Editorial

〔Lab Med Int 2024; 3(1): 1〕

The role and significance of ISO 15189 in incident/accident prevention

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See article volume 3: 8-14

The paper is by Miura et al., entitled: "Analysis of Incident/Accident Reports in the Clinical Laboratory Department at Tohoku Medical and Pharmaceutical University Hospital: Effect of ISO 15189 Implementation on Medical Safety".

Medical safety is a very important issue in medical department of the hospital. ISO 15189 is an international standard that specifies requirements for quality management systems (QMS) specific to clinical laboratories. ISO 15189 accreditation clearly improves the reliability and quality control of inspection results. It also contributes to medical safety by reducing incidents/accidents through repeated review of work.

In this paper, Miura et al. confirmed that the introduction of ISO 15189 is highly effective to reduce the number of recurrent incidents in clinical laboratory, although it will take some time to achieve a full effect.

It is hoped that the introduction of ISO 15189 into clinical laboratories will reduce the number of incidents/accidents and contribute to medical safety.

Review

〔Lab Med Int 2024 3(1): 2-7〕

Mechanism underlying the in vitro formation of urine dysmorphic erythrocytes

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Received November 13, 2019; accepted March 1, 2022

Key Words
dysmorphic erythropoiesis mechanism, glomerular bleeding, urinary acanthocytes, urinary osmolality, hematuria

I. Introduction

In 1979, Birch et al.[1] reported that hematuria can be broadly divided into glomerular (dysmorphic erythrocytes) and urinary tract-derived (isomorphic erythrocytes) based on the morphology of erythrocytes. This classification is also widely used in diagnostics and laboratory tests. On the other hand, various alterations have been reported in the mechanism of erythropoiesis, but no consensus has been reached. There are three of his hypotheses about the formation mechanism of dysmorphic erythrocytes: (1) Mechanical hypothesis: Caused by leakage from damaged glomerular basement membrane, (2) Nephron passage hypothesis: Caused by sudden changes in osmotic pressure, pH, and urinary components in nephron, (3) Mixed hypothesis: First A combination of his two hypotheses. To test these hypotheses, the author searched his original papers and reviews on the mechanism of dysmorphic erythrocyte formation published in his PubMed from 1979 to 2018.

II. Result

1. Documents on which these hypotheses are based

In references [2)3)], dysmorphic erythrocytes leaking from the damaged glomerular basement membrane are observed, but this is not a report on the formation mechanism of dysmorphic erythrocytes. It proves that the cause of hematuria bleeding is the injured glomerulus.

Stapleton [4] reports that mechanical trauma may explain the dysmorphic red blood cells that represent glomerulonephritis. Later, Schramek [5] introduced a hypothesis about the formation mechanism of dysmorphic erythrocytes.

2. About three hypotheses

Hypothesis (1)

Kubota [6] reported that he could produce donut-shaped dysmorphic erythrocytes simply by filtering erythrocytes soaked in saline with a 5 μm fibrin or an untreated 3 μm filter (38℃, 30 minutes). However, the full picture of dysmorphic red blood cells and acanthocytes (ACs) is not displayed.

Daza JL [7] showed an image (Masson's trichrome staining) of erythrocytes passing through the glomerular basement membrane. Daza JL also explained that the causes of erythrocyte malformations are mechanical trauma as cells pass through cracks in the glomerular basement membrane and osmotic trauma as cells pass through nephrons. This indicates that hypothesis (3) is taken into account as the cause of dysmorphic erythropoiesis.

In 2015, Yuste C et al. [8] "Review: Pathogenesis of glomerular hematuria" stated as follows. The presence of dysmorphic red blood cells with irregular contours and shapes in the urine is a characteristic symptom of glomerular hematuria. It also suggests deviations from glomerular capillaries to the urinary tract. Therefore, glomerular hematuria is a marker of glomerular filtration rate barrier (GFB) dysfunction or damage. This indicates that hypothesis (1) is considered to be the formation mechanism of dysmorphic erythrocytes.

Hypothesis (2)

If hypothesis (2) is defined as the case where erythro-

cytes are continuously immersed in the osmotic pressure of simulated urine or nephron solution, Schramek [2], Miura et al [9) 10) 11)], Kitamoto et al. [12] We carried out a nephron simulation. The authors of this method, Miura and Kitamoto, were able to produce dysmorphic red blood cells in patients with glomerulonephritis. Schramek, on the other hand, said that it is not possible to produce dysmorphic erythrocytes with this method, and that after this method, additional hemolytic environments can produce dysmorphic erythrocytes.

Rath [13] soaked erythrocytes in solutions with different osmotic pressure and pH, but no dysmorphic erythrocytes were produced. In response to this result, Rath reported that hypothesis (3) was necessary. When red blood cells are immersed in a single urine or solution, only individual osmotic forms are obtained. Therefore, this does not seem to prove hypothesis (2).

Hypothesis (3)

Briner [14], urine (pH 5-8, osmolality 200-800 mOsm) and blood mixture were aspirated through a 3 μm polycarbonate filter and then incubated at 37 ℃ . for 1 hour. Of these mixtures, **Figure 1** presented by Briner was obtained after aspiration under conditions of resuspension in urine with a pH of 7.0 and an osmotic pressure of 300 mOsm. Also, no dysmorphic red blood cells were produced without filtration. In addition, the debate states that the formation of dysmorphic red blood cells requires two factors: the passage of cracks and the passage of certain suspensions.

From their results, it is unlikely that hypothesis (3) can explain the formation mechanism of modified erythrocytes. It may be the effect of 1 hour of incubation or fixation. Furthermore, glomerular red cells shown by Briner (14) are the same as the "ACs-like cells" experienced by the authors, and care must be taken when classifying the morphology, and it is important to recognize them as isomorphic erythrocytes. This means hypothesis (3), which is a mixture of the mechanical hypothesis and the nephron transit hypothesis, and it is believed that only one solution (pH 7.0, osmotic pressure 300 mOsm) produces dysmorphic erythrocytes. not. There are also ACs, ring cells, and target cells, and from the fact that the overall picture of the morphology is shown, it is considered to be a true image of dysmorphic red blood cells. The author thinks that this image is the dysmorphic red blood cell itself, which is the same as the proof of hypothesis (3).

3. Papers other than the three hypotheses

Halbhuber [15] states that treatment with proteases prior to the nephron simulation increased the number of dysmorphic erythrocytes from 35% to 80% compared to untreated cells (12% -15%). However, the fact that 12% to 15% of dysmorphic erythrocytes were produced without protease treatment proves hypothesis (2). However, Miura and Kitamoto have succeeded in forming dysmorphic erythrocytes without pretreatment with proteases.

Proteases degrade membrane skeletal proteins (ankyrin, band 4.1 protein), band 3 proteins, and glycophorin C, interfering with the interaction of spectrins with membranes and other membrane scaffold proteins. This suggests that as the degradation progresses, the bond be-

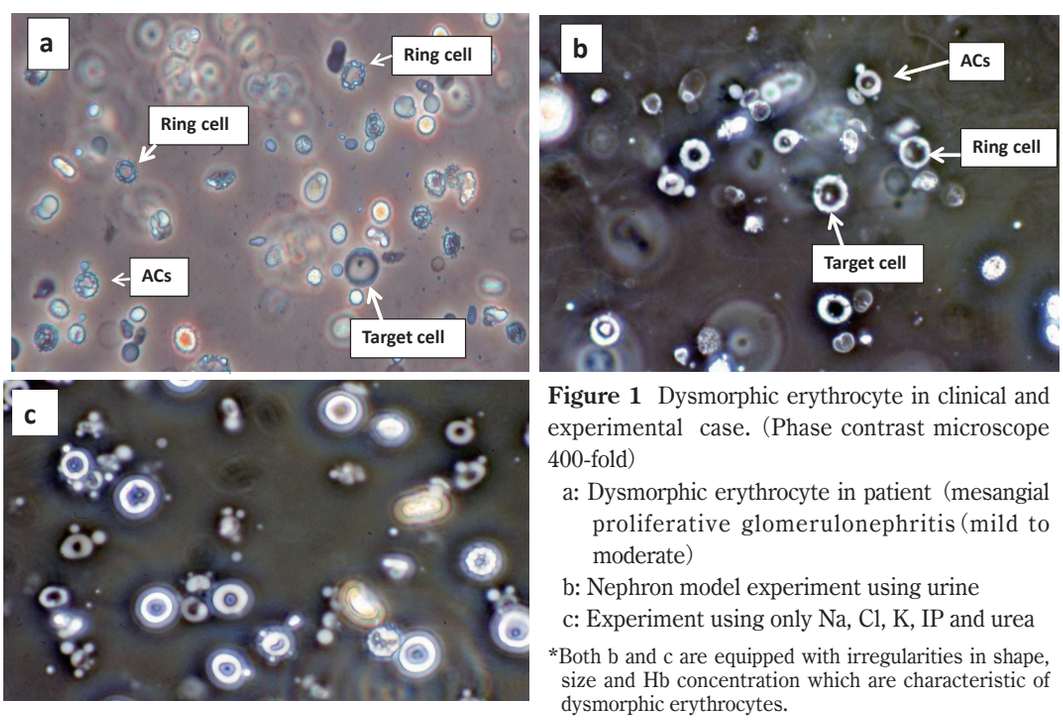

Figure 1 Dysmorphic erythrocyte in clinical and experimental case. (Phase contrast microscope 400-fold)

a: Dysmorphic erythrocyte in patient (mesangial proliferative glomerulonephritis (mild to moderate)

b: Nephron model experiment using urine

c: Experiment using only Na, Cl, K, IP and urea

*Both b and c are equipped with irregularities in shape, size and Hb concentration which are characteristic of dysmorphic erythrocytes.

tween the erythrocyte membrane and the spectrin loosens, and the erythrocyte membrane structure loosens. It is thought that the loosening of these erythrocyte membrane structures results in the formation of protrusions at high osmotic pressure. Therefore, pretreatment with proteases may promote dysmorphic erythropoiesis.

On the other hand, since thrombin is a kind of proteolytic enzyme, thrombin may be involved. In a review entitled "Protease-activated Receptors (PAR) in the Progression of Renal Disease," he reported by Palygin[16] that thrombin is present in the urine of patients with glomerulonephritis rather than in the urine of healthy individuals. In addition, this study showed that excessive PAR stimulation can lead to excessive intracellular Ca2 + levels and cell apoptosis, followed by proteinuria and glomerular damage, which may be directly involved in the progression of renal disease. It was.

In addition, "glomerulonephritis is characterized by carbonyl stress and elevated methylglycoloxol (MGO), where erythrocyte suspensions and MGO accumulate Ca in the body." Degrell et al.[17] We point out that factors may play an important role in the formation of dysmorphic erythrocytes in glomerulonephritis. These facts may fundamentally overturn the hypothesis about the formation mechanism of dysmorphic erythrocytes. However, without treatment with proteases or MGO, Miura et al. Produced dysmorphic erythrocytes in a solution containing only the urinary components Na, Cl, K, IP and urea (see below) and therefore in the urine of patients with glomerulonephritis. It is desirable to measure the content of protease (thrombin) and MGO in the body to clarify the relationship with dysmorphic erythropoiesis, and at the same time, it may be possible to make an early diagnosis of glomerulonephritis by evaluating these factors. Interesting.

Recently, Kitamoto et al.[18] reported that measurement of urinary thrombin enables early diagnosis of crescent glomerulonephritis (CresGN). According to this study, (1) in CresGN, fibrin deposition and thrombinuria were associated with extraglobulinal capillary tissue in which monocytes / macrophages express tissue factor. (2) Fibrin deposition in the CresGN glomerulus indicates thrombin formation. Therefore, the author hypothesized that thrombin was excreted in the urine and that certain he was a CresGN biomarker. (3) Thrombinuria was specific to glomerular inflammation and was not affected by systemic inflammation or coagulation. (4) It has been shown that thrombin produced at the glomerular bleeding site for hemostasis does not significantly affect the measurement of thrombinuria. ⑤ Thrombinuria showed high his CresGN specificity (90.1%) and moderate sensitivity (70.6%). The widespread use of early diagnostic measurement systems will be of great help in treating patients.

Therefore, in addition to the above three hypotheses, proteases or MGO may also be involved, as mentioned above. The mechanism of dysmorphic erythropoiesis remains unclear, justifying future research on this subject.

Finally, when experimenting with the three hypotheses, it is important to note the following:

* Hypothesis (1): Plasma → Bowman's capsule → proximal tubule is isotonic pressure (285 mOsm). Therefore, the osmotic pressure of the immersion liquid before and after filtration should be isotonic urine or solution. We believe that the use of other osmotic pressures does not prove hypothesis (1).
* Hypothesis (2):
  1; The osmotic pressure or pH of the urine or solution used is similar to the osmotic pressure of nephrons, but continuous immersion of red blood cells is essential.
  2; The pH of hyperosmolar urine or solution used after a hemolytic environment must be acidic.
  3; Hemolytic environmental conditions should be determined by the relationship between red blood cell count and osmotic pressure.
  4; Morphological changes that occur after soaking in urine or solution require not only individual images, but also whole images containing dysmorphic red blood cells.

III. Discussion

As described above, various methods and interpretations have been attempted for the three hypotheses, and despite the fact that the electron micrographs and photomicroscopic images of erythrocytes deviating from the glomerular basement membrane have been shown as evidence of the cause of hematuria. However, it is undeniable that it has progressed to three hypotheses by being taken up as the hypothesis (1) of the cause of dysmorphic erythrocytes. Therefore, the factors that cause dysmorphic erythropoiesis are explained below.

1. About nephron osmotic pressure series

The osmotic pressure of each part of the nephron gradually changes in the following order. Bowman's capsule and proximal tubule (285 mOsm), loop of Henle (1,200 mOsm), first distal tubule (50-100 mOsm: hemolytic environment), distal tubule-collecting duct (1,200 mOsm). Therefore, in this order, red blood cells need to be continuously (continuously) immersed in urine or solution.

2. About low osmotic pressure (hemolytic environment)

The osmotic pressure is 50 to 100 mOsm in the textbook, but experimentally it was 161 mOsm or less.

It was full. In addition, urea was needed to obtain different hemolysis levels (various Hb concentrations). Birch [19] states that changes in red blood cell morphology (the pattern of cell morphology is a polymorphism containing at least three different sizes or shapes) and hemoglobin (Hb) levels indicate glomerular hemorrhage. Of these, changes in Hb concentration cannot be explained by hypothesis (1). Regarding this hemolytic environment, Schramek et al. Suggested the existence of an endogenous causal factor released from hemolyzed erythrocytes, Briner et al. [14] found that hemolysis was closely related to the formation of deformation. Kitamoto et al. Report that hemolysis is not essential for the formation of dysmorphic erythrocytes, but the paper produces dysmorphic erythrocytes with only two types, low (140 mOsm) and hyperosmotic solution (1100 mOsm).

3. About urea

Kitamoto et al. Says that urea and other ion-containing solutes are thought to promote dysmorphic erythropoiesis. The authors found that urea caused varying Hb concentrations in dysmorphic erythrocytes, but NaCl did not. The difference in his Hb concentration in dysmorphic red blood cells is due to the difference in the amount of Hb released from hemolyzed red blood cells. It also depends on the degree of damage to the erythrocyte membrane. Dysmorphic erythrocytes produced by experiments using the author's urine and solution met Birch's requirements (**Figure 1b**, **1c**).

4. About hyperosmotic pressure (dysmorphic erythrocyte formation site)

ACs (**Figure 1a**) are the most characteristic of the dysmorphic erythrocyte morphology found in the urine of patients with glomerulonephritis. In an in vitro nephron model experiment using urine, after immersing erythrocytes in his 95 mOsm (hemolytic environment), he developed ACs at a high osmotic pressure of 911 mOsm (**Figure 1b**). In experiments using only Na, Cl, K, IP, and urea, the 104 mOsm (hemolytic environment) solution was simply immersed in a 726 mOsm high osmotic solution (including K: 8.9, IP 9.3 mmol / L, respectively). Can generate ACs (**Figure 1c**). In this way, being able to make his ACs with only his five types of Na, Cl, K, IP, and urea made it easier to elucidate the causes of ACs. Therefore, ACs appeared in hyperosmotic urine or solution after passing through a hemolytic environment. It mimics the in vivo condition in which glomerular hemorrhage induces the formation of dysmorphic erythrocytes in the hyperosmolar region after the erythrocytes have passed through the hemolytic environment. Therefore, for dysmorphic erythropoiesis, it is essential to pass through the hemolytic environment and through the hyperosmolar region. Kitamoto et al. Also stated that conditions of pH 5 and osmotic pressure of 1,000 mOsm simulated in a collecting duct are essential for dysmorphic erythropoiesis. Therefore, it cannot be concluded that dysmorphic erythrocytes were produced simply from the experimental results of immersing erythrocytes in various mono osmotic urine or solutions. Hypothesis (2) is thought to be the result (image) obtained by the final hyper osmolarity in which erythrocytes are continuously immersed according to a nephron simulation. Hyper osmolarity required 425 mOsm or higher, and K or IP required 4.1 mmol / L and 4.3 mmol / L or higher, respectively. Also, no ACs were observed when the composition of the hyperosmolar solution was Na and Cl. However, the number of ACs increased with increasing osmolality or K and IP. These results suggest that the presence of K and IP promotes the formation of AC. However, the question arises as to whether K or IP is the main contributor to ACs formation. In this regard, the K + and PO43- concentrations in erythrocytes are as high as 140 mmol / L and 100 mmol / L, respectively. Some of these are thought to be eluted by hemolysis. For high osmotic solutions, ACs begin to appear at K 4.1 mmol / L and IP 4.3 mmol / L and above, producing large numbers of ACs at osmotic pressures 726 mOsm, K 8.9 mmol / L and IP 9.3 mmol / L and above, respectively. (**Figure 1c**). So far, KH2PO4 solution has been used in experiments, but the question is whether K + or PO43- is involved in the formation of ACs. Therefore, 87 mOsm low osmotic urine, KH2PO4 (pH 4.3), K2HPO4 (pH 8.3), KNO3 (pH 5.6), K2CO3 (pH 10.3), NaH2PO4 (pH 4.3), Na2HPO4 (pH 8.6) are high osmotic solutions. Used as. ACs could not be made with KNO3 (acidic), alkaline and NaCl solutions, but ACs appeared with acidic solutions of KH2PO4 and NaH2PO4 (pH 4.3). Thus, KNO3, which is acidic but does not contain PO43-, could not produce AC, suggesting that PO43- is more important than K + for the formation of AC.

In this regard, anionic drugs are believed to act primarily on the outer membrane surface of the erythrocyte membrane bilayer and expand it. However, the protrusions examined at this point had the same Hb concentration as the ACs body, and it was considered that the entire membrane containing Hb formed protrusions (**Figure 1b** and **1c**). Immersing normal red blood cells in hyperosmotic

fluid allows water to escape and form a sharp, pointed shape. On the other hand, red blood cells soaked in hypotonic fluid increase in volume and loosen the structure of the red blood cell membrane. When erythrocytes are exposed to high osmotic pressure in this state, the transmembrane proteins scattered in the erythrocyte membrane are positively charged by the acidity of the high osmotic solution. As a result, it was considered that a part of the transmembrane protein (+) was pulled out by the phosphate anion (PO43-) eluted from the erythrocytes, and a round protrusion was formed at the tip.

The anatomical changes in osmotic pressure before and after the hemolytic environment are similar. In the nephron simulation using urine, ACs were not observed until the red blood cells reached the hemolytic environment. This is true even after a hemolytic environment. Therefore, it is considered that the erythrocytes formed by bleeding from the glomerulus and renal tubules before reaching the hemolytic environment are degenerative, but the morphology formed by bleeding from the renal tubules after the hemolytic environment is homogeneous. Be done. Red blood cells formed by bleeding from the glomeruli and renal tubules prior to the hemolytic environment are considered to be degenerative, and the morphology formed by bleeding after the hemolytic environment, including the urinary tract, is considered to be uniform.

5. Regarding erythrocyte morphology:

Typical erythrocyte morphologies of deformity include spiny, rinng, and target erythrocytes. Of these, the spiny type is the most characteristic and specific form, and is closely associated with glomerular disease, Köhler [20] states. On the other hand, in non-glomerular hematuria, "ACs-like cells" appear when red blood cells are immersed in the following single urine conditions. Urine: 745 mOsm (pH 5.0, 15 minutes), 273 mOsm (pH 5.0, 2 hours), 273 mOsm (pH 5.0, 2 hours), 385 mOsm (pH 5.5, 2 hours), 607 mOsm (pH 5.5, 2 hours), 607 mOsm (pH 5.0, 2 hours), 607 mOsm (pH 5.0, 2 hours) 5.5, 2 hours), 933 mOsm (pH 5.0, 2 hours), 312 mOsm (pH 5.0, room temperature overnight). These conditions can result in the development of "ACs-like cells" when ureteral stones or hematuria is stagnant in the bladder, and these red blood cells should be classified as uniform red blood cells.

Conclusion

From the above results, the author proposes the following mechanism of his ACs formation in vivo.

Red blood cells due to bleeding from the renal tubules before reaching the glomerulus and hemolytic environment reach the hemolytic environment. Here, erythrocytes become erythrocytes with various Hb concentrations and contract when the hemolyzed erythrocytes reach the hyperosmolar region. This contraction acts disproportionately depending on the degree of damage to the hemolytic erythrocyte membrane, leading to the formation of dysmorphic erythrocytes such as ACs, ring-shaped cells, and target cells.

Acknowledgments

We would like to thank Dr. Hiroshi Sato of Tohoku University for lending us a valuable list of renal biopsies to prepare our paper.

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Original

Analysis of Incident/Accident Reports in the Department of Clinical Laboratory at Tohoku Medical and Pharmaceutical University Hospital: Effect of ISO 15189 Implementation on Medical Safety

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ABSTRACT

Objective: Managing the safety and security of medical treatment information is critical for every clinical department in a hospital, and improving safety and security management is increasingly emphasized. Our laboratory, the Department of Clinical Laboratory, Tohoku Medical and Pharmaceutical Hospital, acquired ISO 15189 accreditation in January 2019. We sought to assess the effect of ISO 15189 quality management on medical safety by analyzing incidents and accidents before 2017 and from ISO 15189 acquisition until 2022.
Methods: We assessed the classification of incident severity, the medical services in which incidents occurred, the specific laboratory test steps in which incidents occurred, the variety and number of recurrences, and the medical services in which recurrent incidents were observed.
Results: No accidents above level 3b were observed over the study period, and the total number of incidents decreased significantly after 2020. Level 1 incidents in particular decreased notably after 2021. Incidents that occurred during the night shift/day duty dramatically decreased after 2019 (2017–2018, 21 cases; 2019–2022, 10 cases, $p < 0.001$). The proportions of incidents by laboratory test step remained unchanged. The number of recurrent incidents was highest in 2018 (12 cases) and then gradually decreased (2019, eight cases; 2020, five cases; and 2021, one case). No recurrent cases were observed after 2022.
Conclusions: Implementing a quality management system that meets ISO 15189 standards is associated with fewer incidents, especially in night shift/day duty services. Reducing recurrent incidents may require additional time, even with the application of ISO 15189 systems.

〔Lab Med Int 2024; 3(1): 8-14〕

Key Words
incidents, ISO 15189, risk management, medical safety

I. Introduction

Achieving medical safety in all the medical departments of a hospital is very important. The 2007 medical service law enforced the preparation of guidelines not only for infection control, operational procedures for pharmaceu-

tical medicines, and maintenance plans for medical equipment but also for medical safety [1]. Therefore, guidelines to ensure medical safety are always required. The errors that occur in clinical diagnostic laboratories are suggested to be fewer than those that occur elsewhere in a hospital setting; however, even a low rate may reflect a large number of errors [2]. Strengthening medical safety has been attracting attention as an approach to delivering conclusive and accurate clinical laboratory data to physicians [3, 4].

In Tohoku Medical and Pharmaceutical University Hospital, an incident report management system, e-power/CLIP (NSD Corp., Tokyo, Japan), has been used since January 2017. By allowing the rapid issue of incident reports, this system contributes to improving the quality of medical services. The following is an example of our practical use of the CLIP system. On 5 July 2021, a clinical specimen sent with a dumbwaiter from the central supply division was ignored for a period beyond that allowed from the time of specimen collection. As a result, the specimen had to be collected again. The CLIP system reported the event on the same day. On July 7, the Medical Safety Managing Department directed that posters displaying "No specimens" be posted on all of the dumbwaiter doors on each floor. On 15 July, a manager from each of the hospital's wards and medical divisions participated in the monthly risk manager meeting and the incident was shared. In summary, only 2 days after the CLIP report, a quick fix was implemented through the collaboration of the Medical Safety Management and the Clinical Laboratory departments, and the poster was posted in all the divisions in the hospital within 8 days. Thereafter, no recurrence of the incident was reported. The CLIP system has enabled a rapid response and efficient communication to address incidents related to medical safety.

ISO 15189 is an international standard that specifies the requirements for quality management systems (QMS) that are specific to medical laboratories. The standard has been reported to play an important role in quality improvement and the prevention of examination errors [5, 6]. Our laboratory, the Department of Clinical Laboratory at Tohoku Medical and Pharmaceutical Hospital, received ISO 15189 accreditation in January 2019 [7]. In this study, we examined the effects of ISO 15189 accreditation and CLIP management on the number and types of incidents. Specifically, we assessed the incident severity classification, the medical services and laboratory testing steps in which incidents occurred, the type and number of recurrences, and the medical services in which recurrences occurred in the fiscal years 2017 to 2022. Furthermore, we discuss the role and significance of ISO 15189 on QMS in the prevention of incidents.

II. Methods

Participants

We determined the number of tests conducted in the Department of Clinical Laboratory, Tohoku Medical and Pharmaceutical University Hospital. In total, 95 incident reports were delivered to the Medical Safety Management Department by the CLIP system between April 2017 and March 2023. The incident severity classification system recommended by the National University Hospital Council of Japan [8] was used, and the risk manager of the Medical Safety Management Department in our hospital objectively determined the final incident level using this classification system. For all of the incidents that occurred during the study period, we determined the incident severity classification, the medical services in which incidents occurred, the laboratory testing steps in which incidents happened, the type and number of recurrences, and the medical services in which recurrences occurred. The following were the numbers of incidents/total numbers of tests observed throughout the study period: 2017: 26/2,201,331, 2018: 27/2,301,813, 2019: 17/2,545,856, 2020: 10/2,670,728, 2021: 9/2,906,752, and 2022: 6/2,873,229.

Statistics

For statistical analysis of the transition of incident occurrence, we first prepared a cross-tabulation table for each fiscal year or setting. We then performed a chi-square test and residual analysis, and statistical significance was calculated. We compared the values for the 2017 or 2018 fiscal year with those for every year after 2019, using the above strategy (**Figure 1**). We noted the p-value when significance was shown ($p < 0.05$, $p < 0.01$, and $p < 0.001$). We used the Holm–Bonferroni method to calculate the revised p-value. In addition, the p-values (**Figures 2** to **4**) were calculated from adjusted residuals.

Ethics

We carefully considered personal information. This study focused on the details of the incidents and ignored the personal information of the persons who reported, caused, or suffered from the incidents. The study was approved by the Ethics Committee of the Tohoku Medical and Pharmaceutical University Hospital (2023-2-012). Informed consent for the handling of personal information and the opportunity to deny participation was obtained in the form of an opt-out sent by e-mail to the Department of Clinical Laboratory staff.

III. Results

Annual change in the number of incident severity classification

The Japan Council for Quality Health Care classifies level 0 and 1 incidents as minor incidents, regarding these incidents as "errors that can be avoided". The Council regards level 2 and 3a incidents as "intermediate errors" and incidents above level 3b as "serious errors"[9]. The number of incidents was highest in the fiscal years 2018 (27 cases) and 2017 (26 cases). Thereafter, 17, 10, nine, and six cases were observed in 2019, 2020, 2021, and 2022, respectively (**Figure 1**). Statistical analysis revealed that the decrease was significant after 2020 (2020: $p < 0.01$, 2021–2022: $p < 0.001$). Among the total 95 incident reports, 31 cases (33%) were level 0, 56 cases (59%) were level 1, two cases (2%) were level 2, six cases (6%) were level 3a, and no accidents above

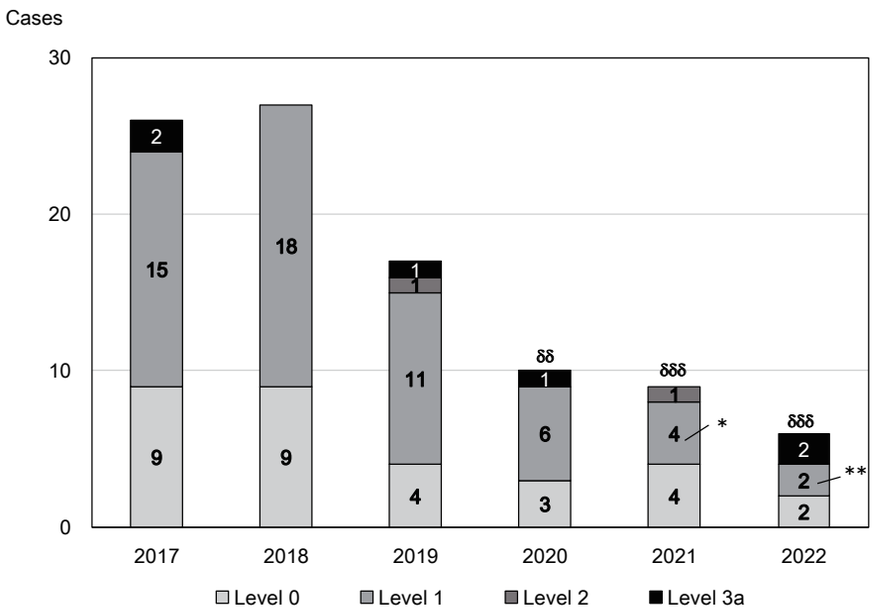

Figure 1 Number of incidents from 2017 to 2022 by incident severity.
The number of incidents in fiscal years 2017 or 2018 were compared with those in each year after 2019, using the chi-square test and residual analysis, and the statistical significance for the total number of incidents (δδ $p < 0.01$, δδδ $p < 0.001$) or each incident level (* $p < 0.05$, ** $p < 0.01$) was calculated. We used the Holm–Bonferroni method to calculate the revised p-value.

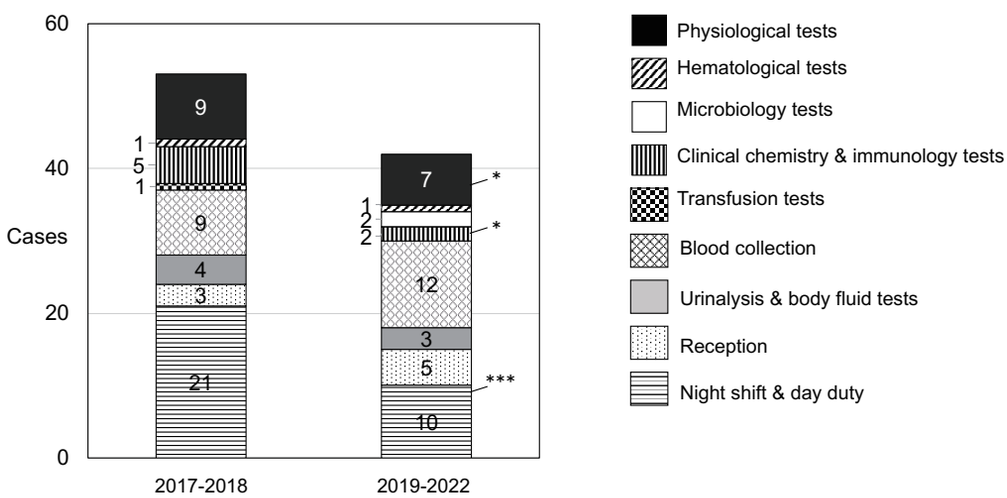

Figure 2 Number of incidents categorized by medical service in 2017–2018 and 2019–2022.
First, a cross-tabulation table that included incidents by medical service was prepared for the periods before 2017–2018 and after 2019–2022. Then the chi-square test and residual analysis were performed, and statistical significance was calculated. We noted the p-value, which was calculated from the adjusted residuals when significance was shown (* $p < 0.05$, *** $p < 0.001$).

level 3b were observed. Level 1 incidents in particular decreased significantly after 2021 (2021: $p < 0.05$; 2022: $p < 0.01$; **Figure 1**); in addition, the chi-square test and residual analysis revealed no significant changes in the remaining levels.

The number of medical services in which incidents occurred before and after ISO 15189 implementation

Next, of the total 95 incidents, we analyzed the number of medical services in which incidents occurred. We compared the change before (2017 and 2018) and after (2019 to 2022) the implementation of ISO 15189 and observed that most of the incidents occurred during laboratory tests (i.e., hematological, microbiology, and clinical chemistry and immunology tests; transfusion tests; urinalysis, and body fluid tests) and not during physiological tests (**Figure 2**). Notably, the incidents related to night shift or day duty were highest in 2017–2018 (21 cases) but dramatically decreased in 2019–2022 (10 cases). In addition, physiological tests and clinical chemistry and immunology tests appeared to decline significantly ($p <$

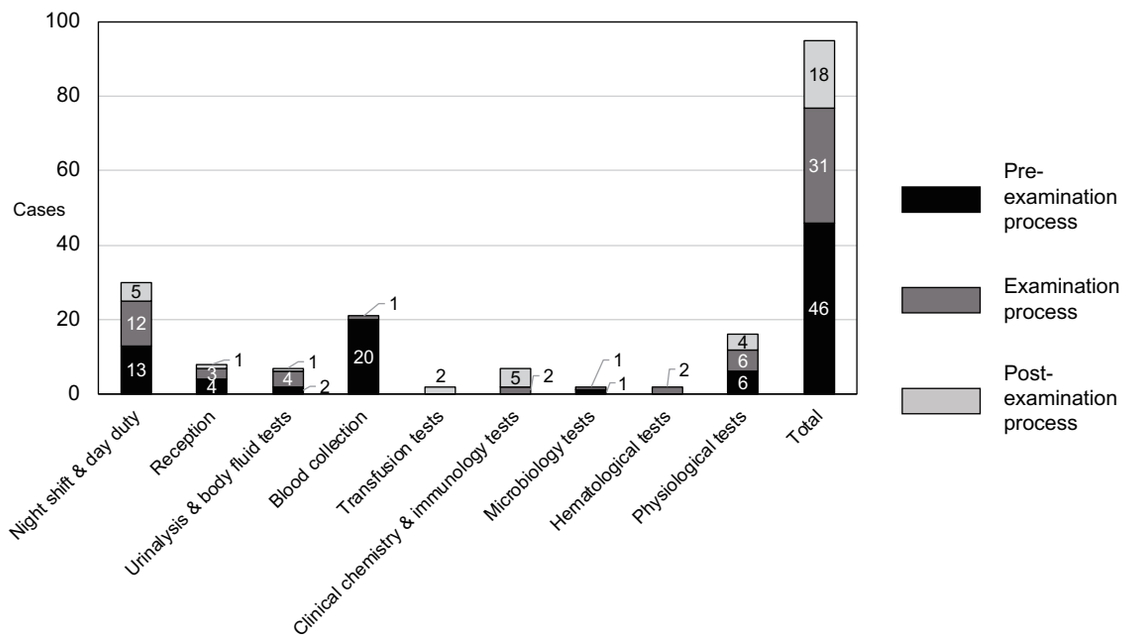

Figure 3 Total number of incidents categorized by examination process.
The total number of incidents that occurred in each medical service, categorized by examination process, is presented.

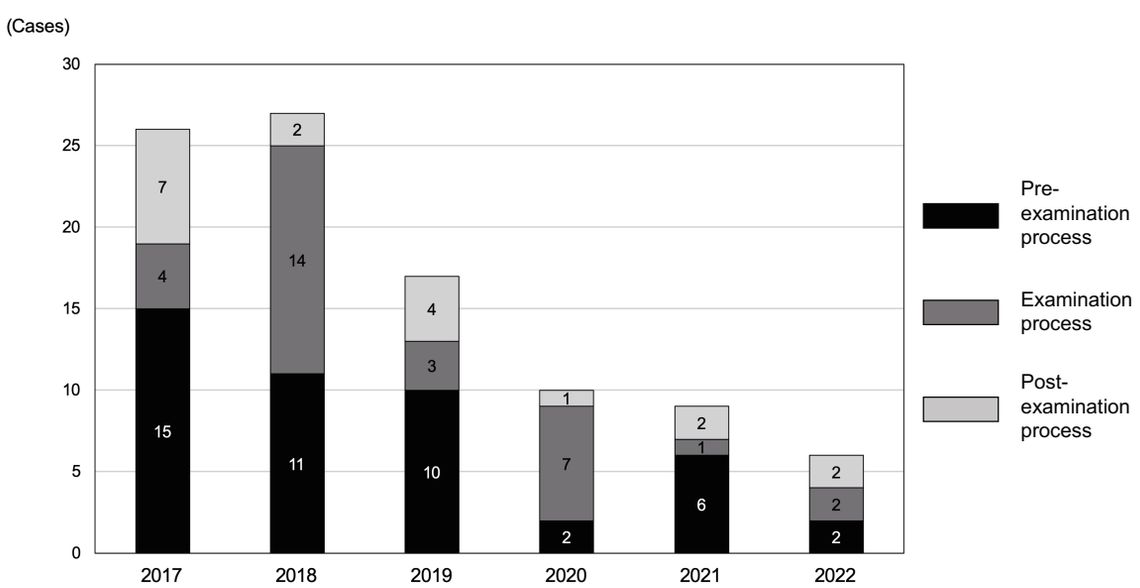

Figure 4 Number of incidents categorized by examination process from 2017 to 2022.
The number of incidents for fiscal years 2017 or 2018 was compared with that of each year after 2019, using the chi-square test and residual analysis. Statistical significance was calculated for examination processes, and no significance was found.

0.05) between 2017–2018 and 2019–2022.

Incidents in each medical service by laboratory testing step

We further analyzed the number of incidents by laboratory testing step in each medical service (**Figures 3** & **4**). Of the total number of incidents, the number of incidents in pre-examination, examination, and post-examination processes was 46, 31, and 18 cases, respectively (**Figure 3**). The proportions of incidents in each process were relatively similar to those in a previous report [10]. We observed that the incidents that occurred in blood collection and reception were mostly during pre-examination processes, whereas in clinical chemistry & immunology tests, incidents occurred mostly in post-examination processes. Despite the significant decrease in the total number of cases after 2020 (**Figure 1**), no significant change was observed in the trend in the proportions of incidents by laboratory testing step (**Figure 4**).

Analysis of recurrent incidents

Figure 5 shows the recurrent incidents among the total 95 incidents throughout the study period. We did not define "recurrence" as the same case of an incident but as the repetition of the same type of incident. The number of recurrent incidents was highest in 2018 (12 cases), and then gradually decreased (2019: 8 cases; 2020: 5 cases; and 2021: 1 case). Recurrences did not occur in fiscal year 2022. In summary, reducing recurrent incidents may take additional time, even with the application of ISO 15189 systems.

IV. Discussion

The ISO 31000 system is an international standard that corresponds to the JIS Q 31000 standard in Japan and is used as a risk management system. The ISO 31000 guidelines are centered on leadership and commitment. The effectiveness of risk management depends on the guideline's integration into all aspects of an organization, including decision making. The remaining components of the framework are design, implementation, evaluation, and improvement. This approach is often represented in management literature as the plan-do-check-act cycle [11]. In the ISO 15189 QMS management requirements, the following is listed as a general requirement: "The laboratory shall establish, document, implement, and maintain a quality management system and continually improve its effectiveness in accordance with the requirements of this international standard" [12]. These concepts of design, implementation, evaluation, and improvement are consistent with those in the ISO 31000 risk management system. We observed an obvious decrease in the number of incidents after 2020, indicating that ISO 15189 was sufficiently effective as a risk management system. The relationships between ISO 15189 and 31000 are as follows. In 2019, ISO 15189 mainly consisted of "4. Management requirements" and "5. Technical requirements." Risk management was only part of management requirement 4.14.6. In contrast, ISO 31000 provides a set of principles and guidelines for the design and implementation of a risk management framework and recommendations for

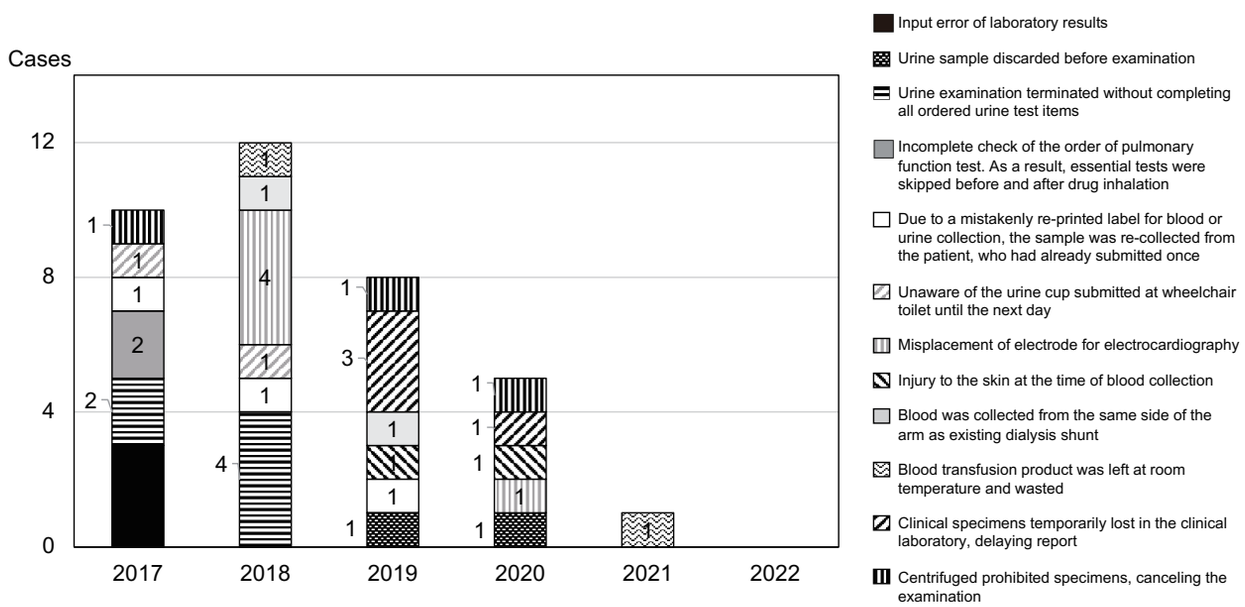

Figure 5 Number and type of recurrent incidents from 2017 to 2022.
The trends in the number and types of recurrent incidents are presented. No recurrent incidents were observed in 2022.

the application of a risk management process [11].

ISO 31000 cannot be used for certification purposes, but guides internal or external audit programs. Organizations that use the standard can compare their risk management practices with an internationally recognized benchmark and have access to sound principles for effective management and corporate governance [13].

Recurrent incidents did not appear in 2022 (**Figure 5**). The number of new incidents significantly decreased after 2020, immediately after ISO 15189 implementation; however, the reduction of recurrent incidents may require additional time. Below, we discuss several possible causes of the decrease in recurrent incidents.

(1) The establishment of a process for informing everyone after incidents occurred: Before ISO 15189 implementation, an informing procedure was not available, and considerable time was required to notify the organization about incidents. However, after ISO 15189 implementation, an informing process was documented and disseminated. Furthermore, "incident sharing reports" allowed the staff to immediately share summaries of incidents, allowing a prompt analysis of causation and the identification of solutions. The establishment of this informing process improves risk management and may result in a reduction in the number of recurrent incidents.

(2) Establishment of an approach for root cause analysis: To allow root cause analysis and the development of incident prevention plans, we scheduled several corrective action workshops during the year.

(3) Development of complete prevention plans and corrective actions: We use an "improvement and prevention plan/report" when incidents occur. Written by a concerned and/or responsible person, this document describes the incident, its cause, and an improvement plan. Next, the document is approved and sometimes corrected by a technical manager who monitors technical aspects in the laboratory, a quality manager who monitors QMS, and a laboratory manager who develops and executes QMS operations. This series of detailed checks ensures the completion of corrective actions, contributing to the reduction of recurrent incidents.

Erroneous reports are known to occur frequently during day or night duty [14]; our results are consistent with this finding (**Figure 2**). This is possible because, during day or night duty, the laboratory technologists who handle sample specimens usually perform physiological tests but not hematological or biochemical tests. Second, the laboratory technologists who usually handle sample specimens have insufficient skills to perform physiological tests such as electrocardiograms. In addition, during day or night duty, less-experienced laboratory technologists must promptly provide services without guidance. The implementation of ISO 15189 includes the preparation of standard operating procedures and day or night duty manuals, which may be especially useful for staff with day or night shift duty who are not familiar with procedures they do not regularly perform. In addition, we observed a significant reduction in incidents involving physiological and clinical chemistry and immunology tests (**Figure 2**). We cannot identify the reasons for the decline in immunology testing incidents. However, we estimate that the reason for the reduction in the number of incidences that involve physiological tests — among which misplacement of electroencephalogram electrodes is frequent — may be associated with our annual check of electrode placement, which may have negated the effect of personnel differences.

Heinrich's law holds that for every workplace accident that causes a major injury, 29 accidents cause minor injuries, and 300 accidents cause no injuries [15]. A similar analysis by Frank E. Bird in 1966 further supported the theory. Bird based his findings on 1,753,498 accident reports from 297 companies. His updated triangle showed the relationship between one serious injury accident and 10 minor-injury accidents, 30 damage-causing accidents, and 600 near-misses [16]. Level 0 and 1 incidents can be regarded as "no injuries," level 2 and 3 a incidents as "minor injuries," and level 3b and higher incidents as "serious injuries." Therefore, the 87:8:0 ratio obtained in our study roughly matched that in Heinrich's law, suggesting that the CLIP system can efficiently detect near-miss incidents.

In conclusion, we analyzed incidents before and after the 2019 implementation of ISO 15189 and observed that the total number of incidents decreased significantly after 2020. In particular, level 1 incidents significantly decreased after 2021. We observed that incidents that occurred during the night shift and day duty declined dramatically after 2019. The proportions of incidents categorized by laboratory testing step remained unchanged. Overall, the implementation of a QMS that meets ISO 15189 standards is effective in reducing incidents.

Acknowledgments

We thank Anahid Pinchis from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Funding

This work was supported in part by the Shino-Test Corporation Research Fund.

Author contributions

R. Miura designed the study, collected and analyzed the data, and wrote the manuscript. R. Kozakai designed the study. S. Suzuki performed the statistical analyses. S. Takahashi wrote the manuscript. All authors have accepted responsibility for the entire content of this manuscript and have approved its submission.

Disclosure of Conflicts of Interest

None.

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Original

Influence of high-fat and high-carbohydrate meals on lipids, apolipoproteins, and coagulation and anticoagulation factors

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ABSTRACT

Background: Nonfasting triglyceride levels have an equal or greater impact on predicting cardiovascular disease events than fasting levels.
Objectives: To examine the effects of consuming a high-fat or a high-carbohydrate meal on the plasma levels of lipids, apolipoproteins, and coagulation and anticoagulation factors.
Methods: In a randomized cross-over study, 12 young non-obese women were served isocaloric meals, predominantly fat (69 E% fat) or carbohydrate (91 E% carbohydrates), on two separate days, and blood samples were taken before and 2.5 and 5.5 h after the meal.
Results: After consuming a high-fat meal, there were significant increases in triglycerides, small dense LDL (sdLDL) cholesterol, and factor VII activity. However, levels of total, LDL, and HDL cholesterol, and apolipoproteins decreased; and levels of total protein S antigen and its activity, free protein S antigen, protein C antigen, and fibrinogen remained unchanged. On the other hand, all these parameters, except for apoC-II and total protein S antigen, decreased after eating a high-carbohydrate meal. Insulin increased postprandially, while glucose levels remained unchanged. Strong positive relationships between triglycerides and the levels of sdLDL cholesterol and apoC-II were observed both at fasting and after consuming meals. However, no correlation was found between triglycerides and coagulation and anticoagulation factors such as factor VII activity and total protein S antigen and its activity.
Conclusions: Consuming a high-fat meal increased factor VII activity during the postprandial elevation of triglycerides and sdLDL cholesterol. However, the activated protein C-dependent anticoagulant system may not regulate this postprandial hypercoagulable state.

〔Lab Med Int 2024; 3(1): 15-21〕

Key Words
High-fat or high-carbohydrate meal, triglycerides, factor VII, protein S, small dense LDL cholesterol

I. Introduction

Plasma lipid profiles, including lipids, lipoproteins, and apolipoproteins, are affected by food intake and show a 24-h cycle. Therefore, they are evaluated in blood drawn after fasting for at least 8 h, which normally only occurs a few hours before breakfast [1]. Elevated low-density lipoprotein (LDL) cholesterol is a well-established independent risk factor for coronary heart disease (CHD) [2]; however, the importance of elevated triglycerides remains to be elucidated. Triglyceride-rich lipoproteins and their remnant lipoproteins are increased in plasma postprandially and may be atherogenic [3] [4]. Prospective cohort studies have reported that elevated levels of nonfasting triglycerides have an equal or greater impact on predicting cardiovascular disease (CVD) events and mortality than fasting levels [5-9].

Postprandial triglyderidemia, particularly after eating

high-fat meals, leads to increased levels of plasma factor VII activity and activated factor VII [10]. In vitro, large very-low-density lipoproteins (VLDL) rather than small VLDL enhance tissue factor (TF)-independent activation of factor VII by factor Xa and factor Xa/Va [11]. In addition, vitamin K-dependent coagulation and anticoagulation factors, such as factors VII, IX, X, prothrombin, protein C, and protein S, as well as C4b-binding protein (C4BP), a complement regulatory protein, have been reported to be associated with triglyceride-rich lipoproteins, chylomicrons, chylomicron remnants, and VLDL, especially after consuming high saturated fat meals; however, there is no association with LDL or high-density lipoprotein (HDL) [12] [13].

Protein S plays an important role in anticoagulation as a cofactor for the activated protein C (APC)-dependent inactivation of factors Va and VIIIa. It also functions as a cofactor for the tissue factor pathway inhibitor (TFPI)-dependent inactivation of factors Xa and TF-VIIa complex [14] [15]. In human plasma, approximately 40% of protein S circulates in its free form, with the remaining 60% of protein S non-covalently binding to C4BP and losing its cofactor activities. Using a cross-sectional study, we previously reported that apolipoprotein (apo) C-II, a critical cofactor of lipoprotein lipase [16], is a significant predictor of fasting total protein S antigen levels in middle-aged obese women, about half of whom exhibited dyslipidemia, as well as in nonobese young women with normolipidemic profiles [17].

Plasma LDL comprises multiple distinct subspecies and has been grouped into four major subclasses based on density, LDL-I through -IV, from the largest, most buoyant to the smallest, most dense [18]. These subspecies differ in their metabolic behavior and pathogenic roles. An elevated level of small dense LDL (sdLDL) particles is accepted as an emerging risk factor for CHD [2] [19]. They are largely formed from the delipidation of triglyceride-rich VLDL catalyzed by lipoprotein lipase and hepatic lipase [18]. Recently, an automated homogeneous assay was developed that accurately measures sdLDL cholesterol [20]. A systematic review and meta-analysis reported a positive association between fasting level of sdLDL cholesterol and CHD [21].

The aim of this study was to investigate the impact of consuming a high-fat meal on the levels of lipids and apolipoproteins, including triglycerides, sdLDL cholesterol, and apo C-II, as well as coagulation and anticoagulation factors such as factor VII and protein S, and to compare the findings with those of an isoenergic high-carbohydrates (low-fat) meal used as a control.

II. Methods

Study subjects and study design

Thirteen healthy Japanese female university students were enrolled in this study. The students were not current smokers, did not have menstruation disorders, nor use oral contraceptives. Gene analysis showed that all subjects were homozygous for the wild-type of protein S Tokushima (p.Lys196Glu, rs121918474), a protein S gene polymorphism prevalent among the Japanese population [22]. One subject dropped out of the study due to feeling sick while consuming a test meal. The basic characteristics (mean ± SD) of the final 12 participants were as follows: age, 21.3 ± 0.5 years; height, 160.8 ± 5.6 cm; weight, 56.6 ± 6.5 kg; BMI, 21.9 ± 2.2 kg/m^2. The present study was performed after approval of the Ethics Committee of Nakamura Gakuen University and written informed consent was obtained from all participants.

The trial was a cross-over study with the order of the meals randomized. The day before each study, the participants were directed to refrain from eating high-fat meals, drinking alcohol, and carrying out intense activity. On the day of the study, all participants consumed an experimental isoenergic high-fat or high-carbohydrate meal after an overnight fast. The meals were served on two separate days (seven to twenty days apart; average: 11 days) at admission to the Health Promotion Center of Nakamura Gakuen University. After measuring weight and height and taking a blood sample, the participants consumed test meals with water under observation between 8:45 and 9:00 in the morning. High-fat meals (total energy 705 kcal: 69 E% fat, 27 E% carbohydrate, and 4 E% protein) consisting of bread, butter, margarine, and pudding, and high-carbohydrate meals (total energy 701 kcal: 2 E% fat, 91 E% carbohydrate, and 5 E% protein) consisting of rice and nonfat agar jelly were consumed. Then the participants were allowed to rest in a sitting position and were instructed not to consume anything except for water (< 500 mL during the study). Blood samples were drawn at 2.5 and 5.5 h after the meal based on a standardized oral fat tolerance test [10], with a slight modification.

Analyses of blood samples

Plasma and serum samples were prepared and stored as reported previously [17]. The analysis of the antigen level and APC-cofactor activity of total protein S was performed by using a total protein S-assay system (Shino-Test Co., Sagamihara, Japan) [23]. Free protein S and protein C antigen were measured using latex agglutination methods (Diagnostica Stago, Inc., New Jersey, USA and LSI Medience. Co., Tokyo, Japan, respectively),

factor VII activity was by a clotting assay (Instrumentation Laboratory Co., Bedford, USA), and fibrinogen by a thrombin-time method (Sysmex Co., Kobe, Japan). Total, LDL, and HDL cholesterol, triglycerides, apoA-I, B (the sum of B-48 and B-100), C-II, C-III, E, glucose, and insulin were measured using previously reported methods [17]. The level of sdLDL cholesterol was assayed by an automated homogeneous assay (Denka Seiken Co., Tokyo, Japan) [20]. Genomic DNA was purified from the buffy coat of citrated blood samples, and the genotype of protein S Tokushima mutation was assessed, as reported previously [23].

Statistical analysis

Kolmogorov-Smirnov test was used to test the normality of data distribution, and all data were found to be normally distributed. Paired Student's t test was used to evaluate the fasting levels of all measures on two different study visits and there was no significant difference. A one-way repeated measures ANOVA followed by multiple comparisons was used to test for differences across time. We performed univariate linear regression analyses to determine whether there were any correlations between fasting values (averaged of two visits), postprandial values, and changes in values during blood sampling, and Pearson's correlation coefficients were calculated. Statistical analyses were performed using PASW Statistics ver. 18 (SPSS Inc., Chicago, IL, USA), and a P value < 0.05 was considered significant.

III. Results

After consuming a high-fat meal, triglycerides significantly increased at 2.5 h and decreased afterward (**Table 1**, **Figure 1**). The level of sdLDL cholesterol increased after 5.5 h; however, total, LDL, and HDL cholesterol, as well as apo A-I, B, C-II, C-III, and E decreased postprandially. Accordingly, the ratio of sdLDL cholesterol to LDL cholesterol showed a significant increase 5.5 h after the meal. A significant increase in factor VII activity was found over time after the meal, whereas the levels of fibrinogen, total PS antigen and its activity, free protein S antigen, and protein C antigen remained unchanged. Insulin levels peaked 2.5 h after meal consumption, while glucose levels remained unchanged. On the other hand,

Table 1 Fasting and postprandial levels of lipids, apolipoproteins, and coagulation and anticoagulation factors.

	High-fat meal				High-carbohydrate meal			
	Fasting	Postprandial		P value[b]	Fasting	Postprandial		P value
		2.5 h	5.5 h			2.5 h	5.5 h	
Triglycerides (mg/dL)	70.7 ± 39.4[a]	124.0 ± 68.4***	97.9 ± 69.6	<0.001	76.2 ± 47.0	67.8 ± 48.7	52.7 ± 37.6***	<0.001
Total cholesterol (mg/dL)	194.4 ± 32.8	187.0 ± 35.2*	188.8 ± 32.9	0.038	191.0 ± 36.9	185.6 ± 31.0	177.9 ± 28.9***	<0.001
LDL cholesterol (mg/dL)	109.8 ± 27.5	102.3 ± 26.4***	104.3 ± 25.9**	<0.001	101.4 ± 28.7	98.5 ± 25.0	94.1 ± 23.6***	<0.001
sdLDL cholesterol (mg/dL)	25.9 ± 12.8	25.6 ± 13.3	28.6 ± 15.3*	0.003	24.6 ± 12.6	22.5 ± 11.2*	20.1 ± 10.0***	<0.001
sdLDL cholesterol/ LDL cholesterol (%)	22.6 ± 6.4	23.9 ± 7.3	26.2 ± 8.4***	<0.001	23.6 ± 7.8	22.2 ± 7.7*	20.6 ± 7.0***	<0.001
HDL cholesterol (mg/dL)	70.7 ± 12.7	65.5 ± 14.2***	65.8 ± 12.2***	<0.001	72.5 ± 15.1	70.7 ± 14.7	68.4 ± 14.7***	<0.001
ApoA-I (mg/dL)	160.8 ± 23.9	154.9 ± 27.7*	156.1 ± 25.1	0.033	167.2 ± 33.6	164.0 ± 33.1	158.8 ± 32.3***	<0.001
ApoB (mg/dL)	79.0 ± 18.7	75.7 ± 19.5*	76.7 ± 18.6	0.012	75.0 ± 18.4	73.2 ± 16.5	69.9 ± 15.8***	<0.001
ApoC-II (mg/dL)	3.2 ± 1.0	3.0 ± 1.0	2.7 ± 0.8**	<0.001	3.2 ± 1.1	3.4 ± 1.1*	3.3 ± 1.1	0.021
ApoC-III (mg/dL)	8.3 ± 2.2	8.1 ± 2.3	7.4 ± 1.9***	<0.001	8.9 ± 2.4	9.2 ± 2.5	8.4 ± 2.3**	<0.001
ApoE (mg/dL)	4.3 ± 0.9	4.1 ± 0.8	3.8 ± 0.8***	<0.001	4.2 ± 0.9	3.9 ± 0.8*	3.5 ± 0.7***	<0.001
Factor VII activity (%)	88.8 ± 19.7	96.1 ± 22.8*	97.9 ± 18.8**	0.005	87.9 ± 21.9	85.7 ± 19.1	77.7 ± 21.5**	0.001
Fibrinogen (mg/dL)	254.8 ± 41.0	251.3 ± 37.5	252.1 ± 38.4	0.598	276.1 ± 41.2	274.5 ± 43.5	264.9 ± 43.4*	0.009
Total protein S antigen (IU/dL)	92.6 ± 7.2	89.8 ± 6.2	90.4 ± 8.1	0.051	90.0 ± 9.2	89.8 ± 8.8	88.3 ± 9.8	0.347
Total protein S activity (IU/dL)	96.1 ± 6.6	94.3 ± 6.7	95.1 ± 8.5	0.321	94.6 ± 10.0	94.3 ± 9.2	92.7 ± 10.0	0.160
Free protein S antigen (%)	103.1 ± 10.9	101.3 ± 12.7	101.3 ± 12.5	0.526	101.0 ± 15.4	98.8 ± 13.7	97.1 ± 12.8*	0.034
Protein C antigen (%)	115.1 ± 26.7	112.3 ± 26.8	113.0 ± 26.1	0.154	113.1 ± 28.5	110.9 ± 27.7	107.2 ± 26.0**	0.008
Insulin (mIU/mL)	5.7 ± 2.0	12.7 ± 6.6**	5.1 ± 2.5	<0.001	6.4 ± 2.4	51.9 ± 21.9***	20.0 ± 9.9	<0.001
Glucose (mg/dL)	89.6 ± 2.8	90.5 ± 13.7	89.0 ± 7.3	0.929	90.7 ± 5.6	90.7 ± 11.5	89.8 ± 11.7	0.967

[a]Mean ± SD, [b]one-way repeated measures ANOVA to test for differences across time. *P<0.05, **P<0.01, ***P<0.001 vs. fasting by multiple comparisons.
Abbreviations, LDL: low-density lipoprotein, sdLDL, small dense LDL; HDL, high-density lipoprotein; Apo, apolipoprotein.

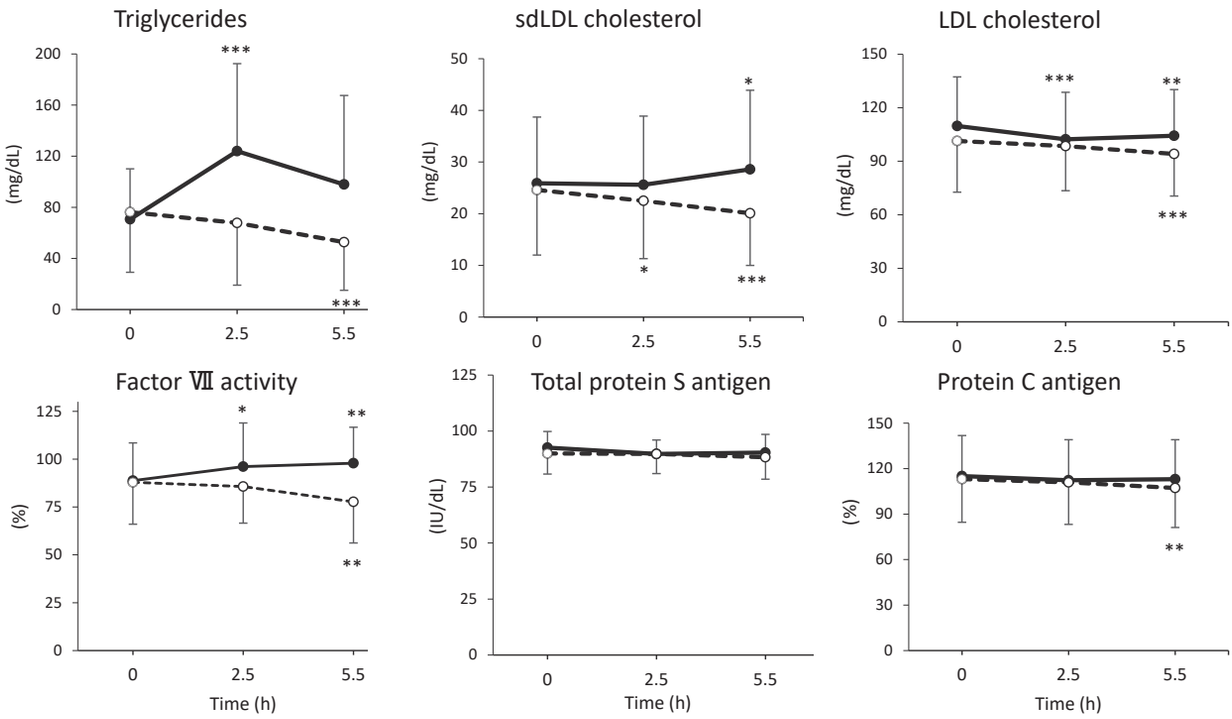

Figure 1 Levels of triglycerides, sdLDL cholesterol, LDL cholesterol, factor VII activity, total protein S antigen, and protein C antigen before and after consuming a high-fat meal (—●—) or a high-carbohydrate meal (--○--). The mean and SD are shown. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. fasting (0 h-value) by multiple comparison.

after consuming a high-carbohydrate meal, all the levels of lipids, apolipoproteins, and coagulation and anticoagulation factors decreased, except for apo C-II and total protein S antigen and its activity. Apo C-II increased 2.5 h after the meal, while total protein S antigen and its activity remained unchanged. Insulin peaked 2.5 h after the meal and the peak was higher than after consuming a high-fat meal, but glucose remained unchanged. The postprandial decrease in total, LDL, HDL cholesterol, and most apolipoprotein may be due to hemodilution [24], because the participants consumed approximately 500 mL of water during the study.

We then conducted univariate linear regression analyses to identify the factors associated with triglycerides (**Table 2**). During fasting, there were strong positive relationships between triglycerides and the levels of sdLDL cholesterol, sdLDL cholesterol/LDL cholesterol ratio, and apo C-II, and these relationships became stronger after consuming a high-fat or high-carbohydrate meal. In contrast, triglyceride levels were positively correlated with total and LDL cholesterol only after consuming a high-fat meal, and with apoB and apo C-III after consuming both high-fat and high-carbohydrate meals. No correlation was found between triglycerides and any other variables, including coagulation and anticoagulation factors such as factor VII activity, protein S antigen and its activity, and protein C antigen in both fasting and postprandial states. Furthermore, correlation analyses did not reveal any statistically significant associations between factor VII activity and the levels of triglycerides or sdLDL cholesterol (data not shown), nor between the postprandial changes in factor VII activity and those in triglycerides and sdLDL cholesterol (**Table 3**).

IV. Discussion

This study demonstrated that consuming a high-fat meal but not a high-carbohydrate (low-fat) meal increased factor VII activity during the postprandial elevation of triglycerides and sdLDL cholesterol. However, total protein S antigen and its APC-cofactor activity, free protein S antigen, and protein C antigen levels did not change postprandially. Strong positive relationships were observed between triglycerides and the levels of sdLDL cholesterol and apoC-II both at fasting and after consuming meals.

Activated factor VII (VIIa) and VIIa-antithrombin complex are reported to significantly increase after eating high-fat meals, indicating postprandial activation of factor VII [10) 25) 26)]. An in vitro study using purified systems reported that large VLDL, rather than small VLDL, enhances TF-independent activation of factor VII by factor Xa and factor Xa/Va [11)]. However, we did not find any significant associations between factor VII activity and the

Table 2 Relationships between triglycerides and the levels of other lipids, apolipoproteins, and coagulation and anticoagulation factors.

			High-fat meal				High-carbohydrate meal			
	Fasting[a]		2.5 h		5.5 h		2.5 h		5.5 h	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Total cholesterol	0.136	0.674	0.559	0.059	0.738	0.006**	0.432	0.161	0.381	0.221
LDL cholesterol	0.140	0.664	0.627	0.029**	0.700	0.011*	0.492	0.104	0.417	0.177
sdLDL cholesterol	0.678	0.015**	0.820	0.001**	0.853	<0.001***	0.876	<0.001***	0.826	<0.001***
sdLDL cholesterol/LDL cholesterol	0.745	0.005**	0.779	0.003**	0.823	0.001**	0.827	<0.001***	0.849	<0.001***
HDL cholesterol	-0.193	0.549	-0.280	0.378	-0.169	0.600	-0.305	0.335	-0.317	0.316
ApoA-I	0.011	0.972	0.057	0.861	0.395	0.204	0.030	0.927	0.002	0.994
ApoB	0.423	0.170	0.746	0.005**	0.743	0.006**	0.746	0.005**	0.670	0.017**
ApoC-II	0.837	<0.001***	0.865	<0.001***	0.824	<0.001***	0.774	0.003**	0.851	<0.001***
ApoC-III	0.518	0.085	0.581	0.048*	0.718	0.009**	0.655	0.021**	0.647	0.023**
ApoE	0.115	0.723	0.430	0.163	0.374	0.232	0.310	0.327	0.078	0.811
Factor VII activity	0.064	0.842	-0.344	0.274	-0.002	0.995	0.042	0.896	-0.072	0.825
Fibrinogen	-0.412	0.183	-0.381	0.222	-0.066	0.838	-0.380	0.223	-0.306	0.333
Total protein S antigen	0.418	0.176	0.493	0.104	0.351	0.263	0.235	0.462	0.122	0.707
Total protein S activity	0.348	0.268	0.432	0.161	0.324	0.304	0.330	0.295	0.238	0.457
Free protein S antigen	0.103	0.750	0.314	0.319	0.316	0.317	0.419	0.175	0.285	0.370
Protein C antigen	0.246	0.440	0.327	0.299	0.463	0.130	0.441	0.152	0.421	0.173
Insulin	0.290	0.361	-0.480	0.115	0.298	0.348	0.243	0.447	-0.029	0.929
Glucose	0.043	0.895	-0.461	0.132	0.452	0.210	0.210	0.512	-0.020	0.951

[a]Fasting levels of all measures were the means of the data obtained at two visits. r, Pearson's correlation coefficient: *P<0.05, **P<0.01, ***P<0.001
Details of the abbreviations used are shown in **Table 1**.

Table 3 Relationships between changes of factor VII activity and those of triglycerides and sdLDL cholesterol during food intakes

	High-fat meal				High-carbohydrate meal			
	FVII:C △ 2.5-0		FVII:C △ 5.5-2.5		FVII:C △ 2.5-0		FVII:C △ 5.5-2.5	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Triglycerides △ 2.5-0	-0.396	0.202			0.485	0.110		
Triglycerides △ 5.5-2.5			-0.130	0.687			0.430	0.163
sdLDL cholesterol △ 2.5-0	-0.236	0.460			-0.454	0.138		
sdLDL cholesterol △ 5.5-2.5			0.371	0.236			0.550	0.064

FVII:C, factor VII activity; △ 2.5-0, changes between fasting and 2.5 h postprandial: △ 5.5-2.5, changes between 2.5 h postprandial and 5.5 h postprandial: sdLDL, small dense LDL; r, Pearson's correlation coefficient.

levels of triglycerides or sdLDL cholesterol, nor between changes in factor VII activity and changes in the levels of triglycerides and sdLDL cholesterol during food consumption. Some in vivo studies have shown that factor IX [10] or kallikrein [27] is essential for the postprandial activation of factor VII; however, the detailed mechanisms are not yet understood.

After consuming high saturated fat meals, protein S, protein C, and C4BP as well as vitamin K-dependent coagulation factors such as factors VII have been reported to be associated with triglyceride-rich lipoproteins, chylomicrons, chylomicron remnants, and VLDL [12) 13)]. However, our study found no significant changes in the levels of total protein S antigen and its APC-cofactor activity, free protein S antigen, and protein C antigen levels after a high-fat meal. Protein S plays a critical role in anticoagulation as a cofactor for the APC-dependent and TFPI-dependent pathways [14) 15)]. Previous research has shown that the consumption of high-fat meals has no effect on plasma levels of free TFPI [26) 27)]. Taken together, the APC-dependent and TFPI-dependent anticoagulation pathways may not regulate the postprandial activation of factor VII after consuming high-fat meals.

Our earlier report demonstrated that apoC-II, a critical cofactor of lipoprotein lipase [16)], is a significant predictor of fasting total protein S antigen levels [17)]. In the pres-

ent study, we observed a weak correlation ($r = 0.522$, $P = 0.082$) between total protein S antigen and apoC-II during fasting; however, this correlation disappeared after consuming meals (data not shown). We also found that of all the apolipoproteins, only apoC-II had a significant correlation with triglycerides, both during fasting and after consuming meals. This may explain why apoC-II has become a potential target for developing new drugs to treat hypertriglyceridemia [16].

In line with a previous report [27], we observed that insulin levels were significantly higher after eating a high-carbohydrate meal compared to a high-fat meal; however, factor VII activity decreased after eating a high-carbohydrate meal, suggesting that there was little or no factor VII activation. A study conducted on healthy individuals who were exposed to 24 h of hyperinsulinemia with or without hyperglycemia reported that factor VII activity decreased the most during high glucose/high insulin and to a lesser degree during selective hyperinsulinemia and selective hyperglycemia [28].

Ogita et al. [29] have reported that sdLDL cholesterol of young adults remains unchanged for up to 4 h after consuming a fat-rich test material (17 g fat/m^2 of body surface area), while triglycerides peak at 2 h after the meal. In our study, the amount of fat consumed was an average of 35 fat/m^2 of body surface area, which was twice as much as their study. As a result, consuming meals containing a high amount of fat tends to raise the levels of sdLDL cholesterol. The homogeneous assay of sdLDL cholesterol measures cholesterol in LDL particles with a density of 1.044-1.63 g/mL [20], which corresponds with very small LDL-IV [18]. The sdLDL particles are formed from the lipolysis of large triglyceride-rich VLDL by lipoprotein lipase and hepatic lipase [18]. This may explain why the increase in sdLDL cholesterol was observed 3 h later than the increase in triglycerides after consuming a high-fat meal. As expected, significant correlations were found between triglycerides and sdLDL cholesterol during fasting and after consuming a high-fat or a high-carbohydrate meal. The atherogenic potential of sdLDL includes prolonged plasma residence, increased susceptibility to oxidative modification, and a greater tendency to penetrate into the arterial wall [18, 30]. Therefore, the postprandial elevation of sdLDL cholesterol may contribute to the relationship between elevated levels of nonfasting triglycerides and the prediction of CVD events and mortality [5-9].

The present study had several limitations. First, the study had a small sample size, which may reduce the ability to detect significant changes from fasting to nonfasting conditions and postprandial changes. Second, the study only included young women as subjects. We previously found that the relationship between protein S and apoC-II is much stronger in middle-aged obese women, about half of whom exhibited dyslipidemia, than in young nonobese women [17]. Third, we analyzed only factor VII and fibrinogen as coagulation factors; however, previous studies reported that prothrombin, factors VII, IX, X are associated with triglyceride-rich lipoproteins in both fasting and postprandial plasma [12]. Fourth, we did not analyze TFPI-cofactor activity of protein S.

Acknowledgments

This study was supported by JSPS KAKENHI Grant Number JP18K07486. We thank Dr. Tomohide Tsuda and Dr. Xiuri Jin of Shinotest Corporation for measuring total protein S antigen and its activity.

Authorship Contributions

H. Tsuda and K. Noguchi designed the research. H. Tsuda, K. Noguchi, and M. M. Kawae performed the study and analyzed the data. H. Tsuda wrote the manuscript. All authors read and approved the final version of the paper.

Disclosure of Conflicts of Interest

The authors declare that they have no conflict of interest.

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