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Endocrine, Metabolic & Immune Disorders-Drug Targets, XXXX, *XX*, 1-8

RESEARCH ARTICLE

Association between Insulin Resistance and Cardinal Rheological Parameters in Young Healthy Japanese Individuals During 75g Oral Glucose Tolerance Test

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Abstract: ***Background*:** Insulin resistance is a well-known predictor and risk factor for type 2 diabetes mellitus (T2DM). Higher hematocrit induced by higher insulin resistance affects blood rheology.

***Objective*:** This study intended to reveal the association between indices of insulin resistance and hemorheological parameters during a 75 g oral glucose tolerance test (75-g OGTT).

***Methods*:** A total of 575 healthy young Japanese participants took 75-g OGTT. We then analyzed the association between insulin resistance indices and hematological parameters.

***Results*:** The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was significantly correlated with hematocrit (Ht), hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelet count, lipid parameters and body mass index (BMI). The Matsuda index was negatively correlated with RBC count, WBC count, platelet count, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), triglyceride (TG), and positively correlated with high-density lipoprotein-cholesterol (HDL-C). The disposition index was negatively correlated with Hb, RBC count, LDL-C and BMI, and positively correlated with HDL-C. The Homeostasis Model Assessment of beta cell (HOMA-β) was positively correlated with WBC count, platelet count, TC, LDL-C and TG. The insulinogenic index was positively correlated with WBC count, platelet count and TC. Multiple regression analysis revealed that HOMA-IR was independently associated with TG, and the Matsuda index was independently associated with TG, WBC count, and platelet count. The insulinogenic index was independently associated with WBC count.

***Conclusion*:** Cardinal rheological parameters reflected insulin resistance and release even in young healthy Japanese individuals within the physiological range of glycemic control.

ARTICLE HISTORY

Received: February 12, 2021
Revised: May 19, 2021
Accepted: May 19, 2021

DOI:
10.2174/1871530321666210720124554

Keywords: OGTT, Insulin secretion, insulin resistance, RBC count, WBC count, platelet count, blood rheology.

1. INTRODUCTION

According to the latest International Diabetes Federation calculations, approximately 463 million adults have diabetes, and this will rise to 700 million by 2045. The proportion of people with type 2 diabetes (T2DM) is increasing in most countries, with 374 million adults having impaired glucose tolerance (IGT) [1]. The prevalence of childhood obesity and consequently T2DM is rising [2-4], hence there is an increasing interest in insulin resistance as a generally recognized predictor and a risk factor for T2DM [5–8]. Insulin resistance is characterized by a decreased uptake and the use of glucose by target cells, resulting in increased insulin production *via* compensatory secretion, leading to sustained hyperinsulinemia [9]. T2DM raises health care costs and is an encumbrance; therefore, the monitoring of insulin resistance in children and adolescents at risk for T2DM is important to prevent T2DM [10-13]. A recent analysis of our data from a 75 g oral glucose tolerance test (75-g OGTT) revealed that there is a sequential decrease in insulin sensitivity and secretion even in young and healthy Japanese individuals within the physiological range of glycemic control [14]. The same study revealed that disposition index, but not fasting circulating insulin or Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), was a good indicator of IGT [14]. HOMA-IR could be evaluated by single blood sampling [10-13], while performing a 75-g OGTT is necessary to evaluate the disposition index and Matsuda index [15-17]. Insulin resistance affected blood rheology in subjects with obesity, hypertension, and metabolic disorders [18-21]. Impairment of blood rheology is associated with lifestyle-related diseases, such as dyslipidemia, hypertension [22, 23], cardiovascular diseases [24, 25], and diabetes [26-29]. Furthermore, insulin resistance evaluated by HOMA-IR was associated with blood rheology in healthy young subjects [30]. In accordance with these reports, we previously showed that

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fasting circulating insulin levels and insulin resistance evaluated by HOMA-IR were associated with blood rheology through modulating hematological and lipid parameters in young adults within the physiological range of glycemic control [31]. Most previous studies, including our report, adopted HOMA-IR to evaluate insulin resistance [10-13, 30, 31]. The HOMA-IR index represents the relationship between pancreatic insulin secretion and the capacity to maintain adequate glycemic levels [32, 33]. In contrast, the Matsuda index represents both hepatic and peripheral tissue sensitivity to insulin, requiring 75-g OGTT for estimation [16, 17]. Therefore, further studies that investigate the association between hemorheological parameters and indices of insulin resistance evaluated by 75-g OGTT, such as Matsuda index, are required. This study used previous data to investigate the association between indices of insulin resistance and hemorheological parameters in young healthy Japanese individuals during a 75-g OGTT.

2. Materials AND Methods

2.1. Study Population

This study is an addition to our previous report which demonstrated that even in young and healthy Japanese individuals within the physiological range of glycemic control, there was a sequential decrease in insulin sensitivity and secretion [14]. We, therefore, reanalyzed our previous data. All participants signed informed consent forms. The Gunma University Ethical Review Board for Medical Research Involving Human Subjects approved the study protocol. Participants were volunteers purely. Participants were 575 medical candidates who practiced at the Gunma University Hospital between May 2010 and July 2016. No subjects were diagnosed as having T2DM or received any medication. As part of their medical practice, they all underwent a comprehensive medical examination, including 75-g OGTT after an overnight fast. All subjects were 22–29 years of age.

2.2. Study Design

The 75-g OGTT was performed after 10-hour fasts with 0-, 30-, 60-, and 120-minutes samplings to establish plasma glucose and insulin levels, and at the pre-load time, serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and hemoglobin A1c (HbA1c) were measured. Hematological parameters were measured at 0-minutes. Associations between indices of insulin resistance and hemorheological parameters were investigated.

We measured height and weight and calculated body mass indices [BMIs] (weight [kg]/height [m^2]). An XE-5000 hematology system (Sysmex, Kobe, Japan) was used to measure hematocrit (Ht), hemoglobin (Hb) levels, red blood cell (RBC) count, white blood cell (WBC) count, and platelet count. We used enzymatic methods with an automatic analyzer (LABOSPECT 008; Hitachi, Tokyo, Japan) to measure serum TC, HDL-C, LDL-C, and TG concentrations. Chemiluminescence immunoassay using an automatic analyzer (AIA-2000 LA; Tosoh, Tokyo, Japan) was used to measure serum insulin concentrations. A hexokinase method was used to measure plasma glucose concentrations, while high-performance liquid chromatography, using automatic analyzers (ADAMS Glucose GA-1170 and ADAMS A1c HA8180, respectively; Arkray, Kyoto, Japan), was used to measure HbA1c levels.

2.3. Statistical Methods

The trapezoid rule was used to calculate areas under the glucose or insulin curves (AUCg and AUCi). We also conducted the homeostasis model assessment of insulin resistance (HOMA-IR, fasting plasma glucose [PG0] (mg/dL) × IRI0 (μU/mL)/405) [15], β cell function (HOMA-β, IRI0 (μU/mL) × 360/[PG0 (mg/dL) - 63]) [15], and Matsuda index of insulin sensitivity (10,000/square root of [fasting glucose (mg/dL) × fasting insulin (μU/mL)] × [mean glucose (mg/dL) × mean insulin (μU/mL) during OGTT])[16], as reported. The insulinogenic index was calculated by dividing the increment in serum insulin (μU/mL) by the increment in plasma glucose (mg/dL) during the 0–30 min time periods of the OGTT [17]. The insulin secretion/insulin resistance (disposition) index was calculated as insulinogenic index × Matsuda index [34].

Because almost all variables, except glucose and insulin, were not normally distributed, data were expressed as median values with a 25th–75th percentile range, rather than as mean values with standard deviations. Spearman's correlation analyses were conducted to determine the relationships between indices of insulin resistance or release and the clinical parameters. Differences and correlations were considered significant when $p < 0.05$. SPSS Statistics version 25.0 (SPSS, Inc., Chicago, Illinois) was used to perform all statistical analyses.

3. RESULTS

Table **1** shows the characteristics of the young and healthy 575 subjects aged 22–29 years. The 75-g OGTT confirmed that all 575 subjects did not have diabetes [14]. We used these results to investigate the association between indices of insulin resistance and hematological parameters during the 75-g OGTT. HOMA-IR was significantly correlated with Ht, Hb, RBC, WBC count, platelet count, TG, TC, HDL-C, LDL-C, and BMI among all of 575 subjects Table **2**. The Matsuda index was significantly correlated with RBC count, WBC count, platelet count, TG, TC, HDL-C, and LDL-C (Table **2**). The disposition index was significantly correlated with RBC count, Hb, HDL-C, LDL-C, and BMI Table **2**. HOMA-β was positively correlated with WBC count, platelet count, TG, TC, and LDL-C (Table **3**). Insulinogenic index was positively correlated with WBC count, platelet count, and TC (Table **2**). All of RBC count, Ht, and Hb were significantly correlated with BMI, AUCg, TC, LDL-C, HDL-C, and TG Table **3**. WBC count was positively correlated with HbA1c, AUCg, AUCi, and TG (Table **3**). Platelet count was significantly correlated with AUCi, TC, LDL-C, HDL-C, and TG (Table **3**). Multiple regression analysis revealed that HOMA-IR was independently associated with TG and Matsuda index was independently associated with TG, WBC count, and platelet count. Also, the insulinogenic index was independently associated with WBC count Table **4**.

Table 1. Characteristics of 575 participants.

Characters	N=575 (Males:359, Females:216)
Age (years)	23 (23-24)
BMI (kg/m^2)	20.7 (19.4-22.3)
HbA1c (%)	5.3 (5.2-5.4)
PG0 (mg/dL)	90 (86-95)
AUCg (mg min/mL)	13095 (11666.3-14715)
IRI0 (mU/mL)	5.7 (4.3-7.8)
AUCi (U min/L)	4430 (3198-6165)
HOMA-β (%)	84 (61.3-142.5)
Insulinogenic index	1 (0.6-1.8)
HOMA-IR	1.3 (0.9-1.8)
Matsuda index	7.1 (5.2-9.5)
Disposition index	6.9 (4.4-11.4)
TC (mg/dL)	180 (162-197.3)
HDL-C (mg/dL)	63 (54.-73)
LDL-C (mg/dL)	97 (80-114.3)
TG (mg/dL)	64.5 (48-86.3)
RBC count (x10^6/mL)	5.0 (4.7-5.3)
Hematocrit (%)	44.3 (41.7-46.5)
Hemoglobin (g/dL)	15.1 (14.1-15.9)
WBC count (x10^3/mL)	5.1 (4.4-6.2)
Platelet count (x10^3/mL)	231 (200-260)

Data are expressed as median (25th-75th percentile). BMI, body mass index; RBC, red blood cell; WBC, white blood cell; PG0, fasting plasma glucose; IRI0, fasting plasma insulin; HbA1c, hemoglobin A1c; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride; HOMA-IR, homeostasis model assessment - insulin resistance; HOMA-β, homeostasis model assessment - beta cell.

Table 2. Spearman's correlation analyses between indices of insulin resistance and hematological parameters among 575 participants.

-		HOMA-IR		Matsuda Index		Disposition Index		HOMA-β		Insulinogenic Index	
		ρ	*p*	*ρ*	*p*	*ρ*	*p*	*ρ*	*p*	*ρ*	*p*
BMI	kg/m^2	**0.089**	**0.033***	-0.042	0.319	**-0.090**	**0.032***	0.017	0.689	-0.064	0.13
HbA1c	%	0.039	0.346	-0.068	0.104	-0.046	0.274	-0.007	0.87	0.002	0.953
TG	mg/dL	**0.230**	**<0.001****	**-0.248**	**<0.001****	-0.074	0.077	**0.185**	**<0.001****	0.071	0.090
TC	mg/dL	**0.110**	**0.008****	**-0.162**	**<0.001****	-0.006	0.885	**0.121**	**0.004****	**0.091**	**0.030***
HDL-C	mg/dL	**-0.115**	**0.006****	**0.126**	**0.003****	**0.157**	**<0.001****	-0.042	0.315	0.056	0.180

Table (2). Contd….

-		HOMA-IR		Matsuda Index		Disposition Index		HOMA-β		Insulinogenic Index	
		ρ	*p*	*ρ*	*p*	*ρ*	*p*	*ρ*	*p*	*ρ*	*p*
LDL-C	mg/dL	**0.161**	**<0.001****	**-0.223**	**<0.001****	**-0.094**	**0.025***	**0.134**	**0.001****	0.053	0.209
RBC count	x10^6/mL	**0.123**	**0.003****	**-0.097**	**0.02***	**-0.091**	**0.029***	0.003	0.948	-0.055	0.190
Ht	%	**0.105**	**0.012***	-0.06	0.153	-0.074	0.075	-0.017	0.681	-0.064	0.130
Hb	g/dL	**0.088**	**0.035***	-0.043	0.304	**-0.088**	**0.035***	-0.02	0.636	-0.082	0.051
WBC count	x10^3/mL	**0.104**	**0.012***	**-0.181**	**<0.001****	0.011	0.787	**0.130**	**0.002****	**0.111**	**0.008****
Platelet count	x10^6/mL	**0.112**	**0.007****	**-0.165**	**<0.001****	-0.021	0.633	**0.155**	**<0.001****	**0.084**	**0.045***

BMI, body mass index; RBC, red blood cell; WBC, white blood cell; HbA1c, hemoglobin A1c; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride; HOMA-IR, homeostasis model assessment - insulin resistance; HOMA-β, homeostasis model assessment - beta cell. *, $p < 0.05$ and **, $p < 0.005$.

Table 3. Spearman's correlation analyses between hematological parameters and metabolic parameters among 575 participants.

-		RBC Count (x10^6/mL)		Hematocrit (%)		Hemoglobin (g/dL)		WBC Count (/x10^3mL)		Platelet Count (x10^6/mL)	
		ρ	*p*	*ρ*	*p*	*ρ*	*p*	*ρ*	*p*	*ρ*	*p*
Age	years	0.093	0.027*	0.077	0.065	0.073	0.08	-0.008	0.84	-0.058	0.167
BMI	kg/m^2	0.322	<0.001**	0.346	<0.001**	0.338	<0.001**	0.046	0.258	-0.059	0.156
HbA1c	%	0.061	0.148	0.03	0.477	-0.044	0.290	0.095	0.023*	0.044	0.292
AUCg	mg min/mL	0.196	<0.001**	0.173	<0.001**	0.197	<0.001**	0.094	0.024*	0.031	0.462
AUCi	U min/L	0.018	0.670	-0.020	0.631	-0.040	0.334	0.192	<0.001**	0.173	<0.001**
TC	mg/dL	0.147	<0.001**	0.125	<0.001**	0.085	0.043*	0.055	0.189	0.173	<0.001**
HDL-C	mg/dL	-0.273	<0.001**	-0.220	<0.001**	-0.253	<0.001**	-0.013	0.759	0.100	0.016*
LDL-C	mg/dL	0.275	<0.001**	0.215	<0.001**	0.196	<0.001**	0.034	0.411	0.128	0.002*
TG	mg/dL	0.268	<0.001**	0.266	<0.001**	0.251	<0.001**	0.161	<0.001**	0.102	0.015*

BMI, body mass index; AUCg, areas under the glucose curve; AUCi, areas under the insulin curve; R BC, red blood cell; WBC, white blood cell; HbA1c, hemoglobin A1c; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride; *, $p < 0.05$ and **, $p < 0.005$.

Table 4. Multiple regression analyses showing the correlation between indices of insulin resistance/release and cardinal rheological parameters among 575 participants.

-		HOMA-IR		Matsuda Index		Disposition Index		HOMA-β		Insulinogenic Index	
		β	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*
BMI	kg/m^2	0.055	0.236	0.014	0.746	0.005	0.914	-0.022	0.634	0.031	0.5
TG	mg/dL	**0.143**	**0.013***	**-0.179**	**0.001****	-0.016	0.789	0.072	0.218	0.083	0.153

Table (4). Contd….

-		HOMA-IR		Matsuda Index		Disposition Index		HOMA-β		Insulinogenic Index	
		β	***p***	***β***	***p***	***β***	***p***	***β***	***p***	***β***	***p***
TC	mg/dL	-0.300	0.183	0.264	0.224	0.232	0.310	-0.036	0.879	-0.001	0.996
HDL-C	mg/dL	0.170	0.196	-0.044	0.726	-0.017	0.896	0.063	0.636	0.132	0.319
LDL-C	mg/dL	0.276	0.183	-0.343	0.087	-0.213	0.312	0.027	0.897	-0.002	0.991
RBC count	$x10^6$/mL	0.028	0.726	-0.047	0.545	0.022	0.79	-0.130	0.113	0.017	0.837
Ht	%	0.080	0.642	-0.159	0.338	-0.025	0.886	-0.073	0.675	0.12	0.487
Hb	g/dL	-0.083	0.603	0.228	0.140	0.015	0.926	0.156	0.338	-0.16	0.317
WBC count	$x10^3$/mL	0.082	0.063	**-0.094**	**0.027***	0.093	0.360	0.021	0.636	**0.107**	**0.016***
Platelet count	$x10^6$/mL	0.045	0.310	**-0.116**	**0.007***	-0.034	0.445	-0.037	0.403	-0.049	0.269

BMI, body mass index; RBC, red blood cell; WBC, white blood cell; HbA1c, hemoglobin A1c; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglyceride; HOMA-IR, homeostasis model assessment - insulin resistance; HOMA-β, homeostasis model assessment - beta cell. *, $p < 0.05$ and **, $p < 0.005$.

4. DISCUSSION

This cross-sectional study found that among healthy young Japanese individuals within the physiological range of glycemic control, TG, WBC count, and platelet count might be independent predictors of IGT and T2DM. The Matsuda index was independently associated with TG, WBC count, and platelet count, while HOMA-IR was independently associated with TG. Additionally, WBC count was independently associated with the insulinogenic index.

In our previously reported hypothesis, whole blood rheology was associated with fasting plasma insulin, HOMA-IR, Ht, Hb, fibrinogen levels, and WBC count [31]. Also, even in a physiologically normal range, both fasting plasma insulin and HOMA-IR were associated with Ht and Hb [31], while whole blood rheology was independently associated with Ht, fibrinogen levels, and WBC count [31]. We repeatedly reported that the whole blood rheology, as evaluated by whole blood passage time, was dependent on physical, hematological, and lipid parameters. A longer whole blood passage time reflected stagnated blood flow, which was dependent on higher values of hematological and lipid parameters, except HDL-C (Table **2**) [31, 35-42]. Fasting insulin levels, insulin resistance, and insulin sensitivity are closely related to hematological parameters such as Ht, Hb and WBC count [26, 27, 30, 31, 43-47]. Higher Ht values decrease blood flow, which leads to insulin resistance by reducing glucose delivery to skeletal muscle [27, 30] and the pancreatic β-cells, resulting in β-cell dysfunction [48]. Insulin resistance-related hyperinsulinemia may induce vasoconstriction through sympathetic neural activation, which subsequently raises whole blood viscosity secondary to an increase in Ht levels and RBC aggregation [30, 49-51]. In accordance with these results, we reported that higher remnant-like lipoprotein particle cholesterol contributed to impaired RBC deformability and whole blood rheology [42]. Longitudinal and cross-sectional analyses supported the hypothesis that insulin resistance is associated with increased RBC count and Ht in young adults within normal ranges of insulin and glucose [52]. Moreover, insulin itself and hyperinsulinemia promote erythropoiesis [53-56]. These suggest that insulin resistance-induced continuous hyperinsulinemia raises RBC count through promoting erythropoiesis. In this study, RBC count, Ht, and Hb were positively associated with BMI, fasting plasma insulin, HOMA-IR, and AUCg during the 75-g OGTT. This suggests that RBC count, Ht, and Hb reflect insulin resistance and glucose tolerance, rather than insulin secretion. RBC count, but not Ht or Hb, was negatively associated with the Matsuda index. Similarly, both WBC and platelet count were associated with HOMA-IR, in accordance with previous reports [28, 31, 37, 38, 41, 42, 57-59]. WBC and platelet count were associated with the Matsuda index, AUCi, HOMA-β, and the insulinogenic index, but not the disposition index. These indicate that WBC and platelet count reflect insulin resistance and secretion. RBC, WBC and platelet count reflect two aspects of insulin resistance, fasting steady-state glucose and insulin levels, and hepatic and peripheral tissue sensitivity to insulin. Moreover, RBC count and Hb were associated with the disposition index, which is an index that indicates the composition of insulin secretion and sensitivity [34]. Thus, among healthy young individuals, RBC count may be a good indicator of insulin resistance and glucose tolerance; in the meantime, WBC and platelet counts reflect insulin resistance and secretion.

In accordance with previous reports [18-29], all fasting TC, HDL, LDL-C, and TG were significantly associated with BMI, fasting plasma insulin, HOMA-IR, and the Matsuda index. This suggests that cardinal lipid parameters reflect two aspects of insulin resistance, same as RBC, WBC and platelet count. HOMA-β was significantly associated with fasting TC, LDL-C, and TG, while fasting HDL-C was significantly associated with the disposition index. These results suggest that TC, LDL-C, and TG reflect insulin resistance and release, and that HDL-C reflects insulin resistance and glucose tolerance. Fasting TC, HDL-C, LDL-C, and TG were significantly associated with RBC count, Ht, Hb, and platelet count. WBC count was significantly associated with TG. Supporting these results, we repeatedly showed the significant association between whole blood rheology and lipid parameters [31, 37, 38, 41, 42]. These results suggest that insulin resistance, within the physiologically normal range, affects blood rheology through influencing erythropoiesis as

well as lipid metabolism. A number of hematologic parameters, particularly the white blood count, the platelet count, and to some extent the hemoglobin/hematocrit/RBC counts, are all impacted by other factors which may be relevant to insulin resistance. The most significant of these factors may be inflammation. Patient hydration status at the time blood was collected may impact the hemoglobin/hematocrit/RBC parameters and iron stores, which have been linked to insulin resistance, and can also affect these parameters in the platelet count.

There are some limitations related to our study. The present cross-sectional study included a relatively small number of participants and was performed in a single center. A prospective multicenter study, with a larger sample size, is necessary to confirm the hypothesis of the present study. It is also necessary to confirm the importance of monitoring physical, hematological and lipid parameters, as well as insulin levels and insulin resistance, to predict the occurrence of metabolic diseases in young adults without diabetes.

CONCLUSION

This study suggests that even in young and healthy Japanese individuals with physiologically normal glycemic indices, higher indices of insulin resistance and impairment of insulin release are associated with higher hematological parameters. This can possibly promote diabetes mellitus by influencing erythropoiesis and lipid metabolism.

AUTHORS' CONTRIBUTIONS

TK and MM designed the study. LM, AY, TA, OA, TO, KT, HS, and MN contributed to data collection. TK contributed to the analysis and interpretation of data, and assisted in the preparation of the manuscript. MM critically reviewed the manuscript and provided final approval of the article. All authors approved the final version of the manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethical Review Board for Medical Research Gunma University, Maebashi, Japan, Protocol number 12-41.

HUMAN AND ANIMAL RIGHTS

No animals were involved in the study. All human procedures were followed in accordance with the Helsinki Declaration of 1975 as revised in 2013.

CONSENT FOR PUBLICATION

A written informed consent was obtained from all patients prior to the publication of the study.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study are available from the corresponding author [TK] on reasonable request and are included in this published article.

FUNDING

This work was supported by a Grant-in-Aid for Gunma University and the Society for Collaboration on Food Science and Wellness to A. Yoshida and T. Kimura. This work was supported, in part, by Grants-in-Aid 17H04109 (to M. Murakami) and 20K07841 (to T. Kimura) for scientific research from the Ministry of Education, Culture, Sports Science, and Technology of Japan.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We are grateful to Mayumi Nishiyama for technical assistance.This work was supported, in part, by Grants-in-Aid 23390146, 26293125 (to M. Murakami) and 20K07841 (to T. Kimura) for scientific research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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