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Original Article

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Exercise With a Novel Digital Device Increased Serum Anti-influenza Antibody Titers After Influenza Vaccination

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ABSTRACT

It has been reported that some exercise could enhance the anti-viral antibody titers after vaccination including influenza and coronavirus disease 2019 vaccines. We developed SAT-008, a novel digital device, consists of physical activities and activities related to the autonomic nervous system. We assessed the feasibility of SAT-008 to boost host immunity after an influenza vaccination by a randomized, open-label, and controlled study on adults administered influenza vaccines in the previous year. Among 32 participants, the SAT-008 showed a significant increase in the anti-influenza antibody titers assessed by hemagglutination-inhibition test against antigen subtype B Yamagata lineage after 4 wk of vaccination and subtype B Victoria lineage after 12 wk ($p<0.05$). There was no difference in the antibody titers against subtype "A." The SAT-008 also showed significant increase in the plasma cytokine levels of IL-10, IL-1β, and IL-6 at weeks 4 and 12 after the vaccination ($p<0.05$). A new approach using the digital device may boost host immunity against virus via vaccine adjuvant-like effects.

Trial Registration: ClinicalTrials.gov Identifier: NCT04916145

Keywords: Digital device; Vaccine; Exercise

INTRODUCTION

Viral infections such as influenza and coronavirus disease 2019 (COVID-19) have a huge detrimental impact on human, which demands for a new approach to enhance host immunity (1). Vaccines which trains the immune system to create humoral and cellular immunity have historically been the mainstay of prevention and control against viral diseases. Vaccine adjuvants are defined as substances added to vaccines to enhance immunogenicity. Especially during the pandemic situation, developing vaccine and adjuvant boosting the host

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
ANS, autonomic nervous system; app, application; COVID-19, coronavirus disease 2019; HI, hemagglutination inhibition; HP, high performer; HR, heart rate; LP, low performer; NSAID, non-steroidal anti-inflammatory drug; RM, repeated-measures.

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Conceptualization: Choi JP, Ham J, Choi SE, Kim ES, Chang YS; Data curation: Choi JP, Ham J, Choi SE, Noh JY, Kim SH, Song JY, Kim ES, Chang YS; Formal analysis: Choi JP, Ayoub G, Ham J, Noh JY, Song JY, Chang YS; Funding acquisition: Ham J, Choi SE, Chang YS; Investigation: Choi JP, Choi SE, Kim SH, Song JY, Kim ES, Chang YS; Methodology: Choi JP, Ayoub G, Ham J, Huh Y, Choi SE, Noh JY, Kim SH, Song JY, Chang YS; Project administration: Choi JP, Ayoub G, Ham J, Huh Y, Choi SE, Hwang YK, Kim SH, Song JY, Kim ES, Chang YS; Resources: Choi JP, Ham J, Choi SE, Kim SH, Kim ES, Chang YS; Software: Choi JP, Ayoub G, Ham J, Chang YS; Supervision: Choi JP, Choi SE, Song JY, Kim ES, Chang YS; Validation: Choi JP, Ham J, Chang YS; Visualization: Choi JP, Ayoub G, Ham J, Noh JY; Writing - original draft: Choi JP, Ayoub G, Ham J, Hwang YK, Kim SH, Song JY, Kim ES, Chang YS; Writing - review & editing: Choi JP, Ayoub G, Ham J, Huh Y, Choi SE, Hwang YK, Kim SH, Song JY, Kim ES, Chang YS.

immunity is very important to prevent viral infections, which need a gigantic resource in order to meet the need for equal delivery of the coincident vaccine to the world (2,3).

Many studies have focused on boosting and strengthening the immunity system (4-6). Physical activities enhancing the host immune system have been studied scientifically in several decades (7). Acute and aerobic exercise has been reported to enhance immunocytes and NK cells in host (8,9). In addition, several studies proved that some exercise activities could enhance the anti-viral antibody titers after vaccination (10). Recently, it has been reported that exercise could enhance the anti-viral antibody titers after vaccination including influenza and COVID-19 vaccines (11). However, the optimal intensity, type, and duration of exercise have not been determined yet.

Other studies found that the host immune system was influenced not only by physical activity but also by an activity related to autonomic nervous system (ANS) (e.g., deep and hold breathing, face cold massage, meditation, etc.). Previous studies suggested that ANS related activities might boost the immunity system through different mechanisms like increasing NK cells or B cells (12).

During the last decade, the world witnessed huge development of digital life including smart devices, social media, mobile applications (apps), cloud data, internet of things, which affected all aspects including the healthcare system. The digital health system has been developed rapidly to encompass technology for better healthcare. A new subdivision of the digital health system has been introduced to manage, prevent and treat disease since 2015 (13). It was formally defined as delivering evidence-based therapeutic interventions to patients driven by software to prevent, manage, or treat a medical disorder or disease (14,15).

A digital device, SAT-008, as a mobile app for enhancing host immune system against viral infection has been developed. SAT-008 was designed based on an algorithm, which is combining systematically the orders of regulation for physical (external) activity and several (internal) activities related to the ANS, translated into programs of performances, which are brought to subjects.

The aim of this work is to assess the feasibility of SAT-008 by measuring changes in immune responses after an influenza vaccination performing the external and internal activities using the digital device in healthy adults.

MATERIALS AND METHODS

Subjects

Healthy adult volunteers who were aged 19 to 50 years, and received influenza vaccination in the previous year were recruited in the present study. We included those who did not exercise regularly assessed by the International Physical Activity Questionnaires. Exclusion criteria included; current infectious disease, history of autoimmune diseases, current immunological compromised diseases, recent vaccination history with any live or killed vaccines. All the participants are informed not to take any drugs which may affect immunity during the study. All participants signed the informed consent form. This study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (No. B-2009-637-306).

Experimental design

A single-center, open label, randomized controlled study was conducted (Clinical Trial Number: NCT04916145). In a 15-wk of the study period, total 5 visits were made for each subject if participants met the recruitment criteria at the screening (**Fig. 1A**). All screened subjects visit the hospital at the day of randomization (visit 2, W-1), where participants were randomly assigned to either a control or a treatment group (SAT-008). The SAT-008 group were prescribed to use a mobile app of the SAT-008 by investigator (physician) and received an account of the mobile app and offered a guide on how to use it. In the other hand, the control group was instructed to maintain their daily activities as the level of visit 2. After a week following the randomization day (visit 3, D0), all the participants received the SKYCellflu Quadrivalent prefilled syringe vaccine (SK Bioscience Co., Seongnam, Korea), including 4 influenza vaccine strains from the 2020–2021 northern hemisphere season: influenza A/Guandong-Maonan/SWL1536/2019 (H1N1), influenza A/HongKong/2671/2019 (H3N2), influenza B/Phuket/3073/2013 (Yamagata lineage), and influenza B/Washington/02/2019 (Victoria lineage). Then, subjects were scheduled to visit 4 wk (visit 4, W4) and 12 wk (visit 5, W12) after the vaccination.

SAT-008, a digital device as an intervention

SAT-008 is a digital device (S-Alpha Therapeutics, Inc., Seoul, Korea) comprises doctor's web portal, server, and user's mobile app. For the SAT-008 group, investigator (doctor in charge) prescribed a SAT-008 app through doctor's web portal, issuing subject's account of the app and scheduling the app programs for the prescribed period. Then, participants can access the SAT-008 app using the account and are required to complete the scheduled activities through the SAT-008 app.

Activity programs of the SAT-008 app consist of 2 types, called external and internal function activity (**Fig. 1B**). Firstly, external function activity is required to be carried out for 30 min (including warming-up and cooling-down activity for 5 min respectively) 2 times a week. Entering the external function activity, the mobile screen presents a subject-adapted targeted range of heart rate (HR). The targeted HR range is estimated as {(207–0.7 × Age) − Resting HR} × Intensity Level + Resting (16). The HR is detected by a wearable device, in the study Mi Smart Band 5.0 was used as a wrist-worn type of sensor. The participant's HR was transferred to the SAT-008 app in real-time, showing the present HR on the screen and displaying visual feedback about whether the level of activity is within the targeted range or not. To complete the activity, subjects need to move their body actively as a level to reach the targeted HR zone, which leads to do physical activity at a moderate intensity level. Secondly, internal function activity consists of 5 static activities (deep breathing, stop breathing, cold face massage, aroma meditation, and listening to white noise), of which 2 activities need to be performed for 10 min every day. It takes 5 min per activity, and 2 activities must be performed in succession to be recorded as completing the activity.

Adherence measurement

Adherence to the SAT-008 app was quantified by calculating the number of completed activities divided by the total number of prescribed activities. The completion of the activity is defined based on a time of use. For the external activity, it is counted as a completed one if the minimum required time (5 min) was spent on physical activity within the targeted HR range, while for the internal activity, only counted if subject finished a planned time (10 min). The total number of completed external and internal activities was divided by prescribed activities to assess adherence to SAT-008. The adherence measures were used for

the subgroup analysis by dividing the SAT-008 group to test the efficacy of the SAT-008 on the immune function. Accordingly, SAT-008 group was divided into 2 subgroups, high performer (HP) and low performer (LP) based on adherence rate for external and internal activities. The HP group consisted of the participants who achieved more than 75% of adherence rate in a 3-month full program. On the other hand, the LP group was defined as the group with less than 75% of adherence rate in the whole program.

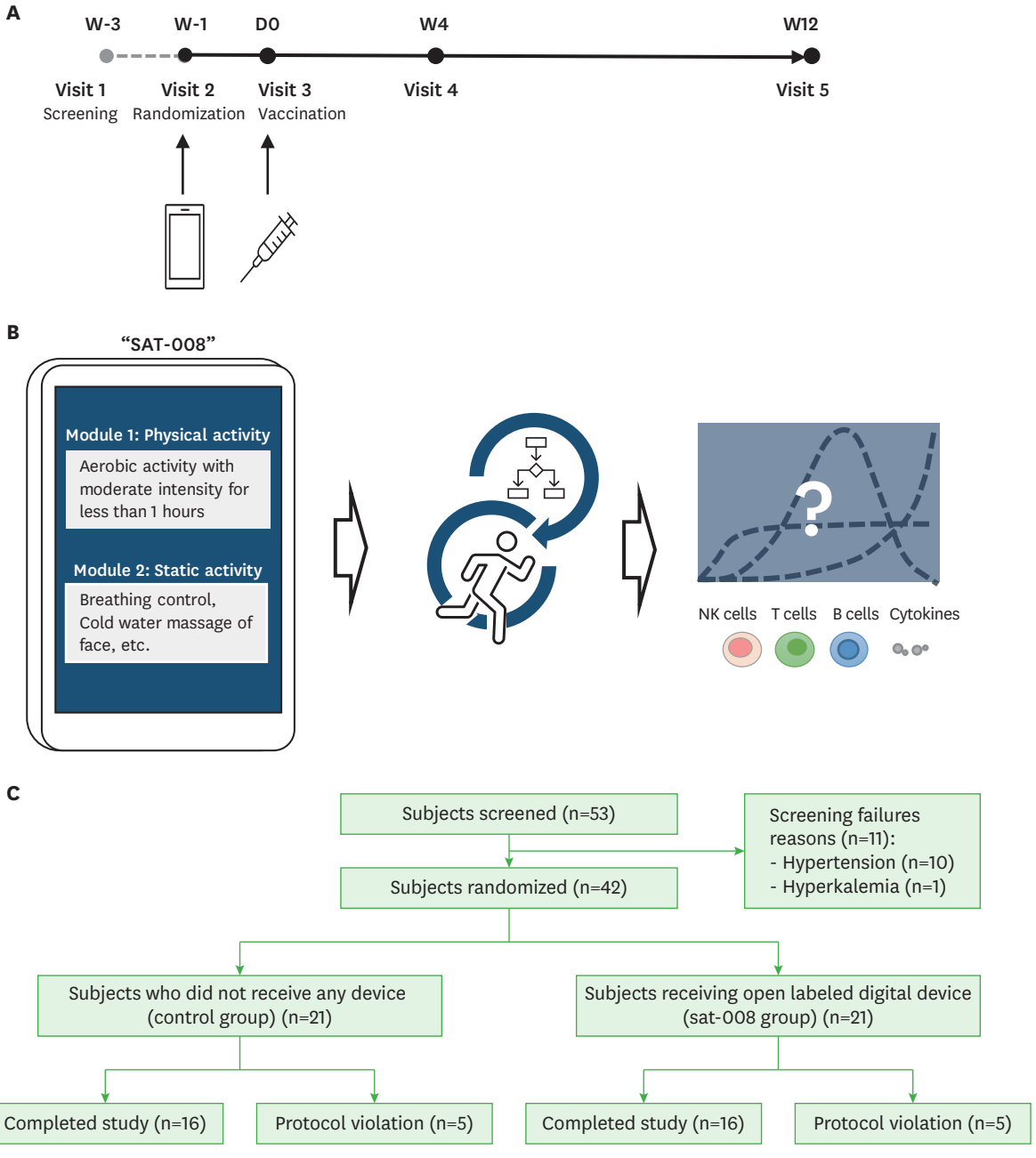

Figure 1. Information of study design and participants. (A) Study design. SAT-008 mobile app was distributed to the experimental group at visit 2 (W-1) to start use it, while the controls required to maintain daily activities without use of SAT-008. A week after randomization on at visit 3 (D0), a single dose of influenza vaccination was administrated to all the subjects. The subjects were asked to visit the institute after 4 wk and 12 wk following the vaccination. (B) Digital device, "SAT-008." (C) Flowchart of disposition of subjects, 42 subjects were enrolled in the present study. After excluding the cases with protocol deviation, 32 subjects were served for data analysis.

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Blood collection of participants

Blood samples of participants were collected using vacuum tube containing cell separation reagent (BD vacutainer CPT mononuclear cell preparation tube contained citrate). All procedures were done as per manufacturer's guideline. Briefly, cells and plasma were isolated by centrifugation of CPT tubes containing collected blood samples. Upper layer, plasma, was collected and saved at −80°C for further analysis. The layer containing PBMC was collected and washed twice with fresh PBS to remove cell separation solution. Isolated cells were dissolved in cell freezing solution (Gibco, Waltham, MA, USA) and saved at −80°C for additional analysis.

Hemagglutination inhibition (HI) assay and immunogenicity assessments

The levels of HI antibodies for influenza virus were measured by a standard microtiter assay for the 4 viruses (A/H1N1, A/H3N2, and B/Phuket (Yamagata) and B/Washington (Victoria)) as we have previously described (17).

NK cell activation & evaluation, and Flow cytometry analysis

For detailed methods, please see the sections of "NK cell activation and evaluation" and "Flow cytometry analysis" in **Supplementary Data 1** and **Supplementary Fig. 1**.

Cytokine evaluation with ELISA

All samples were estimated using human sandwich ELISA system (Duoset; R&D systems, Minneapolis, MN, USA), and all procedures were followed with manufacturer's protocol. Briefly, cytokines including human IL-1b (DY201), IL-6 (DY206), TNF-α (DY210), IFN-γ, (DY285B) and IL-10 (DY217B) were evaluated in collected plasma samples. In the case of culture supernatants derived from co-culture of PBMC and K562 cells, granzyme B (DY2906) and IFN-g were evaluated.

Statistical analysis

We exploratively analyzed the immunity indices as a pilot study, rather than setting a primary or secondary endpoint. Since both groups got influenza vaccination at D0, the antibody production rate was evaluated at week 4 and week 12 after vaccination. The changes in NK cells (**Supplementary Figs. 2** and **3**), adaptive immunocytes such as CD4+ T cells, CD8+ T cells, B cell (**Supplementary Figs. 4** and **5**), and pro-inflammatory cytokines (IL-6, TNF-α and IL-1β), anti-inflammatory cytokine (IL-10, IFN-γ) were compared between groups at week 1, week 5 and week 13 of the study. All the immunity and antibody data were presented as fold change relative to the baseline data (W-1 randomization day, D0 vaccination day), respectively. The descriptive analysis for the fold change was displayed as scattered points supported by error bar of mean value and standard deviation. The GMT-fold (antibody data) were compared between and within groups by repeated-measures (RM) ANOVA model on log transform titer. In addition, the treatment, time, and interaction effects of all immunocytes and cytokine data were obtained by RM ANOVA followed by *post hoc* Tukey analysis to determine the significance. The statistical analysis was performed using MATLAB R2021a (Mathworks, Inc., Natick, MA, USA).

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RESULTS

Characteristics of the participants

We screened 53 subjects for eligibility between October 8 and January 25, 2020. Eleven subjects were screened out due to hypertension or hyperkalemia. Forty-two subjects aged 24 to 46 years were sequentially enrolled and randomly assigned, 21 participants each to control group and SAT-008 group (performing SAT-008 from visit 2). One week after random assignment, all participants received a quadrivalent inactivated influenza vaccine (Sky Cellflu Quadrivalent prefilled syringe; SK Bioscience Co.) at visit 3. Ten participants violated the protocol (5 each in the control and SAT-008 group) because they took non-steroidal anti-inflammatory drugs (NSAIDs) due to dysmenorrhea or other drugs that may affect immunity. A total of 32 participants (76.2%) completed the clinical trial (**Fig. 1A**).

Demographics of the participants are shown (**Table 1**). The median ages were 37 years (26–40 years) in control group (n=16), 36 years in SAT-008 group (n=16). The male and female percent of participants was 19% and 81% respectively in both groups. There were no differences between the groups or subgroups by gender, race, or age. The results of body mass index and complete blood test over several visits are shown (**Table 1**, **Supplementary Table 1**).

Table 1. Demographic information (n=32)

Characteristics	Visit	Control (n=16)	SAT-008 (n=16)		p-value
Age (yr)		37 (26–40)	36 (27–40)		0.960
Female		13 (81)	13 (81)		1.000
Adverse event					1.000
Mild		-	1 (6.3)		
Moderate to severe		-	-		
Vital sign					
Systolic BP	Visit 1	119.0±11.2	120.0±12.1		
	Visit 2	115.0±10.9	117.0±7.7	Group	0.532
	Visit 3	118.0±9.3	121.0±10.3	Visit	0.251
	Visit 4	119.0±9.4	118.0±8.7	Group × Visit	0.343
	Visit 5	116.0±6.8	121.0±11.6		
Diastolic BP	Visit 1	74.0±8.0	77.0±7.8		
	Visit 2	78.0±8.5	79.0±6.0	Group	0.618
	Visit 3	73.0±9.1	75.0±9.4	Visit	0.000 (2>3,4,5)
	Visit 4	73.0±8.7	71.0±10.5	Group × Visit	0.610
	Visit 5	71.0±7.4	74.0±10.9		
Breaths	Visit 1	19.0±1.2	19.0±1.3		
	Visit 2	18.0±1.5	18.0±1.9	Group	0.236
	Visit 3	19.0±1.7	19.0±1.9	Visit	0.063
	Visit 4	18.0±1.9	18.0±2.2	Group × Visit	0.152
	Visit 5	18.0±1.6	20.0±2.0		
Pulse	Visit 1	77.0±8.8	76.0±8.0		
	Visit 2	79.0±10.7	77.0±8.8	Group	0.242
	Visit 3	79.0±11.2	76.0±8.6	Visit	0.809
	Visit 4	81.0±10.4	75.0±8.5	Group × Visit	0.445
	Visit 5	80.0±11.3	75.0±6.5		
Temperature	Visit 1	36.40±0.09	36.40±0.11		
	Visit 2	36.40±0.10	36.40±0.09	Group	0.160
	Visit 3	36.40±0.10	36.40±0.10	Visit	0.000 (1,2,3,4>5)
	Visit 4	36.40±0.07	36.40±0.09	Group × Visit	0.903
	Visit 5	36.30±0.13	36.30±0.08		
Height		165.2±8.8	161.5±7.2		0.204
Weight	Visit 1	62.9±13.8	58.4±13.4		
	Visit 2	61.7±12.8	58.7±13.2	Group	0.396
	Visit 3	63.5±14.1	59.1±13.4	Visit	0.056
	Visit 4	63.2±13.9	59.1±13.3	Group × Visit	0.296
	Visit 5	63.6±14.2	59.2±13.2		

(continued to the next page)

Table 1. (Continued) Demographic information (n=32)

Characteristics	Visit	Control (n=16)	SAT-008 (n=16)	p-value	
BMI	Visit 1	22.8±3.2	22.1±3.4		
	Visit 2	22.4±3.1	22.3±3.3	Group	0.647
	Visit 3	23.0±3.2	22.4±3.4	Visit	0.032 (3>1)
	Visit 4	22.9±3.2	22.4±3.4	Group × Visit	0.296
	Visit 5	23.1±3.3	22.4±3.3		
SF-36					
Physical	Visit 2	95.0±6.6	93.8±8.5	Group	0.738
	Visit 3	96.9±4.8	95.3±6.4	Visit	0.030 (2>1)
	Visit 4	97.5±4.1	96.6±7.7	Group × Visit	0.439
	Visit 5	96.6±6.0	97.8±4.5		
Limit physical	Visit 2	93.8±17.1	98.4±6.3	Group	0.234
	Visit 3	96.9±12.5	100.0±0.0	Visit	0.162
	Visit 4	98.4±6.3	100.0±0.0	Group × Visit	0.501
	Visit 5	100.0±0.0	100.0±0.0		
Limit emotion	Visit 2	93.8±18.1	95.8±11.4	Group	0.294
	Visit 3	95.8±16.7	97.9±8.3	Visit	0.425
	Visit 4	95.8±11.4	100.0±0.0	Group × Visit	0.619
	Visit 5	93.8±13.4	100.0±0.0		
Energy	Visit 2	58.8±15.7	59.7±12.6	Group	0.847
	Visit 3	61.9±15.7	62.5±16.2	Visit	0.406
	Visit 4	61.3±14.9	59.4±13.5	Group × Visit	0.413
	Visit 5	58.4±17.0	62.5±13.5		
Emotion	Visit 2	77.5±13.1	76.0±12.7	Group	0.856
	Visit 3	76.0±15.7	77.8±14.4	Visit	0.873
	Visit 4	78.0±14.2	78.0±11.7	Group × Visit	0.570
	Visit 5	76.0±13.9	79.0±14.8		
Social	Visit 2	88.3±14.8	92.2±13.6	Group	1.000
	Visit 3	90.6±12.5	93.0±13.7	Visit	0.347
	Visit 4	94.5±10.2	93.0±12.0	Group × Visit	0.234
	Visit 5	96.1±7.5	91.4±15.6		
Pain	Visit 2	89.8±14.8	93.0±11.2	Group	0.795
	Visit 3	92.0±9.3	93.0±7.4	Visit	0.378
	Visit 4	91.1±11.2	92.2±11.2	Group × Visit	0.202
	Visit 5	92.2±9.3	83.9±22.4		
General	Visit 2	72.5±14.8	67.8±14.1	Group	0.309
	Visit 3	74.1±18.4	66.3±15.5	Visit	0.828
	Visit 4	74.7±18.1	66.3±15.7	Group × Visit	0.141
	Visit 5	68.8±14.2	68.8±15.7		
IPAQ					
High level of physical activity	Visit 1	35.0±82.5	15.0±43.5		
	Visit 2	45.0±180.0	30.0±70.8	Group	0.200
	Visit 3	150.0±600.0	312.5±327.3	Visit	0.031 (3,4>2; 3,4,5>1)
	Visit 4	180.0±720.0	342.5±341.9	Group × Visit	0.261
	Visit 5	0.0±0.0	260.0±351.2		
Moderate level of physical activity	Visit 1	187.5±489.4	31.8±82.9		
	Visit 2	100.0±243.5	62.5±106.3	Group	0.722
	Visit 3	60.0±181.3	205.0±341.6	Visit	0.201
	Visit 4	430.0±1,265.2	197.5±166.8	Group × Visit	0.291
	Visit 5	137.5±370.6	217.5±195.9		
Low level of physical activity	Visit 1	288.8±248.6	305.3±571.4		
	Visit 2	1,012.7±1,577.3	256.8±169.0	Group	0.066
	Visit 3	1,474.7±1,824.7	317.6±212.0	Visit	0.025 (3,4>2; 3,4,5>1)
	Visit 4	901.3±1,476.0	379.5±236.7	Group × Visit	0.030
	Visit 5	960.1±1,215.0	603.3±664.9		
Total physical activity	Visit 1	511.3±589.4	352.0±653.9		
	Visit 2	1,157.7±1,761.3	349.3±228.3	Group	0.281
	Visit 3	1,684.7±2,365.2	835.1±580.0	Visit	0.024 (5>1; 3>2)
	Visit 4	1,511.3±3,297.2	919.5±445.0	Group × Visit	0.325
	Visit 5	1,097.6±1,404.0	1,080.8±843.9		

Values are presented as median (interquartile range), number (%), or mean ± standard deviation.
BP, blood pressure; BMI, body mass index; SF-36, 36-Item Short Form Health Survey; IPAQ, International Physical Activity Questionnaires.

The subgroup analysis divided the STA-008 group according to the adherence: 6 subjects in HP groups (96%±1.3%) and 10 subjects in LP group (57.3%±12.7%).

Safety

Safety evaluation was performed at each visit. We could evaluate a total of 41 subjects (20 for controls and 21 for the SAT-008 group) including participants who had dysmenorrhea and took NSAIDs. Among the reported adverse event, knee pain reported by one subject was the only case related to the SAT-008 device. It could be 'a data transfer issue' because the subject repeated the physical activity again to record his performance after a data transferring failure, which might cause knee pain. The knee pain lasted for 2 days and recovered without taking medication.

SAT-008 increased anti-influenza antibody titers against type B antigens

SAT-008 significantly increased the HI titers against type B/Phuket (Yamagata) influenza antigen at 4 wk after vaccination as well as another type B/Washington (Victoria) at 12 wk after vaccination than the control group (vaccination only without SAT-008) ($p<0.05$, $p<0.01$, respectively) (**Fig. 2**). Moreover, SAT-008 more enhanced the HI titers against type B/Phuket (Yamagata) at visit 5 in HP group compared with those at visit 4 in HP, at any visits in control, and LP group ($p<0.05$ at each) (**Fig. 3C**). SAT-008 also significantly increased the HI titers against type B/Washington (Victoria) at visit 5 in HP group ($p<0.01$). Interestingly, there was no difference in HI titers against type A of H1N1 and H2N3 between SAT-008 and control groups (**Fig. 2A and B**).

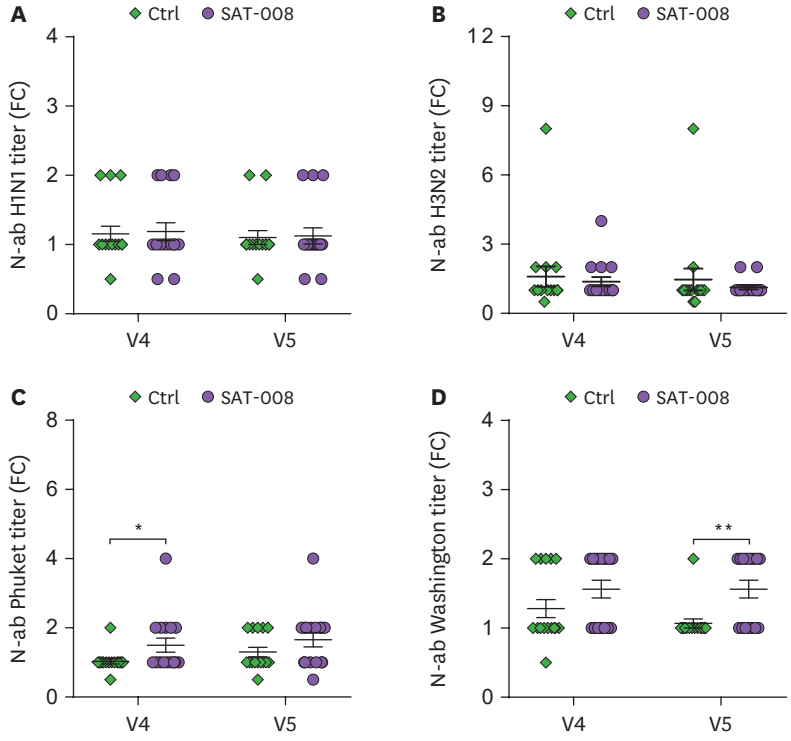

Figure 2. Changes in HI titer at visits 4 and 5. The baseline is a visit 3 (vaccination day, D0). (A) antigen subtype A H1N1, (B) antigen subtype A H3N2, (C) antigen subtype B Phuket, (D) antigen subtype B Washington. The error bars refer to the standard deviation.
The asterisk symbols indicate the significant levels: *$p<0.05$; **$p<0.01$.

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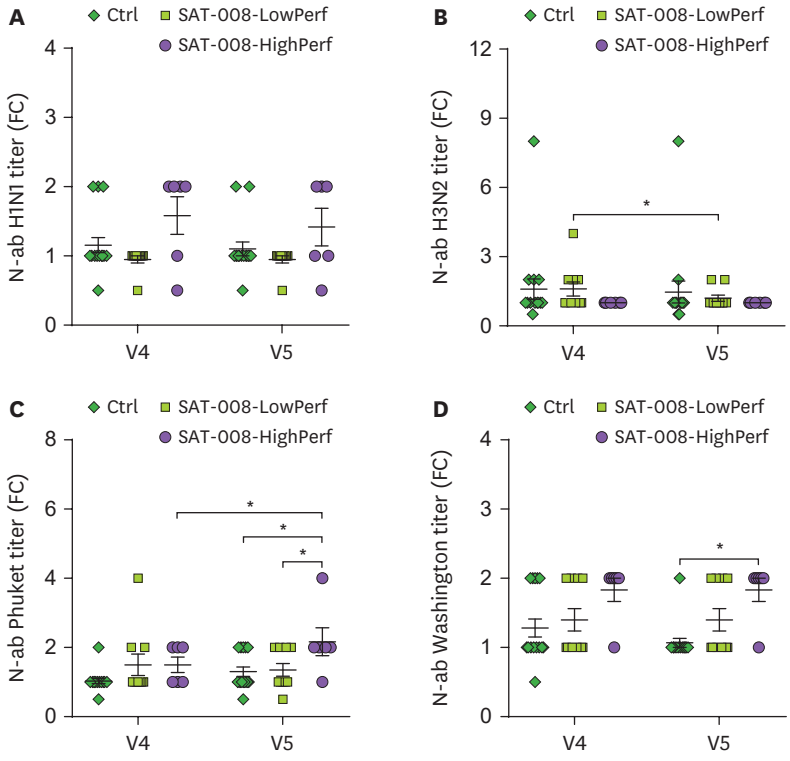

Figure 3. Subgroup analysis of the changes in HI titer at visits 4 and 5. The baseline is a visit 3 (vaccination day, D0). (A) antigen subtype A H1N1, (B) antigen subtype A H3N2, (C) antigen subtype B Phuket, (D) antigen subtype B Washington. The error bars refer to the standard deviation.
The asterisk symbols indicate the significant levels: *p<0.05.

Stimulated NK cells

Stimulated NK cells in the control group decreased significantly between 4 wk (visit 4) and 12 wk after the vaccination (visit 5) (**Fig. 4**, p<0.05). The subsequent analysis showed a significant interaction effect, indicating that the change in stimulated NK cells over time is different depending on group type (p<0.05) (**Supplementary Fig. 2C and D**). Stimulated NK cells in the SAT-008 group slightly increased between 4 wk (visit 5) and 12 wk (visit 5) after the vaccination, but there was no significance (**Supplementary Fig. 2E**). At the time of visit 4 and visit 5 at each, the number of population of stimulated NK cells between the control and SAT-008 groups was not different. A significant decrease of stimulated NK cells in the control group between visit 4 and visit 5 was detected also when it was analyzed as HP, LP, and control groups (p<0.01) (**Supplementary Fig. 2F**).

Non-stimulated NK cells

On the same manner of stimulated NK cells, the non-stimulated NK cell populations were significantly reduced in the control group between visit 4 and visit 5 (p<0.001) although there was no significant difference between the control and SAT-008 group at each visit as displayed in **Supplementary Fig. 3**. In subgroup analysis, both HP and LP did not show significant down-regulation of NK cell population in PBMC compared to visit 3, however, the control group showed decrease of NK cell population.

Plasma cytokine

Fig. 4 illustrated that SAT-008 performed more than 75% of the total program (HP group) worked to elicit IL-10, IL-1β, and IL-6 in visit 4 and visit 5 at each, in comparison to the

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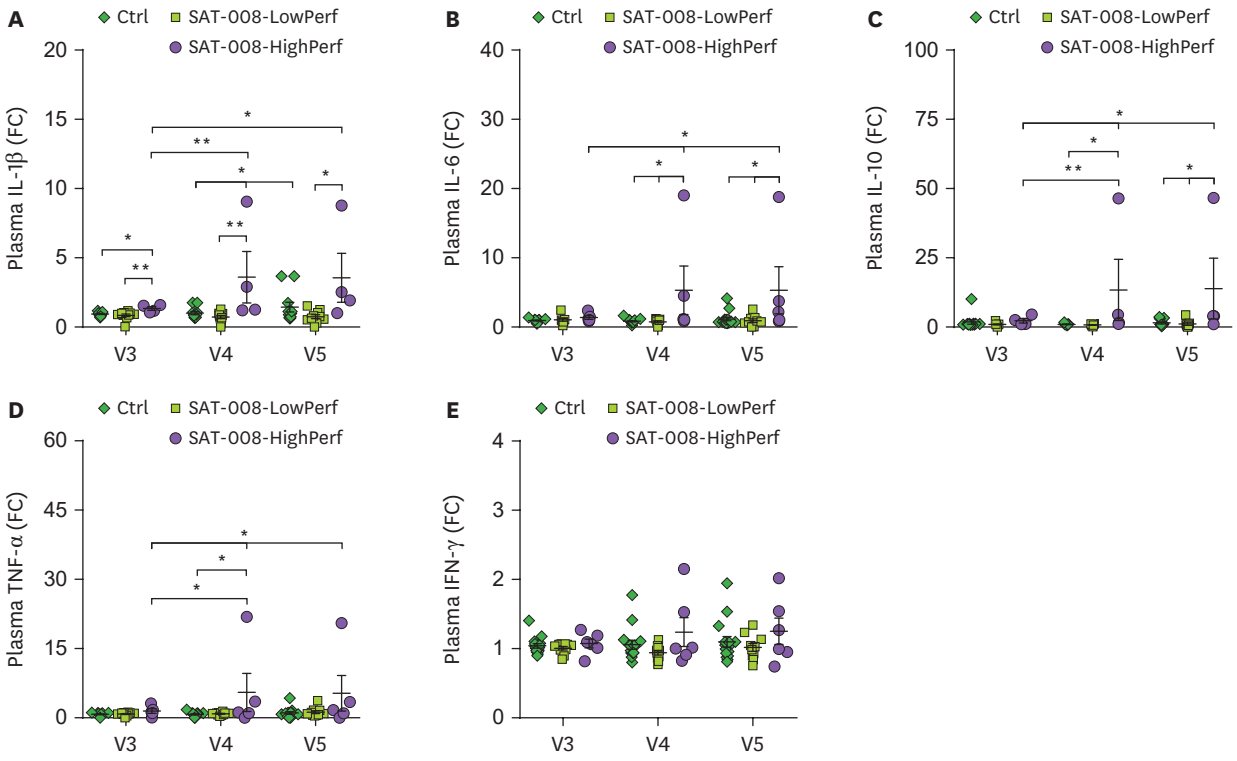

Figure 4. Changes in plasma cytokine between control and SAT-008 of LP and HP groups. (A) IL-1β response, (B) IL-6 response, (C) IL-10 response, (D) TNF-α response, (E) IFN-γ response. The error bars refer to the standard deviation.
The asterisk symbols indicate the significant levels: *p<0.05; **p<0.01.

control group and LP group both (p<0.05) as well as TNF-α in visit 4 (p<0.05) There was no difference of IFN-γ among control, HP and LP group at each visit.

DISCUSSION

This work demonstrated that exercise with a novel digital device, SAT-008, increased serum anti-influenza antibody titers after influenza vaccination. Furthermore, exercise with SAT-008 successfully sustained the stimulated NK cells with a tendency to increase while a significant decline was observed in the NK cells of the control group. The plasma cytokine such as IL-10, IL-6, TNF-α, and IL-1β increased significantly when the adherence to SAT-008 was over 75%.

Viral infections such as influenza and COVID-19 pose a serious threat to human health, which increases the demand for a new approach to enhance the host's immunity. Lambert LC suggested speeding up the production of pipelines to prepare more amount of vaccines and generating antigen-sparing vaccine approaches leading to provide sufficient material to cover the universal population (18). Besides, many strategies focused on the development of novel antigens to elicit the immunity in vulnerability's sites (19), and identify accurate immune correlates of protection against influenza (20). Other researchers center on improving the protective activity of vaccine-induced humoral immunity (21). Vaccine adjuvant constitutes a key factor to boost the immune response to antigen (22). It works on reducing the quantity requirements of antigen and enhancing the vaccine effectiveness (23). Despite the advantages of vaccine adjuvant, several drawbacks still exist (21). Therefor a safer and more reliable vaccine adjuvant is needed.

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We developed SAT-008 as a novel digital device to enhance host immune system against viral infection. The digital device, SAT-008, is software based on an algorithm including functions of regulating physical activity which is named as external activity, and bringing programs of internal activity which is related to the regulation of the ANS. Both internal and external activities may be linked to boosting innate and adaptive immune systems against viral invasion into a human body. Previous studies showed that some exercise enhanced the anti-viral antibody titers after vaccination including influenza and COVID-19 vaccines (24,25). The neuronal components translated into the programs of internal activities were ANS related with vagal nerve (26,27). The ANS is reported to be related to B cell maturation in human organ as well as anti-inflammatory effect (28). SAT-008 delivers the algorithm of the cascading effects of host immune system with internal and external activities, trying to strengthen the host immune system which was aimed to be evaluated in clinical trials.

Our study showed that exercise with SAT-008 had an adjuvant-like effect on influenza vaccine to enhance the anti-influenza virus antibody titer, especially against type B antigens without systemic adverse event. There was no difference in the antibody titer against type A antigen between the groups, which could be explained by following reasons. Usually immunogenicity against type A is better than type B after influenza vaccination. For trivalent influenza vaccines, both of H1N1 and H3N2 are included for type A antigens and either Yamagata or Victoria is included for type B antigen. In this study, because basal titers were already high for type A antigens, seroconversion rate was low. The baseline HI titers to type B antigens were relatively low in the participants although they were vaccinated with influenza vaccines in the previous year. Most of the participants were medical personnel who received repeated influenza vaccinations annually (mostly with trivalent vaccines previously) that may induced more immunogenicity especially against type A antigen (29).

Exercise with SAT-008 influenced NK, T cells, B cells, and plasma cytokines (**Supplementary Figs. 2-5**, **Fig. 4**). NK cell is known as a key player killing virus infected cells and immunoregulating via DC editing and cytokine production (30). This study suggested that exercise with SAT-008 might produce a prolonged NK cell activity. The effect of adherence to SAT-008 was also observed in plasma cytokine, where the HP group showed significant increase in the level of IL-1β, IL-6, IL-10 and TNF-α in visit 4 and visit 5.

Previous studies showed that the activity and mobilization of NK cells changed based on intensity and duration of exercise and that people who did not perform exercise had a high risk for illness (31,32). Vaccination also induce activation of NK cell that increased activity of NK cell enhance the effect of influenza vaccination (33). However, this activation is just sustained less than 3 months (34). Total population of NK cell also decrease 2–3 months after influenza vaccination (34,35). In this study, like previous studies, total population and activation of NK cell were decreased at visit 5 (3 months after vaccination) compared to visit 4 (1 month after vaccination) in the control group with the vaccination only (**Supplementary Figs. 2C and D**, **3C and D**). However, there was no above changes in the SAT-008 performed group at visit 5 compare to control group. These results indicated that SAT-008 might strengthen the effect of influenza vaccine via enhancing NK cell population and activation after the vaccination.

In our study, notable changes in the immunocyte analysis of PBMC were elevation of CD4+ central memory cells and memory B cells. It has been reported that repeated high-intensity exercise induced the reduction of white blood cells in elite athletes (36). However, our results

did not show big difference between the groups. Even central CD4+ memory cells were maintained, and memory B cell population was increased at visit 5 by performing exercise using SAT-008. These data indicated that exercise with SAT-008 after influenza vaccination was able to enhance vaccine effect via up-regulating the memory B and maintaining T cell population (**Supplementary Figs. 4D and L**, **5D and L**). Other points were decrease of plasma B cell and CD4/CD8 effector T cells and CD4 effector memory cells in accordance with increase of CD4 Treg cells (**Supplementary Figs. 4B, C, F, K, and I**, **5B, C, F, K, and I**). It is known that regulatory T cells after influenza vaccination are important in the resolution of immune responses and maintenance of immune-homeostasis (31).

According to the previous study, trivalent influenza vaccine is able to induce cytokines including IL-6, IL-10, TNF-α, and IFN-γ in serum. Most of the cytokines were disappeared within 44 hours after vaccination while IL-6 was detected until 14 days after vaccination (32). In this study, we showed that HP of SAT-008 showed increased serum cytokines until 3 months after the vaccination. Other study also showed that high-intensity exercise was able to induce the elevation of serum cytokines. There could be concerns that increased cytokines might possibly cause chronic inflammation if pro-inflammatory cytokines were continuously increased by chronic exercise (37). However, IL-6 is known as a key player in the immune-defense to viral infection, and antibody production (38). IL-1β is also important on the development of T cell memories to influenza A viruses (39). Considering the increase of memory T cells and B cells, these increased cytokines may play as good side. Increase of IL-10 could play a role for balancing the immunogenicity.

This study was limited with a small number of subjects, which might affect some results, especially for subgroup analysis. However, this study has the strength that showed exercise with a digital device, SAT-008, could enhance anti-viral immune reactions for the first time. Further study with a larger population of subjects is needed to prove SAT-008's effectiveness on influenza vaccines, and possibly other anti-viral vaccines such as COVID-19 vaccines.

Exercise with SAT-008 could increase anti-influenza antibodies especially against type B antigens like an adjuvant. Furthermore, SAT-008 successfully sustained the stimulated NK cells with a tendency to increase while a significant decline was observed in the NK cells of the control group. We noted the importance of the adherence performing more than 75% of the total program. The plasma cytokine such as IL-10, IL-6, TNF-α, and IL-1β increased significantly when the adherence to SAT-008 was over 75%. Our findings indicate a novel approach using digital device may enhance host immune system like a vaccine adjuvant against viral diseases such as influenza.

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SUPPLEMENTARY MATERIALS

Supplementary Data 1
Methods

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Supplementary Table 1
The laboratory test findings (n=32)

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Supplementary Figure 1
FACS gating strategy. Target CD molecules for T & B cell analysis were CD3, CD4, CD8, CD20, CD25, CD27, CD45RA.

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Supplementary Figure 2
Changes in stimulated NK cells. (A) Between control and SAT-008 groups at visits 3, 4, and 5. (B) Between control and SAT-008 LP and HP groups at visits 3, 4, and 5. (C) Between control and SAT-008 groups at visits 4, and 5. (D) Between control and SAT-008 LP and HP groups at visits 4 and 5. (E, F) Representation interaction effect between 2 groups and 3 groups, respectively. The error bars refer to the standard deviation.

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Supplementary Figure 3
Changes in non-stimulated NK cells. (A) Between control and SAT-008 groups at visits 3, 4, and 5. (B) Between ctrl and SAT-008 LP and HP groups at visits 3, 4, and 5. (C) Between control and SAT-008 groups at visits 4, and 5. (D) Between control and SAT-008 LP and HP groups at visits 4 and 5. The error bars refer to the standard deviation.

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Supplementary Figure 4
Changes in immunocytes between control and SAT-008 groups. (A-D) CD4 T cell responses, (E-H) CD8 T cell responses, (I) T-regulatory response, (J-L) plasma B cell response. The error bars refer to the standard deviation.

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Supplementary Figure 5
Changes in immunocytes between control and SAT-008 of LP and HP groups. (A-D) CD4 T cell responses, (E-H) CD8 T cell responses, (I) T-regulatory response, (J-L) plasma B cell response. The error bars refer to the standard deviation.

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