among those genes which were up-regulated above 1.5 fold change (log_2) pro-inflammatory genes were significantly enriched ($p=0.033$, after Bonferroni correction) in L, while this was not the case in F or M. Conversely, women in L showed a strong trend towards down-regulation of anti-inflammatory genes. Some prominent genes like IL6 (coding for interleukin-6), and IL1RN (also termed IL1RA, coding for interleukin-1 receptor antagonist) were clearly regulated in opposite directions in L as opposed to F and M.
In conclusion, women in L showed a distinctly different pattern of gene regulation in response to exercise, compared with women in F or M. The overall direction of

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gene expression changes of women in L is clearly pro-inflammatory. This finding accentuates a need for careful consideration of the female cyclic phase when investigating women in exercise immunology studies. Our results may also have implications relevant to other forms of stress in females.

Keywords: gender, inflammation, gene regulation, aerobic exercise, menstrual cycle, stress response, IL6, IL1RN, IL1RA.

INTRODUCTION

Recent studies have documented that significant gender dimorphisms exist in certain immune responses to different types of exercise (6, 15, 27-29). Gender differences in response to exercise have clear implications for understanding gender-specific adaptations to exercise for athletic performance and overall health. However, while in general the impact of exercise on immune functions has received considerable and increasing attention in recent years, it is still unclear to what extent gender and fluctuations in sex hormones influence immunological responses to exercise.

Several gender-related differences in immune function under non exercise conditions have been identified, and it has been hypothesized that at least some of these differences could be attributed to female sex hormones (7). Numerous clinical studies have demonstrated that immune responsiveness is greater in women than in men (7): women have lower incidence and mortality to several types of infections (7), higher serum concentrations of some immunoglobulins (IgM) (12), a higher absolute number of T-helper lymphocytes (1), and a differential regulation of cytokine production (12, 14). Leukocyte chemotaxis (7) is also sensitive to gender related hormones. Mitochondria from females generate smaller amounts of hydrogen peroxide than those of males and have higher levels of mitochondrial reduced glutathione and antioxidant enzymes (26). Several menstrual cycle associated effects on parameters of the immune system have been described. Compared to the follicular phase (F), the luteal phase (L) of the menstrual cycle was associated with increased concentrations of leukocytes and lymphocyte subsets (5, 9), increased prostaglandin (PG) E2 and PGI2 release by stimulated monocytes (3, 11, 25), a greater capacity of immune cells to produce cytokines (5, 9, 13), a higher plasma cytokine activity (14), but variable effects on plasma cytokine levels (2, 8, 13). In contrast, other studies associate the follicular phase with greater cytokine production from immune cells (14) and higher serum IL-6 levels (2).

The fact that the majority of exercise studies has been done in males does not really come as a surprise. However, in situations where a new hypothesis has to be proven or disproven for the first time, it may be a forgivable or even a wise concept to start off with males only to avoid unforeseeable interferences from fluctuations of sex hormones occurring in women depending on the different phases of their menstrual cycle. Even worse than that, we know that in competitively training female athletes the cycle is often disturbed or abolished. In addition many females take oral contraceptives which again can have an impact on immunological functions as well (27). Thus, it can be tedious and not very easy to find well

defined and willing groups of female volunteers to do meaningful studies. Nevertheless we think that time has come to do exactly that.
A number of studies have reported no differences in cell counts and functions (4, 16-19, 31), plasma cytokine levels (16, 30), and lymphocyte apoptosis (20) between men and women concerning the response to different kinds of exercise. However, it appears that these studies did not control for the menstrual status of the women at the time of testing. In contrast to studies reporting no differences, others have reported gender differences in the immune-related responses to treadmill running (5), cycling (8, 27-29) and eccentric exercise (15, 25). In a recent study (Fehrenbach et al. unpublished), we found out that intracellular HSP70 showed gender and menstrual cycle dependent reactions in lymphocytes and monocytes 24 h after exercise. Timmons et al. (2005) have reported gender and menstrual cycle dependent changes in leukocyte and cytokine responses to cycling (27).
In the present study we used mRNA from the above mentioned HSP study to run a microarray analysis on 789 genes, which were partly selected on the basis of their relation to inflammatory processes. The study had a group of regularly menstruating women who ran twice, once on day 10 (follicular phase) and once on day 25 (luteal phase) of their menstrual cycle and a group of males for comparison.
The first results of this investigation focusing on the differences in gene expression immediately after compared with before a 1 h run close to the individual anaerobic threshold are presented here.

MATERIAL AND METHODS

Subjects

Twelve female (W) and 12 male runners (M) gave informed consent to participate in the study. The investigation was approved by the University Ethics Committee. All were experienced athletes with normal dietary habits. They were not on any medication and they performed endurance training on a regular basis. The W included in the study had regular menstrual cycles and did not use oral contraception. Determination of the cyclic phases was based on a diary, kept by the women, beginning three months prior to the study. To confirm the cyclic phases, the hor-

Table 1: Anthropometric and physical characteristics of the subjects.

	Men (n=12)	Women (n=9)
Age (yrs)	32.6 (28.7 – 36.4)	29.68 (25.4 – 33.7)
Body mass index (kg· m^2)	21.6 (20.9 – 22.3)	20.9 (19.9 – 22.0)
Training sessions (1 · $week^{-1}$)	5.8 (5.3 – 6.2)	4.4 (3.8 – 5.1)*
Training distance (km · $week^{-1}$)	60.8 (53.9 – 67.7)	38.9 (28.6 – 49.2)*
V_{IAT} (km · h^{-1})	14.0 (13.4 – 14.5)	11.8 (11.1 – 12.5)*

V_{IAT}, running velocity at the individual anaerobic threshold. Data are presented as means (95% CI). *p<0.01, men vs. women

monal status of W was determined by measuring oestrogen, progesterone and LH using the ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics, Fernwald, Germany). After hormonal assessment, three women had to be withdrawn from the study due to luteal insufficiency. The physical characteristics of the remaining athletes are shown in Table 1.

Preliminary Testing

Before participating in the main study the athletes performed an incremental exercise test on a treadmill (Saturn, HP Cosmos, Traunstein, Germany) to determine the running velocity (V_{IAT}) at the individual anaerobic threshold (IAT). Capillary blood for lactate measurement (EBIO, Eppendorf, Hamburg, Germany) was obtained from the earlobe after every stage and heart rate was monitored continuously using a heart rate monitor (Polar Electro, Finland). V_{IAT} was calculated by the method of Dickhuth (23) using a PC-routine.

Continuous runs

The main investigation consisted of continuous runs (CR) on the treadmill with duration of 60 min and a running velocity corresponding to 93% V_{IAT}. The exercise procedure started at 09:00 a.m. The W had to perform the identical CR twice: once in the follicular phase (F) of their cycle at day 10 and once in the luteal phase (L) of their cycle at day 25. Capillary blood lactate was determined before and immediately after exercise. Venous blood samples were drawn one hour before (t_0; 8:00 a.m.) and immediately after the end of the CR (t_1; 10:00 a.m.).

PBMC isolation and RNA extraction

EDTA anti-coagulated venous blood samples were used for the isolation of peripheral blood mononuclear cells (PBMC) using the Ficoll-hypaque density gradient technique as described previously (10). After gathering the cells in RLT-buffer total RNA was extracted using an RNeasy minikit (Qiagen, Hilden Germany) in accordance with the manufacturer's protocol. The RNA from M (n=12) and W (L/F; n=9) was pooled using equal amounts of RNA for the corresponding runs for t_0 and t_1. The integrity of extracted RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California, USA).

Microarray data generation and statistical analysis

Microarray data were generated using 65mer oligonucleotide microarrays produced at the IKET, University of Tübingen as previously described (33). We used a 2,402 feature array including transcripts as well as buffers, controls and empty spots. The genes on the array were selected inter alia with a focus on inflammation and regulation of inflammatory processes. Every feature was printed at least in duplicate. The array contained 789 genes in total, while some transcripts were contained up to 12 times in duplicate. For further details of the array used in this study can be obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/geo/) under accession number GPL5676.

An indirect reference design was used with Cy3 labeled uniRNA (Stratagene, La Jolla, California, USA) and Cy5 labeled sample RNA. Amplification of sample RNA was performed using Ambion´s Amino Allyl Message Amp II aRNA Amplification Kit (Ambion Inc., Austin, Texas, USA) together with Amersham CyDye

Post-labeling Reactive Pack (GE Healthcare, Buckinghamshire, UK) following the manufacturer`s protocols, and assessing dye incorporation using a Nano Drop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). After an aRNA fragmentation using Ambion´s Fragmentation reagents (Ambion Inc., Austin, Texas, USA) hybridization was performed for 14 h at 48°C. Subsequently, the hybridized and washed slides were scanned in a microarray scanner (Affymetrix Inc. Santa Clara, California, USA). The photomultiplier tube voltage was set to 100% for both green and red channels. The resulting green and red images were overlaid using ImaGene 5.0 (Biodiscovery Inc. El Segundo, California, USA) as well as for raw data collection.

Data analysis

Further statistical and bioinformatic analysis was performed using the limma (Linear models for microarray) package for R from the bioconductor project (24). The data was normalized using printtip-loess intra-array normalization on the normexp-background corrected expression values followed by inter-array quantile normalization across groups. For further analysis, normalized expression values of duplicate features were averaged. For the different pools (F, L, and M) the fold change ($fc=t_1-t_0$) between $\log_2$ expression values of both time points was computed. On the basis of the fold changes, up-regulated genes ($fc > 1.5$) and down-regulated genes ($fc < -1.5$) were determined. 81 different genes from the array with clearly pro-inflammatory impact and 43 different genes with clearly anti-inflammatory impact were selected for a closer analysis (see addendum). For both pro-inflammatory and anti-inflammatory gene sets and each group, the number of genes exceeding the respective fold change thresholds between t_0 and t_1 was calculated. For significance testing, the same number of genes contained in the respective set was sampled 10,000 times and the fraction of genes exceeding the threshold p value was calculated. A gene set with $p < 0.05$ was considered significantly enriched. Tests were omitted if no genes of the set exceeded the threshold. The result of the analysis of the above mentioned gene sets encompassing the pro- inflammatory and anti-inflammatory genes are listed in the addendum. We are aware that due to pooling the RNA, no classical significance testing could be performed. To control the false-positive rate, we used rather conservative thresholds, requiring absolute fold changes of at least 1.5 ($\log_2$) for genes to be considered significantly regulated.

The raw microarray data is available in GEO (http://www.ncbi.nlm.nih.gov/geo/).

Table 2: Resting hormone levels in women and pre- and post-exercise lactate concentrations.

		Men (n=12)	Women, F (n=9)	Women, L (n=9)
Estrogene (pmol · l^{-1})	Pre-CR	n.d.	370 (111 – 629)	491 (296 – 687)
Progesterone (nmol · l^{-1})	Pre-CR	n.d.	4.0 (1.9 – 6.1)*	23.8 (15.0 – 32.6)
Blood lactate (mmol· l^{-1})	Pre-CR	0.9 (0.7 – 1.2)	1.0 (0.9 – 1.1)	0.9 (0.7 – 1.0)
Blood lactate (mmol· l^{-1})	Post-CR	2.1 (1.5 – 2.6)+	2.4 (1.6 – 3.3)+	2.6 (2.1 – 3.0)+

Data are presented as means (95% CI). F, follicular phase; L, luteal phase; n.d., not detected. *p<0.01, women, F vs. women, L; + p<0.01, post-CR vs. pre-CR. There were no significant differences between F, L and M.

RESULTS

The treadmill runs were performed at a speed which corresponded to 93% V_{IAT}. At the end of exercise, blood lactate concentrations were significantly increased in all groups, but still remained in a range typical for more intensive but still predominantly aerobic exercise. No significant differences were detected between M, F and L (see table 2).

Statistical analysis

The enrichment analysis yielded one enriched gene set. In group L, we found the pro-inflammatory genes enriched among the up-regulated genes (p= 0.0017, after Bonferroni-correction for 10 tests: 0.017).

In general, L showed a high degree of regulation having 129 genes up–regulated and 143 down-regulated, compared with F (48 / 32) and M (34 / 29). This was especially pronounced in the gene sets specifically selected for their strong relation to inflammation. From the 81 genes judged as pro-inflammatory, 20 stood out to be regulated above the mentioned threshold of 1.5 (log_2). Of these, 13 were up-regulated and 7 were down-regulated. 17 of the anti-inflammatory genes were regulated above the threshold, of which 6 were up-regulated and 11 down-regulated. In M and F, much lower regulation was observed (see figure 1).

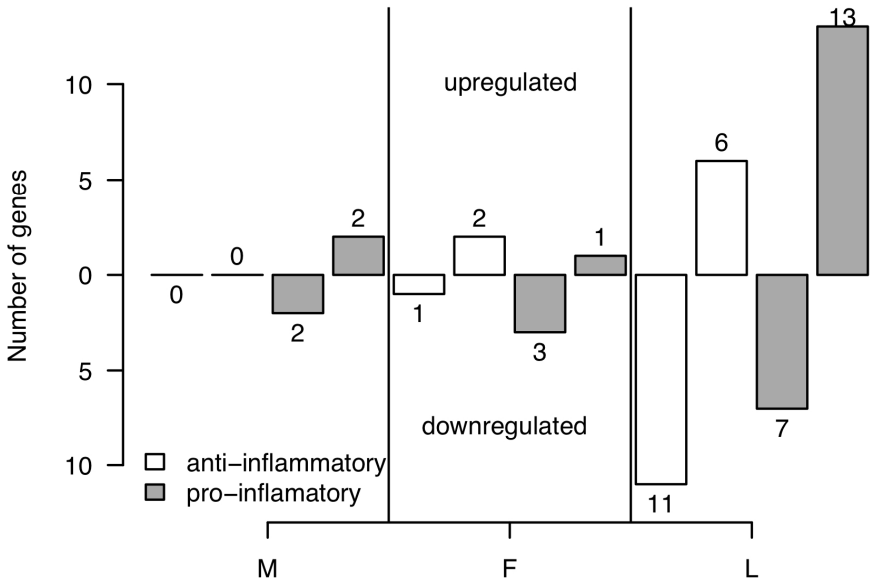

Figure 1: Major changes in expression of anti- (white) and pro (black)- inflammatory genes (see addendum) in the three groups. Bars pointing upwards denote up-regulated genes; bars pointing downwards denote down-regulated genes. A threshold of +/- 1.5 (log_2) was used (see addendum).

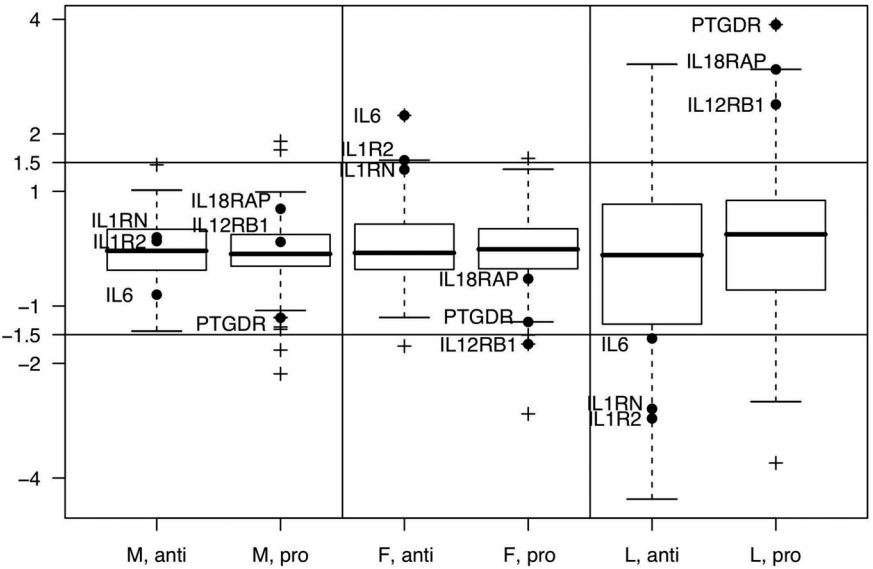

Figure 2: Box plots of $\log_2$ fold change for the selected gene lists, separately for each group. Genes of special interest were marked at their respective positions in the respective boxplot, + denotes outliers (below or above +- 1.5* interquartile range) not considered in this context. For the marked pro- and anti-inflammatory genes, we observe a strong inverse regulation. Note that the variances for both gene sets differ significantly between L and M or F (F-test p value < 10^-9).

By arbitrarily setting another cutoff at $\log_2$ 1.0 (up-regulated (*fc* > 1.0) or down-regulated (*fc* < -1.0)) in either direction, 35 genes from the pro- inflammatory and 25 genes from the anti-inflammatory subset came up in L. Little changes were detected in M (9 / 4) and F (9 / 6).

When aligning the detected genes, according to their pro-inflammatory impact on the one hand (pro-inflammatory genes up-regulated/ anti-inflammatory genes down-regulated) and to their anti-inflammatory impact on the other hand (pro-inflammatory genes down-regulated / anti-inflammatory genes up-regulated), a strong pro-inflammatory response was revealed in L (see figure 1). Neither in F nor in M was a comparable regulation observed.

For some genes of either set, a strong inverse regulation was detected. This was especially pronounced for the anti-inflammatory genes IL6, the decoy receptor interleukin 1 receptor type II (IL1R2) and IL1RN, which were up-regulated during exercise in F, while consistently down regulated in L (see figures 2 and 3). IL1RN codes for the IL-1 receptor antagonist. In the literature the expression IL1RA is used synonymously for the gene. Furthermore, we found several pro-inflammatory genes, including prostaglandin D2 receptor (PTGDR), interleukin 18 receptor accessory protein (IL18RAP) and interleukin-12 receptor beta 1 (IL12RB1) to be down-regulated in F, while strongly up-regulated in L (see figure

2). Some of the remaining genes of both sets exhibited a similar pattern of regulation. A comparable inverse regulation, into the opposite direction (pro- inflammatory impact in F, and anti-inflammatory impact in L) was exhibited by only one anti-inflammatory gene, adrenergic receptor beta 2 (ADRB2) which was down-regulated in F but up regulated in L (for further information see addendum).

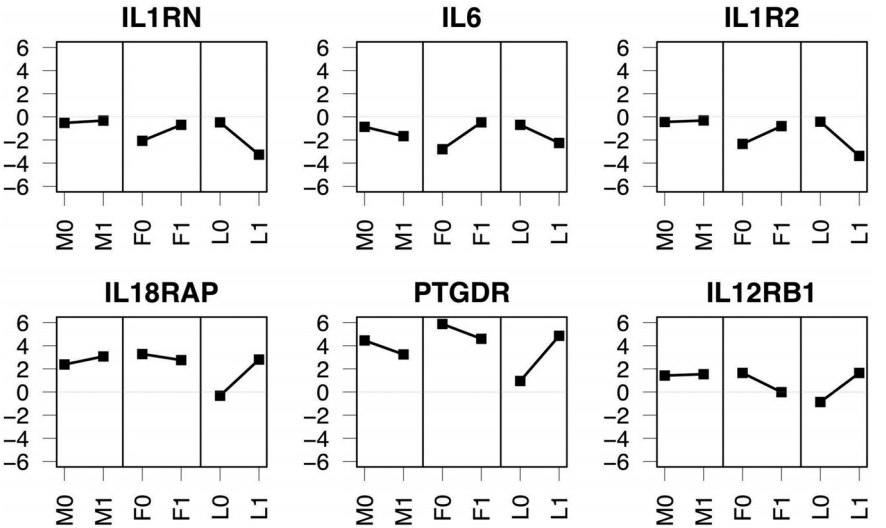

Figure 3: Profile plots for selected pro-inflammatory genes (upper row) and anti-inflammatory genes. The plots show expression values for t_0 and t_1 for each group. The abscissa shows the expression value.

DISCUSSION

Among mammals, very few things are regulated with such a high species-specificity as reproduction. Obviously there is enough flexibility built into this area of physiology to enable each species to adjust optimally to its needs. Conception susceptibility of females decides if newborns arrive all together in spring (typical for favored victims of predators) or several times during the year (like in dogs) or every few weeks (rodents). Human females are disposed to essentially all year long readiness for sexual activity with frequent and regular periods of conception susceptibility.

The situation as described makes animal experiments very tricky to translate to the human situation. Nevertheless, the findings of Nickerson et al. (21), that female rodents did not show elevated myocardial heat shock proteins (HSP) after exercise stress, while males did, prompted us to run a study designed to explore the reaction of HSP to exercise in controlled relation to the female menstrual cycle. To our surprise, females showed strikingly different patterns of regulation, depending on the phase of their menstrual cycle. While at d10 (F), they regulated

HSP upwards (like males), at d25 (L) they regulated downwards (unpublished data). The observation, that the human females seem to take out an important cell protective system during L in reaction to stress induced us to run a gene expression chip analysis focused on genes anyhow related to inflammation or protective anti-inflammatory regulation.

In essence we found an impressive coordinated movement of genes in the direction of a pro-inflammatory impact. It is intriguing – and also reassuring -- that this movement was a combined action of pro-inflammatory genes being up-regulated and anti-inflammatory genes being down-regulated. Although only the pro-inflammatory up-regulation was significant, the down-regulation showed at least a very strong trend and importantly encompassed some key markers which we know from numerous studies as reactive to exercise. Central markers of the protective regulations following exercise like IL6, IL1RN (coding for interleukin receptor 1 antagonist, see addendum) and IL1R2 were significantly down-regulated in L, while they were significantly or borderline significantly up-regulated in F (see figure 2). HSPB (coding for HSP 27), a central gene in the HSP system followed essentially the same pattern. Likewise, important pro-inflammatory genes like PTGDR, IL18RAP, arachidonate 5-lipoxygenase (ALOX5) or IL12 (see addendum) were highly significant up-regulated in L, while they were down-regulated in F. Concerning ALOX5, a gender specific secretion pattern of leukotrienes, governed by androgens, via regulation of extracellular signal related kinases (ERKs) has recently been found (22).

The overall number of genes which were significantly regulated following the exercise challenge underlines the exceptional state of the organism in the luteal phase with females regulating 200+ genes in L while in F only about 70 genes were regulated, similar to the number in males (60).

The question regarding what is behind these striking cycle dependent differences is not easy to answer. It seems safe to say, that, immediately after one hour of exercise, (t_0-t_1) there is a substantial change in gene expression in the direction of an increased pro-inflammatory state in women in the luteal phase. It is also highly likely, that this has to do with reproductive function of women. In the uterine endometrium of adult women a steady increase in the expression of important pro-inflammatory cytokines has already been shown starting in the mid luteal phase and continuing up to the very late luteal phase (32). However, this situation might be different in PBMCs. What we do not know is:

(a) Whether the observed effect is the same at other time points of the luteal phase or whether it is specific for the last few days of the cycle;
(b) Whether the regulation on the mRNA level is accompanied by coordinated translation into the corresponding proteins.

Concerning (a), further analysis of different time points of the cycle should show if the observed phenomenon is characteristic throughout the luteal phase. If not, the observed reaction could rather be understood as something that is related to the initiation of menstruation.

Concerning (b), further studies have to be done to find out to what extent the observed gene expression changes are accompanied by corresponding changes in protein expression. Analysis of serum proteins will be necessary and helpful, but not necessarily sufficient to clarify this point. Fast clearance by the kidney or degradation is likely to occur and might blur the picture. Experiments measuring intracellular, membrane, or ex vivo released proteins will probably be necessary.

There were some indications that a part of the pro-inflammatory genes which were up-regulated in L had quite a low level of expression at rest. Vice versa, part of the anti-inflammatory genes which were down-regulated in L, came from quite high levels of expression at rest. It is therefore possible that the gene expression changes seen in reaction to exercise in L may constitute a fast return to normal from a highly anti-inflammatory state at rest, rather than a truly pro-inflammatory response. Substantially more analysis, including generation of protein data will have to be done to clarify this point. Both possibilities, may, however, make sense.

On the one hand, the organism in L which is prepared for a pregnancy may need a highly anti-inflammatory / immunosuppressive state in order to tolerate the fertilized egg, which, from the standpoint of immunology, is a foreign intruder. A major external stressor like physical exercise might then induce a quick return of this cycle specific expression pattern back to a normal pattern to be prepared for fending off an infection. But even if the observed change of gene expression constitutes a really pro-inflammatory impulse, a second signal (e.g. danger signals) might be necessary to provoke a prolonged inflammatory reaction.

The biological significance of the observed gene expression change can thus not be clearly judged at present. Of course it seems possible that the inflammatory impulse created by substantial exercise is sufficient to induce parturition of an incumbent early pregnancy. Lynch et al. (14) showed in an elegant study that men and women regulate the IL1/ IL1RN system in a completely different way, with women showing differential regulation in F and L. These authors showed that ex vivo monocytes from women secrete high amounts of IL1 and its antagonist IL1RN in balanced amounts during F, so that no bioactivity results, while in L there is a deficit of the antagonist, resulting in bioactivity in the supernatants. They link this finding to the role of IL1 in parturition and during birth. In the light of these experiments, it seems plausible that the pro-inflammatory response of women in L may constitute a mechanism designed to end a very early pregnancy in case of major external stress input. After all, human females get a new chance to conceive in the next month and nature may prefer to destabilize a pregnancy under influence of stress rather than carry it on under high risk.

In conclusion, women in their luteal phase showed a distinctly different pattern of gene regulation in response to exercise, compared with women in their follicular phase or men. This finding accentuates a need for careful consideration of the female cyclic phase when investigating the stress response to exercise in women. Our results may also have implications relevant to other forms of stress in females.

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In memoriam Elvira Fehrenbach †

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Addendum
Anti-inflammatory genes

↑ : Up regulated, fc > 1.5

↓ : Down regulated, fc < -1.5

Gene	Accession Id	Description	M	F	L
ADRB2	NM_000024	adrenergic, beta-2-, receptor, surface			
ADRB2	NM_000024	adrenergic, beta-2-, receptor, surface			↑
ADRB2	NM_000024	adrenergic, beta-2-, receptor, surface			
ADRB2	NM_000024	adrenergic, beta-2-, receptor, surface		↓	↑
ADRBK2	NM_005160	adrenergic, beta, receptor kinase 2			
AHSA1	NM_012111	AHA1, activator of heat shock 90kDa protein			
		ATPase homolog 1 (yeast)			
CD163	NM_203416	CD163 molecule			↑
CD19	NM_001770	CD19 molecule			
CD33	NM_001772	CD33 molecule			
CSF3R	NM_172313	colony stimulating factor 3 receptor (granulocyte)			
CSF3R	M59820.1	Human granulocyte colony-stimulating factor receptor			↑
CYC1	NM_001916	cytochrome c-1			↓
GPX1	NM_201397	glutathione peroxidase 1			
GPX3	NM_002084	glutathione peroxidase 3 (plasma)			↓
GPX4	NM_002085	glutathione peroxidase 4 (phospholipid hydroperoxidase)			
GSS	NM_000178	glutathione synthetase			
GSTM3	NM_000849	glutathione S-transferase M3 (brain)			
GSTP1	NM_000852	glutathione S-transferase pi 1			
HSPB1	NM_001540	heat shock 27kDa protein 1			↓
HSPB1	NM_001540	heat shock 27kDa protein 1			↓
HSPB1	NM_001540	heat shock 27kDa protein 1			↓
IL10RB	NM_000628	interleukin 10 receptor, beta			
IL13	NM_002188	interleukin 13			
IL13RA2	NM_000640	interleukin 13 receptor, alpha 2			
IL16	NM_172217	interleukin 16 (lymphocyte chemoattractant factor)			
IL1R2	NM_173343	interleukin 1 receptor, type II		↑	↓
IL1RN	NM_173843	interleukin 1 receptor antagonist			↓
IL2RB	NM_000878	interleukin 2 receptor, beta			

Gene	Accession Id	Description	M	F	L
IL4R	NM_001008699	interleukin 4 receptor			
IL6	NM_000600	interleukin 6 (interferon, beta 2)		↑	↓
IL6R	NM_181359	interleukin 6 receptor			
IL6ST	NM_175767	interleukin 6 signal transducer (gp130, oncostatin M receptor)			
LILRA2	NM_006866	leukocyte immunoglobulin-like receptor, subfamily A, member 2			↑
MT3	NM_005954	metallothionein 3			
PPARA	BC000052.2	peroxisome proliferator-activated receptor alpha, mRNA			
PPARA	NM_005036	peroxisome proliferator-activated receptor alpha			
PPARG	BC006811.1	peroxisome proliferator-activated receptor gamma			
PRDX4	NM_006406	peroxiredoxin 4			↓
PRDX5	NM_181652	peroxiredoxin 5			↓
PROC	NM_000312	protein C (inactivator of coagulation factors Va and VIIIa)			
PROK2	NM_021935	prokineticin 2			↑
PTGIS	NM_000961	prostaglandin I2 (prostacyclin) synthase			
SOD1	NM_000454	superoxide dismutase 1, soluble			↓
SOD2	AY267901	superoxide dismutase 2, nuclear gene for mitochondrial product.			
SOD3	NM_003102	superoxide dismutase 3, extracellular			
STIP1	NM_006819	stress-induced-phosphoprotein 1			
THBD	NM_000361	thrombomodulin			
TXN	NM_003329	thioredoxin			
TXN2	NM_012473	thioredoxin 2			
TXNIP	NM_006472	thioredoxin interacting protein			

Proinflammatory genes

↑ : Up regulated, fc > 1.5

↓ : Down regulated, fc < -1.5

Gene	Accession Id	Description	M	F	L
ALOX5	NM_000698	arachidonate 5-lipoxygenase			
ALOX5	NM_000698	arachidonate 5-lipoxygenase			↑
CASP1	NM_033295	caspase 1 (interleukin 1, beta, convertase)			
CASP1	NM_033292	caspase 1, transcript variant alpha			↑
CASP1	NM_033294	caspase 1, transcript variant delta			
CASP3	NM_032991	caspase 3 transcript variant beta			
CASP3	NM_032991	caspase 3	↑		
CASP5	NM_004347	caspase 5			
CASP5	NM_004347	caspase 5			
CASP9	NM_001229	caspase 9 transcript variant alpha			
CASP9	NM_032996	caspase 9, apoptosis-related cysteine peptidase			
CCL4	NM_002984	chemokine (C-C motif) ligand 4			
CCR1	NM_001295	chemokine (C-C motif) receptor 1			
CD14	NM_000591	CD14 molecule			↑
CD160	BC014465.1	CD160 molecule			
CD1B	NM_001764	CD1b molecule			
CD1B	NM_001764	CD1b molecule			
CD2	NM_001767	CD2 molecule			
CD44	NM_001001392	CD44 molecule (Indian blood group)	↓		
CD58	NM_001779	CD58 molecule			
CD59	NM_203331	CD59 molecule, complement regulatory protein			
CD69	NM_001781	CD69 molecule			
CD80	NM_005191	CD80 molecule			
CD83	NM_004233	CD83 molecule	↑		
COX7A2	BC100852.1	cytochrome c oxidase subunit VIIa polypeptide 2 (liver)			↓
CSF1	NM_172212	colony stimulating factor 1 (macrophage)			
CSF2	NM_000758	colony stimulating factor 2 (granulocyte-macrophage)			
CX3CR1	NM_001337	chemokine (C-X3-C motif) receptor 1			
CXCL10	NM_001565	chemokine (C-X-C motif) ligand 10			
CYSLTR1	NM_006639	cysteinyl leukotriene receptor 1			↑
DAP	NM_004394	death-associated protein			↓

Gene	Accession Id	Description	M	F	L
DAPK1	NM_004938	death-associated protein kinase 1			↑
FCGR3B	NM_000570	Fc fragment of IgG, low affinity IIIb, receptor (CD16b)			
HIF1AN	NM_017902	hypoxia-inducible factor 1, alpha subunit inhibitor			
HLA-DRA	NM_019111	major histocompatibility complex, class II, DR alpha			
ICAM2	NM_000873	intercellular adhesion molecule 2			
ICAM3	NM_002162	intercellular adhesion molecule 3			↓
ID2	NM_002166	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein			
IFNAR1	NM_000629	interferon (alpha, beta and omega) receptor 1			
IFNG	NM_000619	interferon, gamma			↓
IFNG	NM_000619	interferon, gamma		↓	↑
IFNG	NM_000619	interferon, gamma			
IFNGR1	NM_000416	interferon gamma receptor 1			
IGF2	NM_000612	insulin-like growth factor 2 (somatomedin A)			
IGF2	NM_000612	insulin-like growth factor 2 (somatomedin A)		↓	
IGF2	NM_000612	insulin-like growth factor 2 (somatomedin A)			↓
IHPK3	NM_054111	inositol hexaphosphate kinase 3			↓
IL11	NM_000641	interleukin 11			
IL12RB1	NM_153701	interleukin 12 receptor, beta 1		↓	↑
IL12RB2	NM_001559	interleukin 12 receptor, beta 2			
IL15	NM_172174	interleukin 15			
IL18	NM_001562	interleukin 18 (interferon-gamma-inducing factor)			
IL18R1	NM_003855	interleukin 18 receptor 1			
IL18RAP	BC106765.2	Homo sapiens interleukin 18 receptor accessory protein			↑
IL1A	NM_000575	interleukin 1, alpha			
IL1A	NM_000575	interleukin 1, alpha			
IL1A	NM_000575	interleukin 1, alpha			
IL1B	NM_000576	interleukin 1, beta			
IL1R1	NM_000877	interleukin 1 receptor, type I			
IL21R	NM_181079	interleukin 21 receptor			
IL24	NM_181339	interleukin 24			
IL5RA	NM_175728	interleukin 5 receptor, alpha			
IL8RA	NM_000634	interleukin 8 receptor, alpha			
IL8RA	NM_000634	interleukin 8 receptor, alpha			
INDO	NM_002164	indoleamine-pyrrole 2,3 dioxygenase			
IRAK1	NM_001569	interleukin-1 receptor-associated kinase 1			↓
LBP	NM_004139	lipopolysaccharide binding protein			

Gene	Accession Id	Description	M	F	L
LTA	NM_000595	lymphotoxin alpha (TNF superfamily, member 1)			
LTB	NM_009588	lymphotoxin beta (TNF superfamily, member 3)			
MAP2K4	NM_003010	mitogen-activated protein kinase kinase 4			
MAPK14	BC031574.1	Homo sapiens mitogen-activated protein kinase 14			
MAPK14	NM_139014	mitogen-activated protein kinase 14			
MAPK8	NM_139049	mitogen-activated protein kinase 8			
MAPK8	NM_139049	mitogen-activated protein kinase 8			
MAPKAPK2	NM_032960	mitogen-activated protein kinase-activated protein kinase 2		↑	
MGST2	NM_002413	microsomal glutathione S-transferase 2			
MGST3	NM_004528	microsomal glutathione S-transferase 3			
NGFR	NM_002507	nerve growth factor receptor (TNFR superfamily, member 16)			↑
NOS1	NM_000620	nitric oxide synthase 1 (neuronal)			
NOS2	NM_000625	nitric oxide synthase 2, inducible			
NPY1R	NM_000909	neuropeptide Y receptor Y1			↑
PRKCA	NM_002737	protein kinase C, alpha			
PRKCB	BC036472.1	Homo sapiens protein kinase C, beta 1			
PRKCQ	NM_006257	protein kinase C, theta			
PRKCZ	BC014270.2	protein kinase C, zeta			
PTGDR	U31099.1	Human DP prostanoid receptor (PTGDR)			↑
PTGS1	NM_080591	prostaglandin-endoperoxide synthase 1	↓		
PTGS2	NM_000963	prostaglandin-endoperoxide synthase 2			
SELE	NM_000450	selectin E			
SELL	NM_000655	selectin L			↑
SELP	NM_003005	selectin P (granule membrane protein 140kDa, antigen CD62)			
SMAD5	NM_001001419	SMAD family member 5 (SMAD5), transcript variant 2			
TBXAS1	NM_030984	thromboxane A synthase 1 (platelet)			↑
TGFB1	NM_000660	transforming growth factor, beta 1			
TGFB1	NM_000660	transforming growth factor, beta 1			
TIAM1	NM_003253	T-cell lymphoma invasion and metastasis 1			
TIAM2	NM_012454.	T-cell lymphoma invasion and metastasis 2 transcript variant 1			
TNF	NM_000594	tumor necrosis factor (TNF superfamily, member 2)			