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How to Work
Biomarker and Health Risk Assessment

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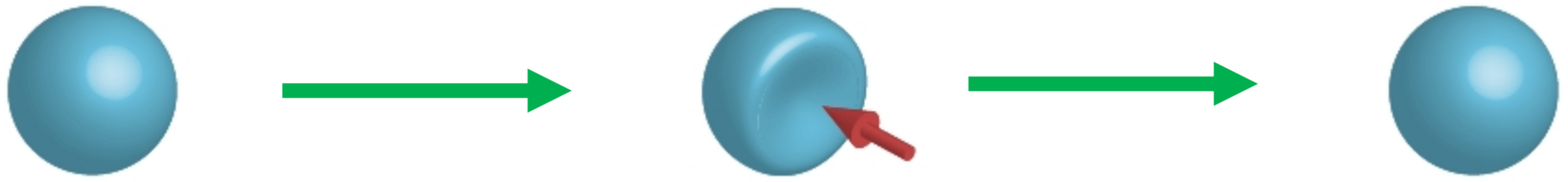
What is the stress?

- **The diagram often used in stress explanation**
  This kinetics is a stress response

When an stressor is added to the ball, it is dented.
But stress occurs to return to the original state.



Stress research is mainly performed from two perspectives.

- **Stressor: external force**
- **Stress: internal force**
  (Force generated from inside against stressor)

Today I will talk about stress and biomarkers.

Representative stress evaluation method

Classification	Inspection item		Measurement and evaluation content
Psychological evaluation	General Health Questionnaire (GHQ)		Physical symptoms, anxiety and insomnia, social activity disorders, depression trends etc.
	Profile of Mood State (POMS)		Mood condition (six factors of tension, depression, anger, liveliness, fatigue, confusion)
	Self-rating Depression Scale (SDS)		Depression trend
	State Trait Anxiety Inventory (STAI)		Anxiety state
Physiological evaluation	Brain waves (alpha waves)		Relax degree
	Heart rate variation (heart rate fluctuation)		Autonomic nervous activity (balance between sympathetic and parasympathetic)
	Acceleration pulse wave		Autonomic function
	Optical topography		Changes in cerebral cortical blood flow
Biochemical evaluation	Blood	Catecholamine	Sympathetic activity
		Cortisol	Hormone secretion level
		DHEA-S	
		NK cell activity	Immune activity
		T cell line surface marker	
		Cytokines	
	Urine	Catecholamines and metabolites	Sympathetic activity
		17-KS-S/17-OHCS ratio	Balance of restoration and abrasion of living body by metabolic products
		8-OHdG	DNA damage
	Saliva	Cortisol	Hormone secretion level
		Amylase activity	Sympathetic activity
		Chromogranin A	
		IgA	Immune activity
		Human herpesvirus type 6 activity	

Stress evaluation notice

We would like to evaluate stress objectively rather than subjectively.

However, there are some important points in objective biomarkers.

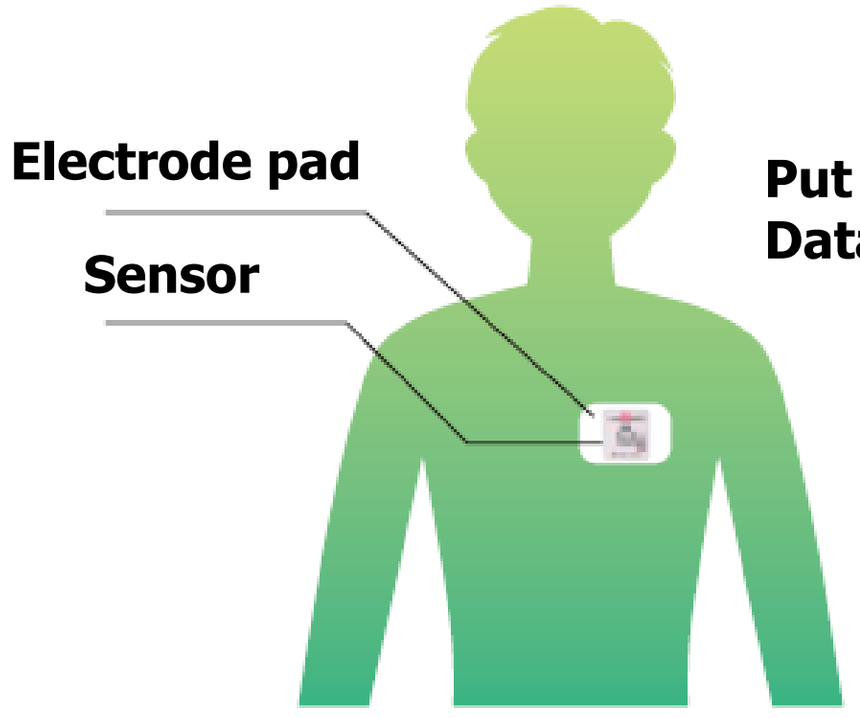
- **Most biomarkers are obtained from experimental research.**
- **The standard value of many biomarkers is not clear.**
- **Reaction time varies depending on the biomarker.**
- **Some biomarkers have circadian rhythms.**
- **Individual differences may affect biomarkers.**

How to evaluate the stress?

Autonomic nerve measuring device

- **Wearable sensor (no pain, no stressor)**
- **Heart rate (number · cycle · waveform), body surface temperature, 3 axis acceleration can be measured simultaneously.**
- **Low frequency [LF] band (reflecting sympathetic and parasympathetic activity) and High frequency [HF] band (reflecting parasympathetic activity) are analyzed.**
- **LF/HF ratio are obtained every 5 seconds.**

myBeat (WHS-1)



Put the sensor on the chest.
Data is recorded on the device.

How to evaluate the stress?

Autonomic nerve measuring device

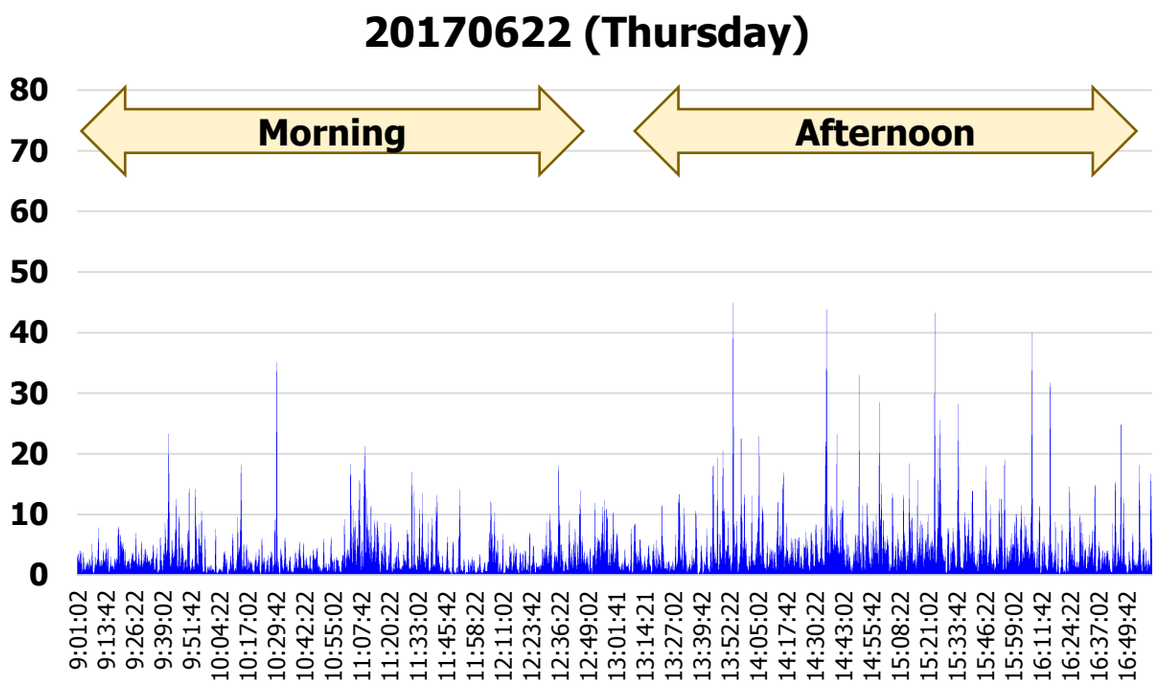
- **Wearable sensor (no pain, no stressor)**
- **Heart rate (number · cycle · waveform), body surface temperature, 3 axis acceleration can be measured simultaneously.**
- **Low frequency [LF] band (reflecting sympathetic and parasympathetic activity) and High frequency [HF] band (reflecting parasympathetic activity) are analyzed.**
- **LF/HF ratio are obtained every 5 seconds.**

LF/HF ratio: stress indicator

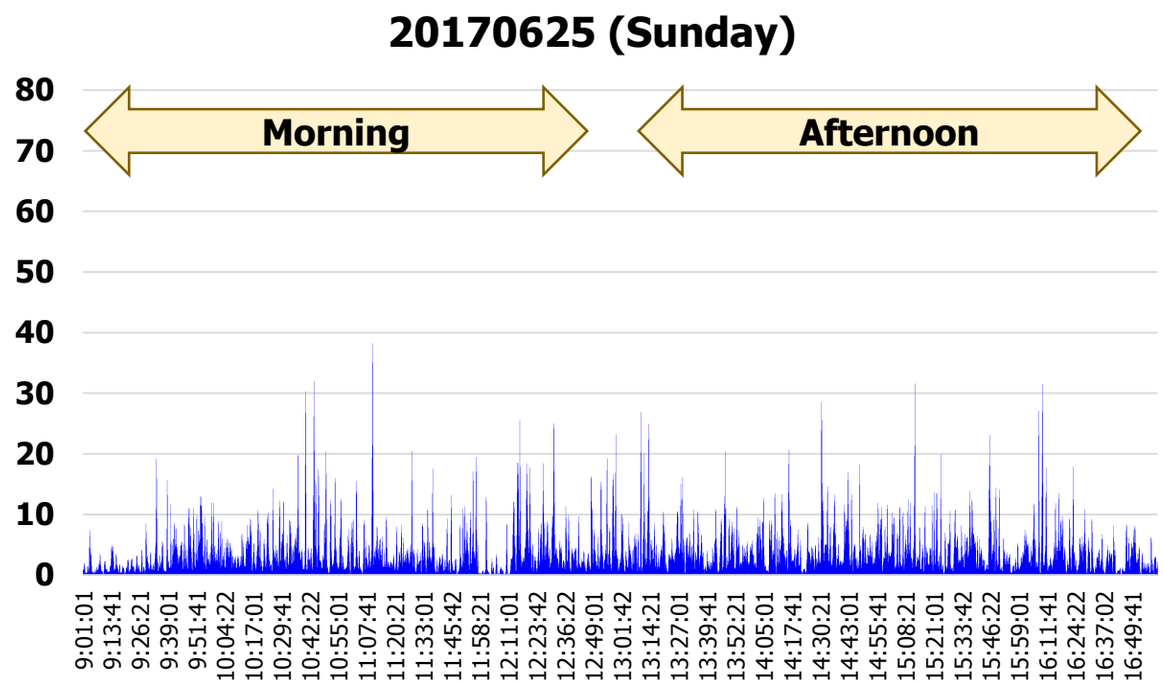
LF/HF ratio
>5 ; high stress
<5 ; low stress

How to evaluate the stress?

Weekday



Weekend



	Morning		Afternoon		All day	
LF/HF ratio	**>5**	**Median**	**>5**	**Median**	**>5**	**Median**
Weekday	**16.5%**	**2.21**	**28.3%**	**3.30**	**22.4%**	**2.67**
Weekend	**21.7%**	**2.55**	**27.2%**	**3.16**	**24.4%**	**2.83**

LF/HF ratio
>5 ; high stress
<5 ; low stress

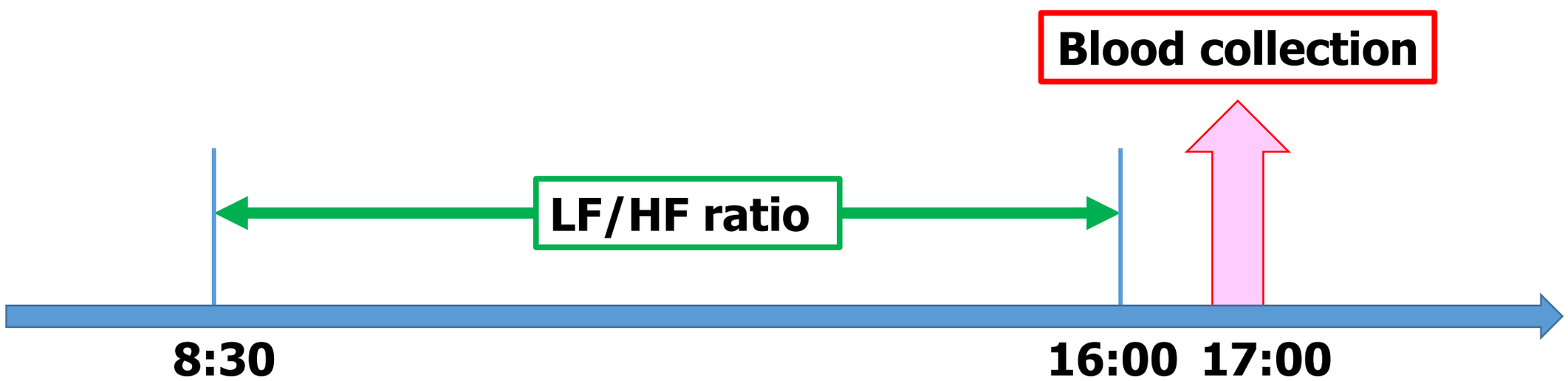
Stress evaluation

Observational research approved by ethical review

- **Staff of medical office**
- **Thirteen females**
- **Check work on medical fee bills**

In Japan, the workload usually increases at the end of the month and early in the month.

- **Observed day: 4 days (busy 2 days and non-busy 2 days)**
- **Physiological evaluation: LF/HF ratio (from 8:30 to 16:00)**
- **Biochemical evaluation (blood collection time is about 17:00)**
  - **Cortisol concentration in serum**
  - **Exosomal microRNAs in serum (to identify new biomarkers)**

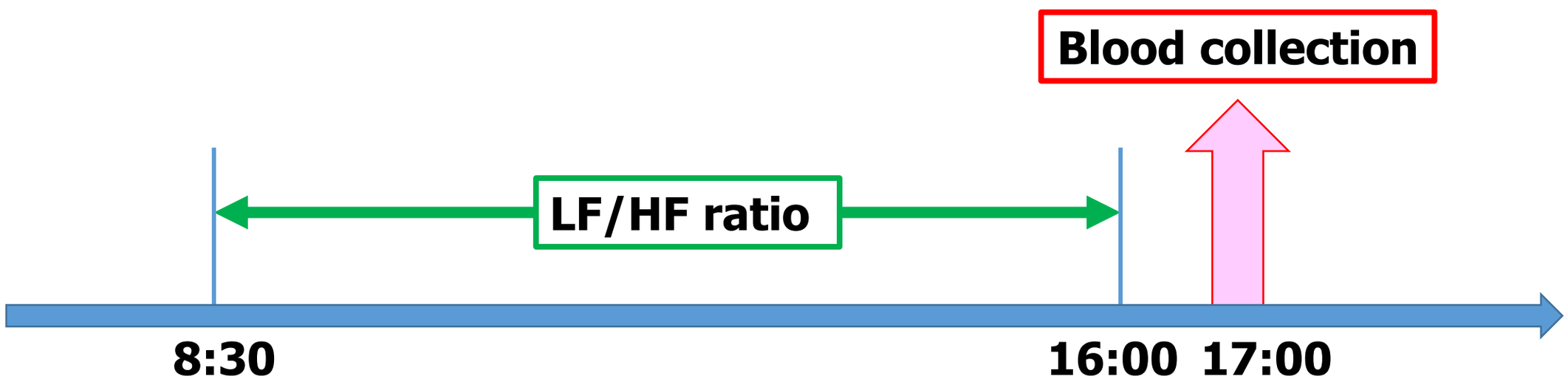


Stress evaluation

Totally, we evaluated 52 results of 13 research subjects

Exclude results of LF/HF ratio:
LF/HF ratios were totally obtained 5,412counts from 8:30 to 16:00.
Counts less than 90% were excluded.

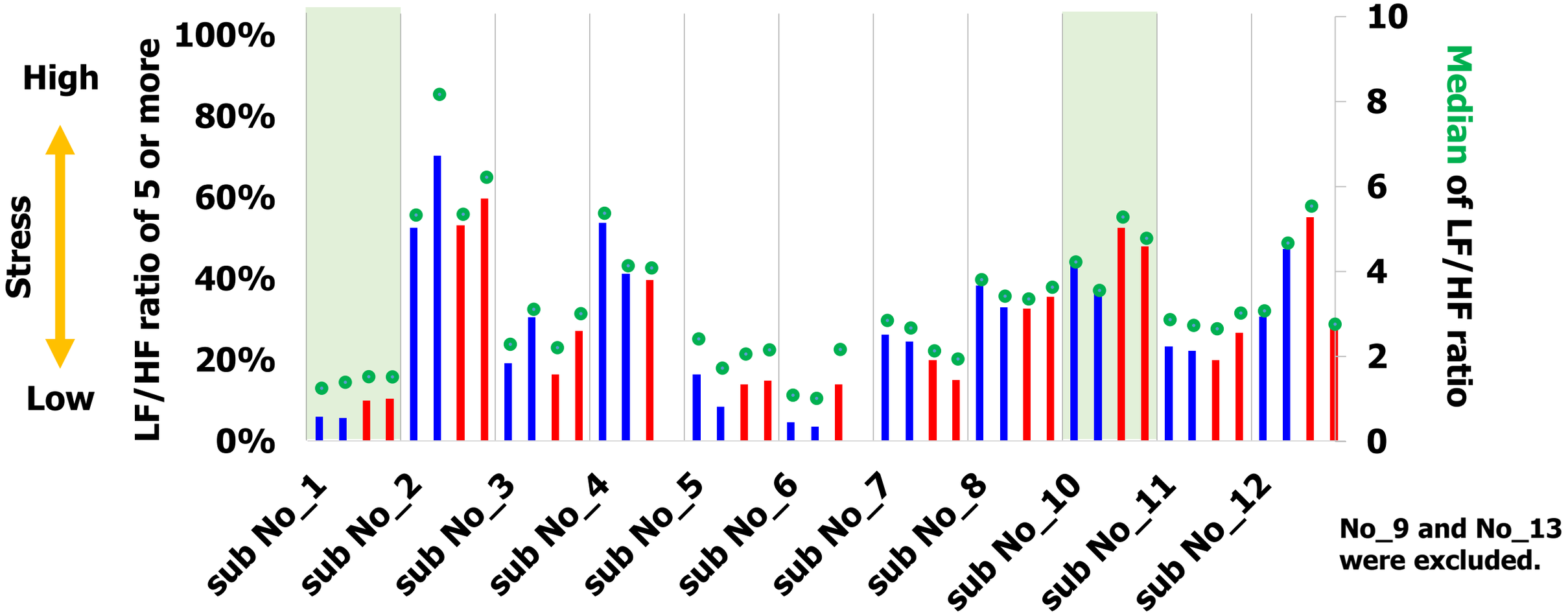
- **Two research subjects were excluded -> 11 research subjects.**
- **Two of 44 results were excluded -> 42 results.**
- **Finally, 42 results of 11 research subjects were evaluated.**



Stress evaluation

- 42 results of 11 subjects
- Non-busy 2 days and busy 2 days

LF/HF ratio of 5 or more (percentage of each day)

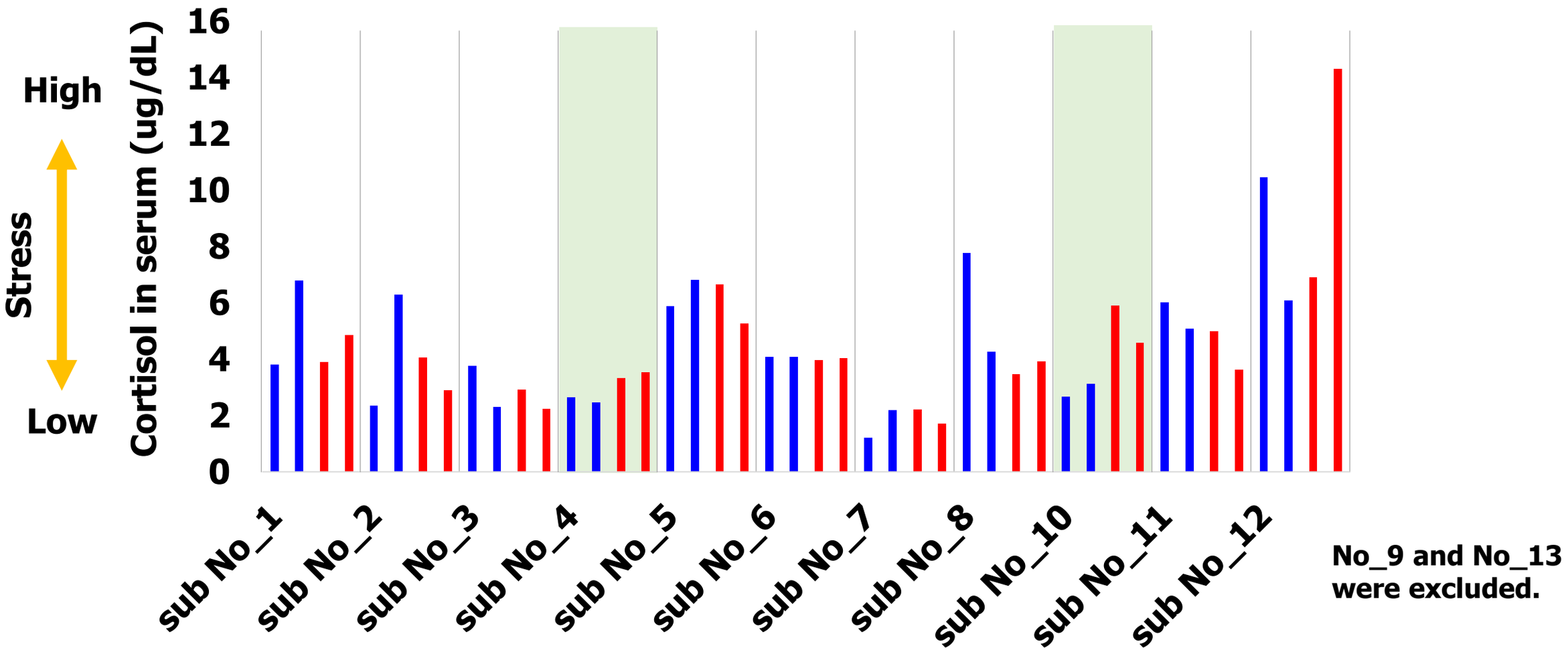


No_9 and No_13 were excluded.

Stress evaluation

- 42 results of 11 subjects
- Non-busy 2 days and busy 2 days

Cortisol in serum



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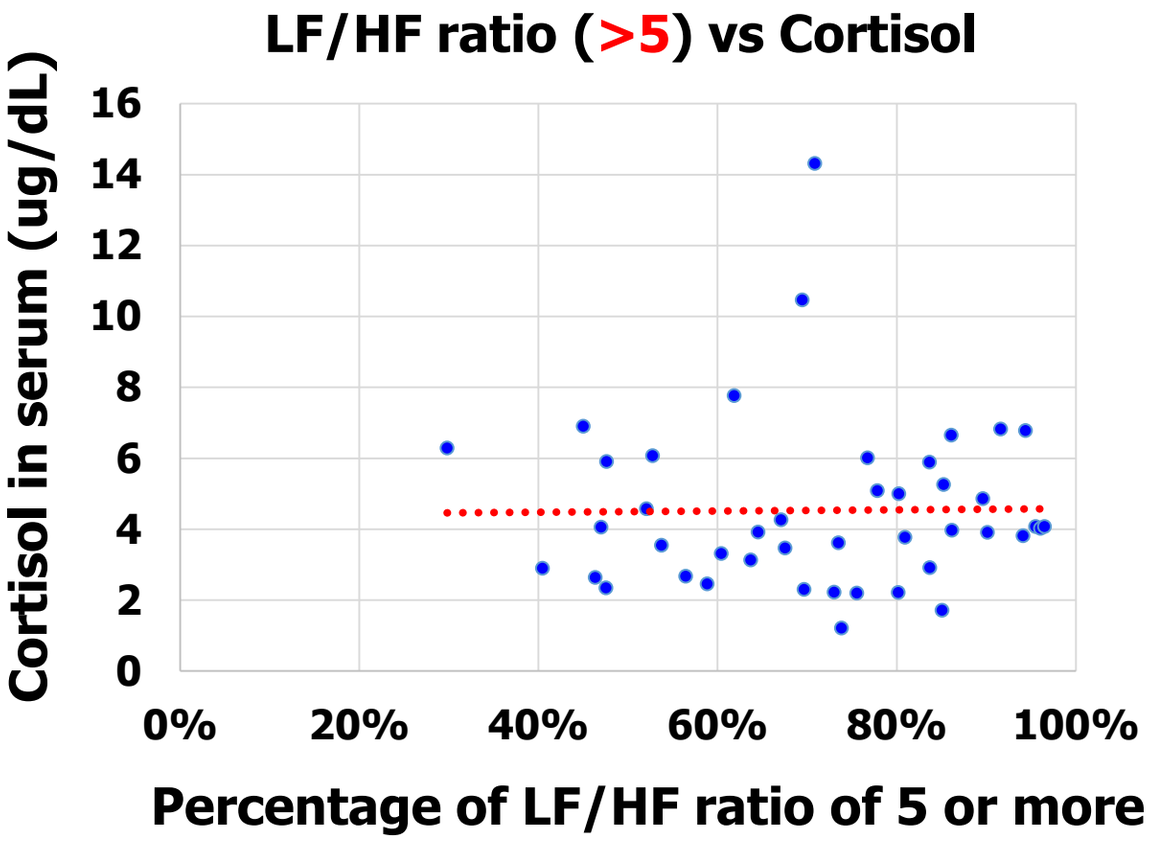
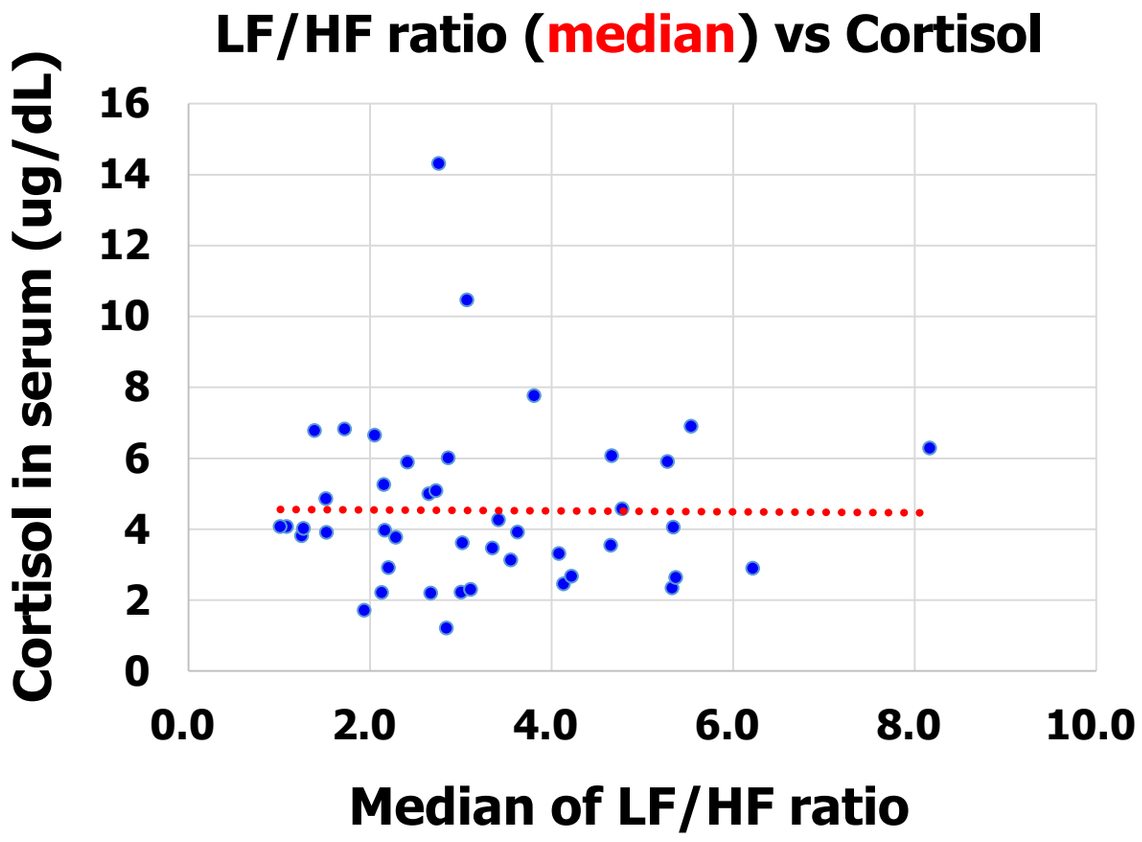
Stress evaluation





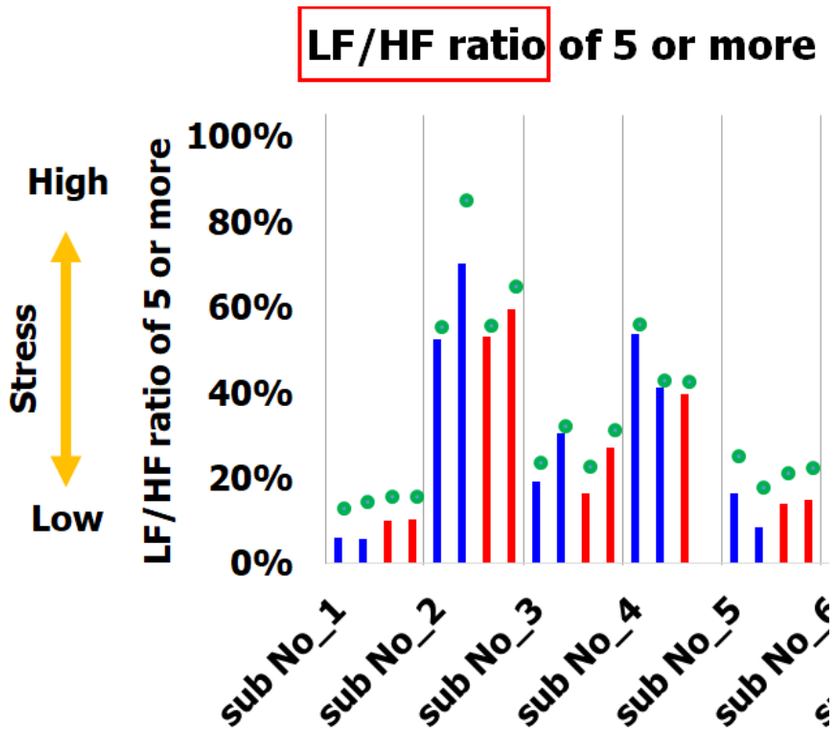
- **There was no correlation between cortisol concentration and percentage of LF/HF ratio of 5 or more, or median of LF/HF ratio.**

Stress evaluation

- **Possible reason why LF/HF ratio and cortisol concentration do not correlate with workload.**

  LF/HF ratio;
  - **Individual differences exist.**

  Cortisol concentration;
  - **Blood collection time may not be appropriate.**



- **My opinion:**
  The increase in workload in this study may not be stressor.

Exosomal miRNAs

- **MicroRNA (miRNA) is a single-stranded RNA molecule of 21-25 bases (nt) in length and is involved in the regulation of post-transcriptional expression of genes in eukaryotes.**

- **Exosome is a particle with a diameter of 50-150 nm secreted from cells and contains nucleic acids (microRNA, messenger RNA, DNA etc.) and proteins.**

- **Exosomes are present in body fluids (blood, cerebrospinal fluid, urine, etc.) and circulate throughout the body**

- **Exosomes are used to transmit information between cells, may contribute to clarifying the molecular mechanism of disease.**

- **Exosomes are widely studied in the field of cancer.**

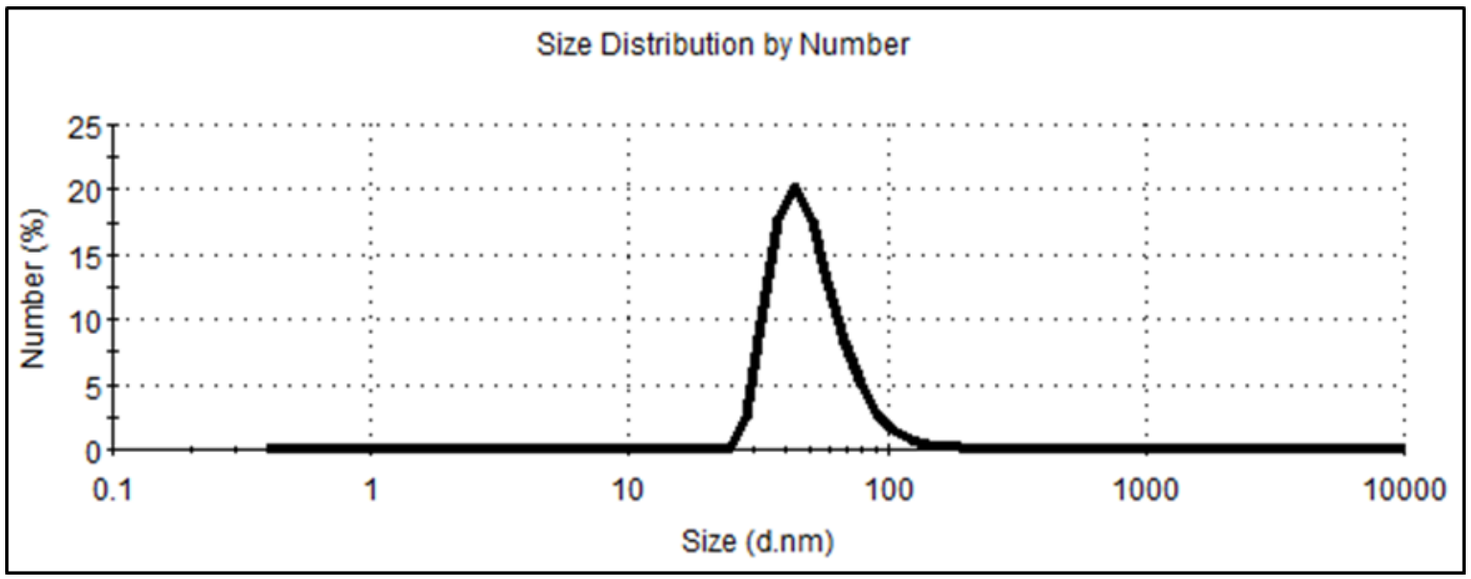
Exosomal miRNAs

- **Purification method of exosome in this study**
  - **Fractionation by size; ExoMir-MINI Kit (Bioo Scientific Corporation)**
  - **Exosome is a particle with a diameter of 50-150 nm**

220 nm

50 nm

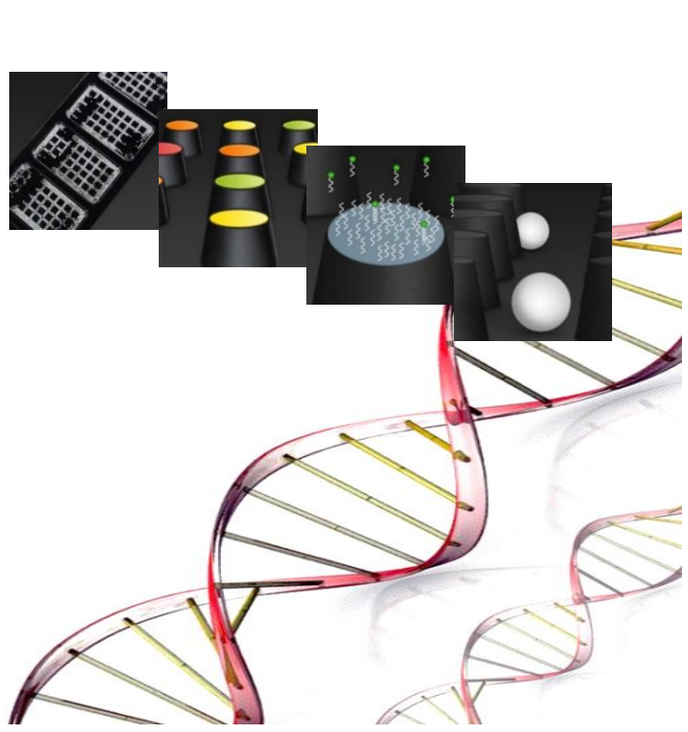
DLS (Dynamic Light Scattering)



	Size (d.nm):	% Number
Peak 1:	52.24	100.0
Peak 2:	0.000	0.0

Exosomal miRNAs

- **Exosomes were collected from serum of 11 research subjects and RNAs were purified.**
- **Comprehensive expression analysis (about 2,600 kinds of microRNAs) was performed by microarray.**
- **We attempted to identify microRNAs related to LF/HF ratio.**

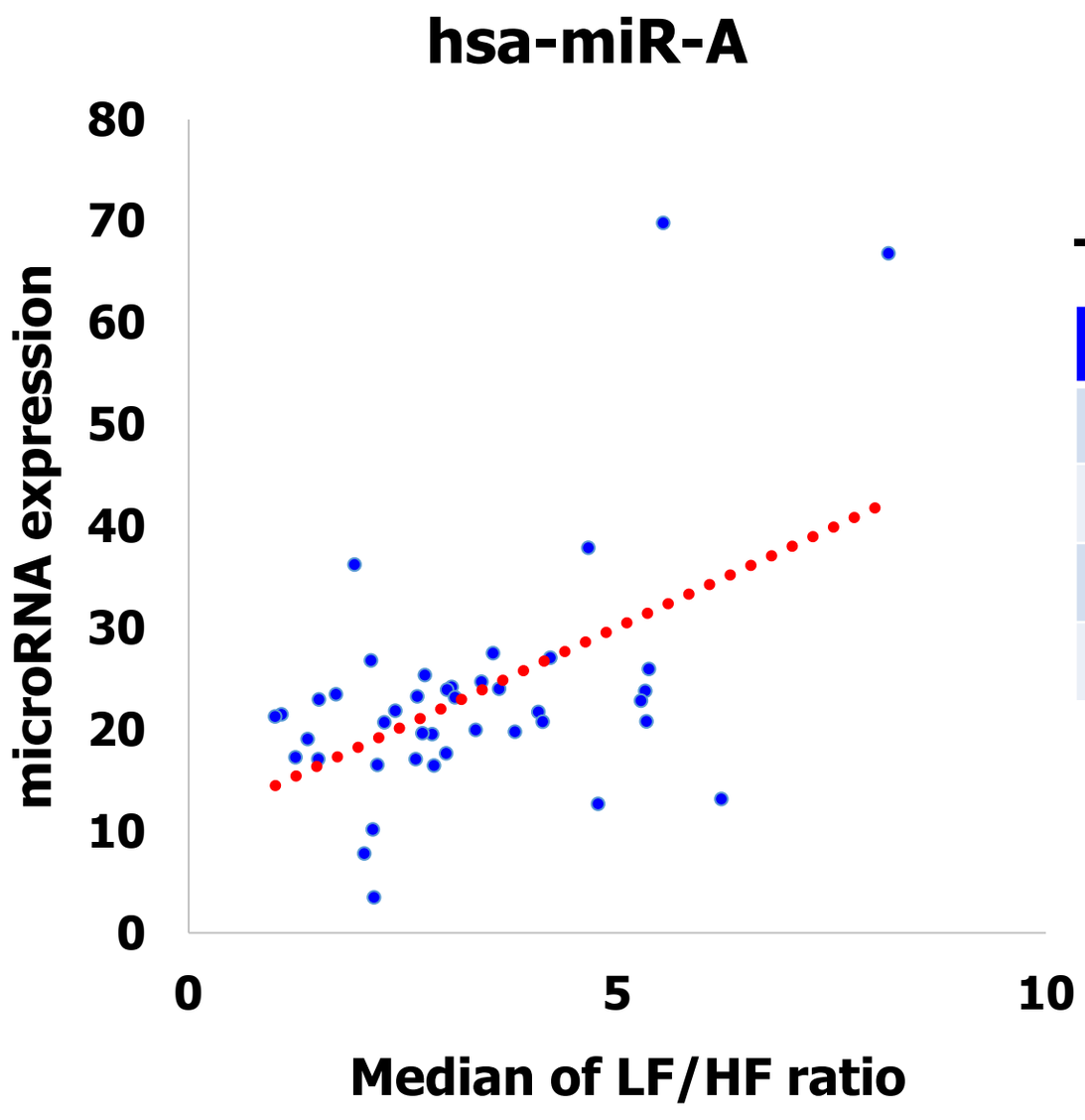


3D-Gene system (TORAY Co, LTD)
About 2,600 kinds of microRNAs are immobilized on the chip.

Exosomal miRNAs

Correlation between median of LF/HF ratio and exosomal microRNA expression was examined with 42 results.

- **Four kinds of exosomal microRNAs were significantly and positively correlated with median of LF/HF ratio.**



The correct names of microRNAs are not open.

microRNA	Correlation	p.value
hsa-miR-A	0.509	0.001
hsa-miR-B	0.414	0.006
hsa-miR-C	0.404	0.007
hsa-miR-D	0.402	0.008

Discussion of exosomal miRNAs

	Number of genes	Estimated target genes of hsa-miR-A: 5531*
Acetylcholine receptor	21	CHRM1, CHRM3, CHRNA4, CHRNA7, CHRNA9
Adrenergic receptor	9	ADRA1B, ADRA2B, ADRB2
Serotonin receptor	17	HTR2A, HTR5A
Dopamine receptor	5	-
Histamine Receptor	4	HRH1, HRH3

*Number of genes predicted by Target Scan: http://www.targetscan.org/vert_72/

Conclusion and discussion

- **There is a possibility that stress can be evaluated by monitoring the autonomic nerve activity with hart rate sensor.**
  -> Many research subjects are necessary.

- **There is an also possibility that stress can be evaluated by exosomal microRNAs.**
  -> Elucidation of molecular mechanism is necessary.