

Combination Effect of Caffeine Intake and Thermotherapy on the Blood Levels of Human Irisin and Fibroblast Growth Factor 21 in Healthy Males

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Abstract

Caffeine is a widely consumed psychoactive substance known to influence physiological processes such as heat generation and autonomic nervous system activity. Fibroblast growth factor 21 (FGF-21) and irisin are the biomarkers associated with thermogenesis and metabolic regulation. The study aimed to determine whether thermotherapy along with caffeine intake could increase the blood levels of FGF-21 and irisin. A total of 87 healthy male subjects were randomly divided into a control group and a caffeine intake group. For heat loading, an experiment was performed in which each subject was given a 30-min half-body bath in hot water ($42^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$), and their tympanic temperature (Tty), mean skin temperature (mTs), and serum FGF-21 and irisin levels were measured. Compared to the control group, the caffeine intake group showed significantly increased Tty, mTs, serum FGF-21, and irisin after thermotherapy. Especially, administration of caffeine led to a significantly amplified response in circulating FGF-21 and irisin levels, showing an additional 22.93% and 28.70% increase, respectively, compared to the control group ($P < 0.001$). The results suggest that as a new form of synergy, the combination of caffeine intake and thermotherapy could potentially be applied to broader clinical and physiological settings.

Keywords: Brown adipose tissue, caffeine, fibroblast growth factor 21, irisin, thermogenesis, thermotherapy

INTRODUCTION

Coffee is one of the most widely consumed drinks in the world with caffeine content, widely known as a psychoactive substance, regardless of age or gender.^[1] Caffeine, which acts as a nerve stimulant, can affect basic physiological processes such as sleep, awakening, cognition, and memory.^[1] Caffeine can induce increased blood pressure and sympathetic activity in a short period.^[2] After caffeine intake, the average body temperature (mTb) and average skin temperature (mean skin temperature [mTs]) increase significantly through heat generation.^[3,4]

This change occurs because caffeine activates neurons in the outer hypothalamus, stimulating the thermal formation of

brown adipose tissue (BAT).^[5] In other words, autonomic neural system activity is activated when caffeine is consumed and is related to the activation of central neural pathways involved in heat generation and regulation.^[6] There is a need for biomarker research to enable the analysis of the thermogenesis reaction control mechanism. According to recent studies, fibroblast growth factor 21 (FGF-21) and irisin are representative substances closely related to heat production in the body.^[7]

FGF-21 is a protein involved in sugar and lipid metabolism and energy homeostasis.^[8] Previous studies have shown that

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energy consumption and body temperature were increased in mice injected with FGF-21 and that FGF-21 mRNA in brown fat was increased when mice were exposed to cold for a short period.^[8,9] In addition, FGF-21 derived from the liver is an endocrine hormone that can act as a nutrient homeostasis regulator in humans. The presence of FGF-21 can improve lipid homeostasis and induce weight loss.^[9] In other words, FGF-21 can stimulate the heat generation of BAT.^[10] It has been reported that stimulation of lipid metabolism by FGF-21 shows great potential for activating energy metabolism and treating type 2 diabetes.^[11]

Irisin is a myokine that can stimulate the “browning” of white adipose tissue, leading to increased energy consumption.^[12] Irisin plays an important role in the treatment of obesity by regulating endothelial precursor cells that are known to be negatively correlated with obesity.^[13] A study measured circulation irisin levels in healthy female subjects and found that irisin levels are increased after thermotherapy as indicated by increases in biomarker levels that are related to several heat generation mechanisms, in addition to increased irisin levels after caffeine intake and heat treatment.^[14] During thermal therapy, circulating irisin is increased as a response to oxidative stress. However, studies conducted so far have mainly used cell lines or experimental animals. Thus, there is a limit to generalization.

The present study was based on the above background and the basic principles are as follows. Caffeine can act as a stimulant, which can affect blood pressure, heart rate, the autonomic neural system, and heat generation.^[6] However, the physiological mechanism that increases body temperature and the sweat gland response following combined caffeine intake and thermotherapy, especially regarding FGF-21, remains unclear. Therefore, the study aimed to examine the effects of caffeine intake and thermotherapy on heat generation and the sweat sensitivity of adult BATs through blood irisin and FGF-21 indicators.

MATERIALS AND METHODS

Sample size and randomization

This study adopted a purposive sampling method. The required sample size was determined based on previous studies and calculated using G-Power 3.1.9.7 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), with an effect size derived from our prior research (statistical significance = 0.05, power = 0.9, effect size = 0.78).^[15,16] As a result, a minimum of 72 participants was deemed necessary. Considering potential dropouts, at least 45 subjects per group were recruited between January 2020 and March 2020.

This research was designed as a double-blind, randomized controlled study. Participants were randomly assigned to either the caffeine or placebo group in a 1:1 ratio using a computer-generated random sequence. To maintain blinding, group allocations were concealed in opaque, sealed envelopes. Neither the participants nor the researchers was aware of the assigned intervention. Furthermore, the caffeine powder was

colorless and odorless, minimizing the risk of unintentional unblinding. The study flowchart outlining the subject recruitment process is shown in Figure 1.

Subjects

A total of 87 healthy male subjects were analyzed, and their general physical characteristics are presented in Table 1. Participants who were habitual smokers or regularly consumed coffee, tea, or energy drinks were excluded. Additionally, individuals with preexisting health conditions, particularly hypertension, heart disease, or cerebrovascular disease, were not eligible for participation.

To control for potential confounding factors, subjects were instructed to abstain from alcohol and medication for 24 h before the trial. Additionally, to prevent dehydration, they were required to consume a placebo drink (5–7 mL·kg⁻¹) four hours before the trial, maintaining an eight-hour fasting with controlled water intake.^[17]

Measurements and experimental procedure

In order to minimize the effect of circadian rhythm on body temperature, this study was conducted by fixing the time from 2 p. m. to 5 p. m., and the place was conducted in the climate chamber at the College of Medicine of Soonchunhyang University in Cheonan (36°48'N, 127°06'E).^[17,18] Subjects were randomly divided via a computer-generated simple randomization into two groups. Those selected as controls were asked to drink 200 mL of placebo (CON) and the other group

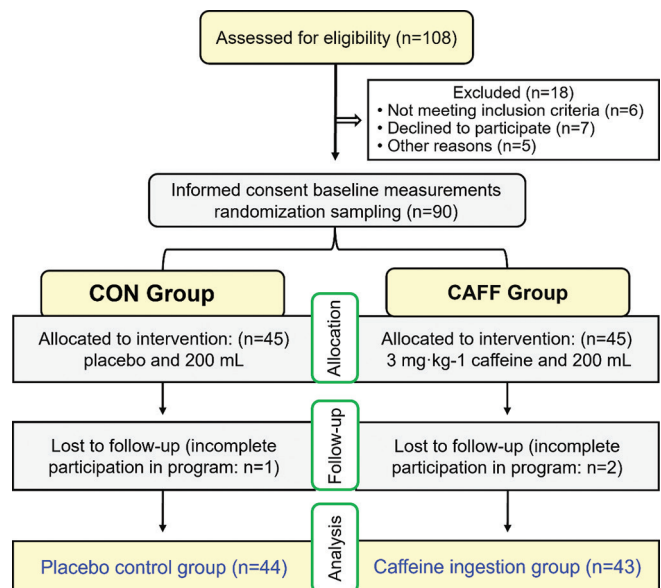


Figure 1: Flow diagram: Subject flow. Between January 2020 to March 2020, 108 eligible subjects were recruited, and 90 of them who met the study criteria were selected through preinterviews by two researchers. They were assigned 1:1 to the experimental group and the control group. Due to difficulties in participating in the ongoing experiment or problems such as the occurrence of COVID-19 infection, 44 subjects were ultimately included in the control group and 43 subjects in the experimental group. CON, control subjects (placebo and 200 mL); CAFF, caffeine ingestion subjects (3 mg·kg⁻¹ caffeine and 200 mL).

was asked to drink a mixture of $3 \text{ mg} \cdot \text{kg}^{-1}$ of caffeine (CAFF) with 200 mL of placebo, with both groups being blinded to the subjects.^[17] The subjects rested in a comfortable position for 60 min before the experiment, followed by passive heat loading for 30 min. Blood was sampled at three different time points during the experiment [Figure 2]. The primary outcome is the serum concentration of irisin and FGF-21, and the secondary outcome includes tympanic temperature (Tty), mean body temperature and mTs.

Supplements (caffeine)

Since the blood caffeine level reaches a maximum of about 30 min after ingestion, water immersion was performed in a hot bath 30 min after caffeine ingestion. In this study, purified 99.9% caffeine powder was used and produced by Scientific Fitness (Oakmont, PA, USA).^[17]

Passive heat loading (exposure)

The conditions of the climate chamber are as follows.

1. Air speed 1 m/s
2. Air temperature $26.5^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$
3. Relative humidity $60 \pm 3.0\%$.

The passive heat loading was performed by immersing the lower body in a hot water bath (temperature: $42^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) for 30 min under the supervision of two researchers, with a thermal intensity similar to that used in our previous studies [Figure 2].^[3,14,17,18] Water immersion is a more efficient method of heat loading than heat lamps or hot packs, as it utilizes heat conduction over a large surface area.

Above setting was chosen based on our previous studies balancing efficiency and experimental duration. While

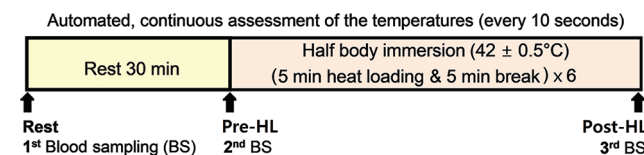


Figure 2: Experimental protocols. At the rest time point, the control group consumed a placebo and 200 mL of water, while the experimental group consumed $3 \text{ mg} \cdot \text{kg}^{-1}$ of caffeine and 200 mL of water. Blood sampling was conducted three times at the rest, preheat loading (HL), and Post-HL time points. Heat loading was performed by immersing half of the body into a hot water bath (temperature: $42^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) in six alternating cycles of 5-min exposure followed by 5-min rest, resulting in a total procedure duration of 60 min. Tympanic and local skin temperature measurements were conducted continuously at 10-s intervals for 60 min. Rest, 30 min before HL procedure; Pre-HL, right before HL; Post-HL, right after HL.

increasing the water temperature or the immersed body surface area can accelerate heat loading, it also increases physiological strain and the risk of adverse effects. Moreover, half-body immersion (rather than full-body immersion) was selected to reduce the unnecessary exposure and allow participants to exit the water more easily during rest periods.

To ensure safety, participants were closely monitored throughout the experiment. If a subject reported nausea, dizziness, or exhibited unstable vital signs, heat loading was to be immediately discontinued, and a medical evaluation would follow. No adverse or unintended symptoms were observed in this experiment.

Tympanic temperature measurements

As in previous studies, the evaluation of Tty was measured by inserting a TSK7 + 1 thermistor probe with a small spring into the ear canal.^[19] This probe is also connected to the K-720 data logger model by connecting to a personal computer.^[18,19] Subsequently, a small cotton ball was filled in the inner pinna to fix the position of the probe to minimize the scratch noise and discomfort that the study subjects could feel as the thermistor probe touched the tympanic membrane.^[14,18]

Mean body temperature and mean skin temperature measurements

Skin temperature measurements were made in eight areas (chest, upper back, lower back, abdomen, upper arm, forearm, thigh, and leg).^[14] Like Tty measurement, the skin temperature was measured with a TSK7 + 1 thermistor probe.^[14,18] Based on the previously measured eardrum temperature and skinny temperature, mTs and mTb were calculated as follows.

$$mTs = 0.3 \cdot (Ts_{\text{chest}} + Ts_{\text{arm}}) + 0.2 \cdot (Ts_{\text{thigh}} + Ts_{\text{leg}})^{[20]}$$

$$mTb = 0.9 \cdot Tty + 0.1 \cdot mTs^{[21]}$$

Blood sampling and analysis of irisin and fibroblast growth factor-21 levels

The blood sample was measured 30 min before the passive heat load (Rest), after the start of the heat load (Pre), and 1 h after the heat load (Post), a total of three times [Figure 2]. Blood collection to confirm changes in serum pyrogen markers was performed in the subjects' antecubital veins.^[17] Immediately after blood collection, blood samples were placed in serum separator tubes and immediately centrifuged for 10 min (3000 rpm, 4°C). After centrifugation, only serum was separated and stored in an amount of 1 mL aliquots at -80°C until analysis.

Table 1: Physical characteristics of subjects

Groups	Age (years)	Height (cm)	Weight (kg)	BSA (m^2)	Percentage fat	BMI (kg/m^2)
CON	24.41 ± 3.85	174.56 ± 4.89	72.16 ± 8.27	1.87 ± 0.12	24.47 ± 3.17	23.68 ± 2.43
CAFF	25.37 ± 4.32	173.48 ± 5.02	73.02 ± 7.10	1.87 ± 0.16	25.15 ± 3.42	24.27 ± 2.08

Physical characteristics of subjects in the CON ($n=44$) and CAFF ($n=43$) groups are presented as mean $\pm$ SD. Statistical analysis confirmed no significant differences between the groups. 87 male volunteers participated in this study. BSA was calculated using the Du bois formula. Body fat percentage was measured using the bio-impedance method (in body 520, Seoul, Korea). CON: Control subjects (placebo and 200 mL), CAFF: Caffeine ingestion subjects ($3 \text{ mg} \cdot \text{kg}^{-1}$ caffeine and 200 mL), % Fat: Body fat percentage, BSA: Body surface area, BMI: Body mass index, SD: Standard deviation

Human FGF-21 and irisin, which are pyrogen, were analyzed with Elisa kit and spectrophotometric reader with the obtained serum. Human FGF-21 Quantikine ELISA Kit was used in this study to confirm FGF-21 levels.^[11] Irisin EIA kit EK-067-16 was used for Irisin blood levels.^[17]

Statistical analysis

The numerical values are expressed in mean $\pm$ standard deviation. The statistical data of this study were analyzed by SPSS for Windows (Ver 24.0) (IBM Corp., New York, USA). Statistical significance was evaluated by Student's *t*-test and two-way measure analysis of variance to determine the differences between and within groups. Statistically significant differences were considered at $P < 0.05$.

Ethics and consent

The study was approved after being reviewed by Soonchunhyang University's Institutional Review Board and Ethics Committee on May 30, 2019 (approval No. 1040875-201611-BR-042, <https://www.r-bay.co.kr/agency/main/Z3dFVjlzT0hRUUIBOUZyc1BoYjdMUT09>) and all procedures complied with the Helsinki Declaration of the World Medical Association (2013).

The researchers explained to the study subjects the purpose of the study, the research protocol, and the potential risks. In addition, the researchers explained to the subjects that the data obtained from the experiment would be anonymized and could be published as a paper. The study subjects were

fully aware of this and agreed and written informed consent was obtained from all participants.

RESULTS

Mean body and mean skin temperature

This study measured mTb and mTsk to assess thermogenic responses in subjects undergoing caffeine intake and passive heat loading [Table 2]. In mTb, the CAFF group showed an increase of $1.69^{\circ}\text{C} \pm 0.21^{\circ}\text{C}$ during thermotherapy, which was significantly greater than the CON group's increase of $1.46^{\circ}\text{C} \pm 0.25^{\circ}\text{C}$ ($P < 0.001$). Similarly, for mTsk, the temperature rise in the CAFF group was $1.27^{\circ}\text{C} \pm 0.21^{\circ}\text{C}$ during thermotherapy, significantly higher than the CON group's increase of $1.09^{\circ}\text{C} \pm 0.24^{\circ}\text{C}$ ($P < 0.001$). The results demonstrated that mTb and mTsk were significantly increased in both groups during thermotherapy and caffeine intake, suggesting a more pronounced thermogenic effect in the CAFF group.

Circulating fibroblast growth factor-21 concentrations

This study aimed to explore the potential synergistic effects of caffeine intake and thermotherapy on circulating FGF-21 levels. Changes in FGF-21 levels across heat loading are shown in Figure 3. The results indicated that circulating FGF-21 levels were significantly increased in both groups after caffeine intake and thermotherapy [Table 3, $P < 0.001$]. Compared to the resting state, the CAFF group showed a $52.59 \pm 17.07\%$

Table 2: Changes in mean body temperature and skin temperature across heat loading

Locations	Groups	Rest	Pre-HL	ΔT , (CI)	Post-HL	ΔT , (CI)
mTb ($^{\circ}\text{C}$)	CON	36.46 $\pm$ 0.21	36.47 $\pm$ 0.20	0.01 $\pm$ 0.03 (-0.00–0.02)	37.92 $\pm$ 0.26***	1.46 $\pm$ 0.25 (1.38–1.53)
	CAFF	36.45 $\pm$ 0.19	36.64 $\pm$ 0.18***,++	0.19 $\pm$ 0.06*** (0.17–0.21)	38.14 $\pm$ 0.29***,++	1.69 $\pm$ 0.21*** (1.63–1.75)
	<i>P</i>	0.406	<0.001	<0.001	<0.001	<0.001
mTsk ($^{\circ}\text{C}$)	CON	31.93 $\pm$ 0.23	31.94 $\pm$ 0.21	0.01 $\pm$ 0.08 (-0.01–0.04)	33.02 $\pm$ 0.24***	1.09 $\pm$ 0.24 (1.02–1.16)
	CAFF	31.91 $\pm$ 0.21	32.08 $\pm$ 0.20***,++	0.17 $\pm$ 0.07*** (0.15–0.19)	33.18 $\pm$ 0.23***,++	1.27 $\pm$ 0.21*** (1.21–1.33)
	<i>P</i>	0.365	0.001	<0.001	<0.001	<0.001

*** $P < 0.001$ Indicates a significant difference compared to the rest time point within the same group, ++ $P < 0.01$ and +++ $P < 0.001$ indicate significant differences between the CON ($n=44$) and CAFF ($n=43$) groups at the corresponding time points. Values ($n=87$) are presented as mean $\pm$ SD, 95% CI are shown as (lower limit, upper limit). Exact *P* values for the comparison between the CON and CAFF groups are presented in the table. Rest: 30 min before heat loading procedure (HL), Pre-HL: Right before HL, Post-HL: Right after HL, ΔT : Change in temperature compared to the rest time point, CON: Control subjects (placebo and 200 mL), CAFF: Caffeine ingestion subjects (3 mg $\cdot$ kg $^{-1}$ caffeine and 200 mL), mTb: Mean body temperature, mTsk: Skin temperature, CI: Confidence interval, HL: Heat loading

Table 3: Changes in fibroblast growth factor - 21 and irisin levels across heat loading

Analysis items	Groups	Rest	Pre-HL	Δ values, CI	Post-HL	Δ Values, CI
FGF-21 (pg/mL)	CON	39.78 $\pm$ 17.53	40.07 $\pm$ 18.03	0.29 $\pm$ 1.28 (-0.09–0.67)	52.64 $\pm$ 24.95***	12.86 $\pm$ 9.21 (10.14–15.58)
	CAFF	40.36 $\pm$ 16.20	47.66 $\pm$ 20.45***,+	7.30 $\pm$ 5.28*** (5.73–8.88)	62.75 $\pm$ 27.94***,+	22.40 $\pm$ 12.60*** (18.63–26.16)
	<i>P</i>	0.437	0.035	<0.001	0.039	<0.001
Irisin (ng/mL)	CON	7.95 $\pm$ 3.15	7.96 $\pm$ 3.26	0.01 $\pm$ 0.60 (-0.17–0.19)	10.41 $\pm$ 4.58***	2.46 $\pm$ 1.83 (1.92–3.00)
	CAFF	7.98 $\pm$ 3.06	9.18 $\pm$ 3.36***,+	1.20 $\pm$ 0.67*** (0.99–1.40)	12.27 $\pm$ 4.45***,+	4.29 $\pm$ 1.74*** (3.77–4.81)
	<i>P</i>	0.482	0.045	<0.001	0.029	<0.001

*** $P < 0.001$ Indicates a significant difference compared to the rest time point within the same group, + $P < 0.05$ and +++ $P < 0.001$ indicate significant differences between the CON ($n=44$) and CAFF ($n=43$) groups at the corresponding time points. Values ($n=87$) are presented as mean $\pm$ SD, 95% CI are shown as (lower limit, upper limit). Exact *P* values for the comparison between the CON and CAFF groups are presented in the table. Rest: 30 min before HL, Pre-HL: Right before HL, Post-HL: Right after HL, Δ value: Change in concentration compared to the rest time point, CON: Control subjects (placebo and 200 mL), CAFF: Caffeine ingestion subjects (3 mg $\cdot$ kg $^{-1}$ caffeine and 200 mL), FGF-21: Fibroblast growth factor 21, CI: Confidence interval, SD: Standard deviation, HL: Heat loading procedure

increase in FGF-21 postmeasurement while the CON group showed $29.66 \pm 19.57\%$ increase [Table 4, $P < 0.001$]. This 22.93% additional increase suggests that caffeine intake amplifies the effect of heat therapy on FGF-21 levels.

Circulating irisin concentrations

Previous studies suggest that irisin levels rise in response to physical and thermal stimuli, which served as the basis for measuring circulating irisin concentrations in this study. Changes in irisin concentrations across heat loading are presented in Figure 4. The circulating irisin levels were significantly increased in both groups after caffeine intake and thermotherapy [Table 3, $P < 0.001$]. Compared to the resting state, the CAFF group showed $17.03 \pm 13.49\%$ increase in Pre-HL and a $58.02 \pm 25.89\%$ increase in Post-HL, whereas the rate of increase was $-0.27 \pm 8.23\%$ and $29.32 \pm 18.72\%$ in the CON group [Table 4, $P < 0.001$]. The CAFF group demonstrated a further 28.70% increase after thermotherapy, underscoring the potential of caffeine intake to amplify irisin production during thermogenic stimulation.

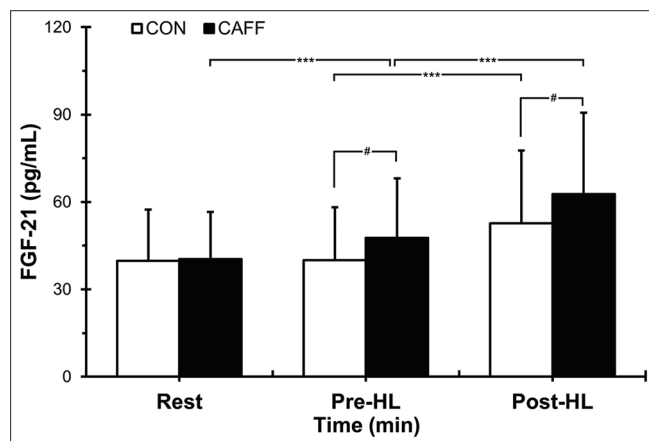


Figure 3: Changes in circulating fibroblast growth factor-21 concentrations before and after heat loading (HL) in the CON and CAFF groups. Values ($n = 87$) are presented as mean $\pm$ standard deviation *** $P < 0.001$ versus the previous point. # $P < 0.05$, between groups. CON (open bar), control subjects (placebo and 200 mL); CAFF (closed bar), caffeine ingestion subjects ($3 \text{ mg} \cdot \text{kg}^{-1}$ caffeine and 200 mL); Rest, 30 min before HL procedure; Pre-HL, right before HL; Post-HL, right after HL.

DISCUSSION

Body temperature is a fundamental physiological indicator of homeostasis. Maintaining an appropriate body temperature is essential for sustaining life, as prolonged exposure to heat stress increases the risk of fever, exhaustion, and fatigue.^[22] Symptoms of heat exhaustion typically appear at 38°C – 40°C .^[23] Thus, maintaining body temperature within an appropriate range and controlling homeostasis are important for maintaining physical activities.

Thermotherapy raising body temperature above the hypothalamic set point, activates the sympathetic nervous system and inducing physiological responses such as sweating, vasodilation, and hyperpnea. The preoptic area and anterior hypothalamus play a key role in this autonomic regulation.^[5] Similarly, cold exposure triggers sympathetic activation, leading to catecholamine release and β -adrenergic receptor signaling. This cascade increases cyclic adenosine monophosphate (cAMP) levels, activates the protein kinase A pathway, and enhances mitochondrial biogenesis. The resulting

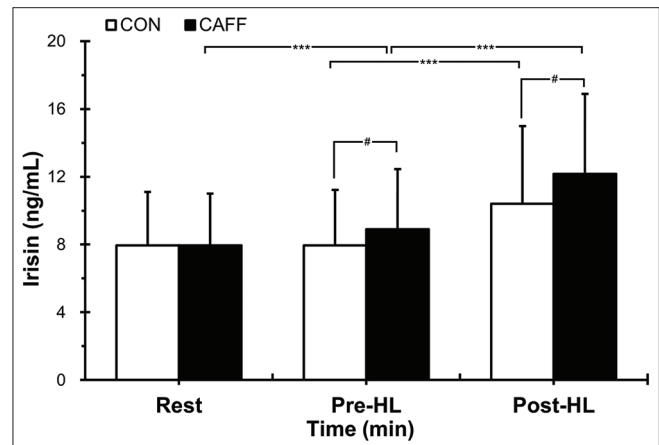


Figure 4: Changes in circulating irisin concentrations before and after heat loading (HL) in the CON and CAFF groups. Values ($n = 87$) are presented as mean $\pm$ standard deviation *** $P < 0.001$ versus the previous point. # $P < 0.05$, between groups. CON (open bar), control subjects (placebo and 200 mL); CAFF (closed bar), caffeine ingestion subjects ($3 \text{ mg} \cdot \text{kg}^{-1}$ caffeine and 200 mL); Rest, 30 min before HL procedure; Pre-HL, right before HL; Post-HL, right after HL.

Table 4: Rate of increase in fibroblast growth factor - 21 and irisin levels across heat loading

Analysis items	Groups	IR (%)		
		Rest-pre	Pre-post	Rest-post
FGF-21 (pg/mL)	CON	0.19 ± 3.38	29.23 ± 17.61	29.66 ± 19.57
	CAFF	$16.97 \pm 9.18^{+++}$	30.36 ± 9.52	$52.59 \pm 17.07^{+++}$
	<i>P</i>	< 0.001	0.356	< 0.001
Irisin (ng/mL)	CON	-0.27 ± 8.23	29.53 ± 14.11	29.32 ± 18.72
	CAFF	$17.03 \pm 13.49^{+++}$	$34.67 \pm 10.16^{+}$	$58.02 \pm 25.89^{+++}$
	<i>P</i>	< 0.001	0.027	< 0.001

⁺ $P < 0.05$ and ⁺⁺⁺ $P < 0.001$ indicate significant differences between the CON ($n = 44$) and CAFF ($n = 43$) groups at the corresponding rate of increase. Values ($n = 87$) are presented as mean $\pm$ SD. Exact *P* values for the comparison between the CON and CAFF groups are presented in the table. Rest: 30 min before HL, Pre: Right before HL, Post: Right after HL, FGF-21: Fibroblast growth factor 21, CON: Control subjects (placebo and 200 mL), CAFF: Caffeine ingestion subjects ($3 \text{ mg} \cdot \text{kg}^{-1}$ caffeine and 200 mL), SD: Standard deviation, HL: Heat loading procedure, FGF: Fibroblast growth factor, IR: Rate of increase

upregulation of uncoupling protein 1 promotes adipose tissue browning and thermogenesis.^[24]

FGF-21 is one of the peptides that can promote heat generation from brown fat, maintain energy homeostasis, increase the browning of white fat, affect energy expenditure increases, and affect metabolic homeostasis improvement.^[25-27] FGF-21 can also induce browning in subcutaneous white fat in cooperation with irisin, which is secreted by muscles as a result of tremors or exercise.^[28] Accelerated lipolysis can lead to excess fatty acids. As a result, lipolysis can lead to increased thermogenesis of BAT and induce genes related to oxidation and heat generation activity.^[28] In other words, the activation of FGF-21, due to thermotherapy and caffeine intake, significantly improved their heat generation ability compared to the control group. This suggests that thermotherapy can cause an increase in FGF-21, which is related to heat generation. It is effective in increasing body temperature and increasing antioxidant effects. In addition, this study observed a synergistic effect between thermotherapy and caffeine intake. When caffeine is consumed during thermal therapy, there is a sufficient combination effect [Figure 5].

Caffeine acts as an adenosine receptor antagonist, enhancing cholinergic activity in the central nervous system. It also inhibits phosphodiesterase, leading to an increase in cAMP levels, which in turn activates the sympathetic nervous system and promotes lipid metabolism.^[2,5,29] In this study, all subjects showed average body temperature and skin temperature

increases of more than 1°C after thermotherapy, but were higher in the CAFF group than in the CON group [Table 2]. These results are consistent with recent research trends. A previous study showed that the positive correlation between irisin and FGF-21 concentrations, along with thermotherapy and caffeine intake based on body temperature, involves factors that affect the amount of oxidative stress corresponding to changes in body temperature under the same thermal stimulation.^[14] Compared to the CON group, the CAFF group showed additional 22.93% and 28.70% increase in FGF-21 and irisin level after thermotherapy [Table 4]. It was found that caffeine intake was more effective when it was combined with thermotherapy. These findings suggest that caffeine exhibits biological similarities to thermotherapy and may exert a synergistic effect on the metabolism and browning of adipose tissue by promoting the additional release of FGF-21 and irisin.

This study was conducted exclusively on healthy adult Korean males, which limits the generalizability of the findings. Sweating response and thermoregulatory function are influenced by factors such as sex, age, body region, and climate.^[30] One study has reported sex-related differences in acetylcholine-mediated activation of the sweat gland.^[31] Considering these biological variations, studying a more diverse population could enhance the understanding of thermoregulatory mechanisms and broaden the applicability of the findings.

Recently, studies suggesting the health benefits of coffee are being presented. Gebeyehu and his team reported that Ethiopian coffee consumption altered lipid metabolism and may contribute to extending the lifespan of patients with cardiovascular disease.^[15] Another report suggested that coffee consumption has health-related effects for modern population especially with sedentary lifestyle.^[32]

Although caffeine has promising health benefits, much consideration is required before implementing caffeine intake for the general population. To increase the reliability of the study findings, future studies should include measurements targeting different hormones and systems in the body. The standardization of caffeine drinks is difficult; therefore, the variability of caffeine intake should be considered.

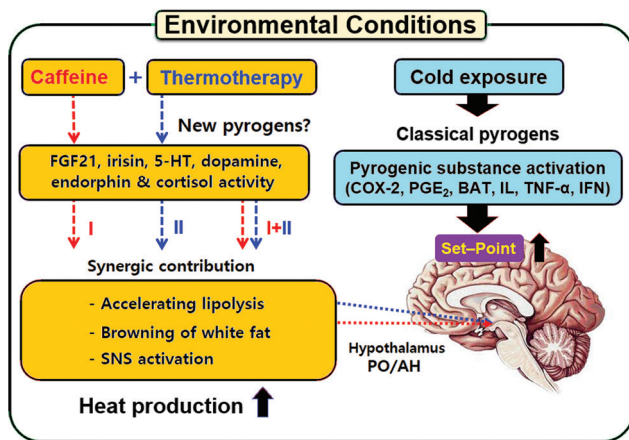


Figure 5: Background and hypothesis of the research scheme for febrile responses. Exposure to cold stimulates the activation of pyrogenic substances, leading to an increase in classical pyrogens such as COX-2 and PGE₂. These pyrogens adjust the hypothalamic set point, resulting in enhanced heat production in the human body. Caffeine intake (I, red dotted arrow) increased the concentrations of substances considered as new pyrogens, such as fibroblast growth factor-21 and irisin, and thermotherapy (II, blue dotted arrow) exhibited a similar effect. Therefore, caffeine may contribute to the increased production of new pyrogens when compared to classical thermotherapy alone. FGF21: Fibroblast growth factor 21; 5-HT: 5-hydroxytryptamine; COX-2: Cyclooxygenase 2; PGE₂: Prostaglandin E₂; BAT: Brown adipose tissue; IL: Interleukin; TNF-α: tumor necrosis factor alpha; IFN: Interferon; PO/AH: Preoptic area and anterior hypothalamus.

CONCLUSION

This study demonstrated that caffeine intake, in combination with thermotherapy, enhances thermogenic responses by increasing the levels of key biomarkers such as FGF-21 and irisin. These findings suggest that caffeine may not only help reduce fatigue but also enhance heat production, potentially contributing to thermoregulation-related interventions. Further research investigating the sustained effects of caffeine and thermotherapy, optimal dosing strategies, and influence on health conditions across different populations would help clarify the broader applicability of these interventions in clinical and physiological settings.

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Author contribution statement

All authors have contributed to this manuscript and confirm that they meet the authorship criteria established by the ICMJE. The manuscript has been read and approved by all the authors, Hye-Jin Lee: Conceptualization, Data curation, Formal analysis, Writing - original draft. Tae-Hwan Park: Data curation, Investigation, Writing - review & editing. Bah-Da Yun: Data curation. In-Ho Lee: Conceptualization, Data curation, Formal analysis, Writing - original draft. Mid-Eum Moon: Data curation. Sang-Hyeon Kim: Data curation, Formal analysis. Yi-Rang Lim: Data curation. Mun-Jeong Kim: Data curation, Investigation. Da-Jeong Bae: Data curation. Jin Kim: Data curation, Investigation. Young-Hyun Jung: Data curation, Investigation. Jeong-Beom Lee: Conceptualization, Investigation, Writing - review & editing.

Data availability statement

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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