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Discrepancies in lipemia interference between endogenous lipemic samples and Smoflipid®-supplemented samples

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ABSTRACT

Background

Manufacturers evaluate lipemia-induced interference using Intralipid®, but it does not contain all lipoprotein types. The aim of this study was to evaluate lipemia-induced interference in biochemical parameters from endogenous lipemic samples and SMOFlipid® supplemented samples, in order to assess if SMOFlipid® can be used in lipemic interference studies.

Methods

Serum pools were supplemented with SMOFlipid® to achieve 800 mg/dL and 1500 mg/dL triglyceride concentration, and analyzed for 25 biochemical parameters both before and after the supplementation. In another independent phase, lipemic serum pools were prepared choosing patient samples of 800 mg/dL

and 1500 mg/dL triglyceride concentration. These lipemic serum pools were ultracentrifugated in order to remove lipids. Biochemical parameters were analyzed before and after ultracentrifugation. The bias between SMOFlipid®-supplemented samples and endogenous lipemic samples were compared. The bias between the lipemic and non-lipemic samples were compared with the reference change value.

Results

At 800 mg/dL triglyceride concentration, we found that total protein and transferrin had been affected only in endogenous lipemic serum samples. Magnesium and creatinine had been affected only in SMOFlipid®-supplemented samples. At 1500 mg/dL triglyceride concentration, we found that total protein, amylase, ferritin and glucose had lipemic interference only in endogenous lipemic samples, and chloride only in SMOFlipid®-supplemented samples.

Conclusions

The use of SMOFlipid®-supplemented samples does not provide suitable data to estimate lipemia-induced interference. Thus, interference studies should be performed using a wide variety of lipemic patient samples that represent the heterogeneity of the lipoprotein particles size.

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BACKGROUND

Lipemia in serum samples is a common problem in the daily practice of clinical laboratories. Analytical results may be perturbed by lipemia, leading to misdiagnosis and unnecessary treatments for patients. The overall frequency of lipemic samples ranges from 0.5% to 2.5%, with the higher percentage in primary care (1, 2).

Lipemia is defined as turbidity in serum samples produced by accumulation of lipoprotein particles. Turbidity in serum samples depends on the lipoproteins´ size and number. Chylomicrons are the largest lipoproteins (70- 1000 nm) and the principal cause of lipemia. Very low-density lipoproteins (VLDL) are classified as: small (27-35 nm), intermediate (35-60nm) and large (60-200 nm), but only intermediate and large VLDL contribute to the turbidity. Small lipoproteins particles such as high-density lipoproteins (HDL) (6-12.5 nm) and low-density lipoproteins (LDL) (20-26 nm) do not cause lipemia (3).

The most frequent cause of lipemia is postprandial hypertriglyceridemia; however, lipids and lipoproteins only change minimally in response to normal food intake. Intravenous lipid emulsion is the most common cause of severe lipemia (4, 5).

Other causes include diabetes mellitus, dyslipidemias, pancreatitis, alcohol abuse, chronic renal failure, hypothyroidism, recent parenteral nutrition (6, 7) and some treatments such as protease inhibitor, oral contraceptives, diuretics, cyclosporine and glucocorticoids (8).

Three mechanisms are mainly responsible for lipemic interference: 1) Spectral interference: lipoproteins absorbs and scatters light in a wide range of wavelengths (300 to 700 nm) and consequently exert profound effects on colorimetric, turbidimetric and nephelometric assays (9). 2) Volume displacement effect: the aqueous fraction of the serum may decrease as a consequence of the high volume of the lipid fraction, causing low values in the concentration of various analytes that are distributed in the aqueous phase of the sample (such as electrolytes) (10). 3) Non-homogeneity of the sample: due to their lower density, chylomicrons and VLDL particles are located at the top of the tube after centrifugation. Hydrophobic analytes are also distributed in that phase. Most analyzers obtain sample

from the upper part of the tube, reporting false values (3, 11).

As the lipemia-induced interference is dependent on the analytical method, manufacturers often provide guidelines for acceptable maximum lipemia established with interference studies based on spiking serum samples with commercial lipid emulsions such as Intralipid®. These interference studies are carried out without considering each parameter's individual biological variability, and using, as criterion of acceptability, only an arbitrary variation set at a 10% bias (12, 13).

Intralipid® is a commercial lipid emulsion used as a component of intravenous nutrition. Its particles' size ranges from 200 to 600 nm and lacks the sizes that mimic large VLDL, as well as the lower and upper ranges for chylomicrons size (12, 14), whereas patient samples contain a complex mixture of macromolecular lipid and protein structures (15). Therefore, lipemia induced by Intralipid® is not identical to lipemia in patient serum samples (16, 17).

Lipemia-induced interference should be verified by all clinical laboratories. SMOFlipid® is commercial available, and is a lipid emulsion of 200 mg/dL for intravenous infusion that contains soybean oil, medium chain triglyceride, olive oil, and fish oil. SMOFlipid® is an electrolyte free solution, it only contains small amounts of sodium (5 mmol/1000 mL emulsion) (18). To the best of our knowledge, there is no study that has determined SMOFlipid®'s lipoprotein size range.

The aim of this study is to determine if SMOFlipid® could be used by manufacturers to evaluate lipemia-induced interference in the analysis of biochemical parameters.

METHODS

Serum samples were taken from those routinely analyzed and frozen at −20 °C until use. Individual serums were mixed to prepare different pools using two methods:

A) Artificial lipids

50 different serum pools were prepared excluding hemolyzed, icteric and lipemic (HIL) samples based on a negative semi-quantitative HIL index. They were analyzed on AU5800 (Beckman Coulter Inc. Brea, CA, USA). Serum pools were divided into two aliquots and SMOFlipid® was added to both of them in order to achieve a final triglyceride concentration around of 800 mg/dL and 1500 mg/mL. The SMOFlipid® volume was calculated to reach the desirable triglycerides concentration, no additional diluent was added. The 800 mg/dL triglyceride concentration pools were prepared by adding 0.5 mL of SMOFlipid® to a 15 mL serum pool. To achieve the 1500 mg/dL triglyceride concentration, 1.1 mL of SMOFlipid® was added to a 15 mL serum pool. Then, biochemical parameters were remeasured to assess lipemia-induced interference. The results were multiplied by the dilution factor (1.03 for 800 mg/dL triglyceride concentration and 1.07 for 1500 mg/dL triglyceride concentration).

B) Endogenous lipids

Lipemic serum pools were prepared collecting patient samples with triglyceride concentrations around 800 mg/dL and 1500 mg/dL from routine clinical care, and rejecting hemolyzed samples based on a negative semi-quantitative haemolysis index. All collected samples had a milky or turbid appearance due to a high triglycerides concentration. To prepare each pool, 8-10 patient samples were used in order to have all lipoprotein size range. Lipemic serum pools were classified into two groups: 25 serum pools with triglyceride concentrations of 800 mg/dL and 20 serum pools with triglyceride concentrations of 1500 mg/dL. All pools were analyzed on AU5800 (Beckman Coulter Inc. Brea, CA,

USA). Consecutively, lipemia was removed by ultracentrifugation (Sorvall™ WX100+, Thermo Scientific) at 108,200x*g* for 20 minutes at 4°C. The clear infranatant was transferred into a clean tube and biochemical parameters were remeasured.

Biochemical parameters analyzed: Albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), total bilirubin, calcium, chloride, creatine kinase (CK), creatinine, ferritin, iron, phosphate, gamma-glutamyl transferase (GGT), glucose, lactate dehydrogenase (LDH), lipase, magnesium, C-reactive protein (CRP), potassium, total proteins, sodium, transferrin, urate and urea. Measurements were analyzed in duplicate. Biochemical parameters, analytical methods and lipemia-induced interference with Intralipid® provided by manufacturers are presented in table 1.

Table 1 Biochemical parameters, analytical methods and lipemia-induced interference (Intralipid®) reported by manufacturers

Biochemical parameters	Analytical methods	Lipemia-induced interference (Intralipid®)
Albumin	Bromocresol green – 600/800 nm	<10% to 800 mg/dL
Alkaline phosphatase (ALP)	p-nitrophenyl phosphate – 410/480 nm (IFCC)	<3% to 1000 mg/dL
Alanine aminotransferase (ALT)	Enzymatic spectrophotometry - 340 nm (IFCC)	<3% to 300 mg/dL
Amylase	p-nitrophenol – 410 nm (IFCC)	<3% to 1000 mg/dL
Aspartate aminotransferase (AST)	Enzymatic spectrophotometry - 340 nm (IFCC)	<5% to 300 mg/dL
Total bilirubin	Dichlorophenyldiazoniumtetrafluoroborate (DPD) – 540/660 nm	<10% to 1000 mg/dL
Calcium	Arsenazo III – 660/700 nm	<10% to 1000 mg/dL
Chloride	Indirect potentiometry	<5% to 500 mg/dL
Creatine Kinase (CK)	NADPH – 340/660 nm (IFCC)	<3% to 1000 mg/dL
Creatinine	Enzymatic spectrophotometry – 600/700 nm	<10% to 1000 mg/dL
Ferritin	Immunoturbidimetry	<10% to 1000 mg/dL

Iron	Tripyridyl-5-triazine – 600 nm	<10% to 100 mg/dL
Phosphate	Phosphomolybdate – 340/380 nm	<10% to 800 mg/dL
Γ-glutamyl transferase (GGT)	Gamma-glutamil-3-carboxilo-4-nitroanilida – 410/480 nm (IFCC)	<5 % to 1000 mg/dL
Glucose	Hexokinase – 340 nm	<10% to 700 mg/dL
Lactate dehydrogenase (LDH)	NADH – 340 nm (IFCC)	<3% to 1000 mg/dL
Lipase	4-aminophenazone – 540 nm	<10% to 500 mg/dL
Magnesium	Xylidyl blue – 520 nm	<10% to 500 mg/dL
C-reactive protein (CRP)	Immunoturbidimetry	<10% to 1000 mg/dL
Potassium	Indirect potentiometry	<5% to 500 mg/dL
Total proteins	Cupric ion – 540 nm	<10% to 1000 mg/dL
Sodium	Indirect potentiometry	<5% to 500 mg/dL
Transferrin	Immunoturbidimetry	<10% to 1000 mg/dL
Urate	Uricase – 660/800 nm	<5% to 1000 mg/dL
Urea	Urease, glutamate-deshydrogenase (GLDH) – 340 nm	<3% to 500 mg/dL

STATISTICAL ANALYSIS

Normality of distributions were analyzed using normal distribution tests. Parametric and non-parametric data for each parameter´s concentration was presented as mean ± standard deviation (mean ± SD) or median with interquartile range (median ± IQR), respectively.

The percentage differences (bias) between the lipemic and non-lipemic samples were calculated, for each parameter, according to the following formula:

$$\textbf{Bias} = (Cx - Cn) / Cn \times 100$$

where Cn represents the arithmetic mean or median from the non-lipemic samples parameters and Cx represents the arithmetic mean or median from the lipemic samples parameters (endogenous lipids or spiked with artificial lipids).

Bias were compared using the independent t-test or Mann-Whitney test depending on population distribution (significance threshold: $p<0.05$).

The reference change values (RCV), defined as the critical differences that must be exceeded between sequential results for a significant change to occur, were calculated for lipemia-induced interference in biochemical parameters, considering unilateral Z statistic with 95% confidence (Z=1.65), according to the following formula:

$$RCV = Z \cdot 2^{1/2} \cdot (CVa^2 + CVi^2)^{1/2}$$

CVa: analytical coefficient of variation. The analytical variation must be less than Cvi/2 (desirable quality specification).

Cvi: within-subject biological variation. Cvi values for the parameters were taken according to the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) biological variation database (19).

The difference between Cx and Cn is not affected by biological variation. Therefore, Cvi is considered as null. The proposed equation to define acceptance criteria by lipemia-induced interference was:

$$RCV = 1.65 \cdot 2^{1/2} \cdot (Cvi/2)$$

RCV calculated using Cvi/2 may be inappropriate depending on biological variation. It´s not possible to assume that the Cva meets the desirable quality specification for some analytes with small Cvi (for example sodium, Cvi= 0.5). Instead, RCV should be calculated using Cva extracted from the quality control level that is closest to the reference interval. Thus, the equation for these parameters was:

$$RCV = 1.65 \cdot 2^{1/2} \cdot (Cva)$$

Bias and RCV were calculated and compared for each biochemical parameter, in order to assess lipemia-induced interference. When bias exceeds RCV, the provided measurements should not be reported, as the error caused by lipemia exceeds the acceptance criteria.

Statistical analyses were performed using MedCalc for Windows version 19.6 (MedCalc Software, Ostend, Belgium).

RESULTS

Triglyceride concentrations in SMOFlipid® supplemented samples were 854 (790 – 919) mg/dL and 1462 (1427 - 1488) mg/dL.

Triglyceride concentrations in lipemic patient samples were 816 (800 - 846) mg/dL and 1520 (1481 - 1553) mg/dL. Results are represented either as: median (interquartile range) or mean ± standard deviation depending on normal distribution tests.

The biochemical parameter results, both before and after adding SMOFlipid® to the samples, are presented in table 2. Moreover, bias and RCV for each parameter are also presented in table 2. Bias exceeded RCV in SMOFlipid® supplemented samples for creatinine, lipase and magnesium at 800 mg/dL triglyceride concentration; and for all previously mentioned parameters as well as chloride and transferrin at 1500 mg/dL triglyceride concentration.

The results for the biochemical parameters from lipemic patient samples both before and after ultracentrifugation are presented in table 3. Bias and RCV can also be found in table 3. Bias exceeded RCV in lipemic patient samples for lipase, total proteins and transferrin at 800 mg/dL triglyceride concentration; and for all previously mentioned parameters as well as amylase, creatinine, ferritin, glucose and magnesium at 1500 mg/dL triglyceride concentration.

The bias in SMOFlipid-supplemented samples and bias in serum samples with endogenous lipemia were compared (table 4). At 800 mg/dL triglyceride concentration we found differences in all parameters, except albumin (p=0.1453),

Table 2 Results for the biochemical parameters both before and after adding SMOFlipid® to the serum samples at 800 mg/dL and 1500 mg/dL triglyceride concentration

Biochemical parameters	800 mg/dL triglyceride concentration			1500 mg/dL triglyceride concentration			Cvi	Cva	RCV (%)
	Mean or median before adding SMOFllipid® (SD or IQR)	Mean or median after adding SMOFlipid® (SD or IQR)	Bias (%)	Mean or median before adding SMOFlipid® (SD or IQR)	Mean or median after adding SMOFlipid® (SD or IQR)	Bias (%)			
Albumin (g/L)	39 (38 - 40)	40 (39 - 41)	2.6	42 (41 - 42)	43 (42 - 43)	2.4	2.5	1.6	2.9
Alkaline Phosphatase (U/L)	85 (78 – 91)	84 (79 - 92)	1.2	77 (73 - 82)	79 (73 - 82)	2.6	10.0	4.5	11.7
Alanine aminotransferase(U/L)	21 (19 - 24)	21 (19 - 23)	0	*	*	*	10.1	3.3	11.8
Amylase (U/L)	80 ± 17	79 ± 17	1.3	75 ± 14	74 ± 13	1.3	6.6	2.2	7.7
Aspartate aminotransferase (U/L)	24 (22 - 25)	24 (22 - 25)	0	*	*	*	9.6	2.8	11.2
Total bilirrubin (mg/dL)	0.6 (0.5 - 0.6)	0.6 (0.5 - 0.6)	0	0.7 (0.6 - 0.7)	0.7 (0.6 - 0.8)	0	21.8	3.2	25.4
Calcium (mg/dL)	9.3 (9.1 - 9.3)	9.2 (9.1 - 9.3)	-1.1	9.8 (9.6 - 9.8)	9.6 ± 0.3	2.0	2.1	1.4	2.5
Chloride (mmol/L)	104 ± 1	103 ± 2	1.0	105 (104 - 106)	103 ± 2	1.9 [b]	1.1	0.7	1.3
Creatine Kinase (U/L)	89 (75 - 113)	89 (73 - 109)	0	90 (84 - 96)	88 (83 - 96)	-2.2	15	3.0	17.5
Creatinine (mg/dL)	0.89 (0.83 - 1.01)	0.79 (0.73 - 0.89)	11.2 [a]	0.92 (0.86 - 0.96)	0.75 (0.71 - 0.78)	18.5 [b]	4.5	3.6	5.3
Ferritin (ng/mL)	185 (141 - 273)	182 (136 - 268)	1.6	143 (113 - 182)	149 (120 - 190)	4.2	12.8	1.1	14.9

Iron (µg/dL)	73 ± 18	58 ± 19	20.6	72 ± 19	55 ± 15	23.6 [b]	26.5	2.4	30.9
Phosphate (mg/dL)	3.6 ± 0.3	3.5 ± 0.3	-2.8	3.8 (3.7 – 4.0)	3.5 (3.4 - 3.6)	7.9 [b]	8.2	2.7	9.6
Γ-glutamyl transferase (U/L)	33 (27 - 46)	32 (26 - 47)	-3.0	29 (26 - 34)	28 (25 - 32)	3.45	9.1	2.6	10.6
Glucose (mg/dL)	114 ± 15.92	110 ± 16	-3.5	108 (105 - 111)	103 (101 - 106)	4.6	5.0	2.5	5.8
Lactate Dehydrogenase (U/L)	192 (182 - 199)	190 (178 - 196)	-1.0	195(183-206)	182 (177 - 188)	6.7	5.2	3.4	14.5
Lipase (U/L)	32 (28 – 38)	46 (41 - 51)	43.8 [a]	29 (26 - 32)	45 (41 - 47)	55.2 [b]	9.2	5.5	10.7
Magnesium (mg/dL)	2.0 ± 0.1	2.1 ± 0.1	5.0 [a]	3.5 (2.1 - 3.8)	2.2 ± 0.1	37.1 [b]	3.6	2.1	4.2
C-reactive protein (mg/L)	13 (8 – 21)	13 (8 - 21)	0	5 (4 - 6)	5 (4 - 6)	0	34.1	2.5	39.8
Potassium(mmol/L)	4.6 ± 0.3	4.6 ± 0.3	0	4.8 (4.7 - 4.9)	4.6 ± 0.3	4.2 [b]	4.1	0.7	4.8
Total proteins (g/L)	67 ± 5	67 (66 - 69)	0	70 (69 - 72)	68 (67 - 69)	2.9	2.6	1.7	3.0
Sodium (mmol/L)	141 ± 2	140 ± 3	-0.7	142 (141 - 143)	139 ± 2	-2.1	0.5	1.4	3.3**
Transferrin (mg/dL)	245 ± 31	236 ± 29	3.7	257 (245 - 267)	244 (240 - 254)	5.1 [b]	3.9	2.0	4.6
Urate (mg/dL)	5.2 ± 0.9	5.0 ± 0.9	-3.9	5.1 (5 - 5.3)	4.7 ± 0.6	7.8 [b]	8.6	2.1	10
Urea (mg/dL)	41 (37 - 44)	41 (37 - 44)	0	40 (37 - 41)	39 (37 - 41)	-2.5	13.9	3.3	16.2

RCV: reference change values; SD: standard deviation; IQR: interquartile range; Cva: analytical coefficient of variation; Cvi: intraindividual coefficient of variation.

[a] The bias exceed RCV at 800 mg/dL triglyceride concentration. [b] The bias exceed RCV at 1500 mg/dL triglyceride concentration.

**incalculable for negative values. **RCV calculated using Cva. 50 samples were analyzed for each variable*

Table 3 The biochemical parameters results from lipemic patient samples before and after ultracentrifugation at 800 mg/dL and 1500 mg/dL triglyceride concentration

Biochemical parameters	800 mg/dL triglyceride concentration			1500 mg/dL triglyceride concentration			Cvi	Cva	RCV (%)
	Mean or median before ultra-centrifugation (SD or IQR)	Mean or median after ultra-centrifugation (SD or IQR)	Bias (%)	Mean or median before ultra-centrifugation (SD or IQR)	Mean or median after ultra-centrifugation (SD or IQR)	Bias (%)			
Albumin (g/L)	43±1	44±1	2.9	43(43-44)	44±1	2.3	2.5	1.6	2.9
Alkaline Phosphatase (U/L)	81±16	85±17	3.9	90(84-100)	93(87-104)	3.2	10.0	4.5	11.7
Alanine aminotransferase (U/L)	27±8	25(22-29)	7.4	30(24-36)	32(25-34)	6.3	10.1	3.3	11.8
Amylase (U/L)	59±15	64±16	7.4	67±21	73±22	8.2 [b]	6.6	2.2	7.7
Aspartate aminotransferase (U/L)	29(26-35)	29(25-35)	0	36(31-39)	33(30-37)	9.1	9.6	2.8	11.2
Total bilirrubin (mg/dL)	0.4(0.3-0.4)	0.4±0.1	7.5	0.3(0.3-0.4)	0.3(0.3-0.4)	0	21.8	3.2	25.4
Calcium (mg/dL)	9.9(9.7-10.0)	10.1(9.9-10.3)	2.0	9.8(9.6-10.0)	10.0(9.9-10.2)	2.0	2.1	1.4	2.5
Chloride (mmol/L)	102±2	103±2	0.8	101(100-102)	102(101-104)	1.0	1.1	0.7	1.3
Creatine Kinase (U/L)	111±35	116±37	4.5	117±49	122±49	4.1	15	3.0	17.5
Creatinine (mg/dL)	0.91(0.86-0.99)	0.96(0.90-1.96)	5.2	0.79(0.73-0.94)	0.91(0.84-1.06)	13.2 [b]	4.5	3.6	5.3
Ferritin (ng/mL)	291(209-412)	327(245-481)	11.0	321±132	380±156	15.5 [b]	12.8	1.1	14.9

Iron (µg/dL)	84±24	91±26	8.2	76±18	85±21	10.6	26.5	2.4	30.9
Phosphate (mg/dL)	3.5±0.3	3.6±0.4	3.1	3.3(3.2-3.4)	3.5(3.4-3.6)	5.7	8.2	2.7	9.6
Γ-glutamyl transferase (U/L)	72(58-112)	79(61-118)	8.9	129±62	140±66	7.9	9.1	2.6	10.6
Glucose (mg/dL)	158(127-169)	163(131-176)	3.1	177±54	188±53	5.9 [b]	5.0	2.5	5.8
Lactate Dehydrogenase (U/L)	158±19	170±23	7.1	176±19	196±20	10.2	5.2	3.4	14.5
Lipase (U/L)	49(43-57)	41(36-51)	18.3 [a]	65(62-74)	50(44-55)	30 [b]	9.2	5.5	10.7
Magnesium (mg/dL)	2.0±0.2	2(1.9-2.0)	0.5	2.2(2.1-2.3)	2.0(1.9-2.0)	10 [b]	3.6	2.1	4.2
C-reactive protein (mg/L)	4(3-5)	4(2-5)	9.7	5(3-8)	4(3-6)	25	34.1	2.5	39.8
Potassium(mmol/L)	4.6(4.4-4.7)	4.7(4.5-4.8)	1.3	4.6(4.5-4.7)	4.7(4.6-4.7)	2.13	4.1	0.7	4.8
Total proteins (g/L)	72(71-73)	75(74-77)	4.5 [a]	71(70-72)	76(75-78)	6.58 [b]	2.6	1.7	3.0
Sodium (mmol/L)	140(139-141)	141(140-141)	0.9	138(137-140)	141(140-142)	2.13	0.5	1.4	3.3**
Transferrin (mg/dL)	278(265-291)	298(282-306)	6.7 [a]	270±26	293±29	7.85 [b]	3.9	2.0	4.6
Urate (mg/dL)	6.3±1.6	6.5±1.6	2.6	6.4(6.1-6.9)	6.7(6.4-7.2)	4.48	8.6	2.1	10
Urea (mg/dL)	37(33-39)	37(33-40)	0	36(33-40)	37(33-40)	2.70	13.9	3.3	16.2

RCV: reference change values; SD: standard deviation; IQR: interquartile range; Cva: analytical coefficient of variation; Cvi: intraindividual coefficient of variation

[a] The bias exceed RCV at 800 mg/dL triglyceride concentration [b] The bias exceed RCV at 1500 mg/dL triglyceride concentration.
***RCV calculated using Cva.*

25 samples with 800 mg/dL triglycerides concentration and 20 samples with 1500 mg/dL triglycerides concentration were analyzed for each variable.

Table 4 Statistical significance of bias between endogenous lipids and artificial lipids at 800 mg/dL and 1500 mg/dL triglyceride concentration

Biochemical parameters	800 mg/dL triglyceride concentration			1500 mg/dL triglyceride concentration		
	Endogenous lipids Bias (%)	Artificial Lipids Bias (%)	p	Endogenous lipids Bias (%)	Artificial Lipids Bias (%)	p
Albumin (g/L)	2,9	2,6	0.1453	2.3	2,4	0.9662
Alkaline Phosphatase (U/L)	3,9	1,2	0.0001	3.2	2,6	0.0391
Alanine aminotransferase(U/L)	7,4	0	0.4584	6.3	*	*
Amylase (U/L)	7,4	1,3	<0.0001	8.2	1,3	<0.0001
Aspartate aminotransferase (U/L)	0,0	0	0.3551	9.1	*	*
Total bilirrubin (mg/dL)	7,5	0	0.1319	0	0	0.4378
Calcium (mg/dL)	2,0	-1,1	0.0100	2,0	2,0	0.0005
Chloride (mmol/L)	0.8	1.0	0.0005	1.0	1.9	<0.0001
Creatine Kinase (U/L)	4.5	0	0.0001	4.1	-2.2	0.0229
Creatinine (mg/dL)	5,2	11,2	<0.0001	13.2	18,5	0.0439
Ferritin (ng/mL)	11,0	1,6	<0.0001	15.5	4,2	0.0001

Iron (µg/dL)	8,2	20,6	<0.0001	10.6	23,6	0.0001
Phosphate (mg/dL)	3,1	-2,8	0.0001	5.7	7.9	<0.0001
Gamma-glutamyl transferase (U/L)	8,9	-3,0	<0.0001	7.9	3,45	0.0017
Glucose (mg/dL)	3,1	-3,5	0.065	5.9	4,6	0.0007
Lactate Dehydrogenase (U/L)	7,1	-1,0	0.0001	10.2	6,7	<0.0001
Lipase (U/L)	18,3	43,8	<0.0001	30	55,2	<0.0001
Magnesium (mg/dL)	0,5	5,0	0.0202	10	37,1	0.0376
C-reactive protein (mg/L)	9,7	0	0.0001	25	0	<0.0001
Potassium(mmol/L)	1,3	0	0.5693	2.13	4,2	0.0591
Total proteins (g/L)	4,5	0	<0.0001	6.58	2,9	<0.0001
Sodium (mmol/L)	0,9	-0,7	0.7356	2.13	-2,11	0.0001
Transferrin (mg/dL)	6,7	3,7	<0.0001	7.85	5,1	0.0231
Urate (mg/dL)	2,6	-3,9	<0.0001	4.48	7,8	0.0054
Urea (mg/dL)	0,0	0	0.0480	2.70	-2,5	0.0268

significance threshold: p<0.05.

ALT (p=0.4584), AST (p=0.3551), total bilirubin (p=0.1319), glucose (p=0.065), potassium (p= 0.5693), and sodium (p=0.7356). At 1500 mg/dL triglyceride concentration we found differences in all parameters, except albumin, total bilirubin (p=0.4378), potassium (p=0.5693).

DISCUSSION

We analyzed whether the lipemia-induced interference was different depending on the method used to induce lipemia: artificial lipids (SMOFlipid®) or endogenous lipids (lipemic serum samples). At 800 mg/dL triglyceride concentration, we found that total protein and transferrin had been affected only in endogenous lipemic serum samples. Magnesium and creatinine had been affected only in artificial lipemic samples (SMOFlipid®). At 1500 mg/dL triglyceride concentration, we found that total protein, amylase, ferritin and glucose had lipemic interference only in endogenous lipemic samples and chloride only in artificial lipemic samples (SMOFlipid®).

Some biochemical parameters have not shown lipemia-induced interference in any assay: albumin, ALP, ALT, total bilirubin, calcium, CK, iron, phosphate, GGT, LDH, CRP, potassium, sodium, urate and urea. Lipase has shown interference induced by both artificial and endogenous lipemia at 800 and 1500 mg/dL triglyceride concentration.

Using artificial lipemic samples it is not possible to calculate bias for ALT and AST at 1500 mg/dL triglyceride concentration due to the fact that the analyzer reports negative values. However, when employing endogenous lipids, there is no lipemia-induced interference for ALT and AST.

Table 1 shows lipemia interference reported by manufacturers, they evaluate the interference using Intralipid® without considering the biological variability of the magnitudes under study. We believe that biological variability is crucial to establish acceptance criteria in many parameters. In addition, manufacturers should perform lipemia interference studies with endogenous lipids and include them in package inserts.

Previous studies have shown discordant interference results between endogenous lipemia and lipemia induced by artificial lipids. Lipemia-induced interference was not observed in some biochemical parameters when artificial lipids were used. Bornhorst et al. compared lipemia interference both using lipemic patient serum and interference induced by Intralipid® supplementation (16). Lipemia interference was evaluated in α1-antitrypsin, ceruloplasmin, haptoglobin, prealbumin and transferrin. Results showed that concentrations of ceruloplasmin, prealbumin and transferrin were significantly different in patient samples and in Intralipid®-supplemented samples (16). Koch et al. compared lipemic interference for sodium using diferents methods: direct ISE and free interference method (indirect ISE) (17). Their results show that endogenous hyperlipidemic samples have significant deviations in sodium concentration compared with Intralipid®-supplemented samples (17).

These studies evaluated lipemia-induced interference using Intralipid®. One of the strengths of the present study is that we used SMOFlipid® instead of Intralipid®. In addition, published studies have evaluated the interference for limited number of parameters (16,17,20), whereas we have evaluated the most common biochemical parameters.

Currently, lipemia interference is being evaluated using a lipemic index instead of using the triglycerides concentration. This may be inappropriate because lipemic index have limitations, they don't correlate with triglycerides concentration (21). Hunsaker et al. evaluated the lipemic index using endogenous lipids and Intralipid®, and concluded that those limits that were defined using endogenous lipids could be different from those derived from spiking studies using Intralipid® (22).

Therefore, in the present study, the endogenous lipemic samples were collected based on

triglycerides concentration instead of lipemic index. We used many lipemic patient samples to create serum pools. This is important because lipemia-induced interference depends on different sizes and types of lipid particles and, consequently, a representative sample must be chosen to ensure that all kinds of lipid particles are represented.

CONCLUSIONS

Lipemia-induced interference studies performed with artificial and endogenous lipids show discrepancies. Laboratories should verify lipemia-induced interference using endogenous lipids. These endogenous lipids should be obtained from a wide variety of lipemic patient samples that represent the heterogeneity of the lipoprotein particles size. This study is useful for laboratories that do not have the possibility of verifying manufactures´ data of lipemia-induced interference, especially in laboratories with low number of lipemic samples.

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