Original Article

Effect of fat ingestion on postprandial oxidative status in healthy young women: a pilot study

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Reactive oxygen species (ROS) and highly reactive oxygen species (hROS) secreted by leukocytes are crucial to innate immunity; however, they pose a risk of oxidative stress. To monitor their balance in daily health check-ups, optical technologies for the simultaneous measurement of ROS (superoxide radicals) and hROS (hypochlorite ions) that utilize only a few microliters of whole blood have been developed. The aim of this study was to clarify whether this system could assess the effects of fat ingestion on postprandial oxidative status. Eight healthy young Japanese women ingested a beverage containing oral fat tolerance test cream. Blood samples were collected before and 0.5, 1, 2, 4, and 6 h after fat ingestion. Blood ROS and hROS levels, oxidative stress markers, and biochemical markers were monitored. Consistent with previous studies, triglyceride levels significantly increased at 4 h ($p<0.01$) and returned to near-baseline levels 6 h after ingestion. ROS levels peaked significantly at 2 h ($p<0.05$), and hROS levels peaked significantly at 1 ($p<0.05$) and 2 h ($p<0.01$) after ingestion. This study offers an insight into the acute effects of fat ingestion on leukocyte activity and provides a methodology for monitoring postprandial oxidative status.

Key Words: **fat ingestion, leukocyte, myeloperoxidase, postprandial oxidative stress, reactive oxygen species**

Leukocytes enzymatically produce reactive oxygen species (ROS) and highly reactive oxygen species (hROS) in the innate immune system.[1,2] As a primary ROS, leukocyte NADPH oxidases generate superoxide radicals ($O_2^{\bullet-}$), a precursor of hydrogen peroxide (H_2O_2).[1,2] Myeloperoxidase (MPO), predominantly present in neutrophil granules, catalyzes the conversion of H_2O_2 to hypochlorite ions (OCl^-), a type of hROS.[1,2] ROS and hROS produced by leukocytes are crucial to host defense; however, they pose a risk of oxidative damage, which may lead to acute and chronic inflammation[3,4] and various diseases.[5–8] To monitor the oxidative balance, optical technologies that can simultaneously measure ROS [chemiluminescence (CL)-$O_2^{\bullet-}$] and hROS [fluorescence (FL)-OCl^-] in a reaction mixture containing 3 µl of whole blood have been developed.[9,10]

Using a fluidic-chip-type system, we previously conducted an observational study of daily ROS and hROS fluctuations in healthy individuals over several months.[10] Our findings demonstrated that (1) each volunteer had a unique steady-state range of CL-$O_2^{\bullet-}$ and FL-OCl^- levels and (2) transient effects such as exercise and acute inflammation possibly deviate CL-$O_2^{\bullet-}$ and FL-OCl^- levels from the steady-state ranges.[10] In another study, the same type of system was used to measure CL-$O_2^{\bullet-}$ and FL-OCl^- levels before and after endovascular treatment (EVT) in 30 cases of peripheral arterial disease (PAD).[11] The ratio of FL-OCl^- to CL-$O_2^{\bullet-}$ levels, which is considered to reflect MPO activity per unit number of leukocytes, significantly decreased approximately 1 month after EVT in a total number of 30 cases, suggesting a reduction in the oxidative status in these patients.[11] Thus, we monitored CL-$O_2^{\bullet-}$ and FL-OCl^- levels in human blood daily and monthly; however, we did not address more short-term hourly fluctuations. One characteristic of our system is the possibility to detect signs of oxidative stress at an earlier stage by monitoring leukocyte activity rather than the consequences of oxidative stress. To verify our system more comprehensively, it is important to monitor the shorter-term dynamics of CL-$O_2^{\bullet-}$ and FL-OCl^- levels in human blood with an appropriate model.

Postprandial oxidative stress is a possible candidate for such an acute model, and it is characterized by an increased susceptibility of the organism to oxidative damage after the consumption of a lipid- and/or carbohydrate-rich diet.[12–14] Postprandial hyperlipidemia and hyperglycemia are related to increased oxidative injury, which may lead to endothelial dysfunction, atherosclerosis, and diabetic complications.[12–14] Triglycerides (TG) can induce the activation of leukocytes *in vitro*[15,16] and *ex vivo* in patients with hypertriglyceridemia.[17] Additionally, postprandial increases in TG and glucose levels are associated with increases in leukocyte counts and activation marker expression, suggesting a close relationship between endothelial dysfunction and leukocyte ROS production.[18–20] Notably, these postprandial changes arise within a few hours after ingestion.[18–20] We therefore selected postprandial oxidative status as a suitable model and verified whether our system could capture the hourly dynamics of postprandial leukocyte activity.

The relationship between fat intake and health is of great concern worldwide.[21,22] Notably, even in young healthy individuals, it requires 6 h for blood TG levels to return to fasting levels after a standard fat load,[23] implying that the postprandial state persists for most of the day if three meals are consumed per day. Therefore, monitoring the oxidative status after fat ingestion is of medical importance, and not just for device validation. Thus, in the present study, a protocol of oral fat tolerance test (OFTT) was used to reproduce the postprandial status.[23] The aim of this pilot study was to reveal the acute effects of fat ingestion on leukocyte activity using our system and to provide a new methodology for postprandial oxidative stress research. To this end, eight healthy young Japanese women ingested a beverage containing OFTT cream (0.35 g/kg as fat) after fasting for more than 12 h. Blood samples were collected at baseline and 0.5, 1, 2,

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4, and 6 h after fat ingestion, and the ROS and hROS production levels were monitored on-site using the fluidic-chip-type system.[10] Blood oxidative stress and biochemical markers were also measured.

Materials and Methods

Study participants. Eight healthy Japanese women with normal ovarian cycles and apoE phenotypes of 3/3 were enrolled in this study. None of the participants had any apparent acute or chronic disease. The baseline physical information of the participants is listed in Table 1. This study was approved by the Institutional Review Board of the Sugiyama Jogakuen University School of Life Studies in accordance with the Helsinki Declaration (Number 2016-15). All participants provided written informed consent.

Fat load test. The individuals ingested a beverage containing OFTT cream (1 g/kg as cream, 0.35 g/kg as fat; Jomo, Gunma, Japan) after more than 12 h of fasting. The OFTT cream was used as described previously.[23,24] During the test, the volunteers avoided exercise and eating; however, they were granted free access to water 1 h after starting the test.

Blood sampling. Venous blood samples were collected before (0 h) and at 0.5, 1, 2, 4, and 6 h after fat ingestion, with the volunteers in the supine position. The blood was stored in an anticoagulant container BD Microtainer (Becton, Dickinson and Company, Franklin Lakes, NJ) for monitoring CL-$O_2^{\bullet-}$ and FL-OCl^- levels and used within 2 h. The serum and plasma samples, isolated by centrifugation, were immediately refrigerated at 4°C or frozen at −80°C until the measurement of oxidative stress and biochemical markers.

Monitoring of CL-$O_2^{\bullet-}$ and FL-OCl^- levels. The production of $O_2^{\bullet-}$ and OCl^- by leukocytes, stimulated by phorbol 12-myristate 13-acetate, was simultaneously measured using the fluidic-chip-type system (CFL-H1200 or -H2200) and calculated as described previously,[10,11] except for the monitoring time in this study being 1,500 s.

Measurements of oxidative stress markers. The serum levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), hexanoyl-lysine (HEL), propanoyl-lysine (PRL), interleukin-6 (IL-6), and plasma MPO activity were measured as described previously.[11,25]

Measurements of biochemical blood markers. The levels of serum TG (Sekisui Medical, Tokyo, Japan) and free fatty acid (FFA; Eiken Chemical, Tokyo, Japan) were measured enzymatically. ApoB levels in the serum were measured using the immunoturbidimetric method (Sekisui Medical). Serum apoB-48 levels were measured using chemiluminescent enzyme immunoassay (Fujirebio, Tokyo, Japan). ApoB-100 levels in serum were calculated by subtracting apoB-48 levels from apoB levels.[26] The apoE phenotypes of the volunteers were determined via the isometric electrophoresis method (Phenotyping ApoE IEF System™; Jokoh, Tokyo, Japan). Serum acetoacetate (ACA) and β-hydroxybutyric acid (β-HB) levels were measured using an enzymatic cycling method (Kainos, Tokyo, Japan). Total ketone body (T-KB) levels were calculated as the sum of β-HB and

Table 1. Physical characteristics of the participants

Participants (*n*)	8
Age (years)	21.3 (0.97)
Height (cm)	158 (5.77)
Weight (kg)	52.0 (6.39)
BMI (kg/m^2)	20.7 (1.67)
Waist (cm)	68.5 (7.81)
W/H	0.76 (0.06)
VFA (cm^2)	29.4 (15.5)
HbA1c (NGSP) (%)	5.28 (0.27)
SBP (mmHg)	110 (5.93)
DBP (mmHg)	66.0 (7.89)
PR (beats/min)	71.1 (9.87)

Mean (SD) values are shown. BMI, body mass index; W/H, waist-hip ratio; VFA, visceral fat area; SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate.

Table 2. Biochemical blood markers, CL-$O_2^{\bullet-}$ and FL-OCl^- levels, and oxidative stress markers of fasting and after fat ingestion

	0	0.5	1	2	4	6
Biochemical blood markers						
TG (mg/dl)	72.6 (49.7)	—	74.2 (49.4)	83.6 (50.9)	91.2 (57.0)**	75.2 (59.2)
ApoB-48 (mg/dl)	0.21 (0.11)	—	0.25 (0.09)	0.35 (0.14)**	0.41 (0.17)**	0.31 (0.18)
ApoB-100 (mg/dl)	63.7 (11.8)	—	63.0 (11.9)	63.0 (12.3)	63.0 (12.3)	65.6 (11.8)**
T-KB (mg/dl)	74.1 (70.5)	—	133 (65.9)	132 (76.4)	270 (208)	395 (423)**
FFA (μEq/l)	431 (154)	—	409 (139)	520 (199)	749 (269)*	884 (399)**
VLDL-C (mg/dl)	22.2 (5.86)	—	22.2 (5.93)	23.0 (6.53)	23.7 (6.90)	25.4 (8.11)**
CM-TG (mg/dl)	11.4 (15.6)	—	10.8 (13.8)	13.8 (14.5)	16.5 (17.3)*	9.62 (16.3)
VLDL-TG (mg/dl)	31.9 (26.3)	—	34.9 (28.0)	40.2 (29.0)*	44.6 (32.8)**	37.8 (36.6)
CL-$O_2^{\bullet-}$ & FL-OCl^- levels						
CL-$O_2^{\bullet-}$ (×10^5)	9.06 (2.84)	9.11 (3.18)	10.2 (4.08)	11.4 (4.56)*	9.54 (3.68)	9.74 (3.64)
FL-OCl^- (×10^3)	2.31 (0.79)	2.91 (1.10)	3.03 (1.21)*	3.24 (1.43)**	2.71 (0.91)	2.62 (0.90)
FL-OCl^-/CL-$O_2^{\bullet-}$ (×10^{-3})	2.71 (0.93)	3.27 (0.69)*	3.15 (0.90)	3.06 (1.05)	3.09 (1.16)	3.02 (1.33)
Oxidative stress markers						
8-OHdG (ng/ml)	6.50 (4.86)	7.12 (5.35)	8.33 (5.40)	6.70 (2.43)	5.62 (2.25)	5.73 (2.27)
HEL (nM)	14.0 (7.93)	16.2 (9.53)	15.0 (11.8)	15.2 (12.4)	15.2 (9.18)	15.6 (11.4)
PRL (μM)	9.03 (9.76)	9.23 (7.84)	5.00 (3.21)	6.79 (5.47)	3.38 (2.56)	3.00 (1.85)
IL-6 (pg/ml)	0.56 (0.09)	0.49 (0.22)	0.43 (0.29)	0.53 (0.11)	0.57 (0.14)	0.66 (0.12)
Plasma MPO (mU/ml)	12.2 (13.9)	5.51 (5.71)	13.2 (11.8)	8.74 (6.38)	11.6 (9.00)	12.2 (23.3)

Mean (SD) values are shown. *p<0.05, **p<0.01. TG, triglyceride; Apo, apolipoprotein; T-KB, total ketone bodies; FFA, free fatty acid; VLDL-C, very low-density lipoprotein cholesterol; CM, chylomicron; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; HEL, hexanoyl-lysine; PRL, propanoyl-lysine; IL-6, interleukin-6; MPO, myeloperoxidase.

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ACA levels. Very low-density lipoprotein cholesterol (VLDL-C) levels in serum and TG levels in TG-rich lipoproteins [chylomicron (CM)-TG and VLDL-TG] were measured using high-performance liquid chromatography LipoSEARCH (Immuno-Biological Laboratories, Gunma, Japan).

Statistical analysis. Differences in the time-course changes from the initial values were analyzed as parametric multiple pairwise comparisons using Dunnett's test. The statistical analyses were conducted using SPSS ver. 26 software package (IBM, Tokyo, Japan). All p values were two-sided, and results with $p<0.05$ were considered statistically significant.

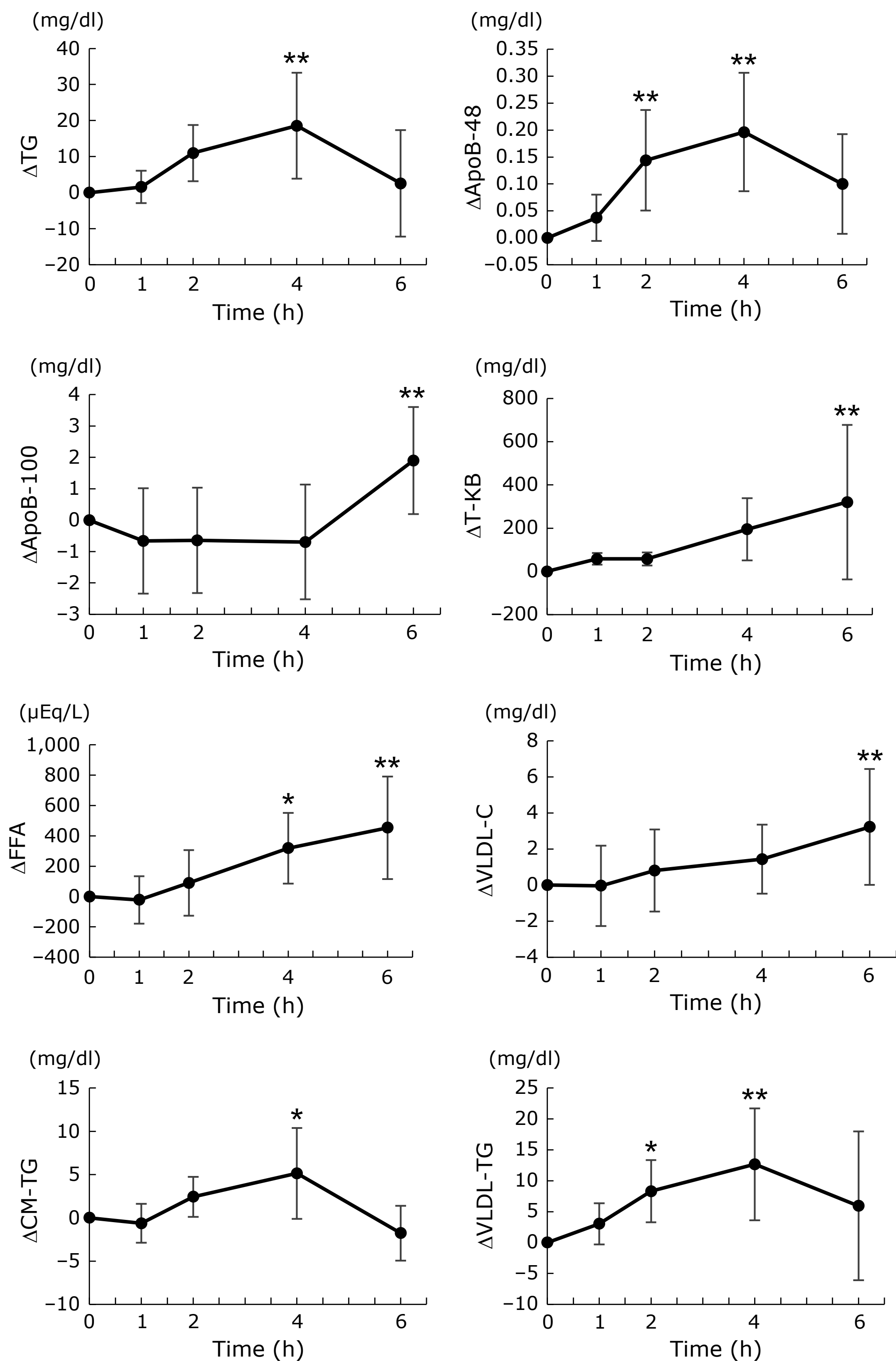

Fig. 1. Postprandial changes in biochemical blood markers. Postprandial ΔTG, ΔapoB-48, ΔapoB-100, ΔT-KB, ΔFFA, ΔVLDL-C, ΔCM-TG, and ΔVLDL-TG are shown. Values are presented as mean (SD). $*p<0.05$, $**p<0.01$.

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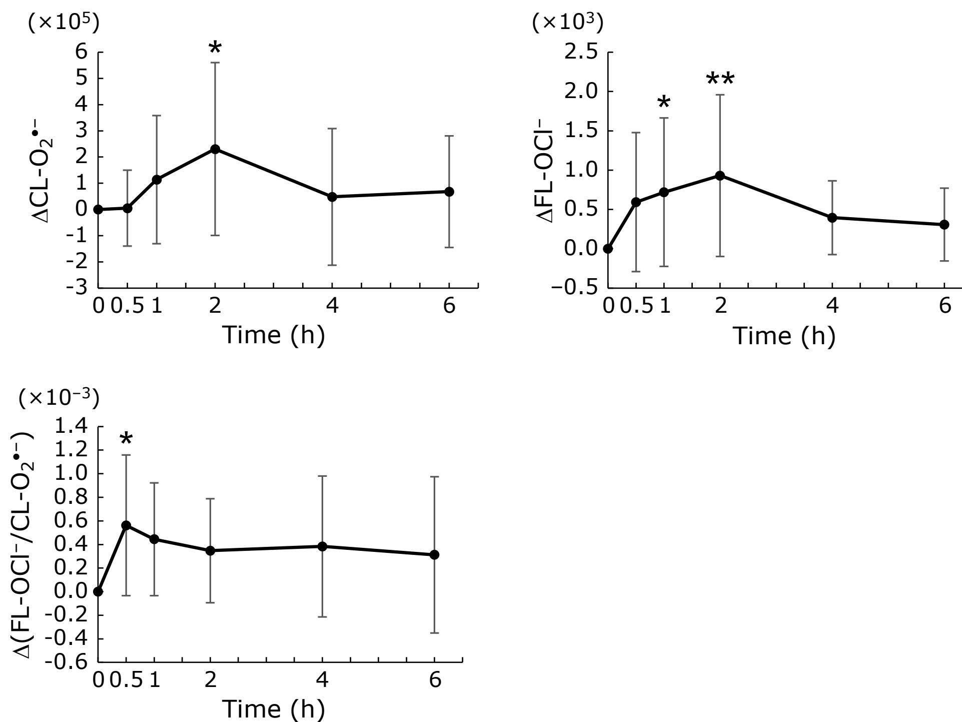

Fig. 2. Postprandial changes in $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels. Postprandial $\Delta CL\text{-}O_2^{\bullet-}$, $\Delta FL\text{-}OCl^-$, and $\Delta(FL\text{-}OCl^-/CL\text{-}O_2^{\bullet-})$ are shown. Values are presented as mean (SD). *$p<0.05$, **$p<0.01$.

Results

Postprandial changes in biochemical blood markers. Serum concentrations of baseline and postprandial TG, apoB-48, apoB-100, T-KB, FFA, VLDL-C, CM-TG, and VLDL-TG are summarized in Table 2. The postprandial differences (Δ) compared to baseline levels are shown in Fig. 1. The serum TG levels peaked significantly at 4 h after fat ingestion ($p<0.01$) and returned to near-baseline levels at 6 h (Fig. 1 and Table 2). The serum apoB-48 levels, which are known to reflect exogenous (dietary) effects,[27] significantly increased at 2 h ($p<0.01$) and peaked 4 h ($p<0.01$) after fat ingestion (Fig. 1 and Table 2). Subsequently, the apoB-48 levels decreased; however, it did not appear to return to baseline levels even at 6 h (Fig. 1 and Table 2). In contrast, the serum apoB-100 levels, which are known to reflect endogenous events,[27] significantly increased at 6 h after fat ingestion ($p<0.01$, Fig. 1 and Table 2). The time-course changes in the serum concentrations of T-KB, FFA, and VLDL-C were similar; they tended to increase gradually over time, with a significant increase at 6 h ($p<0.01$, Fig. 1 and Table 2). The FFA levels also increased significantly at 4 h post-ingestion ($p<0.05$, Fig. 1 and Table 2). Meanwhile, the postprandial changes in serum CM-TG levels were similar to those in TG; they peaked significantly at 4 h ($p<0.05$) and returned to near-baseline levels at 6 h (Fig. 1 and Table 2). The serum VLDL-TG levels significantly increased at 2 h ($p<0.05$) and peaked at 4 h ($p<0.01$) after fat ingestion (Fig. 1 and Table 2). Subsequently, the VLDL-TG levels decreased; however, they did not appear to return to baseline levels even at 6 h post-ingestion, in contrast to the CM-TG levels (Fig. 1 and Table 2).

Postprandial changes in $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels. Baseline and postprandial $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels, as well as the ratio of $FL\text{-}OCl^-$ to $CL\text{-}O_2^{\bullet-}$ levels, which is considered to reflect MPO activity per unit number of leukocytes, are presented in Table 2. The postprandial differences (Δ) compared to baseline levels are shown in Fig. 2. The $CL\text{-}O_2^{\bullet-}$ levels peaked significantly at 2 h after fat ingestion ($p<0.05$) and then decreased, although they did not appear to return to baseline levels even at 6 h post-ingestion (Fig. 2 and Table 2). The $FL\text{-}OCl^-$ levels significantly increased at 1 h ($p<0.05$) and peaked at 2 h ($p<0.01$) after fat intake (Fig. 2 and Table 2), which was slightly earlier than that observed for the $CL\text{-}O_2^{\bullet-}$ levels. Similar to the $CL\text{-}O_2^{\bullet-}$ levels, the $FL\text{-}OCl^-$ levels then decreased, but they did not appear to return to baseline levels even at 6 h (Fig. 2 and Table 2). The $FL\text{-}OCl^-/CL\text{-}O_2^{\bullet-}$ levels, which is the ratio of $FL\text{-}OCl^-$ to $CL\text{-}O_2^{\bullet-}$ levels, significantly increased at 0.5 h after fat ingestion ($p<0.05$, Fig. 2 and Table 2), which was earlier than the increase in the $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels. Similar to the $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels, the $FL\text{-}OCl^-/CL\text{-}O_2^{\bullet-}$ levels then decreased, but they did not appear to return to baseline levels even at 6 h (Fig. 2 and Table 2).

The postprandial differences (Δ) in the $CL\text{-}O_2^{\bullet-}$, $FL\text{-}OCl^-$, and $FL\text{-}OCl^-/CL\text{-}O_2^{\bullet-}$ levels per volunteer are presented in Fig. 3. The postprandial changes in the $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels tended to be categorized into two groups: one with larger fluctuation (participants D, E, F, and G; indicated with solid lines in Fig. 3) and another with a relatively small fluctuation (participants B, K, L, and M; indicated with dotted lines in Fig. 3). Regarding the $FL\text{-}OCl^-/CL\text{-}O_2^{\bullet-}$ levels, their postprandial changes also appeared to be categorized into the same two groups: one that peaked 0.5 h after fat ingestion (participants D, E, F, and G; indicated with solid lines in Fig. 3) and another without this tendency (participants B, K, L, and M; indicated with dotted lines in Fig. 3).

Postprandial behavior of oxidative stress markers. The baseline and postprandial concentrations of serum 8-OHdG, HEL, PRL, IL-6, and plasma MPO activity are listed in Table 2. The postprandial differences (Δ) compared to baseline levels are presented in Fig. 4. The five oxidative stress markers showed no significant postprandial changes when compared to baseline concentrations (Fig. 4 and Table 2).

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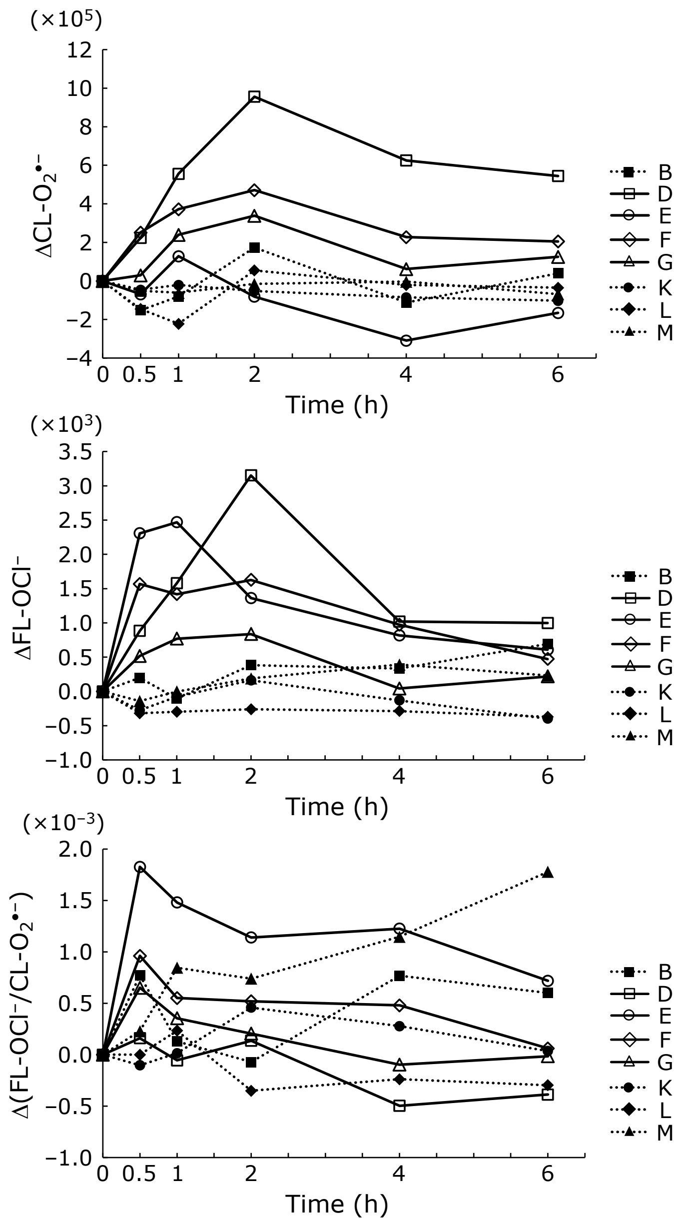

Fig. 3. Postprandial changes in $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels in each volunteer. Postprandial $\Delta CL\text{-}O_2^{\bullet-}$, $\Delta FL\text{-}OCl^-$, and $\Delta(FL\text{-}OCl^-/CL\text{-}O_2^{\bullet-})$ in each volunteer are shown.

Discussion

In this study, the postprandial changes in the serum concentrations of TG and apoB-48 (Fig. 1 and Table 2) were consistent with those reported previously.[18–20,23,24,27–29] Our results indicated that the serum concentrations of exogenous (diet-derived) lipids peaked approximately 4 h after fat intake. They also suggested normal fat metabolism in the participants and ensured fat loading in the current study. The postprandial changes in the serum apoB-100 levels were similar not only to those reported in previous studies,[24,28] but also to the changes in T-KB, FFA, and VLDL-C levels in the present study (Fig. 1 and Table 2). Our results suggested that VLDL-C synthesis in the liver was initiated approximately 6 h after the start of the test because of the fasting state rather than the direct effects of fat intake. In addition, these findings implied normal metabolism in the volunteers.[27] The postprandial changes in serum VLDL-TG levels appeared to slightly lag behind those in CM-TG, and the VLDL-TG levels did not appear to return to baseline levels even at 6 h post-ingestion, which were in contrast to the CM-TG levels (Fig. 1 and Table 2). These results suggested the production of fat ingestion-derived TG (CM-TG) and the subsequent synthesis of endogenous VLDL-C (VLDL-TG).

In our previous study, we found that the leukocyte counts correlated more with the $CL\text{-}O_2^{\bullet-}$ levels than with the $FL\text{-}OCl^-$ levels,[10] implying that the $FL\text{-}OCl^-$ level reflects cellular activity, which is difficult to capture sufficiently based on leukocyte counts alone.[10] The postprandial behavior of $CL\text{-}O_2^{\bullet-}$ levels in this study may also represent changes in leukocyte counts (Fig. 2 and Table 2). In support of this hypothesis, leukocyte counts have been reported to increase gradually after fat ingestion, with a significant increase at 2 h.[20] However, the possibility of increased NADPH oxidase activity in leukocytes should also be considered.[30] The postprandial increase in $FL\text{-}OCl^-$ levels was slightly faster than that in $CL\text{-}O_2^{\bullet-}$ levels, with a significant increase at 1 and 2 h (Fig. 2 and Table 2). Our previous study suggested that the $FL\text{-}OCl^-$ levels per unit number of leukocytes reflect the physical conditions of individuals.[10] In addition, the $FL\text{-}OCl^-/CL\text{-}O_2^{\bullet-}$ levels have been used as a substitute for $FL\text{-}OCl^-$ levels per unit number of leukocytes.[11] Notably, in the present study, the $FL\text{-}OCl^-/CL\text{-}O_2^{\bullet-}$ levels significantly increased 0.5 h after fat intake, implying a rapid postprandial increase in MPO activity (Fig. 2 and Table 2). The $FL\text{-}OCl^-/CL\text{-}O_2^{\bullet-}$ levels may be an earlier marker of postprandial oxidative status that warrants further research, such as monitoring leukocyte counts and increasing the number of participants.

Based on the postprandial changes in $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels, the participants in this study could be divided into two groups (Fig. 3): larger (participants D, E, F, and G) and smaller (participants B, K, L, and M) fluctuation groups. To explore the underlying factors, we examined the differences between these groups based on the data obtained. Notably, the group with larger fluctuations in the $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels showed a typical postprandial change in TG levels, that is, a peak 4 h after fat intake and return to baseline level at 6 h (Fig. 5). In contrast, the smaller fluctuation group tended to show relatively small postprandial changes in TG levels (Fig. 5). Postprandial glucose spikes in the blood have been reported to be linked to various disorders associated with oxidative stress.[31] The results of this study implied that postprandial serum "TG spikes" may also cause oxidative stress,[32] and these findings may provide a basis for further research. Future studies are expected to compare fat and sugar loads as well as investigate the effects of a combinational load of fat and sugar using a protocol similar to that of this study.[28,33]

Meanwhile, no significant postprandial changes were observed in the oxidative stress marker levels in this study (Fig. 4 and Table 2). The serum IL-6 levels tended to increase 6 h after fat ingestion, which is consistent with the results of a previous study.[19] A significant increase in plasma MPO levels after 4 h of high-fat meal intake has been reported.[34] The fat content in our protocol was approximately one-third lower than that used in the previous study.[34] The effects of higher fat loads should be analyzed in the future based on a protocol similar to that of the current study. Diet-derived serum TG levels returned to near-baseline levels 6 h after fat intake in this study (Fig. 1 and Table 2). In contrast, the $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels remained constant from 4 to 6 h (Fig. 2 and Table 2). Although the postprandial $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels peaked earlier than the serum TG levels, their levels might remain higher than the baseline even after 6 h of fat ingestion. Future studies should also focus on changes in oxidative stress markers beyond 6 h after fat intake, as well as $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$ levels.

In this study, significant changes in the $CL\text{-}O_2^{\bullet-}$ and $FL\text{-}OCl^-$

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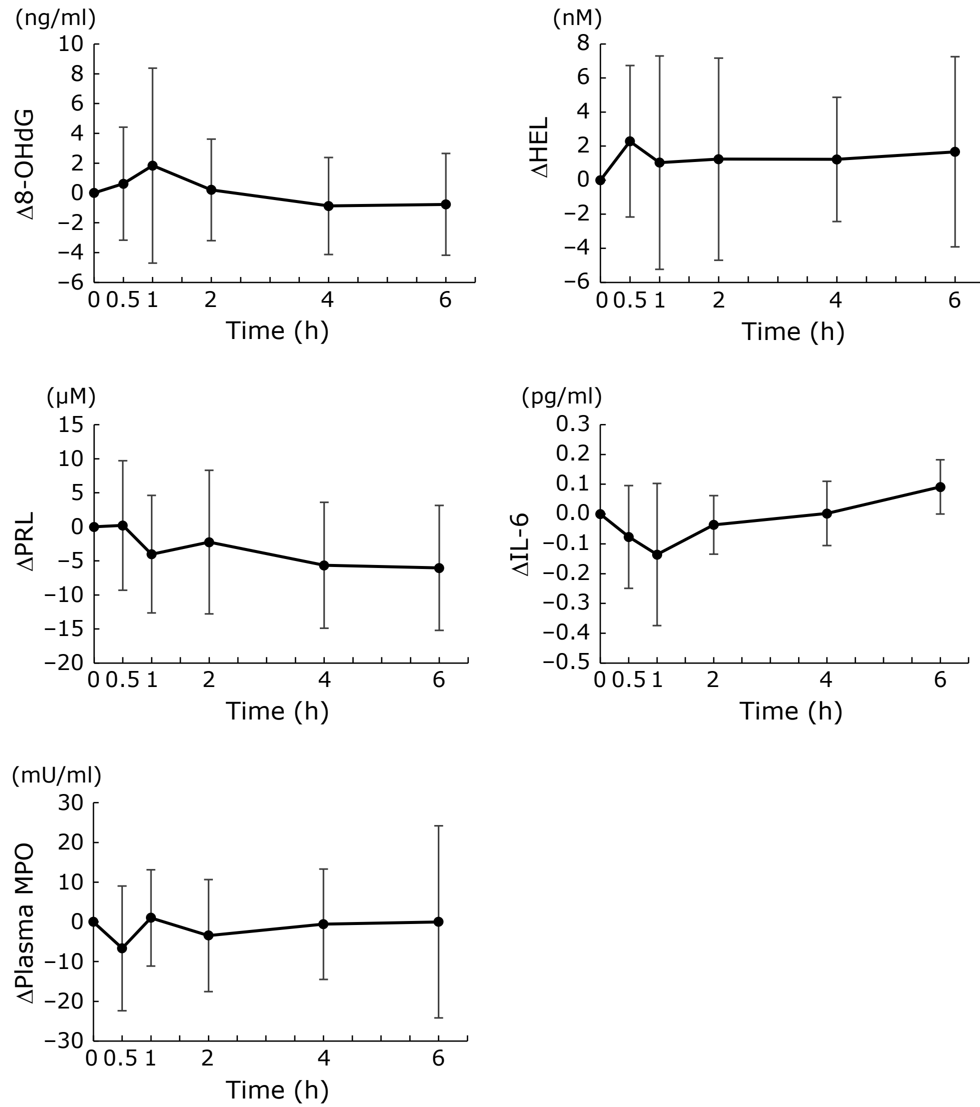

Fig. 4. Postprandial changes in oxidative stress markers. Postprandial Δ8-OHdG, ΔHEL, ΔPRL, ΔIL-6, and Δplasma MPO levels are shown. Values are presented as mean (SD).

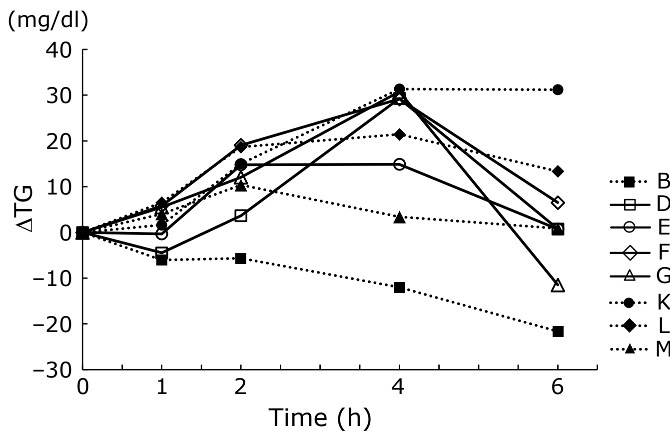

Fig. 5. Postprandial changes in TG levels in each volunteer. Postprandial ΔTG in each volunteer is shown.

levels were observed at an earlier time, that is, 0.5–2 h after fat ingestion, than those of other indices (Fig. 2 and Table 2). Our system detects signs of oxidative status rather than the consequences of oxidative stress, such as conventional oxidative markers.[10] The results of this study also imply the potential of our system to provide an early marker of postprandial oxidative status. In addition, the amount of fat load in this study (0.35 g/kg as fat) is equivalent to that in one Japanese meal, which is approximately one-third lower than the standard fat load in Western countries.[34] The postprandial changes were detectable even under this condition, suggesting that our system could be applied to various fat-loading protocols. One promising application is the evaluation of functional molecules that can suppress acute postprandial oxidative stress. For practical applications, further validation with improved protocols is required, including an increased number of volunteers, various amounts of fat load, and leukocyte count monitoring.

Compared to conventional biochemical-based approaches, our system has the advantage of minimal blood usage. This is particularly useful in studies that require sampling at multiple time points in a short period, from the perspective of reducing the

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burden on study participants and the operations of researchers. Furthermore, this study serves as a foundation for accelerating research in the field of acute oxidative stress.

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Conflict of Interest

KT and KK are employed by Hamamatsu Photonics K.K. YH was employed by Hamamatsu Photonics K.K. when this research was conducted. KT, KK, and YH submitted patent applications for the technologies used in this study. MNagai is employed by Healthcare Systems Co., Ltd. The funders had no role in the data analysis and interpretation. The remaining authors have no potential conflicts of interest to declare.

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