Minireview

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Methods to reduce lipemic interference in clinical chemistry tests: a systematic review and recommendations

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Abstract

Objectives: Lipemia is the presence of abnormally high lipoprotein concentrations in serum or plasma samples that can interfere with laboratory testing. There is little guidance available from manufacturers or professional bodies on processing lipemic samples to produce clinically acceptable results. This systematic review summarizes existing literature on the effectiveness of lipid removal techniques in reducing interference in clinical chemistry tests.

Methods: A PubMed search using terms relating to lipid removal from human samples for clinical chemistry tests produced 1,558 studies published between January 2010 and July 2021. 15 articles met the criteria for further analyses.

Results: A total of 66 analytes were investigated amongst the 15 studies, which showed highly heterogenous study designs. High-speed centrifugation was consistently effective for 13 analytes: albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, creatine kinase (CK), creatinine (Jaffe method), gamma-glutamyl transferase (GGT), glucose (hexokinase-based method), lactate dehydrogenase (LDH), phosphate, potassium, and urea. Lipid-clearing agents were uniformly effective for seven analytes: ALT, AST, total bilirubin, CK, creatinine (Jaffe method), lipase, and urea. Mixed results were reported for the remaining analytes.

Conclusions: For some analytes, high-speed centrifugation and/or lipid-clearing agents can be used in place of ultracentrifugation. Harmonized protocols and acceptability criteria are required to allow pooled data analysis and interpretation of different lipemic interference studies.

Keywords: centrifugation; interference; lipemia; lipemic interference; lipid removal.

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Introduction

Lipemia is defined as an abnormally high concentration of lipoproteins in blood, resulting in visible turbidity of the serum sample. There are five major classes of lipoproteins, which may be distinguished by their densities, size, and lipid/protein composition [1]. The largest lipoproteins, chylomicrons (70–1,000 nm), as well as the large (60–200 nm) and medium (35–60 nm) subclasses of very-low-density lipoproteins (VLDLs), contribute to sample turbidity and therefore lipemic interference, whereas the smaller particles – small VLDLs, low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs) – do not [2]. Estimates of the frequency of lipemia observed in specimens received by clinical laboratories range from 0.16% in the inpatient setting to 7.4% in the outpatient setting [3, 4].

The most prevalent cause of lipemia is inadequate duration of fasting prior to sample collection [5]. The incidence of lipemia is also elevated in patients with medical conditions such as diabetes mellitus and pancreatitis, or certain lifestyle habits e.g. ketogenic diets and alcoholism [6]. Finally, iatrogenic causes such as the use of lipid emulsion therapy in drug overdoses and total parenteral nutrition, as well as medications including steroids, antiviral drugs and propofol, also contribute to lipemia [6].

Several mechanisms of lipemic interference on laboratory testing have been described; the most common of these is the alteration of light absorbance properties of the sample [5]. Light scattering by lipoproteins occurs across the visible light spectrum and increases as the wavelength decreases [5]. Consequently, methods that involve a spectrophotometric readout at lower wavelengths are the most affected. For example, there are numerous clinical

chemistry tests that are based on the reduction/oxidation of NAD(P) which leads to changes in absorbance at 340 nm; these are obscured if lipemia is present [5]. Similarly, tests based on the principle of nephelometry or turbidimetry are affected due to changes in the light scattering properties of the sample. Other mechanisms of lipid interference that have been described include the volume displacement effect, sample non-homogeneity resulting in falsely elevated levels of hydrophobic analytes which are concentrated in the upper lipid phase, and interference with antigen-antibody binding by blocking antibody binding sites [5].

Due to the impact of lipemia on downstream analyses, routine automated assessment of serum indices prior to analyte measurement has become a common feature of modern clinical chemistry analysers. Most laboratories use either the automatically generated lipemia index (L-index), or a combination of visual inspection and L-index [7]. This enables prompt identification of lipemic samples for intervention.

The Clinical and Laboratory Standards Institute (CLSI) recommends ultracentrifugation for the processing of lipemic samples [8], which enables the separation of chylomicrons and VLDLs from serum/plasma based on density. Ultracentrifugation refers to the use of centrifuges capable of generating centrifugal forces up to 200,000×*g* [1]. However, ultracentrifugation remains an impractical option for some laboratories, due to cost, space constraints and the delay in turnaround time. Consequently, some laboratories substitute ultracentrifugation with high-speed centrifugation using benchtop micro-centrifuges, which typically generate much lower centrifugal forces of up to 20,000×*g* [1]. Although high-speed centrifugation is less effective in lowering triglyceride concentration, it may be sufficient to remove the larger lipoproteins and enable analysis of certain analytes [9]. If however the lipemia is caused mainly by VLDL particles which are smaller in size, high-speed centrifugation becomes less effective and has to be repeated several times to obtain a clear sample [5].

The demand for efficient lipid removal without the use of additional instrumentation has also promoted the development of commercial reagents such as Lipoclear, a non-ionic cyclodextrin (StatSpin, Norwood, Massachusetts, USA) [10]. These reagents bind to and precipitate lipids; following high-speed centrifugation, the clarified supernatant may be used for analysis. However, these come with the caveats of causing significant changes in sample matrix due to dilution, or possible direct interference with certain tests.

Analytes that are mainly distributed in the lipid fraction present additional challenges. The lipid removal techniques described above are inappropriate as the sample would be depleted of the analyte during the process [5, 11]. In such circumstances, serum dilution may be a viable alternative, though the extent of dilution that can be performed is limited by the analytical limits of the measurement procedure [5]. Sample blanking has also been attempted in two-step systems that consist of a sample diluent and a trigger reagent. Briefly, the sample is first mixed with the sample diluent – "the sample blank", and an absorbance reading is taken. The trigger reagent is subsequently added, the reaction is allowed to proceed, and a second absorbance reading is taken. The difference between this two readings is used to determine the final result [12].

A 2019 survey done by the European Federation of Clinical Chemistry and Laboratory Medicine Working Group for the Pre-analytical Phase on 1,265 labs across Europe revealed that although the vast majority of laboratories (>90%) monitors samples for lipemia, only 27% attempt delipidation procedures (either for all or specific analytes or only when requested), and nearly 70% reject either the entire sample or only the affected tests if the L-index exceeds the laboratory's specific cut-offs [7]. This may be contributed by the lack of analyte-specific guidelines for lipid removal from manufacturers, who play an especially important role given that the effects of lipemia are platform and analyte specific. Given that lipemia is a fairly common occurrence, identifying which analytes may be amenable to lipid removal is of interest to clinical laboratories, and may reduce the number of samples that have to be rejected because of lipemia.

Here, we report a systematic review of recent studies evaluating the efficacy of different lipid removal methods in analyte recovery in human samples, with the aim of guiding the development of laboratory protocols for handling lipemic samples.

Methods

Search strategy

The literature review was conducted using the PubMed database. The following search terms and their variants were used for the literature search: "lipemia" OR "lipaemia" OR "lipemic" OR "lipaemic" OR "lipid removal" OR "delipidation" OR "lipid interference". Additional references were manually searched using the reference list in the articles reviewed.

Following the initial search results, two reviewers (SXX and LTP) independently reviewed the articles identified using the inclusion and exclusion criteria below. The articles were first screened by the title, followed by the abstract and the full text. Any conflicts were resolved by consensus.

Inclusion and exclusion criteria

The inclusion criteria were:
- Published from 01 January 2010 to 28 July 2021
- Written in English
- Original peer-reviewed study with full text available
- Performed in human samples
- Reported the effect of lipid removal or sample dilution on the recovery of biochemical analytes

The exclusion criteria were:
- Non-original research articles e.g. topic reviews
- Non-chemistry laboratory tests e.g. coagulation tests

Data extraction

Data extraction was performed with a dedicated form in Microsoft Excel. The following variables from each study were obtained: centrifuge model, centrifugation protocol (duration and centrifugal force), source of lipemia (native vs. spiked), range of lipemia concentrations examined, statistical criteria to determine effectiveness of lipid removal, analyte, laboratory method and analytical platform. The analytical principle of each method was either obtained from the study itself if included; otherwise we referred to the manufacturers' websites.

Data summary

In order to present a summary of the conclusions from multiple studies, we tabulated the number of studies that supported or refuted the presence of lipemic interference, and also the number of studies that supported or refuted the use of each lipid removal method, for each analyte and analytical principle used (Supplementary Table 1).

Results

Study selection

In total, 1,558 articles were identified after removal of duplicates, of which 42 were considered potentially eligible after screening the title and/or abstract (Figure 1). Of the 42 articles, 15 articles were eventually shortlisted for this study after full-text review. The remainder were rejected for the following reasons: (i) lipid removal not attempted (n=19), (ii) insufficient details for analysis (n=3), (iii) sample turned out to be non-lipemic (n=2), (vi) full-text article not available (n=2), and (v) non-original article (n=1). The reviewers were able to achieve consensus for all the articles.

Study characteristics

A summary of the attributes of the 15 eligible studies is provided in Table 1. Overall, eight of the studies were performed on native lipemic samples, five used spiked samples only, and the remaining studies used both native and

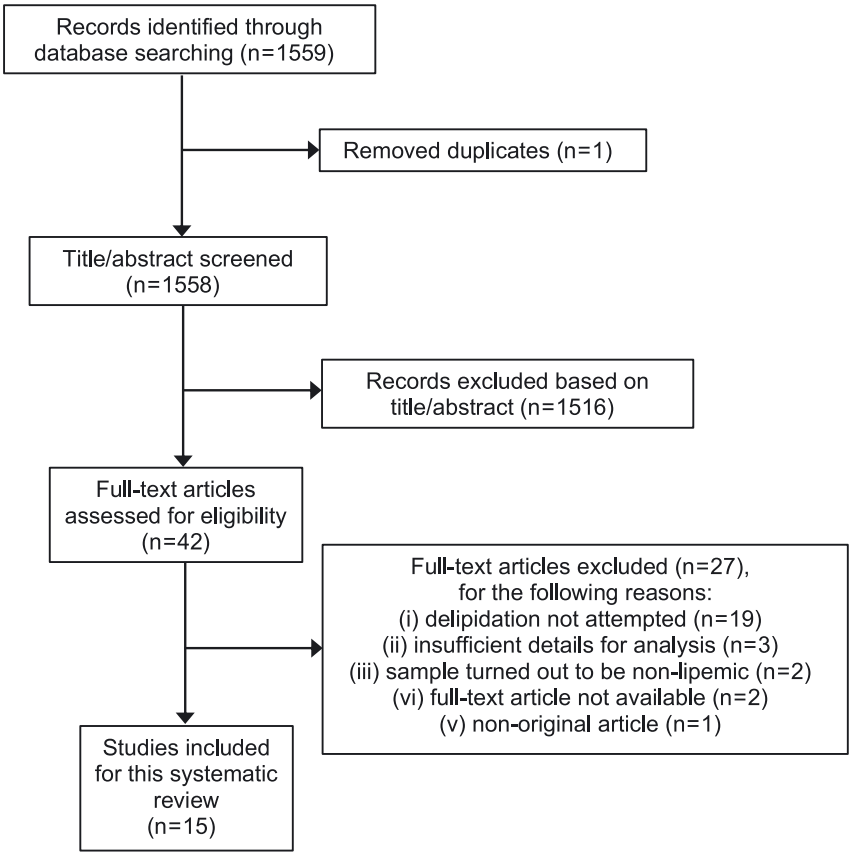

Figure 1: Flowchart showing the process of study selection. 1,558 unique studies were identified, from which 15 studies were eventually shortlisted for this systemic review.

Table 1: Summary of the characteristics of the 15 shortlisted studies.

Reference list no.	First author	Year	Type of sample	No. of samples	Delipidation procedu	Criteria for effectiveness of removal protocol	Analytes	Platform
[9]	Dimeski G	2011	Patient serum pools	10 Serum pools	UC vs. HC	Least significant change (LSC) $=2.77 \times \sqrt{CV_A}$	Creatinine, LDH, Magnesium, Sodium, Total Protein, Urate	Beckman Coulter DXC800
[13]	Calmarza P	2011	Patient serum samples	110 Patients	UC only	Desirable specifications for bias (Westgard)	ALP, ALT, AST, Calcium, Creatinine, GGT, Glucose, Iron, LDH, Phosphate, Total Bilirubin, Total Protein, Urate, Urea	Roche Hitachi Modular D and P
[14]	Hunsaker JJH	2019	Patient serum samples, and AB sera spiked with Intralipid	42 Patients, four pools	UC only	≥10% baseline result	AAT, C3, C4, Ceruloplasmin, Haptoglobin, Prealbumin, Transferrin	Roche Cobas c502
[15]	Koch CD	2021	Patient plasma sample	28 Patients	UC only	≥4 mmol/L (CLIA'88 guidelines)	Sodium	Roche Cobas 8000 Radiometer ABL835 Flex
[16]	Grunbaum AM	2012	Patient serum/ plasma pools spiked with Intralipid	Three serum/ plasma pools	HC only	Significant interference was defined as measurement falling outside the confidence interval, which was calculated as [unsupplemented concentration] ? ± 2x CV_{comb} × [unsupplemented concentration], where $CV_{comb}=\sqrt{(CV_A^2 + CV_W^2)}$	Albumin, ALT, Amylase, Bicarbonate, Calcium, Chloride, CK, Creatinine, Glucose, Lipase, Magnesium, Phosphate, Potassium, Sodium, Total Bilirubin, Total Protein, Troponin I, Urea	Beckman Coulter DXC800 Beckman Coulter DXI Roche Modular P
[17]	Steen G	2011	Serum pools spiked with Intralipid	Two serum pools	HC only	Clinically significant interference: $1.96 \times \sqrt{(CV_A^2 + CV_W^2)}$ Analytically significant interference: $2 \times CV_A$	AFP, Alatop (screening for inhalation allergens), CA-125, CA 15.3, CEA, Cortisol, Estradiol, Ferritin, Folate, Siemens food panel 5, fT4, FSH, hCG, LH, NT-proBNP, Progesterone, Prolactin, PSA, T3, Testosterone, Total IgE, Troponin I, TSH, Vitamin B12	Siemens Immulite 2500
[18]	Tan JG	2021	Patient serum pools spiked with SMOFlipid	Two serum pools	HC only	RCPA allowable limits of performance	Albumin, ALT, AST, Creatinine, Potassium, Sodium, Urea	Roche Cobas 6000

Table 1: (continued)

Reference list no.	First author	Year	Type of sample	No. of samples	Delipidation procedure	Criteria for effectiveness of removal protocol	Analytes	Platform
[19]	Agarwal S	2015	Patient serum pools spiked with Intralipid	Three serum pools	LC only	Total allowable error according to CLIA and CAP	AFP, aHBC, Albumin C3, C4, Ceruloplasmin, CK-MB, Glucose, Ferritin, Haptoglobin, HIV-1/2, IgA, IgG, IgM, hCG, Progesterone, Testosterone, TSH, Vitamin D	Ortho VITROS 5600 Siemens Prospec Siemens Dimension Xpand Siemens ADVIA Centaur Abbott Architect i1000SR
[20]	Van Elslande J	2021	Patient plasma sample	One patient	Dilution only	Not specified	Lipase	Roche Cobas c702
[21]	Castro-Castro MJ	2018	Patient serum/ plasma samples	Phase I: 32 samples	UC vs. HC	Limit for clinically significant interference (LCSI)=(CV_W/2) × (Xc/100), where Xc is the critical or clinical decision concentration of the parameter	Albumin, ALT, ALP, AST, Calcium, Chloride, Creatinine, GGT, Glucose, Potassium, Sodium, Total Bilirubin, Total Protein, Urea	Beckman Coulter AU5400 Roche Cobas c711/c701 Siemens ADVIA 2400
				Phase II: six labs, at least 36 samples each	HC vs. LC vs. 1,1,2-trichlorotrifluoroethane			
[22]	Hunsaker JJH	2018	Patient serum samples, and AB sera spiked with Intralipid	75 Patients, four pools	UC, LC, and dilution	≥10% baseline result	Ceruloplasmin	Abbott Architect ci8200 Beckman Coulter AU5800 Roche Cobas Integra 400 Plus
[23]	Roberts CM	2013	Patient serum/ plasma samples	40 Patients	UC vs. LC	Deming regression comparing ultracentrifuged and Lipoclear-treated samples, ±4 mEq/L (mmol/L) for sodium	Albumin, ALP, ALT, AST, Bicarbonate, Calcium, Chloride, CK, Creatinine, Glucose, Phosphate, Potassium, Sodium, Total Bilirubin, Total Protein, Urea	Beckman Coulter DxC800
[24]	Soleimani N	2020	Patient serum samples	202 Patients	UC vs. serum blank vs. dilution	Desirable specifications for bias (Westgard)	AST, Calcium, Chloride, CK, Creatinine, GGT, Glucose, Iron, LDH, Lipase, Magnesium, Phosphate, TIBC, Total Bilirubin, Total Protein, Urea, Urate	DIRUI CS-800

Table 1: (continued)

Reference list no.	First author	Year	Type of sample	No. of samples	Delipidation procedure	Criteria for effectiveness of removal protocol	Analytes	Platform
[25]	Saracevic A	2014	Patient plasma pool spiked with Intralipid	One plasma pool	HC vs. LC	Desirable specifications for imprecision (Westgard)	Albumin, ALP, ALT, Amylase, AST, Calcium, Chloride, CK, CK-MB, Conjugated Bilirubin, Creatinine, CRP, GGT, Glucose, Iron, LDH, Lipase, Magnesium, Potassium, Phosphate, Procalcitonin, Sodium, Total Protein, Total Bilirubin, Troponin T, Urea	Beckman C[illegible] AU680 Roche Cobas E411
[26]	Radišić VB	2016	Patient serum samples	34 Patients	HC vs. LC, used Roche lipase colorimetric method as reference	Desirable specifications for bias (Westgard)	Lipase	Beckman Co[illegible] AU400 Roche Cobas Integra 400 plus

AAT, alpha1-antitrypsin; AFP, alpha-fetoprotein; aHBC, anti-hepatitis B core antibody; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C3, complement protein 3; C4, complement protein 4; CAP, College of American Pathologists; CEA, carcinoembryonic antigen; CLIA, Clinical Laboratory Improvements Amendment; CK, creatine kinase; CK-MB, creatine kinase MB isoform; CRP, C-reactive protein; CV_A, analytical coefficient of variation; CV_W, coefficient of intraindividual biological variation; CV_{comb}, combined coefficient of variation incorporating both CV_A and CV_W; GGT, gamma-glutamyl transferase; fT4, free thyroxine; FSH, follicle stimulating hormone; HC, high-speed centrifugation; hCG, human chorionic gonadotropin; HIV-1/2, human immunodeficiency virus-1/2; LC, lipid clearing agent; LDH, lactate dehydrogenase; LH, luteinizing hormone; NT-proBNP, N-terminal prohormone BNP; PSA, prostate specific antigen; RCPA, Royal College of Pathologists of Australasia; T3, triiodothyronine; TG, triglyceride; TIBC, total iron binding capacity; TSH, thyroid stimulating hormone; UC, ultracentrifugation.

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spiked samples. With regards to the methods used for lipid removal, eight of the studies investigated single techniques: three studies investigated ultracentrifugation alone [13–15], three studies investigated high-speed centrifugation alone [16–18], one study investigated Lipoclear only [19], and one study studied dilution only [20]. The remaining studies compared multiple methods: of these, five studies used ultracentrifugation as the reference method for comparison with high-speed centrifugation and/or lipid-clearing agents [9, 21–24] and two studies designated high-speed centrifugation as the reference method for comparison with lipid clearing agents [21, 25]. One study used the results from the purportedly interference-free Roche lipase colorimetric assay as the reference method for comparison with high-speed centrifugation and Lipoclear [26]. Another study [24] included serum blanking amongst the methods for reducing interference; however, as serum blanking is strictly speaking not a method of lipid removal but rather a feature of the analyzer itself, the data related to serum blanking was not extracted for this review.

Lipid removal protocols

Next, we compared the protocols used for lipid removal in Table 2. Although the CLSI recommends ultracentrifugation for processing lipemic samples, the specific protocol is not provided [8]. Not surprisingly, a variety of ultracentrifugation conditions was used across the studies (Table 2.1). In general, ultracentrifugation was done at centrifugal forces of ≥100,000×*g* for ≥10 min. One study used a lower centrifugation force of 40,000×*g* but compensated for this with a longer duration of 18 h [13]. For high-speed centrifugation, a single centrifugation of ≥10,000×*g* for ≥5 min was used in all studies, with the exception of two studies that performed multiple consecutive centrifugations [9, 17]

Table 2.1: Summary of centrifugation protocols used in the studies.

Ultracentrifugation							
Reference list no.	**First author**	**Ultracentrifuge model**	**Speed**	**Duration**	**Additional special handling**	**Lipid source**	**Lipemia concentration**
[9]	Dimeski G	Beckman Coulter Airfuge	107,000×*g*	15 min		Native	TG=11.6–42.7 mmol/L
[13]	Calmarza P	Centrikon T-1080 Ultracentrifuge	40,000×*g*	18 h	4 °C	Native	TG=5.11–6.79 mmol/L
[14]	Hunsaker JJH 2019	Beckman Coulter Airfuge	178,000×*g*	10 min		Spiked	Roche Cobas L-index 595.5 ± 16.6 (equivalent to 1,268.8 ± 2.7 mg/dL TG)
[15]	Koch CD	Beckman Coulter Airfuge	167,000×*g*	10 min		Native	Roche Cobas L-index <2,000
[21]	Castro-Castro MJ	Beckman TL-100	108,200×*g*	20 min	6 °C	Native	TG=900–4,300 mg/dL
[22]	Hunsaker JJH 2018	Beckman Coulter Airfuge	178,000×*g*	10 min		Native	Roche Cobas L-index 0-2000
[23]	Roberts CM	Not specified	199,000×*g*	15 min		Native	Beckman Coulter DxC800 L-index 3 to >10
[24]	Soleimani N	Not specified	100,000×*g*	15 min		Native	TG=400–1,000 mg/dL
High-speed centrifugation							
Reference list no.	**First author**	**High-speed centrifuge model**	**Speed**	**Duration**	**Additional special handling**	**Lipid source**	**Lipemia concentration**
[9]	Dimeski G	Abbott Heraeus Biofuge	21,885×*g*	15 min × 2		Native	TG=11.6–42.7 mmol/L
[16]	Grunbaum AM	Sigma 1–14 microcentrifuge	14,000×*g*	10 min		Spiked	TG=1–80 mmol/L
[17]	Steen G	Not specified	16,000×*g*	15 min × 3		Spiked	0–380 mg/dL Intralipid (equivalent to 0–11.7 mmol/L TG)
[18]	Tan JG	Sorvall Legend Micro 21	21,100×*g*	60 min		Spiked	TG<2000 mg/dL
[21]	Castro-Castro MJ	Abbott Heraeus Biofuge	10,000×*g*	10 min		Native	TG=900–4,300 mg/dL
[25]	Saracevic A	Eppendorf Mini Spin	12,100×*g*	5 min		Spiked	300 mg/dL and 500 mg/dL Intralipid (equivalent to 9.6 and 14.2 mmol/L TG)
[26]	Radišić VB	Abbott TDX centrifuge	11,266×*g*	15 min		Native	TG=4.3–124.6 mmol/L

Table 2.2: Summary of dilution protocols used in the studies.

Reference list no.	First author	Diluent used	Dilution factor	Lipid source	Lipemia concentration
[20]	Van Elslande J	0.9% saline	20x	Native	TG=175.9 mmol/L
[22]	Hunsaker JJH 2018	Distilled water	5x	Native	Roche Cobas L-index 0-2000
[24]	Soleimani N	Distilled water	10x	Native	TG=401–3,562 mg/dL

(Table 2.1). Serum dilution was performed using distilled water for two studies [22, 24] and 0.9% 'normal' saline for one study [20] at the dilution factors listed in Table 2.2.

Analytes affected by lipemia

Supplementary Table 1 summarizes study conclusions about the effect of lipemia on analytical results.

For four of the analytes – alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), troponin I, thyroid stimulating hormone (TSH) – no significant interference from lipemia was observed consistently across studies (defined as ≥two studies deriving the same conclusion, and complete absence of contradictory studies). There was also uniform agreement between studies that lipemia adversely affected analytical results for ceruloplasmin, creatinine (Jaffe method), haptoglobin, iron, magnesium and total protein.

For several of the other analytes, there was lack of agreement between studies on whether analyte measurement was affected by lipemia, even amongst papers that used analytical methods based on the same principles. For example, of the four studies that examined lipemic interference in the measurement of phosphate using the same method (ammonium molybdate), three studies showed significant interference [13, 16, 24], but one study showed the contrary [25].

Amongst the studies, there was also significant heterogeneity with regards to the acceptance criteria used to determine if a lipid removal method was effective or not (Table 1). For instance, three studies used the desirable specification of bias as a cutoff [13, 24, 26], whereas another study plotted results obtained from ultracentrifugation and Lipoclear treatment with a Deming regression and used a 4 mmol/L difference in sodium concentration as a cutoff [23]. Others used a variety of formulas that incorporated analytical and/or intra-individual coefficients of variation [16, 17, 21].

Effectiveness of lipid removal protocols

Finally, the effectiveness of each lipid removal method were summarised according to analyte (Supplementary Table 1). Ultracentrifugation, as expected, was effective for all analytes tested (n=17).

As many laboratories use high-speed centrifugation as an alternative for ultracentrifugation, we next looked at the efficacy of high-speed centrifugation across studies. We defined concordance between studies as ≥2 studies showing the same conclusion, and complete absence of contradictory studies. From the conclusions of these studies, high-speed centrifugation was adequate for the following 13 analytes: albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, creatine kinase (CK), creatinine (Jaffe method), gamma-glutamyl transferase (GGT), glucose (hexokinase-based method), lactate dehydrogenase (LDH), phosphate, potassium, and urea.

Lipid-clearing agents were uniformly effective for seven analytes: ALT, AST, total bilirubin, CK, creatinine (Jaffe method), lipase and urea. Finally, serum dilution appeared to be ineffective for the 18 analytes tested; however, the majority of these 18 analytes were tested in single studies.

Discussion

The CLSI recommends that each laboratory establish its own guidelines for handling lipemic samples [8]. Unfortunately, the majority of manufacturer-supplied product inserts are vague about details with regards to how the interference testing was performed, making verification of these claims by individual laboratories a challenge [27, 28]. Furthermore, recommendations on how lipemic samples should be processed are usually absent. Since the extent of lipemic interference is highly dependent on the analyte and analytical method used, analyte- and method-specific information on handling lipemic samples would be invaluable in guiding laboratories on establishing their protocols.

Given this issue, there have been efforts in recent years by individual laboratories to verify reported interference claims from manufacturers. A 2015 systematic review by the Lipid Emulsion Therapy in Clinical Toxicology Workgroup

summarized the data available from these studies, but did not focus on lipid removal methods [29].

Here, we present a systematic review of the studies published since 2010 that evaluated the efficacy of lipid removal methods in patient samples for different analytes. The data obtained from these studies has been organised according to analyte and analytical method. For analytes that are affected by lipemia, we have tabulated the number of studies that either support or refute the lipid removal methods commonly used by laboratories – high-speed centrifugation, lipid-clearing agents and serum dilution. The protocols used in each paper have been also summarized.

There were a number of limitations that were observed during this review. Firstly, this review was limited in scope to papers published in the last 10 years, so that studies would be likely to have been performed on platforms that are currently still in active usage.

Another limitation is the under-representation of certain analytes in this review. Whilst the common clinical chemistry tests e.g. electrolytes and liver function tests were covered by multiple papers, 31 of the 66 analytes/test panels were studied in single papers. Noticeably, analytes such as hormones, cardiac markers and serological markers were under-represented. Therapeutic drug monitoring tests were completely absent, perhaps reflective of the general lipophilic nature of many drugs. For this reason, although serum dilution appeared ineffective for the analytes covered in this review, we note that it is probably the most reasonable approach for analytes that are concentrated in the lipid fraction, such as drugs.

For some of the analytes, we found a lack of agreement between studies with regards to whether a particular analytical method is affected by lipemia, and whether a certain method is viable for lipid removal.

We surmise that one of the reasons is the heterogeneous nature of lipemic samples used in the studies, which included native patient samples with high triglyceride concentrations and patient samples spiked with intravenous fat emulsion. VLDLs and chylomicrons vary widely in terms of particle size as well as triglyceride percentage. Therefore, the measured triglyceride concentration does not correlate well with the L-index, with a R^2 value of 0.2399 for turbid samples reported in a study [30]. Consequently, two samples with the same triglyceride concentration can have different L-indices and light scattering properties due to differences in lipoprotein composition, and therefore can be affected to different extents for the same assay. Furthermore, there is a lack of standardization between manufacturers in reporting the L-index [31].

To compound this problem, it is widely recognized that Intralipid, an intravenous fat emulsion composed of soybean oil, egg yolk phospholipids and glycerin, does not completely recapitulate the properties of native lipemic samples and therefore the effects of assay interference [32]. Specifically, Intralipid has a different refractive index from lipoproteins, and the particles in Intralipid range in size from 200 to 600 nm, therefore missing the lower and upper ranges for chylomicrons [2]. Intralipid also has sodium hydroxide added for pH adjustment, making it suboptimal for interference studies involving osmolality and electrolytes [33]. Despite this, Intralipid continues to be widely used for its convenience, and its ability to mimic lipemia that is caused by intravenous lipid emulsions.

Secondly, the concentrations of analytes tested vary from study to study. It is known that the effect of interference may be dependent on the concentration of analyte. Consequently, the CLSI recommends testing for interference at two medical decision concentrations of the analytes [34].

Furthermore, there is a lack of consensus for defining the acceptance criteria for assay interference, with studies choosing to adopt criteria from a variety of sources including manufacturer-recommended interference acceptance (usually the arbitrary 10% suggested by Glick and colleagues more than 30 years ago [35]), reference change values, external quality assurance (EQA) performance criteria and so on [36]. Some of these are based on the concept of analytically significant interference, whereas others are based on the idea of clinically significant interference. Likewise, there is also no standardized criteria for determining whether a lipid removal method produces acceptable results. This is reflected in the heterogeneity in the cutoffs used by the studies identified in this systematic review, which renders comparison difficult.

Looking ahead, the incidence of lipemic samples is likely to increase given recent trends such as the change in lipid testing guidelines allowing the use of non-fasting specimens and the acceptance of glycated hemoglobin (HbA1c) for diagnosis of diabetes mellitus [37, 38]. Unlike hemolysis or icterus, lipemia is amenable to removal and therefore laboratories can potentially intervene and reduce the number of samples that have to be rejected because of lipemia. It should be noted that rejection of a sample often deprives the clinician valuable information that may be necessary for optimal management of the patient.

From the information summarized in this systematic review, the following general recommendations may be synthesized:

(1) Manufacturers should evaluate lipidemia interference for all assays and provide the following minimum details in the product inserts: sample matrix, analyte concentration, source and concentration of lipid, lipid removal method and acceptability criteria.

(2) Laboratory should verify lipemic interference [39]. Where resources are limited, priority should be given to high impact tests where precise numerical laboratory results play a critical role in patient care, such as troponin. Additionally, laboratories may consider performing the verification exercise in a network to optimize resources.
(3) The impact of lipids on therapeutic drug monitoring should be evaluated, with particular focus on the effectiveness of sample dilution as a strategy to minimize assay interference.
(4) It is preferable to perform lipemia interference studies by spiking patient samples with high triglycerides. Ideally, multiple samples should be examined to account for heterogeneity of lipid composition among different patients.
(5) Where the use of patient samples with high triglycerides is not practical, intravenous fat emulsions may be considered as an alternative.
(6) High-speed centrifugation is an acceptable alternative to ultracentrifugation for some analytes, with a generally accepted setting of centrifugal force of >10,000×*g* for >10 min.
(7) Acceptability criteria should follow the Milan criteria [40], and be based on clinical impact, biological variation data or state-of-the-art performance. At a minimum, acceptability criteria should be based on analytical performance (i.e. state-of-the-art), such as multiples of imprecision (e.g. the reference change value concept). Arbitrary criteria that are not based on clinical or analytical performance should be avoided.

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