Turbid plasma donations in whole blood donors: fat chance?

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BACKGROUND: Blood donations resulting in "nontransparent turbid milky" plasma must be discarded. The aim of this study was to objectively determine opacity and to identify risk factors for turbid plasma donations.

STUDY DESIGN AND METHODS: This case-control study included 238 whole blood donors who provided turbid plasma (cases) and 309 random control subjects with normal plasma. Participants filled in a questionnaire regarding cardiovascular risk factors. Fat intake was assessed using a validated questionnaire. Opacity and lipids were measured. Additional data were retrieved from the blood bank information system. Mean differences and odds ratios were calculated with 95% confidence intervals.

RESULTS: Cases had a less favorable cardiovascular profile compared to control subjects. The usual intake of fat was not associated with turbid plasma donation. In a multivariate model, having dinner before donation (OR, 4.9; 95% CI, 2.2-11.1), triglyceride levels (OR, 7.1; 95% CI, 4.6-10.8), and smoking ($OR_{yes\ vs.\ no}$, 2.5; 95% CI, 0.9-6.7; and $OR_{ever\ vs.\ no}$, 5.7; 95% CI, 1.8-18.4) were associated with an increased risk. Opacity was clearly increased in turbid plasma. Total cholesterol levels were 0.51 (95% CI, 0.35-0.66) mmol/L and triglyceride levels 4.28 (95% CI, 3.92-4.68) mmol/L higher in cases. High-density lipoprotein cholesterol levels were 0.34 (95% CI, −0.39 to −0.30) mmol/L lower. Forty-two percent of all cases had very high triglyceride levels (≥5.65 mmol/L) compared to less than 1% of control subjects.

CONCLUSION: Donors who provided a turbid donation have a less favorable cardiovascular profile compared to other donors. Having dinner, high triglyceride levels, and smoking are independent risk factors for turbid plasma donations.

In the Netherlands more than 400,000 donors provide more than 560,000 whole blood donations and 280,000 plasma donations through apheresis procedures each year.[1] Whole blood is processed into plasma, red blood cells (RBCs), and platelet concentrates. Apheresis plasma is being used for transfusion in patients and for fractionation and processing into therapeutics, such as immunoglobulins, coagulation factors, protease inhibitors, and albumin. Plasma derived from whole blood donations is fractionated. However, not all blood donations are utilizable. According to the European Pharmacopoeia, before freezing, plasma must have a clear to slightly turbid appearance without visible signs of hemolysis.[2] Thus, a blood donation resulting in so-called "nontransparent turbid milky plasma," or, in short, "turbid plasma," must be discarded. In the Netherlands, 3 to 4 per 1000 whole blood donations reveal turbid plasma. Available blood resources make every donation count and discarding donations may have a negative impact on donor satisfaction and retention.

Risk factors for turbid plasma in donors are not completely known and objective measures of turbidity are lacking. Knowing who is at risk and what is causing the risk may provide options for prevention of turbid plasma donation. Several case reports describe patients with turbid-appearing plasma who were diagnosed with familial chylomicronemia.[3-6] However, these inborn errors of metabolism are very rare and therefore unlikely to cause turbid plasma in predominantly healthy donors. It does

ABBREVIATIONS: BMI = body mass index; HDL = high-density lipoprotein; NCEP = National Cholesterol Education Program.

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point us to the direction of elevated triglycerides, contained in intestinally derived chylomicrons and hepatic very-low-density lipoprotein particles, being the contributor of many, if not most, cases of increased turbidity in donors. Two case reports describe the occurrence of turbid plasma in blood donors, who were later found to have elevated levels of triglycerides.[7,8] Temporary conditions could be of particular interest among blood donors, such as eating a fatty meal before donation. The intake of a fatty meal increases the level of triglycerides for several hours and could be one of the underlying causes of turbid plasma.[9,10] Furthermore, donors vary in their characteristics and those who provide turbid plasma may have an unfavorable cardiovascular profile in terms of obesity and diabetes mellitus. One might seize the opportunity to take preventive actions, if results warrant this. The aim of this study was to objectively measure the amount of opacity in turbid plasma donations and to identify risk factors for turbid plasma donations.

MATERIALS AND METHODS

Study design and study population

This case-control study was conducted at Sanquin Blood Bank Southeast Region in the Netherlands between May 13, 2008, and December 18, 2008. Eligible donors were those who had passed the standard medical examination for donation, which includes a questionnaire relating to the donors' health and behavior that may affect the safety of blood products or the safety of the donor. Additionally, donors had to be between 18 and 70 years and had to have a weight above 50 kg. Consecutive whole blood donors who provided a turbid plasma donation as assessed during routine quality checks on every donation were included as cases. Plasma was defined as turbid according to the standard Dutch criterion: a specific white label is invisible through the separated plasma unit on visual inspection by the laboratory technician (Fig. 1). A group of donors who provided normal plasma was included as a control group. These subjects were sampled randomly from the total number of whole blood donations per week in the same time period. None of the control donors were sampled twice. Thus, a representative sample was drawn from all donors with normal plasma. Demographic characteristics of all Dutch whole blood donors have been described before.[11]

Data collection

Within 5 days after donation, both cases and control subjects received a letter with information about the study and a standardized questionnaire. A reminder was sent 3 weeks later. Donors who provided more than one turbid donation were asked to participate only after their first

Fig. 1. Normal plasma (left) and turbid plasma (right). In normal plasma the label is clearly visible. This is not the case in the turbid plasma unit.

turbid plasma donation. Donors were not informed about their case or control status. The questionnaire included items on sex, age, alcohol, smoking, weight, height, medical history, (familial) history of hyperlipidemia, and any medication use. Body mass index (BMI) was calculated by dividing body weight (kg) by height squared (m^2). The medical history included diabetes, hypertension, and renal and thyroid disease. Fat intake was assessed using a validated short food frequency questionnaire, the adapted *Fat List*.[12] This list can be used to classify subjects into broad categories of total fat intake. The list consists of 35 questions covering 19 categories of food items, which have been selected based on their contribution to total and saturated fat intake in the general Dutch population. Individuals were asked how frequently the food items were usually consumed and additional questions on quantity or kind of product were included. For each of the 19 categories of food items a fat score, ranging from zero (lowest fat intake) to five points (highest fat intake), was determined. An individual total fat score was calculated by adding up the 19 fat scores for each subject. Additional questions relating to food intake on the day of donation were included, such as having a warm meal, referred to as dinner, within 4 hours before donation, and type of meal. The questionnaire provided date and time of the donation to remind donors. Data relating to donor status were retrieved from the blood bank information system and included date and time of donation, blood pressure, and

hemoglobin levels as assessed by a finger-stick method (Hemocue, Ängelholm, Sweden) at the standard medical examination. All participants provided written informed consent.

Blood collection and laboratory analyses

Whole blood was collected in a quadruple top-and-bottom bag system (Composelect, Fresenius HemoCare BV, Emmer-Compascuum, the Netherlands) and anticoagulated with 70 mL of citrate-phosphate-dextrose. After centrifugation at 4956 × *g* for 11 minutes and 30 seconds at 22°C, plasma, buffy coat, and RBCs were separated into three separate units (Compomat G4, Fresenius HemoCare BV). From every plasma unit, plasma was drawn into one collection tube, which was divided into several samples. In one sample absorption was measured on a spectrophotometer at 600 nm to objectively assess opacity. One sample was centrifuged at 9503 × *g* for 15 minutes at 4°C and absorption was measured in the supernatant. From August 2008 onward, color, thickness of the white layer on top of the supernatant, and sediment were visually inspected in this sample and each aspect was categorized into groups on a predefined form. Additional samples were stored at –80°C until measurement of lipid levels. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were determined enzymatically in one batch in 1 day following the instructions of the manufacturer (Olympus Life and Material Science Europe, Hamburg, Germany). Lipid levels were categorized according to the National Cholesterol Education Program (NCEP) guidelines into three or four groups.[13] Laboratory technicians were unaware of the case or control status of the sample while performing lipid measurements.

Statistical analyses

Differences of continuous variables between cases and control subjects were tested using the t test and reported as mean differences with 95% confidence intervals (CIs). Differences of categorical variables were assessed using the chi-square test. As triglycerides were skewed, 10log-transformation was applied. These log-transformed values were used in the analyses on mean differences. However, all values presented are converted back to geometric means with the appropriate 95% CI. Total fat score was divided separately for men and women into tertiles based on the distribution of the control subjects. Odds ratios (ORs) were calculated to assess the relative risk of the presence of a risk factor compared to a reference group. Appropriate 95% CIs were calculated using standard errors of the logistic model. Adjustments were made using unconditional logistic regression (multivariate model). All analyses were performed with computer software (SPSS for Windows 15.0, SPSS, Inc., Chicago, IL).

RESULTS

From May until December 2008, 282 turbid plasma donations occurred. During these months, eight donors provided a turbid plasma donation twice, and one donor three times. These nine donors were asked to participate only after their first turbid plasma donation, resulting in 272 invited whole blood donors. Out of these 272 donors who provided a first turbid donation, 238 (87%) participated in the study (cases). A total of 309 out of 335 (92%) invited donors who provided normal plasma participated (control subjects). The mean age of participating cases was 46.1 years, which was comparable to 46.5 years among control subjects (Table 1). Of all cases, 87% were men compared to 59% of the control subjects. Cases had a less favorable cardiovascular profile than control subjects. They had a higher mean BMI compared to control subjects (difference, 1.8 [95% CI, 1.2-2.4] kg/m^2). The percentage of (ever) smokers among cases was also higher, as was the percentage of donors with hypertension and self-reported hyperlipidemia.

Absorption was measured in 530 of 547 samples (Table 2). In 17 samples absorption could not be measured due to practical administrative reasons. Median levels of absorption were much higher in turbid plasma samples (cases) compared to normal plasma samples (controls). In other words, the opacity was increased in turbid plasma samples. The distribution of absorption was clearly different between turbid plasma and normal plasma for centrifuged and noncentrifuged samples (Fig. 2). This distinction was even more apparent in the supernatant of centrifuged samples. Visual inspection of the samples after centrifugation showed clear differences. Turbid samples were more often yellowish or milky white compared to control samples and more often had a white layer on top of the supernatant. A thin, regular, or thick layer was observed in 97.2% of turbid samples and in 22.0% of control plasma samples. Sediment was present in a high percentage of turbid samples, with 27.9% having a regular amount and 7.8% having a large amount of sediment compared to, respectively, only 1.4 and 0.5% in control samples.

Lipid levels were different between the two groups. Total cholesterol was 0.51 (95% CI, 0.35-0.66) mmol/L higher, HDL cholesterol 0.34 (95% CI, –0.39 to –0.30) lower, and triglycerides 4.28 (95% CI, 3.92-4.68) mmol/L higher in cases with turbid plasma compared to control subjects (Table 3). Of all cases almost 20% had borderline high and 5% high cholesterol levels, 91% had low HDL cholesterol levels, and 42% had very high triglyceride levels. Among control subjects, 8% had borderline high and 1% high cholesterol levels, 45% had low HDL cholesterol, and less than 1% had very high triglyceride levels.

Although the usual total fat intake was slightly higher among cases compared to control subjects (a difference of

TABLE 1. Characteristics of 238 participating donors who provided turbid plasma (cases) and 309 control subjects*

Characteristic	238 cases with turbid plasma	309 control subjects with normal plasma	Difference (95% CI)
Men	87.0	59.2	†
Age (years)	46.1 (11.8)	46.5 (12.6)	–0.4 (–2.4 to 1.7)
Hb‡ (mmol/L)	9.3 (0.7)	9.0 (0.7)	0.3 (0.2 to 0.4)
BMI (kg/m^2)	27.4 (3.3)	25.7 (3.8)	1.8 (1.2 to 2.4)
Diabetes	2.9	2.3	
Hypertension	25.2	16.2	†
Blood pressure‡			
Systolic	137.2 (14.9)	131.7 (16.3)	5.5 (2.9 to 8.2)
Diastolic	84.2 (8.4)	81.7 (9.2)	2.5 (1.0 to 4.0)
Alcohol use	91.5	91.6	
Smoking			
Yes	25.4	15.9	†
Ever	15.7	11.7	
No	58.9	72.5	
Hyperlipidemia			
Hypercholesterolemia	19.1	12.0	†
Hypertriglyceridemia	1.3	0.0	
Both	2.6	1.3	
None	77.0	86.7	
Familial hyperlipidemia			
Hypercholesterolemia	27.8	32.7	
Hypertriglyceridemia	0.4	0.0	
Both	3.0	0.7	
None	68.7	66.7	
Renal disease	1.7	0	§
Thyroid disease	1.2	3.2	
Medication use	33.8	29.6	

* Data are reported as percentage or mean (SD).
† p value chi-square < 0.01.
‡ Information retrieved from blood bank information system, other characteristics obtained through questionnaire.
§ $p < 0.05$

TABLE 2. Characteristics of turbid plasma samples and normal samples*

Characteristics	Turbid plasma	Normal plasma
Absorption not centrifuged sample	0.51 (0.17-1.27)	0.09 (0.03-0.26)
Absorption in supernatant, centrifuged sample	1.57 (0.51-2.78)	0.20 (0.06-0.68)
Plasma color		
Milky white	9 (7.1)	5 (2.5)
Yellowish	85 (67.5)	115 (56.9)
Yellow	28 (22.2)	73 (36.1)
Dark yellow	0 (0)	9 (4.5)
Orange	4 (3.2)	0 (0)
White layer		
Thick	6 (4.3)	0 (0)
Regular	65 (46.1)	3 (1.4)
Thin	66 (48.6)	43 (20.6)
None	4 (2.8)	163 (78.0)
Sediment		
Large amount	11 (7.8)	1 (0.5)
Regular	39 (27.9)	3 (1.4)
Some	81 (69.0)	84 (40.2)
None	9 (6.4)	121(57.9)

* Data are reported as median (5th-95th percentile) or number (%).

1.3; 95% CI, 0.2-2.3; Table 4), the intake of a medium or high amount of fat was not associated with the donation of turbid plasma. Similar results were found in men and in women separately.

The time of the day of the donation differed considerably between the two groups. Only 5% of turbid plasma samples were donated in the morning between 8:30 AM and 12:00 PM and 68% were donated in the evening between 6:00 PM and 9:00 PM, whereas the donation of control plasma varied from 24% in the morning to 29% in the evening (Fig. 3). There was a very strong association between time of donation and having dinner. Of those control subjects who donated in the evening, 73% had dinner within 4 hours before donation compared to less than 6% of the control subjects who donated in the morning or afternoon. Analyses showed that 18 of 156 (11.5%) cases had dinner within 4 hours before donation compared to three of 71 (4.2%) control subjects. Of the 18 cases, 11 reported a standard Dutch meal, three donors consumed fast food, and four donors had eaten another type of meal. Two control subjects had a Dutch meal and one had another type of meal.

Of 238 cases, 162 (68.1%) had dinner before donation compared to only 77 of 309 (25.0%) control subjects.

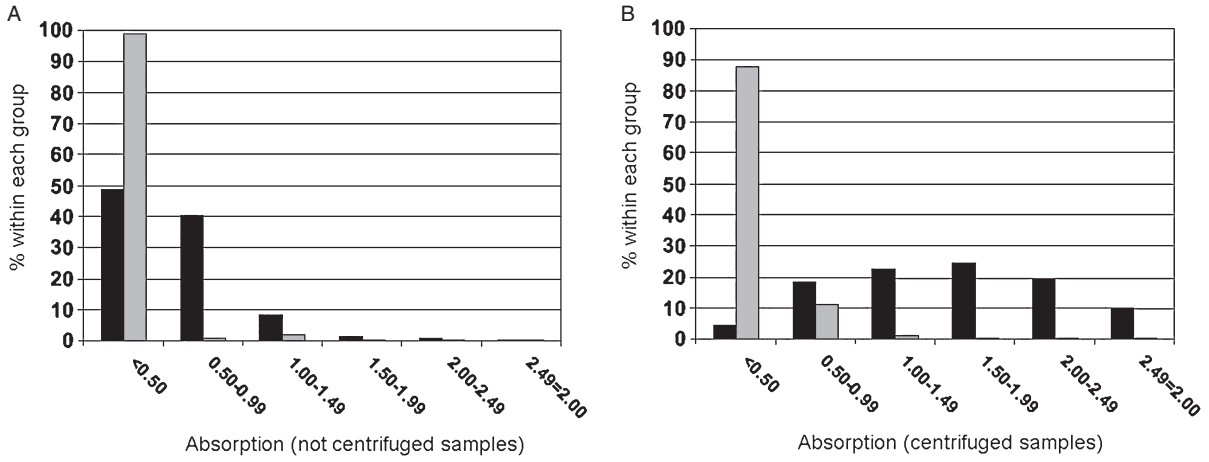

Fig. 2. Absorption of turbid plasma (■) and normal plasma (▒).

TABLE 3. Lipid levels and the percentage of abnormal levels in donors who provided turbid plasma (cases) and control subjects with normal plasma*

Lipid levels	Turbid plasma (n = 238)	Normal plasma (n = 309)	Mean difference (95% CI)
Total cholesterol (mmol/L)	4.67 (1.03)	4.16 (0.79)	0.51 (0.35 to 0.66)
NCEP			
Desirable (<5.2)	176 (75.9)	272 (90.7)	
Borderline high (5.2-6.1)	44 (19.0)	25 (8.3)	
High (≥6.2)	12 (5.2)	3 (1.0)	†
HDL cholesterol (mmol/L)	0.75 (0.20)	1.09 (0.30)	–0.34 (–0.39 to –0.30)
NCEP			
High (≥1.55)	1 (0.4)	20 (6.7)	
Medium (1.03-1.54)	19 (8.2)	144 (48.0)	
Low (<1.03)	212 (91.4)	136 (45.3)	†
Triglycerides (mmol/L)	5.26 (1.69)	1.23 (1.67)	4.28 (3.92 to 4.68)
NCEP			
Normal (<1.70)	8 (3.4)	230 (74.4)	
Borderline high (1.70-2.25)	3 (1.3)	39 (12.6)	
High (2.26-5.64)	128 (53.8)	39 (12.6)	
Very high (≥5.65)	99 (41.6)	1 (0.3)	†

* Data are reported as mean (SD) or number (%). Conversion factors for mmol/L into mg/dL: total and HDL cholesterol (mg/dL) = mmol/L × 38.67; triglycerides (mg/dL) = mmol/L × 88.57.
† p value chi-square < 0.01.

TABLE 4. Total fat score and having dinner before donation as a risk factor for turbid plasma donation*

Risk factor	Cases with turbid plasma (n = 238)	Control subjects with normal plasma (n = 309)	OR (95% CI)
Total fat score	20.1 (6.1)	18.8 (6.2)	
Total fat score category			
High	73 (30.7)	93 (30.1)	1.0 (0.6-1.5)
Medium	75 (31.5)	103 (33.3)	0.9 (0.6-1.4)
Low	90 (37.8)	113 (36.6)	1 (reference)
Dinner before donation			
Yes	162 (68.1)	77 (25.0)	6.4 (4.4-9.3)
No	76 (31.9)	231 (75.0)	1 (reference)

* Data are reported as mean (SD) or number (%).

Donors who had dinner before donation had a sixfold increased risk of providing a turbid plasma donation compared to donors who did not have a meal (OR, 6.4; 95% CI, 4.4-9.3; Table 5). Men, donors with high triglycerides, high total cholesterol levels, low HDL cholesterol levels, high BMI, smokers, and donors with high blood pressure had a higher risk of a turbid donation. When we included these risk factors, apart from triglycerides, total cholesterol, and

HDL cholesterol, into a multivariate model only the OR of sex decreased somewhat. However, when we included all risk factors that were associated with turbid plasma into one multivariate model to disentangle the associations, only a few remained as an independent risk factor. In this multivariate model, only having dinner before donation (OR, 4.9; 95% CI, 2.2-11.1), high triglyceride levels (OR per mmol increase, 7.1; 95% CI, 4.6-10.8), and smoking (OR yes vs. no, 2.5; 95% CI, 0.9-6.7; OR ever vs. no, 5.7; 95% CI, 1.8-18.4) remained independent risk factors. The ORs of all other factors reduced to the null (OR, 1). The very strong effect of sex (OR men vs. women, 4.6; 95% CI, 3.0-7.1) also reduced to the null (OR, 1.0; 95% CI, 0.4-2.6).

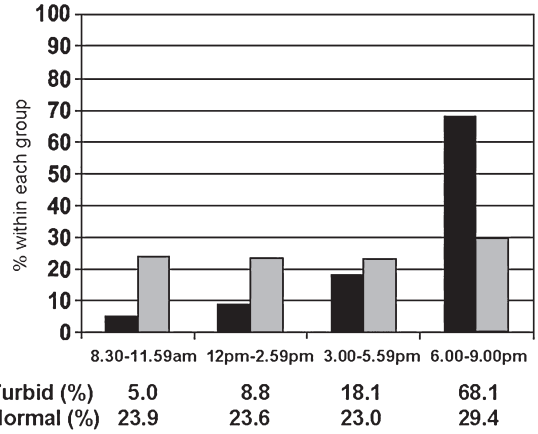

Fig. 3. Time of donation among donors who provided turbid plasma (■) and among donors who provided normal plasma (▒).

DISCUSSION

Donors who provided turbid plasma had a less favorable cardiovascular profile compared to control subjects who donated normal plasma. Opacity was increased in turbid plasma compared to normal plasma and visual aspects were clearly different between plasma samples as were lipid levels. BMI, systolic and diastolic blood pressure, triglyceride and total cholesterol levels, and the percentage of men and smokers were higher. Donors who had dinner before donation more often provided a turbid plasma donation than donors who did not have dinner. After adjustment for all risk factors in a multivariate model, only having dinner before donation, triglyceride levels, and smoking remained independent risk factors.

Turbid plasma samples had an increased opacity as measured by objective methods, especially after centrifugation. The samples were also visually different. The presence of a white layer and sediment was distinctive for turbid plasma samples. More than 50% of turbid plasma samples had a regular or thick white layer and more than 35% had a regular or large amount of sediment. Less than 2% of the control samples had such a layer or sediment after centrifugation. Apparently the subjective method used daily in Dutch blood banks, that is, the visibility of a specific white label through a separated plasma unit by a laboratory technician, can distinguish turbid and normal plasma quite well, although not perfectly. Some plasma samples may have appeared turbid, yet contained normal triglyceride levels (3.4%). This might be due either to the subjective method of identifying turbid plasma samples or to other causes of increased turbidity (i.e., other than triglycerides). In the latter instance, one might think of hemolysis. Whether plasma units are unnecessarily discarded is difficult to conclude from these results. This

TABLE 5. Risk factors for turbid plasma donation, unadjusted (univariate model) and adjusted for all other factors (multivariate model)*

Risk factor	OR unadjusted (95% CI)	OR adjusted (95% CI)
Dinner before donation (yes vs. no)	6.4 (4.4-9.3)	4.9 (2.2-11.1)
Sex (men vs. women)	4.6 (3.0-7.1)	1.0 (0.4-2.6)
Age (years)†	1.00 (0.98-1.01)	0.98 (0.95-1.02)
Triglycerides (mmol/L)†	6.0 (4.5-8.2)	7.1 (4.6-10.8)
Total cholesterol (mmol/L)†	1.6 (1.4-2.0)	0.8 (0.5-1.3)
HDL cholesterol (mmol/L)†	0.01 (0.00-0.01)	0.7 (0.2-2.9)
BMI (kg/m^2)†	1.15 (1.09-1.21)	0.98 (0.87-1.10)
Smoking		
Yes vs. no	2.0 (1.3-3.0)	2.5 (0.9-6.7)
Ever vs. no	1.7 (1.0-2.7)	5.7 (1.8-18.4)
Blood pressure (mmHg)†		
Diastolic	1.03 (1.01-1.05)	0.96 (0.90-1.02)
Systolic	1.02 (1.01-1.03)	0.98 (0.95-1.02)

* Conversion factors for mmol/L into mg/dL: cholesterol (mg/dL) = mmol/L × 38.67; triglycerides (mg/dL) = mmol/L × 88.57. OR adjusted = adjusted for all other factors (multivariate model). For example, the risk of having dinner before donation is adjusted for sex, age, triglycerides, total and HDL cholesterol, BMI, smoking, and diastolic and systolic blood pressure.
† OR per unit increase, for example, per year increase in age.

depends on the reasons for discarding turbid plasma samples. The European guidelines do not give much insight. It may well be that sample turbidity interferes with laboratory methods of viral testing. Because the safety of plasma used for transfusion and production of therapeutics is of paramount importance, these viral tests should be performed in optimal conditions and the turbidity of samples may challenge these conditions. Discarding turbid plasma could in that case be an unfortunate necessity, although the currently applied quarantine method in processing plasma should safeguard against false-negative results. In the United States, visually borderline turbid plasma samples are being tested on excessive triglycerides to salvage plasma samples with triglyceride levels that fall within the specific levels of triglycerides for which the test assays for infectious diseases have been validated (personal communication). Because we found less than 5% of all turbid samples to have normal or borderline high triglyceride levels, this might indicate that many plasma samples need to be tested to be able to salvage only a small proportion of visually turbid plasma samples. Whatever the reasons for discarding turbid plasma, preventing the occurrence of such donations may be preferable, because three to four per 1000 donations reveal turbid plasma.

The difference in triglyceride levels between donors who provided turbid plasma and control subjects is not surprising, considering the fact that they more often had dinner before donation and the nonfasting conditions in which blood samples were drawn. Triglyceride levels are known to be affected by the intake of food before measuring levels, in contrast to total and HDL cholesterol. However, total cholesterol and HDL cholesterol levels were also less favorable; 5% had high total cholesterol levels and 91% had low HDL cholesterol levels according to the NCEP guidelines.[13] Self-reported hyperlipidemia was also more often present in donors who donated turbid plasma than control subjects, supporting the unfavorable lipid profile in these donors. Although the percentage with high total cholesterol levels was higher than among donors who provided normal plasma, this percentage is comparable to that of the general population.[14] The initial high risk of donating turbid plasma among men compared to women completely disappeared after adjusting for triglyceride levels. Men have higher and more prolonged levels of dietary triglycerides after a fatty meal than women, which explains the initial high risk.[15]

Currently, it is the blood bank's role to select donors who are eligible to donate based on standard medical examinations, not to perform a health check. However, good donor management implies that the blood bank takes proper action to ensure or promote the health of its donors in case of adverse findings, including turbid plasma. Therefore, referral of donors who repeatedly donate turbid plasma to a general practitioner appears to be preferable and is already daily practice in the Netherlands. Not only were lipid profiles less favorable among donors who provided turbid plasma, BMI was much higher, and the percentage of donors who smoked or reported hyperlipidemia or hypertension was also increased. Higher blood pressure readings before donation corroborated the latter finding. No large differences were found in diabetes or medication use. However, the estimates also pointed to a higher percentage in donors who provided turbid plasma. Although donors who provided turbid plasma had a less overall favorable cardiovascular profile compared to control subjects who donated normal plasma, they all met the current criteria for donating blood. An overall comparison of cardiovascular risk factors between donors and the general population has been described elsewhere.[14]

Although the usual total fat intake was slightly higher among cases compared to control subjects, the intake of a medium or high amount of fat was not associated with the donation of turbid plasma. Apparently, usual fat intake is not an important risk factor, in contrast to having dinner immediately before donation. Usual fat intake is measured by a validated short food frequency questionnaire that has been filled in within a few days after whole blood donation. The answers have been given by the donor without knowing if turbid plasma or normal plasma had been donated, excluding the possibility of obtaining different answers through this knowledge and thus precluding information bias. Because the questionnaire was sent within 5 days after the donation, it may have been difficult for both cases and control subjects to recall having dinner before donation, especially the contents of the meal. If this was the case, it would probably have had a similar impact on both cases and control subjects.

Having dinner was an important risk factor for donating turbid plasma. Those who had dinner before donation had a fivefold increased risk of providing a turbid plasma donation compared to donors who did not have dinner. This explains why donating in the evening increased the risk of donating turbid plasma. Having a meal increases triglyceride levels for several hours.[9,10] Known risk factors of an increased postprandial triglyceride response include male sex; obesity; older age; the concurrent intake of alcohol; and the carbohydrate, fiber, and protein content of the meal.[16] Some of these risk factors were confirmed in our multivariate analysis that included triglyceride level in the model. Male sex, total cholesterol and HDL cholesterol, BMI, and blood pressure were no longer associated with turbid plasma donations, because triglyceride level is presumably the underlying cause. However, the risk associated with having dinner did not completely disappear after adjusting for triglycerides, pointing to other factors that may explain the high risk after having dinner. Although this study included 238 cases with turbid plasma and 309 control subjects, we cannot exclude the

possibility that we missed the effect of some potential risk factors with a low prevalence such as diabetes.

One may speculate whether advice should be given to donors. The population-attributable risk percentage gives the percentage of turbid donations due to having dinner before donation and is easily calculated using the prevalence of this risk factor and the relative risk of the risk factor involved. The incidence of turbid plasma among whole blood donors was 3.48 per 1000 donations during the study period (data not shown). This means that turbid plasma is a rare occurrence, which justifies the interpretation of an OR as a relative risk. Assuming that the control subjects reflect the overall donor population, on average 25% of whole blood donors have dinner before donation. Together with the adjusted OR of 4.9, the population-attributable risk percentage is

$$0.25\times(4.9-1)/1+(0.25\times[4.9-1])\times 100\% = 49\%.$$

This means that 49%, that is, approximately half of the turbid donations, is due to having dinner before donation. Extrapolation of our case-control study data to the entire population of donations made during the study period shows that only a very small percentage (<1.0%) of all donors having had dinner will actually produce a turbid donation. Because not having a meal increases the risk of fainting, it is questionable if avoiding dinner before donation brings improvement. Instead, we anticipate that advising a low-fat and carbohydrate- and fiber-rich meal could have an appropriate effect.

This study has been performed among whole blood donors, but the results are probably also applicable to plasma donors. However, the incidence of donating turbid plasma may be different. Plasma donors are somewhat older and a higher percentage is men, but they are also somewhat healthier.[14] In any case, the risk factors for turbid plasma donations are likely to be similar as in whole blood donors.

In conclusion, opacity as measured by objective methods is clearly different between turbid plasma samples and normal plasma samples, and current, subjective methods seem to distinguish moderately well between turbid plasma samples and normal plasma samples. The finding that donors of turbid plasma show a higher risk profile for cardiovascular diseases needs further exploration of its consequences.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

REFERENCES

1. Sanquin. Sanquin Blood Bank. Annual Report. 2007.
2. European Pharmacopoeia 6.0. Human plasma for fractionation. 2008;no.0853:2073-5.
3. Lersch F, Fontana S, Nohl F, Taleghani BM. Transfusion medicine illustrated. Pica, peanuts, and plasma exchange. Transfusion 2006;46:1853-4.
4. Mohandas MK, Jemila J, Ajith Krishnan AS, George TT. Familial chylomicronemia syndrome. Indian J Pediatr 2005;72:181.
5. Sugandhan S, Khandpur S, Sharma VK. Familial chylomicronemia syndrome. Pediatr Dermatol 2007; 24:323-5.
6. Röggla G, Fasan M, Kapiotis S. Treating hypertriglyceridemia. CMAJ 2007;177:603-5.
7. Bedi R, Basu S, Kaur P, Chattopadhya A. Transfusion medicine illustrated. Visual inspection of blood units, a necessary practice at blood centers. Transfusion 2005; 45:459.
8. Mbahi M, Reddy V, Narvios A. Transfusion medicine history illustrated. Milky platelet concentrate: a second look. Transfusion 2006;46:877.
9. Groot PH, van Stiphout WA, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofman A, Cresswell SR, Havekes L. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. Arterioscler Thromb 1991;11:653-62.
10. Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. J Lipid Res 2003;44:2065-72.
11. Veldhuizen IJ, Doggen CJ, Atsma F, De Kort WL. Donor profiles: demographic factors and their influence on the donor career. Vox Sang 2009;97:129-38.
12. Van Assema P, Brug J, Ronda G, Steenhuis I. The relative validity of a short Dutch questionnaire as a means to categorize adults and adolescents to total and saturated fat intake. J Hum Nutr Diet 2001;14:377-90.
13. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.

14. Atsma F, Veldhuizen IJ, Doggen CJ, de Vegt F, de Kort WL. Cardiovascular and demographic characteristics of whole blood and plasma donors: results from the Donor InSight study. Transfusion 2011;51:412-20.
15. Knuth ND, Horowitz JF. The elevation of ingested lipids within plasma chylomicrons is prolonged in men compared with women. J Nutr 2006;136: 1498-503.
16. Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br J Nutr 2007;98:458-73.