Title

Circulating Fatty Acid Binding Protein 4 Concentration Increases with an Acute Maximal Exercise Independently of Exercise Training Status

Running title

Fatty acid binding protein 4 concentration during incremental maximal exercise

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Abstract

Limited data on the response of fatty acid binding protein 4 (FABP4) to acute exercise are available. The purpose of this study was to determine the response of FABP4 to incremental maximal exercise in exercise-trained and untrained men. Eleven exercise-trained young men (T group; age, 20.4 ± 1.2 years) and 9 untrained young men (UT group; age, 20.7 ± 0.5 years) performed an incremental maximal exercise after a 12-h fasting period. Before and immediately after the incremental maximal exercise, venous blood was drawn to measure circulating FABP4, free fatty acid, and glycerol concentrations. Body composition, and aerobic capacity were also assessed. Glycerol concentration significantly increased during the exercise in the T group (group $\times$ time interaction: $p = 0.034$; group effect, $p = 0.088$; and time effect, $p = 0.003$), and the percentage increase in glycerol concentration was greater in the T group than in the UT group ($p = 0.046$; ES(r): 0.45). Although circulating FABP4 concentration significantly increased in the two groups (group $\times$ time interaction: $p = 0.766$; group effect, $p = 0.114$; and time effect, $p = 0.001$), the percentage increase in FABP4 concentration was similar ($p = 0.210$; ES(r): 0.28). Additionally, the change of FABP4 concentration was not correlated with that of glycerol ($r = -0.04$, $p = 0.872$). These results indicated that circulating FABP4 concentration increased during incremental maximal exercise regardless of difference in lipolysis and exercise training status in healthy young men.

Keywords: lipolysis, acute exercise, training status

1. Introduction

The fatty acid binding protein (FABP) family regulates lipid trafficking and response in cells (Furuhashi, 2019). Fatty acid binding protein 4 (FABP4), also known as adipocyte FABP or adipose protein 2, belongs to the FABP family and is highly expressed in adipocytes and macrophages (Baxa et al., 1989; Hunt et al., 1986; Spiegelman et al., 1983). FABP4 is secreted mainly from adipocytes to the blood (Cao et al., 2013; Mita et al., 2015). FABP4 secretion is enhanced via lipolysis induced by catecholamines and natriuretic peptides (Lafontan et al., 2008; Mita et al., 2015). Moreover, circulating FABP4 clearance is regulated by the kidney, and circulating FABP4 is mainly eliminated from the circulation by the kidney (Furuhashi et al., 2011; Ishimura et al., 2013; Shrestha et al., 2018; Yeung et al., 2007).

Circulating FABP4 concentration has been known as marker of various diseases, such as atherosclerosis (Furuhashi et al., 2018; Yeung et al., 2007), insulin resistance (Xu et al., 2006), type 2 diabetes (Haluzik et al., 2009), hypertension (Ota et al., 2012; Xu et al., 2006), dyslipidemia (Furuhashi et al., 2016b; Xu et al., 2006), and cardiovascular diseases (Chow et al., 2013; von Eynatten et al., 2012). Exogenous FABP4 affects various physiological responses, such as modification of the inflammatory response (Makowski et al., 2005), enhancement of insulin and hepatic glucose production (Cao et al., 2013; Wu et al., 2014), and promotion of endothelial dysfunction (Furuhashi et al., 2016a) and breast cancer cell proliferation (Guaita-

Esteruelas et al., 2017). Thus, FABP4 is considered to be involved in the pathogenesis of metabolic disorders.

Exercise training has been well established to improve the metabolic disorders (Haskell et al., 2007). However, it remains unknown whether potential changes in circulating FABP4 concentrations induced by an acute exercise are dependent on exercise training status. In addition, there is also little information on the effect of maximal exercise load on circulating FABP4 concentrations. To the best of our knowledge, only one study has described the response of circulating FABP4 concentration to acute exercise (Iso et al., 2017). This previous study showed that low-intensity acute exercise (below anaerobic threshold [AT]) did not change the circulating FABP4 concentration, whereas moderate-intensity acute exercise (above AT) increased it in healthy young adults (Iso et al., 2017). Moreover, exercise-trained men have a greater whole-body lipolytic rate during high-intensity acute exercise than untrained men (Coggan et al., 2000; Klein et al., 1996). Based on these facts, we anticipated that the increase in circulating FABP4 concentrations during acute exercise would be dependent on exercise intensity, and that the increase in circulating FABP4 concentration would be higher in exercise-trained men than in untrained men. However, no studies have verified the issues listed above.

The present brief report demonstrated that the differences in the response of circulating FABP4 concentration during acute incremental maximal exercise in exercise-trained and untrained men. The data may be helpful in understanding the association between circulating

FABP4 and fat metabolism during acute exercise, and the effect of exercise training on the response of circulating FABP4 to acute exercise.

2. Materials and methods

2.1. Participants

Overall, 20 healthy men participated in this study: 11 exercise-trained individuals (exercise-trained group, T), and 9 untrained individuals (untrained group, UT). All participants were recruited from the undergraduate and graduate student populations using an advertisement. This study included individuals who had more than 1-year of aerobically competitive sports careers, and more than 3 strenuous exercise-training session per week. The effect of exercise training on lipolytic response has been demonstrated for training that consists of high-intensity exercise more than 3 times/week and has been performed for more than 12 weeks (Thompson et al., 2012). To ensure this effect, the abovementioned inclusion criteria were adopted. In fact, all the exercise-trained individuals belonged to intercollegiate middle-distance runners ($n = 4$), and soccer ($n = 7$) teams. Their competitive sports careers exceeded 3 years (range, 3–15 years). The ranges of training frequency, volume, and intensity (Borg's scale) were 6 session/week, 540-1800 min/week, and 15-17, respectively. None of the UT group had regular exercise training (< 3 sessions/week) for at least one year. None of the participants had a history of any metabolic or cardiovascular diseases. All participants were nonsmokers and were currently not

taking any medications, anabolic steroids, or other performance-enhancing drugs or supplements. The purpose, design, and risks of this study were explained to all participants, and each provided written informed consent. The study conformed to the principles outlined in the Helsinki Declaration and was approved by the ethics committee of the National Institute of Fitness and Sports in Kanoya (approval number: 11-94).

2.2. Study procedure

The participants reported to our laboratory. They completed the measurements of blood pressure, body composition, blood collection, and incremental maximal exercise test. They were instructed to refrain from intense physical activity for 24 h, and to consume carbohydrate at least 250 g the day prior to the measurements to replete glycogen for maximal exercise performance (Coggan et al., 2000). Additionally, they restricted food consumption except water for 12 h, because food consumption influences circulating FABP4 concentration (Ciardi et al., 2010; Mita et al., 2015).

2.3. Anthropometry and body composition

For each participant, height was measured to the nearest 0.1 cm using a stadiometer. Weight, fat mass (FM), fat-free mass (FFM), and skeletal muscle mass (SMM) were measured to the nearest 0.1 kg using a dual frequency body composition monitor (impedance method) (RD-800; Tanita Corp., Tokyo, Japan). These measurements were performed prior to blood collection and incremental maximal exercise. Body mass index (BMI) was calculated as the

weight in kilograms divided by the square of the height in meters.

2.4. Blood pressure

Systolic and diastolic blood pressures were measured using an automatic sphygmomanometer (HEM-1040, Omron Corp., Kyoto, Japan) after the participants rested in a sitting position for 5 min.

2.5. Incremental maximal exercise test

An incremental maximal exercise protocol consisted of 30-watt (W) increases every 2 min at 60 W until 6 min and thereafter 30-W increases every 1 min until exhaustion after a brief individual warm-up period on a cycle ergometer (Aerobike 75XLIII, Konami Sports Life, Kanagawa, Japan). During the test, ventilation and gas exchange were measured using indirect calorimetry (K4b2, COSMED; Rome, Italy). Peak oxygen uptake ($\dot{V}O_{2peak}$) was determined using this test. The criteria for achieving $\dot{V}O_{2peak}$ were as follows: (1) $\dot{V}O_2$ plateaued despite increasing exercise intensity (<100 mL O_2 /min), (2) the highest respiratory exchange ratio during the final stage of the incremental exercise was >1.10 , and (3) the highest heart rate (HR) measured at the end of exercise was $>90\%$ of the predicted maximal HR ($220 - \text{age} [\text{years}]$). The highest $\dot{V}O_2$ achieved over 30 s was determined as the $\dot{V}O_{2peak}$. Before and immediately after this test, blood samples were collected from the antecubital vein.

2.6. Blood sampling and analysis

Blood samples were collected into 9- and 2-mL tubes containing thrombin before and

immediately after the incremental maximal exercise, respectively. These were centrifuged at 3,000 g for 10 min at room temperature after 30 min of collection. After centrifugation, the serum samples were transferred into a plastic tube and immediately stored at -80 °C until further analysis. Hemoglobin and hematocrit values were measured to assess plasma volume changes (Dill and Costill, 1974).

The serum creatinine concentration was measured according to the endogenous creatinine elimination reaction method (KANTO CHEMICAL Corporation, Tokyo, Japan). The estimated glomerular filtration rate (eGFR) was calculated using the equation for Japanese adult men ($194 \times \text{creatinine concentration [mg/dl]}^{-1.094} \times \text{age [years]}^{-0.287}$). The serum free fatty acid (FFA) concentration was measured using an enzymatic method (LabAssay NEFA, FUJIFILM Wako Pure Chemical Corp., Osaka, Japan). The serum glycerol concentration was analyzed via a coupled enzymatic reaction (Glycerol Colorimetric Assay Kit, Cayman Chemical, MI, USA). The serum FABP4 concentration was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine ELISA DFBP40, R&D Systems Inc., MN, USA). To eliminate inter-assay variation, samples from each participant were analyzed in the same run.

2.7. Statistical analysis

All data are presented as mean $\pm$ standard deviation. Sample size was calculated to detect a large effect ($f = 0.40$). It was determined that an estimated total sample size of 16 would be

required to have approximately an 80% power needed to detect a large effect at 0.05 significance. The Kolmogorov–Smirnov test and Levene tests were used to confirm normality and homoscedasticity, respectively. FFA, glycerol, and FABP4 concentrations were log transformed. An unpaired t-test was used to compare differences in anthropometry, blood pressure, and body composition between the T and UT groups. Two-way repeated-measures analysis of variance (group $\times$ time) was used to compare changes in blood parameters between the groups over time. Moreover, the Wilcoxon signed-rank test was used to determine %change in blood parameters between the groups over time. The Mann–Whitney U test was performed to determine differences in %change in blood parameters between the groups at a specific point in time. The effect size was calculated using Cohen’s d (ES(d): small, ≥ 0.20 ; medium, ≥ 0.50 ; or large, ≥ 0.80) for comparison of anthropometry, blood pressure, and body composition between the groups. Moreover, the effect size was calculated using r (ES(r): small, ≥ 0.10 ; medium, ≥ 0.30 ; or large, ≥ 0.50) for %change in blood parameters in each group. The Pearson’s correlation coefficients between changes in blood parameters were calculated for all participants. In addition, the Spearman rank correlation coefficients between changes in blood parameters were calculated for each T and UT group. Statistical analyses were performed using SPSS version 24 software (IBM Corporation, Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Participant characteristics

The characteristics of the participants in the T and UT groups are shown in Table 1. The %fat ($p = 0.021$, $ES(d)$: 1.19) differed significantly between the two groups. FM, FFM, and SMM were not significant, but ESs indicated large effects ($ES(d)$: 0.93-1.19).

3.2. Parameters of the incremental maximal exercise test

The parameters of incremental maximal exercise in the T and UT groups are shown in Table 2. Although maximal HR did not differ ($p = 0.28$), exercise duration ($p < 0.001$, $ES(d)$: 2.60) and maximal load ($p < 0.001$, $ES(d)$: 2.62) were significantly higher in the T group than in the UT group. The T group had a greater $\dot{V}O_{2peak}$ than the UT group ($p < 0.001$, $ES(d)$: 3.69).

3.3. Kinetics of blood parameters during incremental exercise

As changes in plasma volume during the exercise were small, blood parameters were not adjusted. Changes in plasma volume did not differ between the T and UT groups ($p > 0.05$). The kinetics and %changes of blood parameters during incremental maximal exercise in the T and UT groups are shown in Table 3 and Fig. 1, respectively. No significant group $\times$ time interaction and main effects were observed in FFA concentration (interaction, $p = 0.180$; group effect, $p = 0.840$; and time effect, $p = 0.129$). The %change in FFA concentration also did not differ significantly between the T and UT groups ($p = 0.370$). There were significant group $\times$

time interaction and time effect in glycerol concentration (interaction, $p = 0.034$; group effect, $p = 0.088$; and time effect, $p = 0.003$). The %change in glycerol concentration increased significantly in the T group (T group, $p < 0.004$ [ES(r): 0.86], and UT group, $p = 0.314$ [ES(r): 0.34], respectively), and the increment was significantly greater in the T group than in the UT group ($p = 0.046$, ES(r): 0.45). Significant time ($p < 0.001$) effects in FABP4 concentration were observed, and the %change in FABP4 concentration increased in both the groups (T group, $p < 0.001$ [ES(r): 0.88], and UT group, $p < 0.001$ [ES(r): 0.89], respectively). However, the increment in FABP4 concentration was similar between the T and UT groups ($p = 0.230$, ES(r): 0.28).

3.4. Correlation between FABP4 concentration, $\dot{V}O_{2peak}$, and blood parameters

In all participants, FABP4 concentration was not significantly correlated with $\dot{V}O_{2peak}$ ($r = 0.30$, $p = 0.199$). Moreover, Δ FABP4 concentration (immediately after exercise concentration – resting concentration) was not significantly correlated with Δ FFA ($r = 0.17$, $p = 0.480$), Δ glycerol ($r = -0.04$, $p = 0.872$), and eGFR ($r = -0.35$, $p = 0.135$). In each the T and UT group, there were no significant correlations between Δ FABP4 concentration, Δ FFA, Δ glycerol, and eGFR (Table 4).

4. Discussion

To the best of our knowledge, the present study is the first to investigate the response of

1 circulating FABP4 concentration to an incremental maximal exercise in exercise-trained and
2 untrained men. Our data demonstrated that despite that exercise-trained men had a greater
3 lipolysis during the incremental maximal exercise, circulating FABP4 concentration increased
4 similarly during this exercise in both exercise-trained and untrained men. Moreover, the change
5 in circulating FABP4 concentration during the incremental maximal exercise was not correlated
6 with that of FFA and glycerol. These findings suggest that circulating FABP4 concentration
7 increases during high-intensity acute exercise regardless of the exercise training status, and the
8 increase in circulating FABP4 concentration during high-intensity acute exercise is
9 accompanied by the increases in blood level of glycerol in the trained but not in the untrained
10 men.

11 A previous study reported that despite no response of circulating FABP4 concentration
12 during low-intensity acute constant-load exercise, circulating FABP4 concentration increased
13 during moderate-intensity acute constant-load exercise in healthy young individuals (Iso et al.,
14 2017). Our results also indicated that circulating FABP4 concentration increased during acute
15 incremental maximal exercise in both exercise-trained and untrained men. Additionally, an
16 increase in circulating FABP4 concentration was observed in all participants. Thus, high-
17 intensity acute exercise is more likely to increase circulating FABP4 concentration in healthy
18 young individuals. Our findings provide further evidence of the response of circulating FABP4
19 concentration to acute exercise.

The response of glycerol and FFA concentration during short high-intensity exercise is not necessarily parallel in exercise-trained and untrained men. FFA concentration during short high-intensity exercise tends to decrease in both exercise-trained and untrained men, and the changes in FFA concentration do not differ between exercise-trained and untrained men (Bloom et al., 1976; Coggan et al., 2000; Romijn et al., 1993). However, our data do not support this tendency, and FFA concentration during acute maximal exercise did not change in either exercise-trained or untrained men. FFA concentration during exercise represents the balance between its release from adipocytes and uptake by working skeletal muscle. In the present study, it is likely that FFA release from adipocytes and FFA uptake by working skeletal muscle were equivalent during the maximal exercise. In contrast, the difference in glycerol concentration during short high-intensity exercise appears to be apparent. The glycerol concentration during short high-intensity exercise increased in exercise-trained men, whereas it did not change in untrained men (Bloom et al., 1976; Coggan et al., 2000). Our data is in agreement with the results of the previous studies (Bloom et al., 1976; Coggan et al., 2000). This suggests that lipolytic response during short high-intensity exercise is higher in exercise-trained than in untrained men.

FABP4 is released from adipocytes into the blood by lipolysis activation via phosphorylation of adipose triglyceride, hormone-sensitive, and monoacylglycerol lipases through mainly β -adrenergic receptor-mediated adenylyl cyclase–protein kinase A (AC–PKA)

1 (Cao et al., 2013; Ertunc et al., 2015; Mita et al., 2015) and natriuretic peptide receptor-A-
2 mediated guanylyl cyclase–protein kinase (GC–PKG) pathways (Mita et al., 2015). FABP4 also
3 interacts with hormone-sensitive lipase to regulate these pathways (Coe et al., 1999; Scheja et
4 al., 1999; Shen et al., 1999). Moreover, FABP4 may transport fatty acids produced by lipolysis
5 to utilize inside and/or outside cells (Furuhashi et al., 2014). Because acute exercise accelerates
6 lipolysis through the AC–PKA and GC–PKG pathways (Arner et al., 1990; Lafontan et al.,
7 2008), we anticipated that circulating FABP4 concentration would increase during acute
8 exercise. In addition, the lipolytic response to acute exercise is higher in exercise-trained men
9 than in untrained men (Coggan et al., 2000; Klein et al., 1996) even during a high-intensity
10 exercise (Coggan et al., 2000) or maximal exercise (Bloom et al., 1976), because exercise
11 training induces a higher β -adrenergic responsiveness of catecholamines (Moro et al., 2005;
12 Stich et al., 2000), a lower α_2 -antilipolytic action of catecholamines (De Glisezinski et al.,
13 2001), and high sensitivity to ANP (Moro et al., 2005). Moreover, although blood flow in
14 adipose tissue is expected to decrease during high-intensity exercise due to catecholamine-
15 induced vasoconstriction of adipose tissue (Heinonen et al., 2013), blood flow in adipose tissue
16 in response to physiological epinephrine infusion is higher in exercise-trained than in untrained
17 men (Stallknecht et al., 1995). Thus, we expected that the exercise-trained men would exhibit
18 a greater increase in glycerol and FABP4 concentrations after a acute maximal exercise
19 compared to the untrained men. In the present study, glycerol concentration and %change in

glycerol concentration increased during the incremental maximal exercise in exercise-trained men, whereas FABP4 concentration during the incremental maximal exercise increased similarly in both the exercise-trained and untrained men. Additionally, no correlations between the change in FABP4 and glycerol concentration was observed in either exercise-trained or untrained men, which supports the findings of a previous study (Iso et al., 2017). Thus, it is likely that the response of FABP4 concentration to maximal exercise cannot necessarily be explained by lipolysis activation estimated from blood glycerol concentration.

FABP4 is expressed in macrophages as well as adipocytes (Makowski et al., 2001; Shum et al., 2006), and is also secreted by macrophages into the circulation. Although the expression of FABP4 has been shown to be lower in macrophages than in adipocytes (Makowski et al., 2001; Shum et al., 2006), the effect of maximal exercise on circulating FABP4 via macrophages should also be taken into account. The functions of macrophage are partly enhanced after acute exhaustive exercise (Woods, 2000). However, the acute exercise-induced effects on macrophage functions are somewhat attenuated after exercise training (Woods, 2000). Thus, enhancement of macrophage functions may be attenuated after maximal exercise in exercise-trained men compared to that in untrained men. This suggests that suppression of macrophage functions leads to attenuation of the secretion of FABP4 from macrophages in exercise-trained men. However, lack of the data specified for macrophages do not allow us a deeper discussion regarding their roles in FABP4 release during acute exercise. Further studies are required to

1 identify the origin of circulating FABP4 during increment maximal exercise.

2   In the present study, the incremental maximal exercise protocol was adopted. The
3 relative intensity at the end of the maximal exercise was considered to be similar, because the
4 HR at the end of the maximal exercise did not differ between the exercise-trained and untrained
5 men. Meanwhile, exercise duration differed between exercise-trained and untrained men. This
6 difference in exercise duration is not surprising, and it reflects training status. Nevertheless, the
7 difference in exercise duration (approximately 3 min) may influence catecholamines secretion
8 at the end of maximal exercise. Acute exercise-induced changes in circulating catecholamines
9 are not only strongly associated with the response of FABP4 concentration to acute exercise
10 (Iso et al., 2017), but also influence lipolytic response (Arner et al., 1990; Lafontan et al., 2008),
11 adipose tissue blood flow (Heinonen et al., 2013), and macrophage functions (Suzuki et al.,
12 2020; Ortega, 2003), which promote the secretion of FABP4 into the circulation. Unfortunately,
13 although catecholamine concentrations were not measured in the present study, one study has
14 reported the difference in catecholamine secretion between trained and untrained men during
15 incremental maximal exercise (Lehmann et al., 1984). A previous study reported that the
16 secretion of catecholamines was similar between trained and untrained men during the
17 incremental exercise, despite differences in exercise duration (approximately 4 min) (Lehmann
18 et al., 1984). Based on this fact, catecholamine concentration might not differ between trained
19 and untrained men during the incremental maximal exercise. It was speculated that the

1 difference in exercise duration of maximal exercise had little influence on the difference in
2 catecholamine secretion between exercise-trained and untrained men. It is likely that the effect
3 of catecholamine secretion during maximal exercise on the response of circulating FABP4
4 concentration was minimal.

5 As FABP4 may be eliminated from the blood by renal clearance (Furuhashi et al., 2011;
6 Ishimura et al., 2013; Shrestha et al., 2018; Yeung et al., 2007), differences in the renal function
7 may influence the response of circulating FABP4 concentration during acute exercise. However,
8 creatinine and eGFR did not differ between exercise-trained and untrained men. Moreover, they
9 were not correlated with the parameters of circulating FABP4 concentration. Therefore, the
10 renal function could not necessarily be involved in the response of circulating FABP4
11 concentration during acute incremental maximal exercise.

12 Circulating FABP4 concentration has been known as biomarker of metabolic disorders
13 (Furuhashi, 2019). However, physiological roles of increase in circulating FABP4
14 concentration during acute exercise remain unclear. Because the increase in circulating FABP4
15 concentration induced by acute exercise almost return to baseline at post-exercise (Iso et al.,
16 2017), it seems to be a physiological process of metabolic response rather than an adverse
17 response. In fact, Iso et al. (2017) discussed that circulating FABP4 concentration could be
18 surrogates for exercise-induced generalized sympathetic nervous system activation. Further
19 studies are warranted to elucidate the physiological roles of the response of circulating FABP4

concentration to acute exercise.

The present study has several limitations. First, the participants were healthy young men; therefore, our findings may not be applicable to women, adolescents and older adults with or without diseases. Second, the control trial was not assigned. The responses of FABP4 concentration during resting could not be observed. However, the circulating FABP4 concentration did not change during the 6-h post-absorptive state in a previous study, because of no change in insulin and FFA concentration (Ciardi et al., 2010). Thus, we believe that circulating FABP4 concentration is unlikely to change during approximately 10 min of resting. Third, the post-exercise responses of circulating FABP4 concentration could not be observed. The increase in circulating FABP4 concentration during acute moderate-intensity exercise almost return to baseline after 10 min after the end of exercise in young adults (Iso et al., 2017). However, the post-exercise responses of circulating FABP4 concentration to an acute maximal exercise is unclear. Moreover, it is not to be rule out that the post-exercise responses of circulating FABP4 concentration to an acute exercise differ between trained and untrained men.

5. Conclusion

Our study indicated that despite glycerol concentration increased in exercise-trained men, circulating FABP4 concentration increased during incremental maximal exercise in both exercise-trained and untrained men. These suggest that circulating FABP4 concentration

increased during incremental maximal exercise regardless of the exercise training status in healthy young men. Moreover, the increased circulating FABP4 concentration seems not to be attributed to the increased lipolysis during incremental maximal exercise. Further studies regarding the response of circulating FABP4 concentration to long-duration and low-to-moderate-intensity acute exercise are required to clarify the physiological roles of circulating FABP4 during exercise.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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References

- Arner, P., Kriegholm, E., Engfeldt, P., and Bolinder, J. (1990). Adrenergic regulation of lipolysis in situ at rest and during exercise. *J. Clin. Invest.*, 85: 893-898.
- Baxa, C. A., Sha, R. S., Buelt, M. K., Smith, A. J., Matarese, V., Chinander, L. L., Boundy, K. L., and Bernlohr, D. A. (1989). Human adipocyte lipid-binding protein: purification of the protein and cloning of its complementary DNA. *Biochemistry*, 28: 8683-8690.
- Bloom, S. R., Johnson, R. H., Park, D. M., Rennie, M. J., and Sulaiman, W. R. (1976). Differences in the metabolic and hormonal response to exercise between racing cyclists and untrained individuals. *J. Physiol.*, 258: 1-18.
- Cao, H., Sekiya, M., Ertunc, M. E., Burak, M. F., Mayers, J. R., White, A., Inouye, K., Rickey, L. M., Ercal, B. C., Furuhashi, M., Tuncman, G., and Hotamisligil, G. S. (2013). Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metab.*, 17: 768-778.
- Chow, W. S., Tso, A. W., Xu, A., Yuen, M. M., Fong, C. H., Lam, T. H., Lo, S. V., Tse, H. F., Woo, Y. C., Yeung, C. Y., Cheung, B. M., and Lam, K. S. (2013). Elevated circulating adipocyte-fatty acid binding protein levels predict incident cardiovascular events in a community-based cohort: a 12-year prospective study. *J. Am. Heart Assoc.*, 2: e004176.
- Ciardi, C., Tatarczyk, T., Tschoner, A., Kranebitter, M., Niederwanger, A., Ebenbichler, C. F., Patsch, J. R., and Pedrini, M. T. (2010). Effect of postprandial lipemia on plasma concentrations of A-FABP, RBP-4 and visfatin. *Nutr. Metab. Cardiovasc. Dis.*, 20: 662-668.
- Coe, N. R., Simpson, M. A., and Bernlohr, D. A. (1999). Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J. Lipid Res.*, 40: 967-972.
- Coggan, A. R., Raguso, C. A., Gastaldelli, A., Sidossis, L. S., and Yeckel, C. W. (2000). Fat metabolism during high-intensity exercise in endurance-trained and untrained men. *Metabolism*, 49: 122-128.
- De Glisezinski, I., Marion-Latard, F., Crampes, F., Berlan, M., Hejnova, J., Cottet-Emard, J. M., Stich, V., and Riviere, D. (2001). Lack of alpha(2)-adrenergic antilipolytic effect during exercise in subcutaneous adipose tissue of trained men. *J. Appl. Physiol.*, 91: 1760-1765.
- Dill, D. B., and Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J. Appl. Physiol.*, 37: 247-248.
- Ertunc, M. E., Sikkeland, J., Fenaroli, F., Griffiths, G., Daniels, M. P., Cao, H., Saatcioglu, F., and Hotamisligil, G. S. (2015). Secretion of fatty acid binding protein aP2 from adipocytes through a nonclassical pathway in response to adipocyte lipase activity. *J. Lipid Res.*, 56: 423-434.
- Furuhashi, M. (2019). Fatty Acid-Binding Protein 4 in Cardiovascular and Metabolic Diseases. *J. Atheroscler. Thromb.*, 26: 216-232.

- 1 Furuhashi, M., Fuseya, T., Murata, M., Hoshina, K., Ishimura, S., Mita, T., Watanabe, Y., Omori,
2 A., Matsumoto, M., Sugaya, T., Oikawa, T., Nishida, J., Kokubu, N., Tanaka, M.,
3 Moniwa, N., Yoshida, H., Sawada, N., Shimamoto, K., and Miura, T. (2016a). Local
4 Production of Fatty Acid-Binding Protein 4 in Epicardial/Perivascular Fat and
5 Macrophages Is Linked to Coronary Atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.*,
6 36: 825-834.
- 7 Furuhashi, M., Ishimura, S., Ota, H., Hayashi, M., Nishitani, T., Tanaka, M., Yoshida, H.,
8 Shimamoto, K., Hotamisligil, G. S., and Miura, T. (2011). Serum fatty acid-binding
9 protein 4 is a predictor of cardiovascular events in end-stage renal disease. *PLoS One*,
10 6: e27356.
- 11 Furuhashi, M., Omori, A., Matsumoto, M., Kataoka, Y., Tanaka, M., Moniwa, N., Ohnishi, H.,
12 Yoshida, H., Saitoh, S., Shimamoto, K., and Miura, T. (2016b). Independent Link
13 Between Levels of Proprotein Convertase Subtilisin/Kexin Type 9 and FABP4 in a
14 General Population Without Medication. *Am. J. Cardiol.*, 118: 198-203.
- 15 Furuhashi, M., Saitoh, S., Shimamoto, K., and Miura, T. (2014). Fatty Acid-Binding Protein 4
16 (FABP4): Pathophysiological Insights and Potent Clinical Biomarker of Metabolic and
17 Cardiovascular Diseases. *Clin. Med. Insights Cardiol.*, 8: 23-33.
- 18 Furuhashi, M., Yuda, S., Muranaka, A., Kawamukai, M., Matsumoto, M., Tanaka, M., Moniwa,
19 N., Ohnishi, H., Saitoh, S., Shimamoto, K., and Miura, T. (2018). Circulating Fatty
20 Acid-Binding Protein 4 Concentration Predicts the Progression of Carotid
21 Atherosclerosis in a General Population Without Medication. *Circ. J.*, 82: 1121-1129.
- 22 Guaita-Esteruelas, S., Bosquet, A., Saavedra, P., Guma, J., Girona, J., Lam, E. W., Amillano,
23 K., Borrás, J., and Masana, L. (2017). Exogenous FABP4 increases breast cancer cell
24 proliferation and activates the expression of fatty acid transport proteins. *Mol. Carcinog.*,
25 56: 208-217.
- 26 Haluzik, M. M., Anderlova, K., Dolezalova, R., Adamikova, A., Haluzikova, D., Housova, J.,
27 Svacina, S., and Haluzik, M. (2009). Serum adipocyte fatty acid binding protein levels
28 in patients with type 2 diabetes mellitus and obesity: the influence of fenofibrate
29 treatment. *Physiol. Res.*, 58: 93-99.
- 30 Haskell, W. L., Lee, I. M., Pate, R. R., Powell, K. E., Blair, S. N., Franklin, B. A., Macera, C.
31 A., Heath, G. W., Thompson, P. D., and Bauman, A. (2007). Physical activity and public
32 health: updated recommendation for adults from the American College of Sports
33 Medicine and the American Heart Association. *Circulation*, 116: 1081-1093.
- 34 Heinonen, I., Wendelin-Saarenhovi, M., Kaskinoro, K., Knuuti, J., Scheinin, M., and
35 Kalliokoski, K. K. (2013). Inhibition of alpha-adrenergic tone disturbs the distribution
36 of blood flow in the exercising human limb. *Am. J. Physiol. Heart Circ. Physiol.*, 305:
37 H163-172.
- 38 Hunt, C. R., Ro, J. H., Dobson, D. E., Min, H. Y., and Spiegelman, B. M. (1986). Adipocyte P2
39 gene: developmental expression and homology of 5'-flanking sequences among fat cell-

- specific genes. *Proc. Natl. Acad. Sci. U. S. A.*, 83: 3786-3790.
- Ishimura, S., Furuhashi, M., Watanabe, Y., Hoshina, K., Fuseya, T., Mita, T., Okazaki, Y., Koyama, M., Tanaka, M., Akasaka, H., Ohnishi, H., Yoshida, H., Saitoh, S., and Miura, T. (2013). Circulating levels of fatty acid-binding protein family and metabolic phenotype in the general population. *PLoS One*, 8: e81318.
- Iso, T., Sunaga, H., Matsui, H., Kasama, S., Oshima, N., Haruyama, H., Furukawa, N., Nakajima, K., Machida, T., Murakami, M., Yokoyama, T., and Kurabayashi, M. (2017). Serum levels of fatty acid binding protein 4 and fat metabolic markers in relation to catecholamines following exercise. *Clin. Biochem.*, 50: 896-902.
- Klein, S., Weber, J. M., Coyle, E. F., and Wolfe, R. R. (1996). Effect of endurance training on glycerol kinetics during strenuous exercise in humans. *Metabolism*, 45: 357-361.
- Lafontan, M., Moro, C., Berlan, M., Crampes, F., Sengenès, C., and Galitzky, J. (2008). Control of lipolysis by natriuretic peptides and cyclic GMP. *Trends Endocrinol. Metab.*, 19: 130-137.
- Lehmann, M., Dickhuth, H. H., Schmid, P., Porzig, H., and Keul, J. (1984). Plasma catecholamines, beta-adrenergic receptors, and isoproterenol sensitivity in endurance trained and non-endurance trained volunteers. *Eur. J. Appl. Physiol. Occup. Physiol.*, 52: 362-369.
- Makowski, L., Boord, J. B., Maeda, K., Babaev, V. R., Uysal, K. T., Morgan, M. A., Parker, R. A., Suttles, J., Fazio, S., Hotamisligil, G. S., and Linton, M. F. (2001). Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. *Nat. Med.*, 7: 699-705.
- Makowski, L., Brittingham, K. C., Reynolds, J. M., Suttles, J., and Hotamisligil, G. S. (2005). The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor gamma and IkappaB kinase activities. *J. Biol. Chem.*, 280: 12888-12895.
- Mita, T., Furuhashi, M., Hiramitsu, S., Ishii, J., Hoshina, K., Ishimura, S., Fuseya, T., Watanabe, Y., Tanaka, M., Ohno, K., Akasaka, H., Ohnishi, H., Yoshida, H., Saitoh, S., Shimamoto, K., and Miura, T. (2015). FABP4 is secreted from adipocytes by adenylyl cyclase-PKA- and guanylyl cyclase-PKG-dependent lipolytic mechanisms. *Obesity (Silver Spring)*, 23: 359-367.
- Moro, C., Pillard, F., De Glisezinski, I., Harant, I., Riviere, D., Stich, V., Lafontan, M., Crampes, F., and Berlan, M. (2005). Training enhances ANP lipid-mobilizing action in adipose tissue of overweight men. *Med. Sci. Sports Exerc.*, 37: 1126-1132.
- Ortega, E. (2003). Neuroendocrine mediators in the modulation of phagocytosis by exercise: physiological implications. *Exerc. Immunol. Rev.*, 9: 70-93.
- Ota, H., Furuhashi, M., Ishimura, S., Koyama, M., Okazaki, Y., Mita, T., Fuseya, T., Yamashita, T., Tanaka, M., Yoshida, H., Shimamoto, K., and Miura, T. (2012). Elevation of fatty

- 1 acid-binding protein 4 is predisposed by family history of hypertension and contributes
- 2 to blood pressure elevation. *Am. J. Hypertens.*, 25: 1124-1130.
- 3 Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E., and Wolfe,
- 4 R. R. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to
- 5 exercise intensity and duration. *Am. J. Physiol.*, 265: E380-391.
- 6 Scheja, L., Makowski, L., Uysal, K. T., Wiesbrock, S. M., Shimshek, D. R., Meyers, D. S.,
- 7 Morgan, M., Parker, R. A., and Hotamisligil, G. S. (1999). Altered insulin secretion
- 8 associated with reduced lipolytic efficiency in aP2^{-/-} mice. *Diabetes*, 48: 1987-1994.
- 9 Shen, W. J., Sridhar, K., Bernlohr, D. A., and Kraemer, F. B. (1999). Interaction of rat hormone-
- 10 sensitive lipase with adipocyte lipid-binding protein. *Proc. Natl. Acad. Sci. U. S. A.*, 96:
- 11 5528-5532.
- 12 Shrestha, S., Sunaga, H., Hanaoka, H., Yamaguchi, A., Kuwahara, S., Umbarawan, Y.,
- 13 Nakajima, K., Machida, T., Murakami, M., Saito, A., Tsushima, Y., Kurabayashi, M.,
- 14 and Iso, T. (2018). Circulating FABP4 is eliminated by the kidney via glomerular
- 15 filtration followed by megalin-mediated reabsorption. *Sci. Rep.*, 8: 16451.
- 16 Shum, B. O., Mackay, C. R., Gorgun, C. Z., Frost, M. J., Kumar, R. K., Hotamisligil, G. S., and
- 17 Rolph, M. S. (2006). The adipocyte fatty acid-binding protein aP2 is required in allergic
- 18 airway inflammation. *J. Clin. Invest.*, 116: 2183-2192.
- 19 Spiegelman, B. M., Frank, M., and Green, H. (1983). Molecular cloning of mRNA from 3T3
- 20 adipocytes. Regulation of mRNA content for glycerophosphate dehydrogenase and
- 21 other differentiation-dependent proteins during adipocyte development. *J. Biol. Chem.*,
- 22 258: 10083-10089.
- 23 Stallknecht, B., Simonsen, L., Bulow, J., Vinten, J., and Galbo, H. (1995). Effect of training on
- 24 epinephrine-stimulated lipolysis determined by microdialysis in human adipose tissue.
- 25 *Am. J. Physiol.*, 269: E1059-1066.
- 26 Stich, V., de Glisezinski, I., Berlan, M., Bulow, J., Galitzky, J., Harant, I., Suljkovicova, H.,
- 27 Lafontan, M., Riviere, D., and Crampes, F. (2000). Adipose tissue lipolysis is increased
- 28 during a repeated bout of aerobic exercise. *J. Appl. Physiol.*, 88: 1277-1283.
- 29 Suzuki, K., Tominaga, T., Ruhee, R. T., and Ma, S. (2020). Characterization and Modulation of
- 30 Systemic Inflammatory Response to Exhaustive Exercise in Relation to Oxidative
- 31 Stress. *Antioxidants (Basel)*, 9.
- 32 Thompson, D., Karpe, F., Lafontan, M., and Frayn, K. (2012). Physical activity and exercise in
- 33 the regulation of human adipose tissue physiology. *Physiol. Rev.*, 92: 157-191.
- 34 von Eynatten, M., Breitling, L. P., Roos, M., Baumann, M., Rothenbacher, D., and Brenner, H.
- 35 (2012). Circulating adipocyte fatty acid-binding protein levels and cardiovascular
- 36 morbidity and mortality in patients with coronary heart disease: a 10-year prospective
- 37 study. *Arterioscler. Thromb. Vasc. Biol.*, 32: 2327-2335.
- 38 Woods, J. A. (2000). Exercise and neuroendocrine modulation of macrophage function. *Int. J.*
- 39 *Sports Med.*, 21 Suppl 1: S24-30.

- 1 Wu, L. E., Samocha-Bonet, D., Whitworth, P. T., Fazakerley, D. J., Turner, N., Biden, T. J.,
2 James, D. E., and Cantley, J. (2014). Identification of fatty acid binding protein 4 as an
3 adipokine that regulates insulin secretion during obesity. *Mol. Metab.*, 3: 465-473.
- 4 Xu, A., Wang, Y., Xu, J. Y., Stejskal, D., Tam, S., Zhang, J., Wat, N. M., Wong, W. K., and Lam,
5 K. S. (2006). Adipocyte fatty acid-binding protein is a plasma biomarker closely
6 associated with obesity and metabolic syndrome. *Clin. Chem.*, 52: 405-413.
- 7 Yeung, D. C., Xu, A., Cheung, C. W., Wat, N. M., Yau, M. H., Fong, C. H., Chau, M. T., and
8 Lam, K. S. (2007). Serum adipocyte fatty acid-binding protein levels were
9 independently associated with carotid atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.*,
10 27: 1796-1802.

Table 1. Characteristics of participants

	Trained group (n = 11)			Untrained group (n = 9)			<i>p</i> value	ES
Age (years)	20.4	±	1.2	20.7	±	0.5	0.461	0.33
Height (cm)	175.0	±	5.9	170.6	±	4.9	0.091	0.85
Weight (kg)	66.4	±	6.8	64.2	±	5.2	0.433	0.03
BMI (kg/m ²)	21.6	±	1.5	22.1	±	1.5	0.545	0.35
%fat (%)	12.5	±	4.3	17.2	±	4.0	0.021	1.19
Fat mass (kg)	8.3	±	3.2	11.1	±	2.9	0.058	0.96
Fat free mass (kg)	58.1	±	6.3	53.1	±	4.1	0.055	0.97
Skeletal muscle mass (kg)	55.4	±	6.6	50.5	±	3.9	0.062	0.93
Systolic blood pressure (mmHg)	113.1	±	6.6	118.1	±	10.7	0.215	0.61
Diastolic blood pressure (mmHg)	67.7	±	9.1	74.3	±	11.7	0.171	0.67
Creatinine (mg/dl)	0.75	±	0.11	0.78	±	0.07	0.413	0.34
eGFR (ml/min/1.73m ²)	115.5	±	19.9	107.4	±	10.6	0.258	0.52

Value are presented as mean ± SD. ES, effect size; BMI, body mass index; eGFR, estimated glomerular filtration rate.

Table 2. Parameters of the incremental maximal exercise

	Trained group (n = 11)			Untrained group (n = 9)			<i>p</i> value	ES
Exercise duration (min)	12.4	±	0.9	9.7	±	1.3	< 0.001	2.60
Maximal load (watts)	316.4	±	28.0	233.3	±	39.1	< 0.001	2.62
Maximal heart rate (beat/min)	185.8	±	5.1	188.8	±	6.9	0.283	0.55
Peak oxygen uptake (ml/kg/min)	58.9	±	2.8	45.9	±	4.6	< 0.001	3.69

Value are presented as mean ± SD.

Table 3. The kinetics of blood parameters during incremental maximal exercise

	Rest		Exhaustion		<i>p</i> value		
					Group	Time	Interaction
FFA (mmol/l)							
Trained men	0.40	± 0.18	0.39	± 0.14	0.840	0.129	0.180
Untrained men	0.48	± 0.32	0.35	± 0.12	(0.974)	(0.149)	(0.179)
Glycerol (mg/l)							
Trained men	3.3	± 1.5	6.9	± 2.0	0.088	0.003	0.034
Untrained men	7.9	± 5.7	8.6	± 6.0	(0.065)	(0.007)	(0.035)
FABP4 (ng/ml)							
Trained men	4.32	± 1.17	5.69	± 1.92	0.114	0.001	0.766
Untrained men	6.07	± 2.63	7.66	± 4.17	(0.289)	(< 0.001)	(0.315)

Value are presented as mean ± SD. FFA, free fatty acid; FABP4, fatty acid binding protein 4. Values in parentheses are *p* values analyzed by log-transformed value.

Table 4. Spearman rank correlation coefficients between Δ FABP4 and Δ FFA, Δ glycerol, and eGFR

	Δ FFA	Δ glycerol	eGFR
<i>All participants</i>			
Δ FABP4	0.17 ($p = 0.480$)	-0.04 ($p = 0.872$)	-0.35 ($p = 0.135$)
<i>Trained group</i>			
Δ FABP4	-0.40 ($p = 0.223$)	-0.26 ($p = 0.450$)	-0.17 ($p = 0.612$)
<i>Untrained group</i>			
Δ FABP4	0.283($p = 0.460$)	0.38 ($p = 0.308$)	0.15 ($p = 0.700$)

Δ : immediately after exercise concentration – resting concentration, FABP4: fatty acid binding protein 4, FFA: free fatty acid, eGFR: estimated glomerular filtration rate.

Figure 1

Figure Legend

Figure 1. Percentage change in free fatty acid (FFA)(A), glycerol (B), and fatty acid binding protein 4 (FABP4)(C) concentration during incremental maximal exercise. Time and group effects were analyzed using the Wilcoxon signed-rank test and Mann–Whitney U test, respectively. The solid circles and squares (bars) are presented as mean (standard deviation) of the exercise-trained and untrained group, respectively. The open circles and squares are presented as individual values of the exercise-trained and untrained group, respectively. The gray circle and square are presented as median values of the exercise-trained and untrained group, respectively. This figure was secondarily constructed using Table 3 data.

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Main Works

- Numao S, Katayama Y, Nakata Y, Matsuo T, Nakagaichi M, Tanaka K. (2020) Association of abdominal fat with metabolic syndrome components in overweight women: effect of menopausal status. *J. Physiol. Anthropol.*, 39: 12.
- Numao S, Kawano H, Endo N, Yamada Y, Takahashi M, Konishi M, Sakamoto S. (2016) Short-term high-fat diet alters postprandial glucose metabolism and circulating vascular cell adhesion molecule-1 in healthy males. *Appl. Physiol. Nutr. Metab.*, 41: 895-902, 2016.
- Numao S, Kawano H, Endo N, Yamada Y, Konishi M, Takahashi M, Sakamoto S. (2012) Short-term low carbohydrate/high fat diet intake increases postprandial blood glucose and glucagon-like peptide 1 levels during an oral glucose tolerance test in healthy men. *Eur. J. Clin. Nutr.*, 66: 926-931.

Membership in Learned Societies

- Japanese Society of Physical Fitness and Sports Medicine
- Japan Society of Physical Education, Health and Sports Sciences

- 1 • Japan Society of Health Promotion
- 2 • Japanese Society of Clinical Sports Medicine
- 3 • Japanese Association of Cardiac Rehabilitation
- 4 • Japan Society of Physiological Anthropology
- 5 • Japan Society for the Study of Obesity
- 6 • Japanese Society of Education and Health Science
- 7 • Japanese Society of Clinical Nutrition
- 8 • American College of Sports Medicine
- 9