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Red Cell Oxygen Affinity in Severe Hypertriglyceridemia[1] (40365)

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The observed relationship between hyperlipemia and angina by Kuo *et al.* led to his hypothesis that hypertriglyceridemia might impair both oxygen uptake from the lungs and oxygen delivery to the tissues (1). Subsequent studies on oxygenation in hyperlipemia focused on the finding of arterial hypoxemia (2, 3), until Ditzel (4, 5) recently described a series of severely hyperlipemic patients with a markedly increased affinity of hemoglobin for oxygen (low P_{50}). This abnormality was unusual in that red cell 2–3 diphosphoglycerate (DPG) levels were normal, and the high oxygen affinity could be corrected by incubating the patient's red cells with normal plasma. The authors suggested that this abnormality would interfere with oxygen delivery to active muscle, providing an explanation for the observations of Kuo. Since there is no known mechanism to explain a reversible reduction in standard P_{50} (temp = 37°, pH = 7.40, PCO_2 = 40 torr) by as much as 6–10 torr in the presence of normal DPG levels, the present study was performed to further investigate the relationship between hypertriglyceridemia and increased red cell oxygen affinity.

Methods and materials. Blood was obtained from seven subjects with hypertriglyceridemia due to a variety of causes (Table I) at times when their serum was lipemic (TG = 3515 ± 2213 mg/dl, $\bar{X}$ ± SD) and from two normal subjects with triglyceride levels less than 100 mg/dl. Hemoglobin oxygen affinity (P_{50}) was measured for all subjects by the mixing technique (6) using a rotating flask tonometer (7) and Radiometer blood gas electrodes. A blood-gas O_2 correction factor (tonometer gas PO_2/tonometer blood PO_2) calculated from normal blood was measured daily and applied to all P_{50} calculations. Results were expressed as P_{50} standardized to pH of 7.40, PCO_2 of 40 torr, and temperature of 37.0° by the standard correction factors for human blood (8). Patients 5, 6 and 7 (Table I) also had P_{50} measured by the dissociation curve apparatus (DCA) of Duvelleroy *et al.* (9). Both techniques are performed routinely in our laboratory, with standard deviation of 0.5 torr by the mixing technique and 0.4 torr with the DCA apparatus from eleven aliquots of the same sample of human blood. DPG concentrations, expressed as μg/ml of packed red cells, were measured by the method of Detter *et al.* (10). The blood–gas oxygen correction factors for normal and hypertriglyeridemic blood were compared by tonometering samples for 30 min with 21%, 7%, or 4.5% O_2 prior to blood PO_2 measurement, using the flask tonometer and blood gas electrodes described.

Incubation studies comparing normal blood (plasma TG=72 mg/dl) with hypertriglyceridemic blood (plasma TG=1625 mg/dl) were performed after the separated red cells were washed and spun three times in buffered normal saline. Three serial twofold saline dilutions of both the normal and lipemic plasma were prepared. One volume of packed normal red cells was added to 1 vol of each normal plasma sample, and 1 vol of packed lipemic red cells was added to 1 vol of each lipemic plasma sample. The mixed samples were tonometered for 30 min with room air prior to blood gas measurements. Spectrophotometric measurements of hemoglobin concentration and oxygen saturation were made on a model 182 Cooximeter (Instrumentation Laboratories) calibrated with normal human blood. Oxygen content of the tonometered resuspended mixtures was measured directly with a Lex-O_2-Con (Lexington) oxygen analyzer.

Results. In this group of severely hypertriglyceridemic subjects, the mean P_{50} measured

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TABLE I. RBC OXYGEN AFFINITY IN LIPEMIA.

Subject	Cause of hypertriglyceridemia	Plasma triglyceride mg/dl	P_{50} STD (torr) mixing technique	P_{50} STD (torr) duvelloroy apparatus	DPG μg/ml packed RBC
1	Familial and untreated diabetes	6600	28.6	—	4.4
2	Primary lipoprotein lipase deficiency	6048	27.3	—	4.3
3	Broad beta disease and untreated diabetes	2190	27.0	—	6.6
4	Primary lipoprotein lipase deficiency	970	29.0	—	4.3
5	Primary lipoprotein lipase deficiency	4560	27.7	19.1	3.6
6	Familial and untreated diabetes	2475	26.2	20.8	4.9
7	Familial and estrogen therapy	1764	28.2	22.0	5.2
		3515 ± 2213	27.7 ± 1.0	20.6 ± 1.5	4.8 ± 1.0

by the mixing technique was not different from normal (Table I). A substantial discrepancy was observed in P_{50} values measured concurrently by the DCA technique in subjects 5–7. The mean DPG concentration was normal, although there was considerable scatter in this value which did not correlate with the measured P_{50}. This variability may be related to the need to express DPG values per unit of packed red cells rather than per gram hemoglobin, since lipemic plasma causes an artifact in the spectrophotometric measurement of hemoglobin concentration (11). Although the plasma from the DCA chamber after a run showed evidence of hemolysis, in no case did the hematocrit fall by more than 2%. It thus appears that there was insufficient free hemoglobin to account for the decrease in P_{50} by the DCA measurement.

The blood–gas oxygen correction factors calculated at three PO_2 values on the tonometered lipemic blood (Table II) show that the correction value in the PO_2 range of the measured P_{50} is only about 5% greater than that for normal blood. Although these differences are relatively small, a second experiment demonstrated that this error is magnified considerably when lipemic blood remains in contact with the PO_2 electrode for fifteen minutes. In this study either lipemic blood or normal blood was held on the electrode for 15 min and then a sample of normal blood tonometered in 5% CO_2 and 20% O_2 was drawn into the chamber and the measured PO_2 was recorded. The baseline correction factor was 1.07, the correction factor after 15 min of incubation with normal blood was 1.31, and the factor with lipemic blood was 2.00. Thus prolonged contact between lipemic blood and the PO_2 electrode such as occurs during a DCA measurement can result in a measurement error of sufficient magnitude to account for the P_{50} differences observed between the mixing technique and the DCA.

TABLE II. PO_2 CORRECTION FACTORS FOR NORMAL AND LIPEMIC BLOOD.

Tonometer PO_2 torr	Normal blood correction	Lipemic blood correction
148	1.07	1.21
50	1.03	1.08
32	1.03	1.08

The final experiment was performed to expand on the previous observation that the low (DCA) P_{50} of lipemic blood could be corrected by incubation with normal plasma (5). The washed red cells of normal and lipemic blood were incubated in a tonometer with serial saline dilutions of the normal and lipemic plasma, respectively. Measurement of O_2 content, O_2 saturation and PO_2 for each dilution of the blood samples (Fig. 1) shows that while the O_2 content of the lipemic blood remains unchanged with saline dilution of the plasma, there was a progressive increase in both measured O_2 saturation and measured PO_2. This discrepancy was not seen with serial dilution of normal plasma. (The blood from the normal subject had a higher hematocrit, accounting for the higher measured oxygen content at all dilutions.)

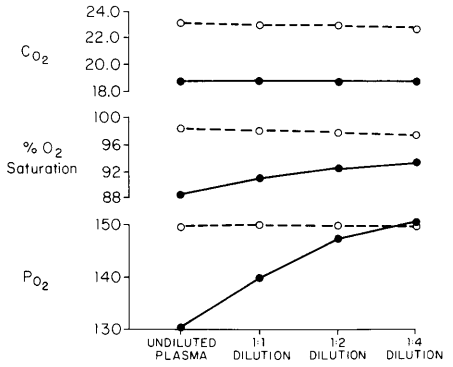

FIG. 1. Effects of serial dilution of lipemic (·—·) and normolipidemic (0– – –0) plasma with saline on measured oxygen content (CO_2, by Lex-O_2-Con), saturation (by Co-oximeter) and partial pressure (by PO_2 electrode.

Discussion. The affinity of hemoglobin for oxygen in blood from severely hypertriglyceridemic patients is normal, and our findings suggest that the previous reports of low P_{50} values measured with the DCA apparatus in these patients are incorrect because of the inaccuracies associated with measurement of PO_2 in lipemic plasma. Lipemic plasma interferes with measurement by the standard methods of both PO_2 and hemoglobin oxygen saturation. The DCA apparatus is particularly susceptible to this PO_2 measurement artifact, as the inscription of a full dissociation curve requires that the oxygen electrode be in contact with the blood for up to 15 min. This problem with the measurement of PO_2 in hypertriglyceridemic blood was noted by Sundstrom *et al.* (12) in a study of patients receiving infusions of a triglyceride emulsion (Intralipid), which has physiological properties similar to chylomicrons *in vivo.* They also reported that there was an artifactual decrease in spectrophotometrically measured oxygen saturation, although the relation of these effects to triglyceride levels was not discussed. Blood with added Intralipid gives a falsely elevated hemoglobin concentration by spectrophotometric measurement (11), and thus the per cent oxygen saturation calculated from this measurement is underestimated. Neither we nor others (5, 12) could demonstrate any effect on the measured PO_2 of Intralipid added to tonometered samples. The reason for this discrepancy may be related to uncharacterized physical chemical differences between chylomicrons and Intralipid particles.

Ditzel (5) had suggested that the increase oxygen affinity could be related to defects in the red cell membrane. Clinical reports have suggested that red cells from some severely lipemic patients are susceptible to hemolysis ("Zieve's Syndrome"), but in fact *in vivo* hemolysis has not been demonstrable in these patients (13). Lipemic blood is susceptible to *in vitro* hemolysis however (13), and this was apparent from the appearance of the lipemic plasma after exposure to the magnetic stirrer in the DCA apparatus in the present study. When the P_{50} was measured in lipemic blood by the mixing technique following 15 min of stirring in the DCA apparatus, the value was 2–3 torr less than the initial mixing technique value, suggesting that *in vitro* erythrocyte damage could also decrease the P_{50}. Nevertheless the DCA induced red cell trauma was not sufficient to decrease the hematocrit by more than 2%, and presumably the major portion of the low P_{50} artifact was related to the oxygen electrode problems.

These results coupled with the findings of Sundstrom *et al.* (12) suggest that previous reports of low arterial PO_2 values or low arterial O_2 saturation related to high triglyceride concentrations should be reevaluated with careful attention to the blood–gas correction factors for lipemic plasma. At present any abnormality of either arterial oxygenation or tissue oxygen delivery remains unestablished, and other factors need be sought to explain the clinical findings of lipemia associated angina or decreased exercise tolerance (14).

Summary. The clinical manifestations of impaired oxygen transport in severely hypertriglyceridemic patients have been attributed to a reversible increase in red cell oxygen affinity (low P_{50}) in recent studies. In seven patients with comparably lipemic plasma (triglyceride levels 970–6600 mg/dl) the mean standard P_{50} measured by the mixing technique was normal. However when measurements were repeated on three of the samples using the Duvelleroy dissociation curve apparatus, the measured P_{50} was decreased by 5–9 torr. This difference was secondary to a time dependent interference of the lipemic plasma with the blood O_2 electrode, increas-

ing the blood–gas O_2 correction factor. The red cell oxygen affinity of subjects with severe hypertriglyceridemia is normal and other explanations need be sought for the clinical observations suggesting a decrease in tissue oxygen delivery.

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